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Original Article

Toxicity of repeated 28-day oral administration of acenaphthylene in rats

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ABSTRACT — To assess the toxicity of acenaphthylene, Sprague-Dawley rats were repeatedly administered the chemical *via* oral gavage at daily doses of 0, 4, 20, or 100 mg/kg/day for 28 days, followed by a 14-day recovery period. Decreases in body weight, food consumption, and body weight gain were observed in males and females in the 100 mg/kg/day group. Additionally, increases in water consumption and urine volume, and decreases in osmolality were observed in both males and females in this group. Moreover, this highest dose was linked to decreases in the reticulocyte percentage and increases in platelet counts in males and females, and females additionally exhibited increases in the hemoglobin concentration, mean corpuscular hemoglobin concentration, and activated partial thromboplastin time. Meanwhile, total cholesterol and phospholipid levels were elevated in males and females treated with 100 mg/kg/day acenaphthylene, with males additionally displaying increased total protein and albumin levels. Increased relative liver weights and changes in liver histopathology were observed in males and females treated with 20 or 100 mg/kg/day acenaphthylene. Additionally, organ weight and/or histopathological changes were observed in the thymus, heart, femoral and sternal bones including bone marrow, urinary bladder, kidneys, spleen, and adrenal gland in both sexes, in the stomach in males, and in the uterus, ovaries, and mesenteric lymph nodes in females in the 100 mg/kg/day group. Some changes exhibited plasticity in the recovery period. Based on these results, the no-observed-effect-level of acenaphthylene after repeated 28-day oral administration was 4 mg/kg/day.

Key words: Acenaphthylene, Body weight loss, Hematological toxicity, Hepatotoxicity, Urinalysis

INTRODUCTION

The toxicity of acenaphthylene was evaluated at the request of the Office of Chemical Safety, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (MHLW), Japan. The results of repeated 28-day oral administration of acenaphthylene in rats are presented in the present report. Acenaphthylene, which is used in the electronic industry (NITE, 2017), is classified by the United States Environmental Protection Agency (US EPA) as a 16 polycyclic aromatic hydrocarbon, the concentration of which in edible mushrooms ranges from 0.02 to 3.37 mg/kg (Igbiri *et al.*, 2017). According to a carcinogenicity assessment for lifetime exposure by the Integrated Risk Information System, acenaphthylene was not classifiable as a human carcinogen based on data indicating no carcinogenicity in mice and a mutagenesis study

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using *Salmonella typhimurium* (US EPA, 1991; Cook, 1932; Kaden *et al.*, 1979; Nagpal, 1993). The data for human toxicity of acenaphthylene have not been reported (US EPA, 1991). Previous findings for the subchronic oral administration of acenaphthylene in rats include considerable body weight loss, unspecified changes in the peripheral blood pattern, changes in renal function, and increased serum aminotransferase activity (Knobloch *et al.*, 1969).

This study was designed to meet the Test Guidelines for Toxicology Studies issued by the "Notification test methods of New Chemical Substances" of MHLW, Ministry of Economy, Trade and Industry (METI), Ministry of the Environment (MOE) (Yakuhatsu No. 1121002, 2003, Seikiyoku No. 2, 2003, and Kanpoki No. 031121002, 2006), and OECD Guidelines for the Testing of Chemicals for "repeated dose 28-day oral toxicity study in rodents" (TG 407), and it was conducted in compliance with the Good Laboratory Practice Standard of MHLW, METI, and MOE (Yakuhatsu No. 1121003, 2003, Seikiyoku No. 3, 2003, and Kanpoki No. 031121004, 2008) for criteria for test facilities for conducting tests on a new chemical substance in Japan.

MATERIALS AND METHODS

The present study was conducted from 2009 to 2010 at BoZo Research Center Inc. (Shizuoka, Japan). This experiment was approved by the institutional animal care and use committee and conducted in compliance with the Act on Welfare and Management of Animals (Act No. 105, 1973), Standards for the Care, Keeping and Pain Reduction of Laboratory Animals (MOE Notification No. 88, 2006), and Guidelines for Proper Conduct of Animal Experiment (Science Council of Japan, 2006).

Test substance and reagent

Acenaphthylene (Lot No. 7-MOM, purity 96.3%, CAS No. 208-96-8) was obtained from Nippon Steel Chemical Co., Ltd. (Tokyo, Japan) and stored in a test substance storage room (light-shielded) at 3-7°C (Gotenba Laboratory, BoZo Research Center Inc. Shizuoka, Japan). Methylcellulose (MC), 400 cP (Lot No. EWM1974) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), and 0.5 w/v% MC solution was prepared in distilled water (Lot No. 8K74, Otsuka Pharmaceutical Factory, Inc. Tokushima, Japan) as the vehicle.

Animals and breeding condition

Specific-pathogen-free Sprague-Dawley (SD) rats (Crl: CD [SD]) of both sexes were purchased from the Atsugi Breeding Center of Charles River Laboratories Japan, Inc. (Kanagawa, Japan) at 5 weeks of age. After arrival, 47 male and 47 female rats were acclimatized to the testing environment for 8 days. After observation (once/day), body weight measurements (three times), and detailed observation (once), 36 healthy rats of each sex (24 rats for the main group and 12 rats for the recovery group for each sex) were selected for the test. Administration of the test substance was initiated at 6 weeks of age for both sexes. The body weight ranges for males and females at the initiation of treatment were 209-234 and 147-177 g, respectively. We configured each group so that the average body weight of each group was as similar as possible. Assignment of an individual rat was based on a randomized block design using a computer. Animals were individually housed in bracket type wire-mesh steel cages (W250 × D350 × H200 mm) and kept in a room under controlled conditions as follows: temperature, 21-25°C; humidity, 46-61%; ventilation, 10-15 times/hr; and illumination, 12 hr/day (lights on: 7:00-19:00). The animals were fed a pellet diet (Lot Nos. 090309 and 090407, CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and provided *ad libitum* access to tap water in bottles. The animals were individually identified by ear tag upon arrival.

Selection of dose levels

The doses of acenaphthylene used in the present study were based on the results of a 14-day dose range-finding study conducted in the same strain of rats (five males and five females for each group) at doses of 0 (0.5 w/v% MC solution as vehicle control), 100, 300, and 1000 mg/kg/day. In the dose range-finding study, all males and females that received 1000 mg/kg/day died, and changes in organ weights were observed in rats treated with 100, 300, and 1000 mg/kg/day acenaphthylene. Therefore, in the present study, 100 mg/kg/day was set as the highest dose, and the middle and low doses were set at 20 and 4 mg/kg/day, respectively, using a common ratio of 5.

Experimental design

Rats were administered acenaphthylene *via* oral gavage once daily at doses of 0 (0.5 w/v% MC solution), 4, 20, or 100 mg/kg/day for 28 days. The 0 and 100 mg/kg/day groups each included 12 males and 12 females, whereas the remaining groups included six males and six females. The administered volume was 5 mL/kg body weight. One day after the end of the treatment period, six males and six females from each group were euthanized to assess hematology, blood biochemistry, and organ weights and conduct macroscopic and microscopic examinations of

tissues and organs. The remaining animals in the 0 and 100 mg/kg/day groups were permitted a 14-day recovery period before a full examination.

Animal observation and assessments

All animals were observed for clinical signs of toxicity at three timepoints (before administration, after administration, and 2 hr after administration) during the administration period and once during the recovery period. Detailed clinical observations including observations in the home cage (posture, convulsions, and abnormal behavior), in-the-hand observations [ease of removal from the cage, condition of the fur and skin, eye and nose secretion, eye ball (exophthalmos and palpebral closure), visible mucous membrane, autonomic function (lacrimation, piloerection, pupil diameter, salivation, abnormal breathing, and handling reactivity)], and open-field observations [wakefulness, convulsions, abnormal behavior, stereotyped behavior, walking, posture, grooming, rising number of times, and excrement (number of bowel movements and urination)] were recorded *via* scoring before treatment and weekly during the administration and recovery periods. In week 4 of the administration period (Day 26 for males and day 27 for females) and week 2 of the recovery period (Day 13), functional observations including auditory response, approaching response, contact reaction, pain reaction, pupillary reflex, aerial righting reaction, and landing foot splay were recorded. In addition, grip strength (forelimb/hindlimb) was measured using CPU gage model-9502A (Aikoh Engineering Co., Ltd., Osaka, Japan), and spontaneous motor activity was recorded for 1 hr at 10-min using model NS-AS01 (Neuroscience, Inc., Tokyo, Japan). Body weight was recorded before treatment on days 1, 4, 7, 10, 14, 17, 21, 24, and 28 of the administration period and days 1, 3, 7, 10, and 14 of the recovery period. Food consumption was measured before chemical administration on days 1, 7, 14, 21, and 28 of the administration period and on days 7 and 14 of the recovery period.

Urinalysis, hematology, and clinical biochemistry

Urinalysis was performed during week 4 (Day 23) of the administration period and week 2 (Day 9) of the recovery period. The 4-hr urine output after fasting was collected and analyzed using a dipstick for parameters such as pH, protein, ketone bodies, glucose, occult blood, bilirubin, urobilinogen, color, sediments, and urine volume. The volume and osmolality of the 20-hr urine output after non-fasting was measured using a Spitz tube and automatic osmometer (OM-6030, Arkray, Inc., Kyoto, Japan). One-day water intake from the previous day was measured using a water bottle under conditions in which animals remained in cages equipped for urine collection.

One day after the end of the administration and recovery periods, blood was collected from the abdominal aorta under deep ether anesthesia after overnight fasting. One portion of the blood was treated with EDTA-2K and examined for hematologic parameters such as red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte percentage (Reticul), platelets (PLTs), white blood cells (WBCs), and differential leukocyte counts [lymphocytes (LYMP), neutrophils (NEUT), eosinophils (EOS), basophils (BASO), monocytes (MONO), and large unstained cells (LUC)]. Another blood sample was treated with 3.8% sodium citrate, and blood clotting parameters, such as prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB), were analyzed.

Blood serum was analyzed for alkaline phosphatase (ALP), total cholesterol (T-CHO), triglyceride (TG), phospholipid (PL), total bilirubin (T-BIL), glucose, blood urea nitrogen (BUN), creatinine (CRNN), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), inorganic phosphorus (P), total protein (TP), and albumin (ALB) levels and the albumin/globulin ratio (A/G). Meanwhile, plasma isolated from heparinized blood was analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH), and gamma-glutamyl transpeptidase (γ-GTP) content.

Organ weights, gross necropsy, and histopathology

After blood collection, all animals were sacrificed by exsanguination, and organs and tissues including the head, breasts, and abdomen were observed macroscopically. The brain, adrenal glands, thymus, spleen, heart, liver, kidneys, testes/epididymides (for males), and ovaries/uterus (for females) were weighed before fixation. In addition, relative organ weights (ratio of organ weight to body weight) were calculated.

The cerebrum, cerebellum, spinal code (chest), sciatic nerve, eye ball, pituitary gland, thyroid, parathyroid, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, trachea, lungs (including the bronchium), stomach, duodenum, jejunum, ileum (including Peyer's patches), cecum, colon, rectum, liver, kidneys, bladder, testes, epididymides, prostate, ovaries, uterus, sternum (including bone marrow), femur (including bone marrow), and skeletal muscle in the

femur were fixed in 10 v/v% phosphate-buffered formalin. In addition, optic nerve and eyeballs were fixed with phosphate-buffered 3 v/v% glutaraldehyde/2.5 v/v% formalin solution, the lungs were injected with phosphate-buffered 10 v/v% formalin, and testes and epididymides were filled with Bouin's solution and stored in 10 v/v% phosphate-buffered formalin. The Harderian gland, thoracic aorta, tongue, esophagus, submandibular gland, sublingual gland, pancreas, vagina, seminal vesicles, mammary gland (groin), skin (groin), individual recognition site (pinna), and throat were stored in 10 v/v% phosphate-buffered formalin. Sections of paraffin-embedded tissues for microscopic examination were stained with hematoxylin-eosin. For bilateral organs, one side was examined *via* microscopy.

Data analysis

The results for open-field observation, functional observation, body weight, food consumption, urinalysis, hematological and blood biochemistry findings, and organ weight analysis (including relative organ weight) were analyzed using Bartlett's test ($P < 0.01$ was considered significant) for homogeneity of distribution. If homogenous, Dunnett's multiple comparison test was conducted, and if not homogenous, Dunnett's type mean rank sum test was conducted to compare the control and individual treatment groups. Data obtained during or after the recovery period were analyzed using the *F*-test for homogeneity of distribution. If the data were homogenous, Student's *t*-test was conducted, and in the absence of homogeneity, Aspin-Welch's *t*-test was conducted for comparison (*, $P < 0.05$; **, $P < 0.01$).

RESULTS

General clinical observation

Salivation was sporadically observed after day 16 in females in the 100 mg/kg/day group, and unkempt fur and emaciation were observed in two and one female, respectively, in this group after day 24 during the administration period. No changes in clinical signs were observed in any animals during the recovery period.

Detailed clinical and functional observations, grip strength, and spontaneous motor activity

Detailed clinical observation: During the administration period, no significant changes were observed in treated groups compared with the findings in control rats in week 1. Slight salivation was observed in one female in the 100 mg/kg/day group. A significant decrease in rising times was observed in females in the 100 mg/kg/day group in week 2 in open-field observation. In week 3, slight or moderate salivation was observed in three females in the 100 mg/kg/day group, and rising times were significantly depressed in females in this group. Slight unkempt fur and salivation were observed in two and four female rats, respectively, in the 100 mg/kg/day group in week 4. Additionally, rising times were significantly decreased in males in the 100 mg/kg/day group in week 1 of the recovery period. No significant changes were observed in either sex in the 100 mg/kg/day group compared with the findings in control rats in week 2 of the recovery period.

Functional observation: In week 4 of the administration period, weak auditory responses were observed in three males in the 100 mg/kg/day group, and a weak tail pinch response was noted in one female. No significant changes were observed in males or females in the 100 mg/kg/day group relative to control rats in week 2 in the recovery period.

Grip strength: Significant decreases in forelimb grip strength were observed in females in the 100 mg/kg/day group in week 4 in the administration period, and significant decreases in forelimb and hind limb grip strength were observed in both sexes in this group in week 2 of the recovery period.

Spontaneous motor activity: During the administration period, significant increases in spontaneous motor activity were observed in females treated with 20 mg/kg/day acenaphthylene after 20-30 min of measurement in week 4 of the administration period, whereas spontaneous motor activity was significantly depressed in males in the 100 mg/kg/day group during the same observation period. Significant decreases in activity were observed in both sexes in the 100 mg/kg/day group during 0-10 and 10-20 min of measurement in week 4, and significant decreases were observed in males in this group during 0-60 min of measurement in week 4. Spontaneous motor activity was significantly increased in females in the 100 mg/kg/day group after 40-50 min of measurement in week 2 of the recovery period.

Body weight and food consumption

Significant decreases in body weight were observed in males and females in the 100 mg/kg/day on days 4-28 and 10-28 during the administration period, respectively. Moreover, significant decreases in body weight gain were observed in both sexes in the 100 mg/kg/day group during the administration period. Significant decreases in body weight were observed in both sexes in the 100 mg/kg/day group throughout the entire recovery period. Significant decreases in food consumption were noted for both sexes

in the 100 mg/kg/day on days 7-28 during the administration period and day 7 during the recovery period.

Urinalysis and water intake

Small round epithelial cells were observed in the urine sediment of 1 of 12 females in the control group, 1 of 6 males in the 20 mg/kg/day group, and 4 of 12 males and 2 of 12 females in the 100 mg/kg/day group. A trend toward significance was noted for males in the 100 mg/kg/day group versus the control group. Increases in water intake and urine volume and significant decreases in osmolality were observed in both sexes in the 100 mg/kg/day group at the end of the administration period. At the end of the recovery period, small round epithelial cells were observed in two males and two females in the 100 mg/kg/day group, which aligned with the trend of increased numbers of animals with small round epithelial cells in urine sediment. Significant increases in urine volume and decreases in osmolality were observed in females and both males and females, respectively, in the 100 mg/kg/day group at the end of the recovery period.

Hematology

Hematological results are summarized in Table 1. Significant decreases in RBC counts were noted in males in the 4 mg/kg/day group and females in the 20 mg/kg/day group. Significant increases in HGB levels and the MCHC in females, significant decreases in Reticul and increases in PLT counts in both sexes, and a prolonged APTT in females were observed in the 100 mg/kg/day group. Significant decreases in LYMP counts were observed in females in the 4 mg/kg/day group at the end of administration period. Significant increase in Reticul in both sexes, increases in PLT counts and decreases in FIB levels in males, and decreases in the BASO rate and count in males were observed in the 100 mg/kg/day group at the end of the recovery period.

Clinical biochemistry

Clinical chemistry results are summarized in Table 2. At the end of the administration period, significant decreases in AST and CRNN levels were observed in females in the 100 mg/kg/day group, and increases in T-CHO and PL levels were observed in both sexes in this group. In the 4 mg/kg/day group, significant increases in TG levels and decreases in P levels were observed in males and females, respectively. Significant decreases in BUN levels were observed in females in the 20 mg/kg/day group. Additionally, TP and ALB levels were significantly elevated in males in the 100 mg/kg/day group. At the end of the recovery period, significant increases were noted for P levels in both sexes in the 100 mg/kg/day group compared to the control group levels, whereas GLU levels were decreased in males. Additionally, increases in T-CHO and PL levels, and decreases in TP and ALB levels and A/G were observed in females in this group.

Organ weights

The results of organ weight analyses are summarized in Table 3. At the end of the administration period, significant decreases in final body weight were observed in both sexes in the 100 mg/kg/day group. Significant increases in the relative brain weight in females, decreases in the absolute and relative thymus weights and absolute ovary weight in females, decreases in the absolute heart weight in both sexes, decreases in the absolute spleen and relative heart weights in males, increases in the relative kidney weight in both sexes, increases in the relative adrenal gland weight in males, were observed in the 100 mg/kg/day group. The relative liver weight was significantly increased in males and females treated with doses of 20 or 100 mg/kg/day. At the end of the recovery period, the final body weight was significantly decreased in both sexes in the 100 mg/kg/day group. Significant increases in the relative brain weight in both sexes, decreases in the absolute thymus weight in males, increases in the relative thymus weight in females, and decreases in the absolute heart weight in both sexes were recorded in the 100 mg/kg/day group. Other changes in this group included increases in the relative heart weight in males, decreases in the absolute liver, absolute adrenal gland, absolute testis, absolute epididymis, and absolute kidney weights in males, increases in the relative liver, relative adrenal gland, and relative spleen weights in females, increases in the relative kidney weight in both sexes, and increases in the relative testis and relative epididymis weights in males.

Gross necropsy

Unkempt fur and undernourishment were observed in two and one female in the 100 mg/kg/day group, respectively. Small uterus size was noted in two females in the 100 mg/kg/day group at the end of the administration period. Renal pelvic dilatation and small thyroid size were each observed in one female in the 100 mg/kg/day group.

Histopathology

Treatment-related histopathological changes are summarized in Table 4. Effects of the test substance were observed in the adrenal gland, femur, sternum, kidneys, liver, mesenteric lymph nodes, spleen, stomach, thymus, bladder, and uterus at the end of the administration peri-

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

Dose (mg/kg/day)	At the end of the administration period				At the end of the recovery period	
	0	4	20	100	0	100
Male						
Number of animals:	6	6	6	6	6	6
RBC, $\times 10^4/\mu L$	819 ± 30	784 ± 14*D	804 ± 19	848 ± 19	851 ± 33	853 ± 44
HGB, g/dL	16.0 ± 0.5	15.5 ± 0.4	15.9 ± 0.4	16.5 ± 0.4	15.6 ± 0.4	15.2 ± 0.2
HCT, %	45.2 ± 2.1	43.4 ± 1.1	45.3 ± 0.6	45.7 ± 1.3	44.2 ± 1.6	43.2 ± 0.6
MCV, fL	55.2 ± 2.5	55.3 ± 0.7	56.5 ± 1.3	53.9 ± 0.9	51.9 ± 1.7	50.8 ± 2.9
MCH, pg	19.6 ± 0.8	19.7 ± 0.4	19.7 ± 0.5	19.4 ± 0.3	18.3 ± 0.4	17.9 ± 0.8
MCHC, g/dL	35.4 ± 0.6	35.6 ± 0.4	35.0 ± 0.5	36.0 ± 0.6	35.3 ± 0.5	35.3 ± 0.6
Reticul, %	2.3 ± 0.6	2.4 ± 0.5	2.5 ± 0.2	1.5 ± 0.4*D	2.3 ± 0.4	3.3 ± 0.7*T
PLT, $\times 10^4/\mu L$	122.9 ± 24.1	124.7 ± 3.2	133.2 ± 9.9	157.2 ± 13.6**DT	111.3 ± 11.0	125.3 ± 8.3*T
PT, sec.	15.7 ± 3.0	13.8 ± 1.2	16.7 ± 2.2	15.4 ± 2.2	14.7 ± 1.6	14.5 ± 1.0
APTT, sec.	24.9 ± 3.8	22.9 ± 3.0	26.4 ± 7.2	25.4 ± 4.6	21.2 ± 2.6	23.2 ± 3.7
FIB, mg/dL	305 ± 19	299 ± 26	311 ± 30	322 ± 6	304 ± 14	275 ± 19*T
WBC, $\times 10^2/\mu L$	106.5 ± 45.1	114.9 ± 44.1	129.4 ± 43.0	96.6 ± 14.5	120.4 ± 27.3	103.7 ± 31.4
Differential leukocyte count (%)						
LYMP	78.0 ± 4.3	78.3 ± 6.8	79.2 ± 1.8	82.5 ± 3.5	82.4 ± 4.2	76.5 ± 5.3
NEUT	18.1 ± 4.7	17.6 ± 6.4	16.4 ± 1.7	13.6 ± 3.9	13.8 ± 3.6	19.3 ± 5.2
EOS	0.9 ± 0.2	1.0 ± 0.4	1.1 ± 0.1	0.9 ± 0.2	1.1 ± 0.4	1.1 ± 0.3
BASO	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.1*T
MONO	2.1 ± 1.2	2.3 ± 0.6	2.2 ± 0.6	2.0 ± 0.5	1.7 ± 0.4	2.1 ± 0.5
LUC	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.4	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Differential leukocyte count ($\times 10^2/\mu L$)						
LYMP	83.6 ± 36.9	91.9 ± 42.1	102.9 ± 35.4	79.9 ± 13.8	99.8 ± 27.3	78.7 ± 21.4
NEUT	18.8 ± 9.0	18.6 ± 5.3	21.0 ± 6.3	12.9 ± 3.5	16.0 ± 3.7	20.6 ± 10.8
EOS	0.9 ± 0.4	1.1 ± 0.3	1.4 ± 0.4	0.9 ± 0.2	1.3 ± 0.5	1.1 ± 0.5
BASO	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1*T
MONO	2.1 ± 1.0	2.4 ± 0.3	2.7 ± 0.8	1.9 ± 0.5	2.0 ± 0.4	2.2 ± 0.8
LUC	0.7 ± 0.4	0.6 ± 0.2	1.0 ± 0.6	0.6 ± 0.2	0.9 ± 0.2	0.7 ± 0.3
Female						
Number of animals	6	6	6	6	6	6
RBC, $\times 10^4/\mu L$	796 ± 34	771 ± 26	745 ± 29*D	836 ± 45	818 ± 24	806 ± 28
HGB, g/dL	15.2 ± 0.7	15.1 ± 0.3	14.8 ± 0.2	16.2 ± 0.8*D	15.2 ± 0.2	14.9 ± 0.6
HCT, %	42.0 ± 2.1	41.6 ± 0.9	41.2 ± 0.6	43.2 ± 2.0	42.2 ± 0.5	41.6 ± 2.0
MCV, fL	52.8 ± 2.4	54.0 ± 1.0	55.4 ± 1.8	51.8 ± 1.9	51.7 ± 1.1	51.6 ± 1.4
MCH, pg	19.1 ± 0.9	19.5 ± 0.4	19.9 ± 0.8	19.4 ± 0.5	18.6 ± 0.4	18.5 ± 0.4
MCHC, g/dL	36.1 ± 0.4	36.2 ± 0.3	35.9 ± 0.6	37.4 ± 0.8**D	36.0 ± 0.4	35.9 ± 0.6
Reticul, %	2.0 ± 0.4	2.0 ± 0.5	2.8 ± 0.8	1.2 ± 0.4*D	1.8 ± 0.6	2.6 ± 0.5*T
PLT, $\times 10^4/\mu L$	150.4 ± 8.3	143.6 ± 10.5	140.3 ± 4.5	175.0 ± 15.5*D	125.9 ± 13.6	138.2 ± 20.2
PT, sec.	12.0 ± 0.2	11.8 ± 0.5	11.6 ± 0.4	12.1 ± 0.6	12.0 ± 0.5	11.7 ± 0.5
APTT, sec.	18.1 ± 2.7	19.5 ± 3.3	18.2 ± 2.0	24.2 ± 2.9**D	17.5 ± 3.2	19.1 ± 2.9
FIB, mg/dL	227 ± 28	237 ± 33	229 ± 34	228 ± 14	218 ± 11	226 ± 16
WBC, $\times 10^2/\mu L$	69.1 ± 17.8	74.1 ± 24.7	88.7 ± 44.0	65.3 ± 22.6	58.0 ± 14.4	73.3 ± 21.0
Differential leukocyte count (%)						
LYMP	81.9 ± 5.3	74.1 ± 5.8*D	78.1 ± 5.9	80.5 ± 4.1	74.0 ± 10.4	74.4 ± 9.1
NEUT	14.5 ± 4.8	21.4 ± 5.5	17.5 ± 5.9	16.3 ± 4.4	21.3 ± 9.8	21.1 ± 8.1
EOS	1.1 ± 0.4	1.5 ± 0.7	1.2 ± 0.6	0.5 ± 0.2	1.4 ± 0.5	1.4 ± 0.7
BASO	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
MONO	1.5 ± 0.6	2.1 ± 0.6	2.0 ± 0.4	1.8 ± 0.8	2.2 ± 0.6	1.9 ± 0.6
LUC	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.6 ± 0.3	0.9 ± 0.3	0.9 ± 0.1
Differential leukocyte count ($\times 10^2/\mu L$)						
LYMP	56.2 ± 12.7	55.8 ± 21.9	70.2 ± 38.4	53.1 ± 20.2	43.0 ± 12.9	54.2 ± 16.9
NEUT	10.3 ± 5.6	15.1 ± 4.3	14.8 ± 6.0	10.0 ± 1.8	12.2 ± 6.5	15.7 ± 7.6
EOS	0.7 ± 0.5	1.0 ± 0.3	0.9 ± 0.2	0.3 ± 0.2	0.8 ± 0.4	1.1 ± 0.7
BASO	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
MONO	1.1 ± 0.5	1.5 ± 0.4	1.7 ± 0.6	1.2 ± 0.8	1.3 ± 0.6	1.4 ± 0.5
LUC	0.6 ± 0.3	0.6 ± 0.3	0.8 ± 0.5	0.5 ± 0.4	0.6 ± 0.3	0.7 ± 0.2

Values are expressed as the mean ± standard deviation.
*: $P < 0.05$; **: $P < 0.01$ (significant compared to the control group).
D: Dunnett's multiple comparison test. DT: Dunnett-type rank test. T: Student's *t*-test.

Table 2. Biochemical findings in male and female rats in repeated 28-day oral administration of acenaphthylene and the 14-day recovery period.

Dose (mg/kg/day)	At the end of the administration period				At the end of the recovery period	
	0	4	20	100	0	100
Male						
Number of animals	6	6	6	6	6	6
AST, IU/L	63 ± 7	60 ± 7	61 ± 6	56 ± 10	67 ± 8	62 ± 6
ALT, IU/L	27 ± 2	26 ± 3	26 ± 5	28 ± 7	29 ± 6	28 ± 2
LDH, IU/L	52 ± 7	49 ± 8	60 ± 18	67 ± 17	64 ± 9	61 ± 9
γ-GTP, IU/L	1 ± 0	1 ± 0	1 ± 0	1 ± 1	1 ± 0	1 ± 1
ALP, IU/L	770 ± 163	644 ± 129	695 ± 183	558 ± 146	531 ± 97	655 ± 199
T-CHO, mg/dL	48 ± 12	56 ± 8	49 ± 5	72 ± 17**D	65 ± 19	68 ± 10
TG, mg/dL	53 ± 15	94 ± 27*D	67 ± 19	69 ± 23	92 ± 48	52 ± 9
PL, mg/dL	86 ± 17	101 ± 12	95 ± 11	121 ± 19**D	110 ± 21	111 ± 12
T-BIL, mg/dL	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
GLU, mg/dL	126 ± 15	128 ± 11	140 ± 17	120 ± 8	143 ± 12	123 ± 14*T
BUN, mg/dL	13 ± 2	12 ± 1	11 ± 1	12 ± 2	12 ± 1	13 ± 1
CRNN, mg/dL	0.24 ± 0.02	0.25 ± 0.03	0.22 ± 0.02	0.26 ± 0.03	0.26 ± 0.01	0.28 ± 0.04
Na, mmol/L	144 ± 1	144 ± 1	144 ± 1	143 ± 1	144 ± 1	144 ± 1
K, mmol/L	4.7 ± 0.2	4.6 ± 0.3	4.9 ± 0.4	4.8 ± 0.2	4.5 ± 0.2	4.8 ± 0.4
Cl, mmol/L	107 ± 2	106 ± 1	107 ± 1	107 ± 2	105 ± 2	106 ± 1
Ca, mg/dL	9.8 ± 0.3	9.7 ± 0.3	9.9 ± 0.2	10.0 ± 0.3	9.4 ± 0.3	9.6 ± 0.3
P, mg/dL	7.7 ± 0.6	8.1 ± 0.5	7.9 ± 0.5	7.2 ± 0.7	7.2 ± 0.4	8.2 ± 0.6**T
TP, g/dL	6.1 ± 0.2	6.0 ± 0.1	6.3 ± 0.2	6.5 ± 0.2*D	6.4 ± 0.2	6.4 ± 0.2
ALB, g/dL	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.3 ± 0.1*D	3.2 ± 0.1	3.3 ± 0.1
A/G	1.05 ± 0.07	1.05 ± 0.06	1.03 ± 0.06	1.05 ± 0.06	0.99 ± 0.10	1.10 ± 0.10
Female						
Number of animals	6	6	6	6	6	6
AST, IU/L	64 ± 6	68 ± 7	62 ± 3	48 ± 2**D	59 ± 4	56 ± 4
ALT, IU/L	21 ± 3	25 ± 6	22 ± 3	20 ± 3	22 ± 6	21 ± 3
LDH, IU/L	52 ± 16	54 ± 8	53 ± 12	63 ± 9	49 ± 9	46 ± 7
γ-GTP, IU/L	1 ± 0	1 ± 1	1 ± 0	1 ± 1	1 ± 0	1 ± 0
ALP, IU/L	423 ± 80	407 ± 75	421 ± 154	335 ± 65	231 ± 49	291 ± 45
T-CHO, mg/dL	57 ± 8	71 ± 12	70 ± 10	88 ± 11**D	63 ± 13	87 ± 7**T
TG, mg/dL	15 ± 7	16 ± 7	20 ± 7	24 ± 6	23 ± 14	25 ± 5
PL, mg/dL	99 ± 12	121 ± 15	120 ± 16	130 ± 17**D	115 ± 23	138 ± 11*T
T-BIL, mg/dL	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
GLU, mg/dL	105 ± 8	103 ± 8	101 ± 12	114 ± 17	122 ± 12	116 ± 13
BUN, mg/dL	17 ± 3	15 ± 1	13 ± 2*D	14 ± 3	16 ± 2	16 ± 3
CRNN, mg/dL	0.31 ± 0.04	0.31 ± 0.04	0.28 ± 0.03	0.24 ± 0.03**D	0.32 ± 0.04	0.31 ± 0.03
Na, mmol/L	143 ± 1	142 ± 1	143 ± 1	142 ± 1	143 ± 1	143 ± 1
K, mmol/L	4.7 ± 0.4	4.7 ± 0.3	4.4 ± 0.3	5.0 ± 0.6	4.1 ± 0.2	4.4 ± 0.5
Cl, mmol/L	110 ± 1	110 ± 1	110 ± 2	109 ± 1	109 ± 1	108 ± 2
Ca, mg/dL	9.8 ± 0.2	9.9 ± 0.2	9.8 ± 0.2	9.6 ± 0.3	9.9 ± 0.4	9.5 ± 0.2
P, mg/dL	8.0 ± 0.9	6.9 ± 0.5*D	7.5 ± 0.6	7.1 ± 0.9	6.7 ± 0.6	7.8 ± 0.6**T
TP, g/dL	6.2 ± 0.2	6.4 ± 0.2	6.1 ± 0.4	6.4 ± 0.5	6.7 ± 0.3	6.3 ± 0.2*T
ALB, g/dL	3.2 ± 0.1	3.4 ± 0.2	3.2 ± 0.1	3.4 ± 0.2	3.6 ± 0.2	3.2 ± 0.2**T
A/G	1.09 ± 0.06	1.11 ± 0.15	1.11 ± 0.10	1.16 ± 0.09	1.17 ± 0.10	1.03 ± 0.08*T

Values are expressed as the mean ± standard deviation. *: $P < 0.05$; **: $P < 0.01$ (significant compared to the control group).
D: Dunnett's multiple comparison test. T: Student's *t*-test.

od.

Specifically, minimal diffuse hypertrophy of the zona glomerulosa in the adrenal gland was observed in one female in the 20 mg/kg/day group and three males and two females in the 100 mg/kg/day group. Minimal or mild decreases in the hypocellularity of femoral bone marrow were observed in one male and two females treated with 100 mg/kg/day acenaphthylene. Minimal decreases in the hypocellularity of sternal bone marrow were observed in one male and two females in the 100 mg/kg/day group. Minimal-to-moderate basophilic changes and minimal single cell necrosis in renal tubules were observed in all rats in the 100 mg/kg/day group. Additional changes observed in the 100 mg/kg/day group included minimal pigmentation in Kupffer cells in two males and one female, minimal single cell necrosis of hepatocytes in two males and one female, and minimal hypertrophy of centrilobular hepatocytes in five males and all females. Meanwhile, minimal hypertrophy of centrilobular hepatocytes was observed in five males in the 20 mg/kg/

Table 3. Absolute and relative organ weights in male and female rats in repeated 28-day oral administration of acenaphthylene and the 14-day recovery period.

Item			Absolute, g				Relative, g/100 g BW			
Dose (mg/kg/day)			0	4	20	100	0	4	20	100
At the end of the administration period	Male	Body weight	371 ± 54	406 ± 26	370 ± 20	297 ± 44**D	-	-	-	-
		Heart	1.27 ± 0.18	1.30 ± 0.07	1.24 ± 0.12	0.92 ± 0.17**D	0.34 ± 0.02	0.32 ± 0.01	0.33 ± 0.02	0.31 ± 0.03*D
		Liver	10.88 ± 2.17	12.18 ± 1.47	12.22 ± 1.17	11.69 ± 1.70	2.92 ± 0.22	2.99 ± 0.22	3.30 ± 0.19**D	3.94 ± 0.17**D
		Spleen	0.77 ± 0.18	0.77 ± 0.12	0.71 ± 0.12	0.58 ± 0.06*D	0.21 ± 0.03	0.19 ± 0.02	0.19 ± 0.03	0.20 ± 0.03
		Kidney (R+L)	2.73 ± 0.44	2.96 ± 0.25	2.85 ± 0.15	2.53 ± 0.28	0.74 ± 0.07	0.73 ± 0.06	0.77 ± 0.01	0.86 ± 0.08**D
	Female	Body weight	217 ± 18	226 ± 10	225 ± 21	164 ± 26**D	-	-	-	-
		Heart	0.79 ± 0.05	0.78 ± 0.11	0.80 ± 0.09	0.57 ± 0.08**D	0.36 ± 0.01	0.34 ± 0.03	0.36 ± 0.03	0.35 ± 0.01
		Liver	5.85 ± 0.64	6.31 ± 0.68	6.71 ± 0.76	5.65 ± 0.83	2.70 ± 0.08	2.79 ± 0.20	2.98 ± 0.14*D	3.45 ± 0.21**D
		Spleen	0.44 ± 0.08	0.46 ± 0.07	0.52 ± 0.12	0.33 ± 0.06	0.20 ± 0.02	0.21 ± 0.03	0.23 ± 0.03	0.20 ± 0.02
		Kidney (R+L)	1.56 ± 0.19	1.65 ± 0.06	1.75 ± 0.13	1.67 ± 0.20	0.72 ± 0.05	0.73 ± 0.04	0.78 ± 0.02	1.04 ± 0.17**DT
At the end of the recovery period	Male	Body weight	480 ± 33	-	-	334 ± 22**T	-	-	-	-
		Heart	1.41 ± 0.08	-	-	1.06 ± 0.06**T	0.29 ± 0.02	-	-	0.32 ± 0.01*T
		Liver	13.69 ± 1.36	-	-	10.05 ± 1.13**T	2.85 ± 0.22	-	-	3.01 ± 0.21
		Spleen	0.79 ± 0.08	-	-	0.66 ± 0.14	0.17 ± 0.01	-	-	0.20 ± 0.04
		Kidney (R+L)	3.14 ± 0.41	-	-	2.58 ± 0.30*T	0.65 ± 0.06	-	-	0.77 ± 0.08*T
	Female	Body weight	250 ± 24	-	-	210 ± 5**AT	-	-	-	-
		Heart	0.83 ± 0.05	-	-	0.73 ± 0.02**AT	0.33 ± 0.03	-	-	0.35 ± 0.01
		Liver	6.68 ± 0.69	-	-	6.29 ± 0.33	2.68 ± 0.12	-	-	3.00 ± 0.11**T
		Spleen	0.45 ± 0.07	-	-	0.45 ± 0.06	0.18 ± 0.02	-	-	0.22 ± 0.03*T
		Kidney (R+L)	1.68 ± 0.15	-	-	1.78 ± 0.16	0.67 ± 0.03	-	-	0.85 ± 0.07**AT

Values are expressed as the mean ± standard deviation of six rats.
*: $P < 0.05$; **: $P < 0.01$ (significant compared to the control group).
D: Dunnett's multiple comparison test. DT: Dunnett-type rank test. AT: Aspin-Welch *t*-test.

Table 4. Histopathological findings of repeated 28-day oral administration of acenaphthylene in rats at the end of the administration period and the recovery period.

Item		At the end of the administration period								At the end of the recovery period			
Organs	Sex:	Male				Female				Male		Female	
Findings	Dose (mg/kg/day):	0	4	20	100	0	4	20	100	0	100	0	100
	Number:	6	6	6	6	6	6	6	6	6	6	6	6
Adrenal													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		6	6	6	3	6	6	5	4	5	3	6	5
Hypertrophy, glomerular, diffuse		0	0	0	3	0	0	1	2	1	3	0	1
Minimal		0	0	0	3	0	0	1	2	1	3	0	1
Bone + Bone marrow, femoral													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		6	6	6	5	6	6	6	4	6	6	6	6
Hypocellularity, bone marrow		0	0	0	1	0	0	0	2	0	0	0	0
Minimal		0	0	0	1	0	0	0	1	0	0	0	0
Mild		0	0	0	0	0	0	0	1	0	0	0	0
Bone + Bone marrow, sternal													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		6	6	6	5	6	6	6	4	6	6	6	6
Hypocellularity, bone marrow		0	0	0	1	0	0	0	2	0	0	0	0
Minimal		0	0	0	1	0	0	0	2	0	0	0	0
Kidney													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		3	3	2	0	5	5	4	0	3	0	5	0
Dilatation, tubular		0	0	0	0	0	0	0	1	0	0	0	0
Minimal		0	0	0	0	0	0	0	1	0	0	0	0
Dilation, pelvic		0	0	0	0	0	0	0	0	0	0	0	1
Moderate		0	0	0	0	0	0	0	0	0	0	0	1
Regeneration, tubular		3	1	3	1	1	0	1	2	2	1	0	1
Minimal		3	1	3	1	1	0	1	2	2	1	0	0
Mild		0	0	0	0	0	0	0	0	0	0	0	1
Basophilic change, tubular		0	0	0	6	0	0	0	6	0	6	0	6
Minimal		0	0	0	1	0	0	0	0	0	4	0	5
Mild		0	0	0	3	0	0	0	1	0	2	0	1
Moderate		0	0	0	2	0	0	0	5	0	0	0	0
Mineralization, corticomedullary		0	1	1	0	0	0	0	0	0	0	1	0
Minimal		0	1	1	0	0	0	0	0	0	0	1	0
Cell infiltration, interstitial		0	1	2	1	0	0	1	1	2	3	0	0
Minimal		0	1	2	1	0	0	1	1	2	3	0	0
Necrosis, single cell, tubular		0	0	0	6	0	0	0	6	0	0	0	0
Minimal		0	0	0	6	0	0	0	6	0	0	0	0
Nephroblastoma		0	0	0	0	0	1	0	0	0	0	0	0
Present		0	0	0	0	0	1	0	0	0	0	0	0
Liver													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		1	0	0	0	0	1	0	0	3	0	1	2
Vacuolation, hepatocyte, periportal		2	3	1	1	2	3	5	1	0	0	3	0
Minimal		2	3	1	1	2	2	5	1	0	0	2	0
Mild		0	0	0	0	0	1	0	0	0	0	1	0
Pigmentation, Kupffer cell		0	0	0	2	0	0	0	1	0	4	0	0
Minimal		0	0	0	2	0	0	0	1	0	4	0	0
Hematopoiesis, extramedullary		0	0	0	1	0	0	0	0	0	1	0	1
Minimal		0	0	0	1	0	0	0	0	0	1	0	1
Hemorrhage, focal		0	0	1	0	0	0	0	0	0	0	0	0
Minimal		0	0	1	0	0	0	0	0	0	0	0	0
Microgranuloma		4	6	5	4	6	5	5	2	3	5	5	4
Minimal		4	6	5	4	6	5	5	2	3	5	5	4
Necrosis, single cell hepatocyte		0	0	0	2	0	0	0	1	0	0	0	0
Minimal		0	0	0	2	0	0	0	1	0	0	0	0
Hypertrophy, hepatocytic, central		0	0	5	5	0	0	0	6	0	3	0	1
Minimal		0	0	5	5	0	0	0	6	0	3	0	1

Table 4. (Continued).

Item		At the end of the administration period								At the end of the recovery period			
Organs	Sex:	Male				Female				Male		Female	
Findings	Dose (mg/kg/day):	0	4	20	100	0	4	20	100	0	100	0	100
	Number:	6	6	6	6	6	6	6	6	6	6	6	6
Lymph node, mesenteric													
Number examined		6	0	0	6	6	6	6	6	0	0	6	6
Not remarkable		6	0	0	6	6	6	6	3	0	0	6	6
Atrophy		0	0	0	0	0	0	0	3	0	0	0	0
Minimal		0	0	0	0	0	0	0	3	0	0	0	0
Spleen													
Number examined		6	0	0	6	6	6	6	6	0	0	6	6
Not remarkable		5	0	0	3	6	6	6	4	0	0	5	5
Atrophy, lymphoid		0	0	0	0	0	0	0	2	0	0	0	0
Minimal		0	0	0	0	0	0	0	2	0	0	0	0
Hematopoiesis, extramedullary		1	0	0	3	0	0	0	0	0	0	1	1
Minimal		1	0	0	3	0	0	0	0	0	0	1	1
Stomach													
Number examined		6	6	6	6	6	0	0	6	6	6	0	0
Not remarkable		6	6	6	4	6	0	0	6	6	6	0	0
Erosion, glandular stomach		0	0	0	2	0	0	0	0	0	0	0	0
Minimal		0	0	0	2	0	0	0	0	0	0	0	0
Thymus													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		6	6	6	3	6	6	6	2	6	6	6	6
Atrophy		0	0	0	3	0	0	0	4	0	0	0	0
Minimal		0	0	0	2	0	0	0	3	0	0	0	0
Mild		0	0	0	1	0	0	0	0	0	0	0	0
Moderate		0	0	0	0	0	0	0	1	0	0	0	0
Urinary bladder													
Number examined		6	6	6	6	6	6	6	6	6	6	6	6
Not remarkable		6	6	6	2	6	6	6	0	6	6	6	6
Hypertrophy, umbrella cell		0	0	0	4	0	0	0	6	0	0	0	0
Minimal		0	0	0	4	0	0	0	6	0	0	0	0
Uterus													
Number examined		-	-	-	-	6	6	6	6	-	-	6	6
Not remarkable		-	-	-	-	6	6	6	4	-	-	6	6
Atrophy		-	-	-	-	0	0	0	2	-	-	0	0
Mild		-	-	-	-	0	0	0	2	-	-	0	0

-: Not applicable.

day group. Minimal atrophy in mesenteric lymph nodes and minimal atrophy in lymph follicles of the spleen were observed in three and two females treated with 100 mg/kg/day acenaphthylene, respectively. Minimal erosion in the glandular stomach was observed in two males treated with the highest dose. Minimal-to-moderate atrophy was observed in the thymus in three males and four females in the 100 mg/kg/day group. Minimal hypertrophy in umbrella cells of the urinary bladder was observed in four males and all females in the 100 mg/kg/day group. Mild atrophy in uterus was observed in two females in the 100 mg/kg/day group, in which small uteri were observed on gross necropsy. The other findings were determined to be incidental considering the histopathological characteristics or the situation.

The test substance also had evident effects on the adrenal gland, kidneys, and liver at the end of the recovery period, including minimal diffuse hypertrophy of the zona glomerulosa of the adrenal gland in three males and one female in the 100 mg/kg/day group, although this finding was also observed in one control male. Minimal or mild basophilic change in renal tubules was observed in all rats (including one female with renal pelvic dilatation on gross necropsy) in the 100 mg/kg/day group. Minimal pigmentation in Kupffer cells in four males and minimal centrilobular hypertrophy in hepatocytes in three males and one female were observed in the 100 mg/kg/day group. The other findings in the liver, kidneys, spleen, and thyroid were all determined to be incidental considering the histopathological characteristics or the situation.

DISCUSSION

This report examined the toxicity of acenaphthylene,

including its plasticity. Crl: CD (SD) rats were administered acenaphthylene in 0.5 w/v% MC solution by gavage at doses of 0, 4, 20, or 100 mg/kg/day for 28 days, and animals in the control and highest dose groups were subjected to a 14-day recovery period after treatment.

Regarding general clinical observations including detailed clinical signs, salivation was observed in females treated with 100 mg/kg/day acenaphthylene, and some unkempt fur and emaciation were observed. Moreover, decreases in rising times were observed, and these changes were reversed after the treatment was halted. The sporadic weak auditory response in males, and the weak tail pinch response in a female were observed in the 100 mg/kg/day group. Decreases in the spontaneous activity in both sexes were observed in 100 mg/kg/day acenaphthylene. Decreases in the grip strength of the forelimbs were observed in females treated with 100 mg/kg/day acenaphthylene. Moreover, decreases in the grip strength of the forelimbs and hindlimbs were observed in both sexes in week 2 of the recovery period in the 100 mg/kg/day group. No histopathological effects of acenaphthylene were observed in the peripheral and the central nervous systems; however, the influence of the test substance in these changes was suspected. In female rats, increases in spontaneous motor activity were observed in the 20 mg/kg/day group in week 4 of the administration period and in the 100 mg/kg/day group in week 2 of the recovery period. However, these changes were considered incidental because they were slight and temporary.

Decreases in body weight and food consumption were observed in both sexes in the 100 mg/kg/day group, and decreases in body weight gain were also noted. These findings are consistent with a previous report illustrating that the oral administration of 600 mg/kg acenaphthylene for 32 days or 300 mg/kg acenaphthylene for 30 days resulted in considerable body weight loss (Knobloch *et al.*, 1969). Body weight declined in both sex in the 100 mg/kg/day group during the recovery period. However, body weight gain and food consumption on day 14 of the recovery period in this group were comparable to the findings in the control group. Therefore, these changes appeared to exhibit plasticity.

In the urinalysis, a trend toward increases in small round epithelial cell numbers in urine sediment was observed in males in the 100 mg/kg/day group. Increases in water intake and urine volume and decreases in osmolality were observed in both sexes in the 100 mg/kg/day group, and effects of the test substance on the kidneys were suspected at the end of the administration. A trend toward increases in small round epithelial cell numbers, an increase in urine volume, and decreases in osmolality were observed at the end of the recovery period in both sexes in the 100 mg/kg/day group. Therefore, these changes were not plastic in the 14-day recovery period.

Regarding hematology, decreases in Reticul and increases in PLT counts were observed in both sexes in the 100 mg/kg/day group, and increases in HGB levels and the MCHC and a prolonged APTT were additionally observed in females in this group. The mechanisms underlying these changes are unknown; however, the test substance was suspected to be responsible. Increases in PLT counts were observed in males in the 100 mg/kg/day group at the end of recovery period. However, because the increasing trend was reduced and increased Reticul were observed, plasticity of the effects of acenaphthylene was demonstrated. Decreases in RBC counts were observed in males in the 4 mg/kg/day group and females in the 20 mg/kg/day group, and decreases in LYMP counts were observed in females in the 4 mg/kg/day group. However, these changes were considered incidental because they were slight and absent in the highest dose groups. A previous report in subchronic oral administration of 600 mg/kg acenaphthylene showed decreasing trend in LYMP, however, these changes may be unspecific, since the LYMP counts (%) showed decrease in the control group (Knobloch *et al.*, 1969). Decreases in FIB levels and BASO rate or counts were observed at the end of the recovery period in males that received 100 mg/kg/day acenaphthylene. However, these changes were deemed incidental because they were slight and were not observed at the end of the administration period.

Concerning clinical biochemistry, increases in T-CHO and PL levels were observed in both sexes in the 100 mg/kg/day group, and increases in TP and ALB levels were observed in males in this group; thus, the test substance was suspected to have effects on the liver. At the end of the recovery period, increases in T-CHO and PL levels were observed in females in the 100 mg/kg/day group; however, the effects of the test substance were plastic because these changes were diminished in the recovery period. Decreases in AST and CRNN levels were observed at the end of the administration period in females in the 100 mg/kg/day group; however, these changes were not considered important because they were slight and the values were small, indicating that the test substance was not involved. Moreover, a previous report of subchronic administration of 300 mg/kg acenaphthylene showed the significant increase in AST activity (Knobloch *et al.*, 1969). Increases in TG levels, decreases in BUN levels, and decreases in P levels were observed in males in the 4 mg/kg/day group, females in the 20 mg/kg/day group, and females in the 4 mg/kg/day group, respec-

tively. However, these changes were considered incidental because they were slight and were not observed in the highest dose groups. Moreover, at the end of the recovery period, increases in P levels in both sexes, decreases in GLU levels in males, and decreases in TP and ALB levels and A/G in females were observed in the 100 mg/kg/day group. However, these changes were considered incidental because they were slight and were not observed at the end of the administration period.

On pathological examination, unkempt fur, small uteri, and malnutrition were macroscopically observed in females in the 100 mg/kg/day group. Hypertrophy of centrilobular hepatocytes was observed in males treated with 20 or 100 mg/kg/day acenaphthylene and females treated with 100 mg/kg/day acenaphthylene. Pigmentation in Kupffer cells and single cell necrosis in hepatocytes were observed in both sexes in the 100 mg/kg/day group. Moreover, trends of increases in atrophy of the thymus, decreases in hypocellularity of the femur, femoral bone marrow, sternum and sternal bone marrow, basophilic changes in tubules and single cell necrosis in renal tubules, hypertrophy in umbrella cells of urinary bladder, and hypertrophy of zona glomerulosa cells in the adrenal gland were observed in both sexes in the 100 mg/kg/day group. Erosion in the glandular stomach was observed in males in the 100 mg/kg/day group, and atrophy of mesenteric lymph nodes, lymph follicles of the spleen, and the uterus was observed in females in this group. In the organ weight analyses, increases in the relative liver weight was observed in both sexes treated with acenaphthylene doses of 20 or 100 mg/kg/day, and decrease in the absolute heart weight in both sexes, decreases in the relative heart and absolute spleen weights in males and decreases in the absolute and relative thymus and absolute ovary weights in females were observed in the 100 mg/kg/day group at the end of the administration period. At the end of the recovery period, a trend of increasing incidences of lesions, centrilobular hypertrophy in hepatocytes in both sexes, pigmentation in Kupffer cells in males, basophilic changes in tubules in both sexes, and hypertrophy of the zona glomerulosa of the adrenal gland in males were observed in the 100 mg/kg/day group. However, these lesions except those in the adrenal gland were reduced or reversed during the recovery period, suggesting plasticity of the effects. Increases in the relative brain, kidney, and adrenal gland weights at the end of the administration period; changes in the absolute and relative thymus, heart, liver, kidney, adrenal gland, testis, and epididymis weight; and increases in the relative brain and spleen weights at the end of the recovery period were considered due to the repression of body weight gain. Dilatation in the renal pelvis and small thymus were observed in females in the 100 mg/kg/day group; however, these changes were considered incidental. The aforementioned changes in hematology and clinical biochemistry might be related to previous findings indicating changes in the peripheral blood pattern and serum aminotransferase activities following subchronic oral administration of acenaphthylene (Knobloch *et al.*, 1969).

Based on these results, especially considering that the administration of acenaphthylene increases relative liver weight in both male and female rats treated with a dose of 20 or 100 mg/kg/day and hypertrophy in centrilobular hepatocytes in male rats treated with these doses, the no-observed-effect-level of acenaphthylene was determined to be 4 mg/kg/day for both sexes. In addition, although the changes in grip strength and urinalyses in both sexes and adrenal gland lesions in male rats were observed during or at the end of the recovery period, the other findings were reduced or reversed, indicating the plasticity of the effects of acenaphthylene.

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

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