J Toxicol Pathol 2024; 37: 151–161

Original Article

A 13-week subchronic toxicity study of linalool oxide in Crl:CD(SD) rats

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Abstract: Linalool oxide is frequently used as a flavoring agent, however, data on its toxicity is limited. In this study, we performed a 13-week subchronic toxicity study of linalool oxide (furanoid) in male and female Crl:CD(SD) rats. Doses of 0, 80, 250, and 800 mg/kg body weight (bw) per day were orally administered by gavage, using corn oil as the vehicle. Abnormal gait in both sexes and decreased locomotor activity in males were observed in the 800 mg/kg group. Reduced body weight gain was noted in both sexes at 800 mg/kg and at 250 mg/kg in males. In the 800 mg/kg group, serum biochemistry showed increased γ-glutamyl transpeptidase and decreased glucose in both sexes, increased total protein in males, and increased total cholesterol and phospholipids in females, suggesting that linalool oxide may have adverse effects on the liver. Increased relative and/or absolute liver weights, centrilobular hepatocellular hypertrophy in both sexes, and periportal microvesicular fatty changes in females were observed in the 800 mg/kg group. Increased relative liver weights and decreased serum glucose levels were observed in the 250 mg/kg male and female groups, respectively. Increased serum magnesium levels and relative kidney weights were observed in both sexes in the 800 mg/kg group, suggesting possible adverse effects of linalool oxide. Although histopathology showed accumulation of hyaline droplets in the male kidneys, immunohistochemistry revealed α_{2u}-globulin nephropathy, which was not considered toxicologically significant. These results indicate that the no-observed-adverse-effect level of linalool oxide was 80 mg/kg bw/day for both sexes. (DOI: 10.1293/tox.2024-0012; J Toxicol Pathol 2024; 37: 151–161)

Key words: linalool oxide, food additive, flavoring agent, subchronic toxicity, Sprague-Dawley rat

Introduction

Linalool oxide is a colorless to pale-yellow liquid with a floral odor and is widely used as a flavoring agent. It is officially registered on the list of designated additives in Japan and is listed in the Code of Federal Regulations (CFR) as one of the food additives permitted for direct addition to food for human consumption (21 CFR 172.515) in the United States of America (USA). The Joint Food and Agricultural Organization/Word Health Organization Expert Committee on Food Additives has evaluated linalool oxide (CAS no. 1365-19-1, including both furanoids and pyranoids) as a flavoring agent, categorized as a tetrahydrofuran and furanone derivative[1]. Linalool oxide was assigned to Class II based on the structural class of flavoring agents, and the estimated intake levels in the USA and Europe are 85 and 14 μg/person/day, respectively; these values are below the Class II threshold of 540 μg/person/day. Based on these estimates, linalool oxide is not considered a safety concern during its routine use as a flavoring agent.

As part of the risk assessment, linalool oxide was shown to be nonmutagenic and nonclastogenic in the Ames test and chromosomal aberration test[2]. Only few *in vivo* studies have been performed to date, some of which are oral acute toxicity tests in male and female rats (LD50:1,150–2,210 mg/kg body weight [bw])[1]. Despite the use of linalool oxide as a flavoring agent, there are limited data concerning its repeated-dose toxicity. Our laboratory has used rodent models to investigate the toxicity of several food additives, including representative flavoring agents from each category[3–9]. Here, we conducted a 13-week subchronic toxicity study of linalool oxide orally administered to Crl:CD(SD) rats by gavage to clarify the toxicological profile and establish a no-observed-adverse-effect level (NOAEL) for this chemical compound.

Received: 9 February 2024, Accepted: 17 June 2024
Published online in J-STAGE: 2 July 2024
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Materials and Methods

Test chemical

Linalool oxide (furanoid; CAS no. 60047-17-8; lot no. 170605; purity 99.5%) produced by T. Hasegawa Co., Ltd. (Tokyo, Japan) was provided by the Division of Standards and Evaluation, Department of Food Safety, Ministry of Health, Labour and Welfare, Japan, with support from the Japan Flavor & Fragrance Materials Association (Tokyo, Japan). Gavage with corn oil (lot no. WDL0579; Wako Pure Chemical Industries, Osaka, Japan) was selected as the vehicle for administration owing to the volatility of linalool oxide and its insolubility in water. Linalool oxide solutions were freshly prepared every 3–7 days and refrigerated until further use. After storage for 10 days at 4°C and then 24 h at room temperature, the residual ratios of linalool oxide in 16 and 160 mg/mL solutions were confirmed as 99.3% and 101.7%, respectively, by high-performance liquid chromatography.

Experimental animals

In total, 40 male and 40 female specific pathogen-free rats (Crl:CD(SD), 5 weeks old) were purchased from Jackson Laboratory Japan, Inc. (Kanagawa, Japan) and acclimated for 1 week. During the study period, the animals were housed in plastic cages (two rats per cage) in a room with a barrier system controlled with respect to the light/dark cycle (12 h), ventilation (air-exchange rate of >10 times/h), temperature (22 ± 3°C), and relative humidity (55 ± 15%). The cages were exchanged two or three times per week. Each animal had free access to tap water and basal diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan). At the beginning of the experiment, the animals were randomly allocated to four groups of 10 male and 10 female rats based on their body weights, which were measured the day before starting the chemical treatment.

Study design

The doses of linalool oxide were determined based on the results of a preliminary 14-day repeated oral administration study at doses of 0, 200, 400, and 800 mg/kg bw/day (5 rats/sex/group). In a preliminary study, abnormal gait, decreased locomotor activity, and increased liver weight after administration were observed in the 400 and 800 mg/kg groups for both sexes (data not shown). Based on these results, we selected doses of 0, 80, 250, and 800 mg/kg bw/day for administration to both the male and female rats in a subsequent 13-week toxicity study. The study was designed in accordance with the Guidelines for Designation of Food Additives and Revision of Standards for Use of Food Additives of Japan (1996) and approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences, Japan. The stability test for the linalool oxide solution in corn oil described above and the 13-week subchronic toxicity study were conducted in compliance with the GLP regulations at the DIMS Institute of Medical Science, Inc. (Aichi, Japan).

Linalool oxide dissolved in corn oil at doses of 0, 80, 250, and 800 mg/kg bw/day was administered intragastrically by oral gavage for 91 days (male) or 92 (female) days. General condition and mortality rates were checked daily. Body weight and the amounts of supplied and residual diet and water were measured once a week during the experimental period. Ophthalmological examinations were performed in the 13th week of administration. After macroscopic observation, the anterior ocular segment, optic media, and ocular fundus were examined under mydriatic conditions using indirect binocular ophthalmoscopy. Urine samples for urinalysis were collected from 6 rats/group using metabolic cages over periods of 4 and 20 h during the last week of administration. Urine samples were tested for pH, glucose, bilirubin, ketones, occult blood, protein, urobilinogen, color, urine sediment (epithelial cells, crystals, casts, erythrocytes, and leukocytes), specific gravity, volume, and electrolytes (Na, K, and Cl). All rats were fasted overnight on completion of treatment, and blood samples for hematology and serum biochemistry were collected from the abdominal aorta under deep anesthesia by isoflurane inhalation.

Hematology and serum biochemistry

The following hematological parameters were analyzed using XT-2000i and CA-530 automatic hematology analyzers (Sysmex, Kobe, Japan): red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelet count (PLT), reticulocyte (RET), white blood cell count (WBC), differential leukocyte counts (including neutrophils, eosinophils, basophils, monocytes, and lymphocyte), prothrombin time (PT), and activated partial thromboplastin time (APTT). Serum biochemical analysis was performed using a Hitachi 7070 automated analyzer (Hitachi, Ltd., Tokyo, Japan) to assess the following parameters: total protein (TP), albumin (ALB), ALB/globulin ratio (A/G), glucose (GLU), total cholesterol (T-CHO), triglyceride (TG), phospholipid (PL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), urea nitrogen (BUN), creatinine (CRE), total bilirubin (T-BIL), alkaline phosphatase (ALP), inorganic phosphorus (IP), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and chlorine (Cl).

Organ weights and histopathological assessment

A complete necropsy was performed on all animals, and the brain, thymus, lungs, heart, spleen, liver, kidneys, pituitary gland, thyroid glands with parathyroid glands, adrenal glands, testes, prostate, seminal vesicles, ovaries, uterus, and salivary glands (submandibular and sublingual glands) were weighed. These organs and tissues from the spinal cord, nasal cavity, Zymbal gland, tongue, esophagus, trachea, aorta, stomach, small and large intestines, pancreas, submandibular and mesenteric lymph nodes, urinary bladder, epididymis, vagina, oviducts, femur and sternum

with bone marrow, skeletal muscle, sciatic nerve, skin, and mammary gland were fixed in 10% neutral-buffered formalin, and paraffin-embedded sections were prepared and stained with hematoxylin and eosin for histopathological examination. The testes and eyes with the optic nerve and Harderian gland were fixed in a glutaraldehyde formalin acetic acid solution and a glutaraldehyde-formaldehyde fixative solution, respectively. Histopathological assessment was performed on all tissues from animals in the control group and the group administered the highest dose unless any treatment-related lesions were observed.

Immunohistochemistry for α_{2u}-globulin

As a significant increase in accumulation of hyaline droplets in tubular epithelial cells was observed in the kidneys of male rats, immunohistochemical analysis for α_{2u}-globulin with a primary antibody (R&D Systems, Minneapolis, MN, USA) was performed according to a previous report[10].

Statistical analysis

Variances in body weight data during the experimental period, food and water intake, urinalysis (numerical data), hematology, serum biochemistry, and organ weights were checked for homogeneity using Bartlett's test. To determine statistically significant differences, Dunnett's and Steel's multiple comparison tests were used to compare homogeneous and heterogeneous data between the control and treatment groups, respectively. To compare several parameters from urinalysis and histopathological findings, incidences and grades were analyzed using Fisher's exact probability test and the Wilcoxon signed-rank test, respectively. Results with p-values of less than 0.05 were considered statistically significant.

Results

In-life parameters

All animals survived until the scheduled necropsy and the clinical signs throughout the experimental period are summarized in Table 1. Abnormal gait immediately after administration was observed in both sexes in the 800 mg/kg group in week 1. In week 2, a decrease in locomotor activity after administration was found in the male 800 mg/kg group. Salivation was also observed in both sexes in the 800 mg/kg group from weeks 3 to 13 and in the male 250 mg/kg group at week 11. The clinical signs resolved within a few hours.

In males, a significant suppression of body weight gain was observed in the 250 and 800 mg/kg groups from weeks 9 and 5, respectively (Fig. 1a). In females, significant suppression of body weight gain was observed in the 800 mg/kg group at week 12. Although statistically significant changes in daily food intake were sporadically observed during the experimental period, no dose-related differences were detected between groups of either sex (Fig. 1b). In the 800 mg/kg group, water consumption significantly increased during weeks 1 to week 5 in males, and weeks 11 and 12 in females (Fig. 1c and 1d). However, although a significant decrease in water consumption was noted in the female 250 mg/kg group between weeks 3 to week 8, lack of a dose relationship suggests that these differences were not associated with exposure to the test substance. Moreover, no ophthalmic findings were observed in any of the rats examined.

Urinalysis

Urinalysis results are summarized in Table 2. In males, significant decreases in K and Cl were observed in the 250 and 800 mg/kg groups. In female rats, a significant decrease in pH was observed in the 250 and 800 mg/kg groups. Although a significant decrease in Na in the male 250 mg/kg group, a decrease in urine volume in the female 250 mg/kg group, and an increase in protein in the female 80 mg/

Table 1. General Condition of Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

Groups	Findings	Week												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Males	Cumulative total no. of animals per week	70	70	70	70	70	70	70	70	70	70	70	70	70
0 mg/kg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80 mg/kg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250 mg/kg	Salivation	0	0	0	0	0	0	0	0	0	0	1	0	0
800 mg/kg	Decrease in locomotor activity	0	7	0	0	0	0	0	0	0	0	0	0	0
	Abnormal gait	14	0	0	0	0	0	0	0	0	0	0	0	0
	Salivation	0	0	26	37	43	36	40	35	29	28	36	29	20
Females	Cumulative total no. of animals per week	70	70	70	70	70	70	70	70	70	70	70	70	80
0 mg/kg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80 mg/kg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
250 mg/kg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
800 mg/kg	Abnormal gait	15	0	0	0	0	0	0	0	0	0	0	0	0
	Salivation	0	0	18	22	29	18	14	31	27	25	27	24	13

Values are the total number of occurrences each week. -: no abnormalities detected.

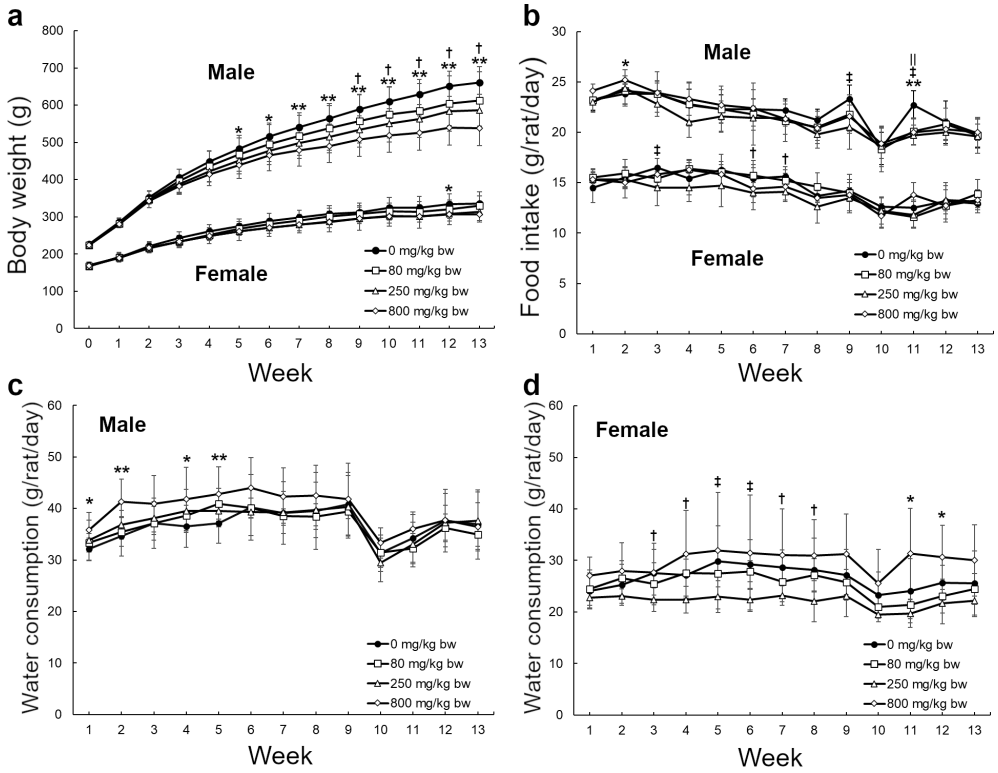

Fig. 1. Body weight (a), daily food intake (b), and water consumption (c and d) of male and female Crl:CD(SD) rats treated with the indicated doses of linalool oxide for 13 weeks. Each group contains 10 animals. The error bars represent the standard deviations of the experimental groups. * and ** (800 mg/kg), † and ‡ (250 mg/kg), § and || (80 mg/kg): significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

kg groups were noted, the lack of any dose relationship suggested that these differences were not associated with exposure to the test substances.

Hematology and serum biochemistry

Hematological and serum biochemistry data are shown in Tables 3 and 4, respectively. A significant increase in PLT levels was detected in the male 800 mg/kg group (Table 3). In the female 800 mg/kg group, significant decreases in HGB, MCHC, PT, and APTT levels were observed. In serum biochemistry, significant increases in γ-GTP and Mg were noted in both sexes in the 800 mg/kg group (Table 4). Significant decreases in GLU levels were observed in the female 250 mg/kg group and in both sexes of the 800 mg/kg groups. In the 800 mg/kg group, a significant increase in TP in males, significant increase in T-CHO and PL, and decrease in A/G, CRE, and ALP levels in females were observed. A significant decrease in the A/G ratio was observed in the female 250 mg/kg group. Although significant increases in AST and T-BIL levels were noted in the female 250 mg/kg group, lack of a dose relationship suggests that these differences were not associated with exposure to the test substance.

Organ weights

Organ weights are summarized in Table 5. Significant decrease in the final body weight were observed in both sexes in the 800 mg/kg group and only in male in the 250 mg/kg group (Table 5). In the male 800 mg/kg group, a significant decrease in the absolute weights of the brain, lungs, heart, and spleen was observed. Significant decreases in the absolute weights of the salivary glands were observed in the male 250 and 800 mg/kg groups. Significant increase was detected in the relative weights of the liver in all male-treated groups; the brain, lung, pituitary gland, and kidneys in the male 250 and 800 mg/kg groups; and the heart, thyroid glands, and adrenal glands in the male 800 mg/kg group. In the female 800 mg/kg group, the absolute and relative weights of the liver and adrenal glands significantly increased. A significant increase in the relative weights of the heart and kidneys was observed in the female 800 mg/kg group. Although a significant increase in the relative weight of the adrenal glands and a decrease in the absolute weight of the salivary glands were noted in the male 80 mg/kg and female 250 mg/kg groups, respectively, lack of a dose-dependent relationship suggests that these differences were not associated with exposure to the test substance.

Table 2. Urinalysis Data for Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

Dose (mg/kg/day)	0	80	250	800
No. of animals examined	6	6	6	6
Males				
pH (6.0/6.5/7.0/7.5/8.0/8.5)	0/0/1/3/1/1	0/0/0/1/2/3	0/1/2/1/0/2	0/2/1/3/0/0
Glucose (-)	6	6	6	6
Bilirubin (-)	6	6	6	6
Ketones (-/±/+)	1/4/1	2/4/0	0/3/3	2/4/0
Occult blood (-)	6	6	6	6
Protein (-/±/+)	2/1/3	0/1/5	1/2/3	1/3/2
Urobilinogen (0.1)	6	6	6	6
Color (Pale yellow/light yellow)	0/6	0/6	0/6	1/5
Epithelial cells (-/±)	4/2	6/0	5/1	6/0
Crystals (-/±/+/++/+++)	2/1/1/0/2	2/1/0/0/3	1/0/4/0/1	1/1/0/1/3
Casts (-)	6	6	6	6
Erythrocytes (-)	6	6	6	6
Leukocytes (-)	6	6	6	6
Specific gravity	1.057 ± 0.019	1.058 ± 0.008	1.047 ± 0.017	1.055 ± 0.023
Urine volume (g)	12.4 ± 5.2	11.9 ± 2.2	13.3 ± 5.9	15.7 ± 9.7
Na (mEq/L)	117.8 ± 71.6	82.4 ± 21.2	29.8 ± 12.8*	52.8 ± 28.4
K (mEq/L)	273.5 ± 95.0	261.5 ± 54.5	132.1 ± 44.6**	105.9 ± 46.6**
Cl (mEq/L)	131.1 ± 65.7	107.5 ± 21.3	43.0 ± 7.6*	51.1 ± 22.0*
Females				
pH (6.0/6.5/7.0/7.5/8.0/8.5)	0/0/0/2/2/2	1/0/1/1/2/1	4/0/0/1/1/0*	1/2/1/1/1/0*
Glucose (-)	6	6	6	6
Bilirubin (-)	6	6	6	6
Ketones (-)	6	6	6	6
Occult blood (-)	6	6	6	6
Protein (-/±/+)	4/1/1	0/1/5*	1/0/5	1/4/1
Urobilinogen (0.1)	6	6	6	6
Color (Pale yellow/light yellow)	2/4	0/6	0/6	2/4
Epithelial cells (-/±)	5/1	6/0	6/0	6/0
Crystals (-/±/+/++/+++)	2/3/1/0/0	2/1/3/0/0	4/1/0/1/0	3/0/0/2/1
Casts (-)	6	6	6	6
Erythrocytes (-)	6	6	6	6
Leukocytes (-)	6	6	6	6
Specific gravity	1.046 ± 0.021	1.060 ± 0.020	1.072 ± 0.017	1.054 ± 0.014
Urine volume (g)	14.5 ± 9.1	9.4 ± 5.8	5.3 ± 1.6*	11.6 ± 4.4
Na (mEq/L)	80.8 ± 44.6	104.4 ± 33.2	99.8 ± 52.9	74.4 ± 27.7
K (mEq/L)	233.7 ± 109.1	304.2 ± 110.9	226.1 ± 86.6	136.9 ± 36.5
Cl (mEq/L)	94.2 ± 49.5	127.2 ± 64.7	96.8 ± 41.8	62.4 ± 24.1

Values are means ± standard deviations. ±, minimal; +, mild; ++, moderate; +++, marked. * and **: significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

Histopathology and immunohistochemistry

Histopathological and immunohistochemical data are summarized in Tables 6 and 7, respectively. In the liver, significantly increased incidences of centrilobular hepatocellular hypertrophy in both sexes in the 800 mg/kg group, and periportal microvesicular fatty changes were observed in the female 800 mg/kg group (Fig. 2 and Table 6). In males, the incidence of granular casts and basophilic tubules, and the degree of hyaline droplet accumulation in the kidneys significantly increased in all treated groups (Fig. 3). Immunohistochemical analysis revealed significant increase in the degrees of α_{2u}-globulin deposition in males of all treated groups compared with those in the control group (Fig. 3 and Table 7). Although several lesions were sporadically detected in other organs, no significant treatment-dependent alterations in the incidence of these lesions were apparent.

Discussion

In the current 13-week subchronic toxicity study of linalool oxide, no toxicological changes in food intake or ophthalmology were observed. As clinical signs immediately after administration, abnormal gait and salivation were observed in both sexes and decreased locomotor activity was detected in males. Because the monoterpene linalool (CAS no. 78-70-6), which is found in essential oils and has a structure closely related to that of linalool oxide, shows sedative effects via *N*-methyl-D-aspartate (NMDA) receptor antagonistic activity[11], the observed gait abnormalities and decreased locomotor activity were considered adverse effects. Tolerance to the anesthetic effects of an NMDA receptor antagonist has been reported to be acquired by chronic administration in rats[12], which may explain the early disappearance of these clinical signs in the present study. Howev-

Table 3. Hematology Data for Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

Dose (mg/kg/day)		0	80	250	800
No. of animals examined		10	10	10	10
Males					
RBC	$\times 10^4/\mu L$	854 ± 33	864 ± 28	880 ± 45	858 ± 43
HGB	g/dL	15.1 ± 0.8	15.2 ± 0.5	15.0 ± 0.4	14.8 ± 0.7
HCT	%	41.7 ± 1.9	41.6 ± 1.6	41.0 ± 1.7	41.4 ± 2.4
MCV	fL	48.8 ± 1.6	48.2 ± 2.2	46.7 ± 3.4	48.2 ± 1.7
MCH	pg	17.7 ± 0.6	17.6 ± 0.7	17.0 ± 0.9	17.2 ± 0.5
MCHC	g/dL	36.3 ± 0.3	36.5 ± 0.5	36.6 ± 0.8	35.8 ± 0.6
PLT	$\times 10^4/\mu L$	103.7 ± 5.7	108.0 ± 10.4	113.8 ± 10.8	117.5 ± 13.8*
RET	$\times 10^2/\mu L$	27.9 ± 4.1	29.0 ± 3.2	28.5 ± 2.6	28.2 ± 3.7
WBC	$\times 10^2/\mu L$	112.5 ± 36.3	109.7 ± 32.0	115.8 ± 27.8	107.6 ± 20.5
Neutrophil	$\times 10^2/\mu L$	22.1 ± 9.5	19.8 ± 7.6	22.0 ± 7.6	21.5 ± 9.0
Eosinophil	$\times 10^2/\mu L$	1.3 ± 0.5	1.4 ± 0.6	1.8 ± 0.5	1.4 ± 0.7
Basophil	$\times 10^2/\mu L$	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Monocyte	$\times 10^2/\mu L$	4.3 ± 1.7	4.4 ± 1.7	3.6 ± 0.7	3.9 ± 0.9
Lymphocyte	$\times 10^2/\mu L$	84.8 ± 29.5	84.1 ± 26.7	88.3 ± 23.1	80.8 ± 14.2
PT	sec	12.5 ± 1.4	13.1 ± 2.6	13.0 ± 2.2	11.1 ± 0.5
APTT	sec	16.0 ± 2.6	16.7 ± 3.3	17.1 ± 2.1	15.0 ± 1.4
Females					
RBC	$\times 10^4/\mu L$	752 ± 32	751 ± 59	763 ± 20	722 ± 31
HGB	g/dL	14.3 ± 0.3	14.1 ± 0.6	14.3 ± 0.3	13.7 ± 0.4**
HCT	%	39.1 ± 0.4	38.9 ± 1.8	39.4 ± 0.9	38.5 ± 1.1
MCV	fL	52.1 ± 2.2	51.9 ± 2.4	51.6 ± 1.7	53.3 ± 1.9
MCH	pg	19.1 ± 0.6	18.8 ± 0.9	18.8 ± 0.4	19.0 ± 0.6
MCHC	g/dL	36.6 ± 0.5	36.1 ± 0.5	36.4 ± 0.6	35.7 ± 0.6**
PLT	$\times 10^4/\mu L$	106.1 ± 8.1	107.9 ± 12.5	113.2 ± 12.9	111.6 ± 7.8
RET	$\times 10^2/\mu L$	24.8 ± 4.0	26.1 ± 4.0	23.2 ± 4.2	27.0 ± 4.7
WBC	$\times 10^2/\mu L$	65.1 ± 15.6	63.4 ± 21.5	84.3 ± 29.7	72.4 ± 21.8
Neutrophil	$\times 10^2/\mu L$	10.5 ± 3.6	11.1 ± 5.9	11.1 ± 3.8	11.6 ± 4.6
Eosinophil	$\times 10^2/\mu L$	0.9 ± 0.3	0.9 ± 0.4	1.0 ± 0.3	1.0 ± 0.3
Basophil	$\times 10^2/\mu L$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	$\times 10^2/\mu L$	1.6 ± 0.7	1.8 ± 0.7	2.1 ± 0.8	2.0 ± 0.9
Lymphocyte	$\times 10^2/\mu L$	52.1 ± 12.2	49.5 ± 18.0	70.2 ± 28.8	57.9 ± 18.6
PT	sec	9.4 ± 0.1	9.3 ± 0.1	9.3 ± 0.1	9.1 ± 0.2**
APTT	sec	12.5 ± 1.0	12.4 ± 0.9	12.9 ± 0.9	11.3 ± 1.4*

Values are means ± standard deviations. * and **: significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

er, salivation and water consumption were stimulated by linalool oxide as a flavoring agent and were considered to have low toxicological significance. Body weights in the male 250 and 800 mg/kg groups at week 13 and in the female 800 mg/kg group at week 12 were 88.8%, 81.5%, and 91.6%, respectively, compared to those of the controls. There were no obvious changes in food intake among the groups. Thus, the suppression of body weight gain was considered an adverse effect of linalool oxide related to reduced dietary efficiency, although the detailed mechanism could not be determined. In addition, significant changes in the absolute and/or relative weights of the following organs: brain, lungs, heart, spleen, pituitary gland, thyroid glands, adrenal glands, and salivary glands in males, and heart and salivary glands in females, were associated with suppression of body weight gain and were considered of little toxicological significance.

The observed significant decrease in electrolyte levels in urinalysis was considered to have no toxicological significance because the fluctuation patterns were not consistent with those of renal toxicity or were associated with increased water consumption. A significant decrease in urine pH observed in female rats was considered incidental owing to the lack of urine crystals and histopathological findings in the kidney. Significant increase in PLT in males and decrease in HGB and MCHC in females detected by hematological analysis and decrease in the A/G ratio in females observed by serum biochemistry were considered to have no toxicological significance as there were no abnormalities in related parameters or any histopathological findings in the hematopoietic tissues. Moreover, the observed decrease in PT and APTT in hematological analysis and in CRE and ALP in serum biochemistry analysis in females contradicted the toxicity-related changes.

Serum biochemistry analyses, organ weight measurements, and histopathological assessments revealed several findings related to adverse effects on the liver in both sexes. Increased relative and/or absolute weights of the liver; an increase in serum γ-GTP; and centrilobular hepatocellular hypertrophy in both sexes in the 800 mg/kg groups were considered to reflect the possible adverse effects of linalool

Table 4. Serum Biochemistry Data for Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

Dose (mg/kg/day)		0	80	250	800
No. of animals examined		10	10	10	10
Males					
TP	g/dL	5.9 ± 0.3	6.0 ± 0.2	6.1 ± 0.3	6.2 ± 0.2*
ALB	g/dL	2.6 ± 0.2	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
A/G		0.81 ± 0.06	0.83 ± 0.06	0.82 ± 0.07	0.77 ± 0.06
GLU	mg/dL	134 ± 10	138 ± 19	131 ± 19	115 ± 18*
T-CHO	mg/dL	54 ± 12	54 ± 7	58 ± 12	65 ± 11
TG	mg/dL	52 ± 18	53 ± 30	51 ± 14	41 ± 12
PL	mg/dL	94 ± 14	97 ± 13	97 ± 14	105 ± 14
AST	U/L	87 ± 20	84 ± 28	84 ± 26	82 ± 19
ALT	U/L	33 ± 8	28 ± 7	29 ± 7	38 ± 5
γ-GTP	U/L	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.4 ± 0.1	0.8 ± 0.3**
BUN	mg/dL	11.8 ± 1.2	12.2 ± 1.6	13.0 ± 1.5	12.6 ± 2.2
CRE	mg/dL	0.31 ± 0.03	0.33 ± 0.04	0.31 ± 0.06	0.29 ± 0.05
T-BIL	mg/dL	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.01
ALP	U/L	377 ± 155	295 ± 60	328 ± 61	308 ± 78
IP	mg/dL	6.4 ± 0.4	6.2 ± 0.3	6.2 ± 0.4	6.4 ± 0.4
Ca	mg/dL	10.1 ± 0.2	10.2 ± 0.3	10.1 ± 0.4	10.2 ± 0.2
Mg	mg/dL	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.2	2.3 ± 0.1**
Na	mEq/L	143.1 ± 0.5	143.4 ± 0.9	142.7 ± 1.3	143.1 ± 0.8
K	mEq/L	4.51 ± 0.16	4.43 ± 0.22	4.60 ± 0.25	4.77 ± 0.34
Cl	mEq/L	105.7 ± 1.6	105.2 ± 1.6	104.2 ± 2.1	104.5 ± 1.7
Females					
TP	g/dL	6.5 ± 0.2	6.6 ± 0.4	6.5 ± 0.4	6.5 ± 0.3
ALB	g/dL	3.3 ± 0.2	3.3 ± 0.3	3.2 ± 0.3	3.1 ± 0.1
A/G		1.03 ± 0.08	1.02 ± 0.10	0.94 ± 0.06*	0.92 ± 0.06**
GLU	mg/dL	134 ± 21	128 ± 9	109 ± 12**	113 ± 14*
T-CHO	mg/dL	72 ± 10	80 ± 14	81 ± 14	111 ± 16**
TG	mg/dL	46 ± 24	48 ± 21	43 ± 31	62 ± 24
PL	mg/dL	148 ± 23	158 ± 24	157 ± 25	190 ± 20**
AST	U/L	66 ± 9	81 ± 27	90 ± 14**	66 ± 20
ALT	U/L	29 ± 6	29 ± 8	34 ± 11	34 ± 6
γ-GTP	U/L	0.3 ± 0.1[a]	0.4 ± 0.1	0.5 ± 0.3[a]	0.8 ± 0.3**
BUN	mg/dL	14.7 ± 1.9	13.2 ± 2.1	13.6 ± 1.2	15.3 ± 2.6
CRE	mg/dL	0.40 ± 0.04	0.38 ± 0.03	0.38 ± 0.04	0.34 ± 0.04**
T-BIL	mg/dL	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02*	0.06 ± 0.02
ALP	U/L	145 ± 16	156 ± 49	170 ± 75	115 ± 25*
IP	mg/dL	4.9 ± 0.6	4.8 ± 0.8	5.0 ± 0.6	5.1 ± 0.5
Ca	mg/dL	10.3 ± 0.3	10.3 ± 0.4	10.2 ± 0.3	10.3 ± 0.3
Mg	mg/dL	2.1 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1**
Na	mEq/L	143.1 ± 1.1	143.0 ± 0.8	142.8 ± 1.1	143.1 ± 1.0
K	mEq/L	4.05 ± 0.28	4.10 ± 0.24	3.98 ± 0.19	4.16 ± 0.34
Cl	mEq/L	105.3 ± 2.2	104.8 ± 2.0	103.9 ± 1.5	103.6 ± 1.9

Values are means ± standard deviations. * and **: significantly different from the control at $p<0.05$ and $p<0.01$, respectively. [a]The number of effective animals was reduced to nine because the sample below the detection limit was excluded.

oxide on the liver. In females, adverse effects on lipid metabolism were also suggested from increase in serum T-CHO and PL levels and increased periportal microvesicular fatty changes in hepatocytes[13]. Moreover, decreased serum GLU observed in both sexes and increased TP observed in males could be related to functional alterations of the liver. Although a significant increase in the relative liver weight was also observed in the male low-dose group, this change was considered to be within the range of adaptation, as it was not accompanied by fluctuations in other related parameters or histopathological findings.

Although histopathological findings, including granular casts, basophilic tubules, and accumulation of hyaline droplets, were noted in the kidneys of male rats, immunohistochemical analysis revealed that these renal lesions were associated with α_{2u}-globulin nephropathy, which is a condition specific to male rats and is not relevant to human risk assessment[14]. Therefore, it was reasonable to exclude the histopathological changes associated with chronic nephropathy observed in males from determination of the NOAEL. However, increased relative kidney weights and significant increases in serum Mg levels were observed not only in males but also in females. Since increased serum Mg levels are a well-established blood biochemical indicator related to renal failure in humans[15], these commonly observed findings in male and female rats suggest the possible adverse effects of linalool oxide on the kidney apart from α_{2u}-globulin nephropathy.

Table 5. Organ Weight Data for Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

Dose (mg/kg/day)		0	80	250	800
No. of animals examined		10	10	10	10
Males					
Body weight	g	640.8 ± 38.7	591.1 ± 75.6	565.2 ± 39.0**	513.7 ± 42.7**
Absolute					
Brain	g	2.26 ± 0.09	2.24 ± 0.13	2.25 ± 0.07	2.16 ± 0.05*
Thymus	g	0.296 ± 0.061	0.246 ± 0.054	0.263 ± 0.042	0.258 ± 0.088
Lung	g	1.61 ± 0.10	1.55 ± 0.13	1.57 ± 0.09	1.48 ± 0.10*
Heart	g	1.72 ± 0.12	1.71 ± 0.21	1.64 ± 0.14	1.50 ± 0.12**
Spleen	g	0.908 ± 0.174	0.821 ± 0.168	0.756 ± 0.109	0.711 ± 0.135*
Liver	g	16.3 ± 1.7	16.8 ± 2.8	16.1 ± 1.3	16.8 ± 1.7
Kidneys	g	3.69 ± 0.32	3.76 ± 0.43	3.91 ± 0.42	3.84 ± 0.36
Pituitary gland	mg	14.1 ± 1.3	14.5 ± 1.9	14.1 ± 1.1	13.2 ± 1.8
Thyroid glands	mg	25.9 ± 4.6	25.6 ± 5.4	27.1 ± 5.0	30.0 ± 3.3
Adrenal glands	mg	58.5 ± 5.8	66.1 ± 11.2	61.6 ± 14.8	60.3 ± 7.3
Testes	g	3.81 ± 0.25	3.50 ± 0.40	3.53 ± 0.53	3.48 ± 0.37
Prostate	g	1.93 ± 0.32	1.94 ± 0.33	1.87 ± 0.26	1.66 ± 0.21
Seminal vesicles	g	1.42 ± 0.21	1.39 ± 0.10	1.35 ± 0.12	1.21 ± 0.25
Salivary glands	g	0.825 ± 0.113	0.750 ± 0.083	0.722 ± 0.061*	0.695 ± 0.051**
Relative					
Brain	%	0.354 ± 0.020	0.383 ± 0.038	0.400 ± 0.030**	0.423 ± 0.033**
Thymus	%	0.046 ± 0.009	0.042 ± 0.008	0.047 ± 0.007	0.051 ± 0.018
Lung	%	0.251 ± 0.014	0.263 ± 0.019	0.279 ± 0.027**	0.289 ± 0.018**
Heart	%	0.268 ± 0.020	0.292 ± 0.038	0.291 ± 0.024	0.293 ± 0.014*
Spleen	%	0.141 ± 0.023	0.139 ± 0.019	0.134 ± 0.017	0.138 ± 0.020
Liver	%	2.54 ± 0.19	2.83 ± 0.23**	2.86 ± 0.17**	3.26 ± 0.16**
Kidneys	%	0.576 ± 0.050	0.640 ± 0.068	0.692 ± 0.066**	0.748 ± 0.054**
Pituitary gland	10^{-3}%	2.21 ± 0.21	2.46 ± 0.30	2.50 ± 0.22*	2.57 ± 0.23**
Thyroid glands	10^{-3}%	4.05 ± 0.72	4.35 ± 0.76	4.78 ± 0.75	5.87 ± 0.78**
Adrenal glands	10^{-3}%	9.2 ± 1.1	11.3 ± 1.8*	11.0 ± 3.2	11.8 ± 1.1**
Testes	%	0.597 ± 0.059	0.600 ± 0.090	0.630 ± 0.119	0.681 ± 0.078
Prostate	%	0.301 ± 0.044	0.331 ± 0.060	0.332 ± 0.049	0.326 ± 0.051
Seminal vesicles	%	0.222 ± 0.030	0.238 ± 0.036	0.239 ± 0.024	0.237 ± 0.057
Salivary glands	%	0.128 ± 0.013	0.128 ± 0.017	0.128 ± 0.008	0.136 ± 0.011
Females					
Body weight	g	324.9 ± 28.9	314.8 ± 26.5	300.7 ± 26.5	292.3 ± 18.5*
Absolute					
Brain	g	2.01 ± 0.07	1.99 ± 0.08	2.00 ± 0.13	1.99 ± 0.05
Thymus	g	0.286 ± 0.030	0.255 ± 0.056	0.271 ± 0.098	0.259 ± 0.034
Lung	g	1.18 ± 0.06	1.13 ± 0.07	1.12 ± 0.08	1.13 ± 0.09
Heart	g	0.98 ± 0.08	0.99 ± 0.10	0.97 ± 0.10	0.98 ± 0.08
Spleen	g	0.544 ± 0.049	0.533 ± 0.074	0.570 ± 0.074	0.531 ± 0.053
Liver	g	8.4 ± 0.8	8.6 ± 0.8	8.3 ± 0.5	10.1 ± 1.0**
Kidneys	g	1.90 ± 0.14	1.92 ± 0.12	1.92 ± 0.20	2.03 ± 0.21
Pituitary gland	mg	16.7 ± 1.6	18.4 ± 3.8	17.8 ± 2.7	17.2 ± 2.8
Thyroid glands	mg	20.4 ± 2.6	21.8 ± 4.0	22.2 ± 6.1	21.7 ± 3.5
Adrenal glands	mg	63.1 ± 11.3	66.2 ± 7.5	69.8 ± 9.7	75.8 ± 14.8*
Ovaries	mg	122.5 ± 12.5	116.5 ± 16.6	122.7 ± 26.3	104.9 ± 16.8
Uterus	g	0.569 ± 0.182	0.615 ± 0.193	0.598 ± 0.198	0.634 ± 0.181
Salivary glands	g	0.486 ± 0.068	0.446 ± 0.053	0.421 ± 0.052*	0.451 ± 0.057
Relative					
Brain	%	0.624 ± 0.062	0.634 ± 0.054	0.669 ± 0.069	0.684 ± 0.042
Thymus	%	0.088 ± 0.009	0.082 ± 0.020	0.089 ± 0.026	0.089 ± 0.015
Lung	%	0.364 ± 0.022	0.362 ± 0.031	0.375 ± 0.023	0.386 ± 0.035
Heart	%	0.301 ± 0.020	0.313 ± 0.030	0.323 ± 0.026	0.334 ± 0.026*
Spleen	%	0.168 ± 0.017	0.171 ± 0.030	0.190 ± 0.022	0.182 ± 0.017
Liver	%	2.58 ± 0.14	2.73 ± 0.16	2.77 ± 0.17	3.46 ± 0.28**
Kidneys	%	0.589 ± 0.060	0.613 ± 0.059	0.639 ± 0.052	0.696 ± 0.073**
Pituitary gland	10^{-3}%	5.17 ± 0.60	5.87 ± 1.25	5.94 ± 0.87	5.88 ± 0.78
Thyroid glands	10^{-3}%	6.28 ± 0.67	6.96 ± 1.35	7.34 ± 1.57	7.47 ± 1.35
Adrenal glands	10^{-3}%	19.6 ± 4.3	21.1 ± 2.8	23.4 ± 4.0	26.0 ± 4.9**
Ovaries	10^{-3}%	37.9 ± 4.7	37.4 ± 7.0	41.0 ± 9.3	36.0 ± 6.1
Uterus	%	0.179 ± 0.073	0.198 ± 0.070	0.203 ± 0.084	0.218 ± 0.066
Salivary glands	%	0.150 ± 0.022	0.142 ± 0.019	0.141 ± 0.017	0.154 ± 0.017

Values are means ± standard deviations. * and **: significantly different from the control at $p<0.05$ and $p<0.01$, respectively.

Table 6. Histopathological Findings for Crl:CD(SD) rats Treated with Linalool Oxide for 13 Weeks

		Male				Female			
Dose (mg/kg/day)		0	80	250	800	0	80	250	800
Organs and findings	No. of animals examined	10	10	10	10	10	10	10	10
Liver	Fatty change, macrovesicular, periportal (±,+)	0	2 (0, 2)	0	0	1 (1, 0)	1 (0, 1)	2 (0, 2)	1 (0, 1)
	Fatty change, microvesicular, periportal (±,+)	7 (3, 4)	4 (1, 3)	3 (1, 2)	6 (2, 4)	1 (1, 0)	2 (1, 1)	3 (2, 1)	6 (4, 2)*
	Hypertrophy, hepatocellular, centrilobular (+)	0	0	0	10**	0	0	0	10**
	Infiltrate, mononuclear cell (±)	1	0	1	0	3	0	1	1
	Necrosis, focal (±,+)	0	0	0	0	1 (0, 1)	1 (1, 0)	0	0
	Tension lipidosis (±,+)	0	0	1 (0, 1)	0	2 (0, 2)	1 (0, 1)	1 (0, 1)	1 (1, 0)
Kidney	Accumulation, hyaline droplets (±,+,++)	10 (9, 1, 0)	10 (0, 7, 3)**	10 (1, 6, 3)**	10 (1, 5, 4)**	0	0	0	0
	Cast, granular (±,+,++)	0	6 (3, 3, 0)**	5 (2, 3, 0)*	4 (1, 2, 1)*	0	0	0	0
	Cast, hyaline, pelvis (+)	0	1	0	0	0	0	0	0
	Cyst (+)	1	0	0	0	0	0	0	0
	Dilatation, pelvis (+)	0	2	0	0	0	0	0	0
	Dilatation, tubule (±)	0	0	0	1	0	0	0	0
	Microabscess, renal papilla (+)	0	0	1	0	0	0	0	0
	Mineralization, cortico-medullary junction (±)	0	0	0	0	1	0	0	0
	Pyelitis (+)	0	1	0	0	0	0	0	0
	Basophilic tubule (±,+)	1 (1, 0)	9 (6, 3)**	7 (4, 3)**	7 (3, 4)**	0	0	0	0
Lung	Alveolar macrophage aggregation (±)	1	-	-	0	0	-	-	0
Heart	Fibrosis/infiltrate, mononuclear cell, myocardium (±, +)	3 (1, 2)	-	-	0	0	-	-	0
Spleen	Accessory spleen	1	-	-	0	0	-	-	0
Pituitary gland	Cyst, pars intermedia	1	-	-	0	0	-	-	0
	Cyst, pars nervosa	1	-	-	0	0	-	-	0
	Cyst, pars nervosa, multiple	1	-	-	0	0	-	-	1
	Dilatation, Rathke's cleft	0	-	-	0	0	-	-	1
Thyroid gland	Cyst, congenital	0	3	2	2	1	0	2	0
	Ectopic thymic tissue	0	0	1	1	2	0	0	0
	Hyperplasia, follicular cell, focal (±)	0	0	0	1	0	0	0	0
	Inflammation, acute (±)	0	0	0	0	1	0	0	0
Parathyroid gland	Cyst, congenital	0	0	0[a]	0	0	0	0	1
	Syncytial giant cell	0	0	2[a]	0	1	1	0	0
Adrenal gland	Accessory adrenocortical tissue	0	0	0	0	0	0	0	1
	Vacuolization, cytoplasmic, cortex (+)	3	4	5	2	0	0	0	0
Prostate	Infiltrate, lymphocyte (±,+)	3 (1, 2)	-	-	0	-	-	-	-
	Inflammation (+)	1	-	-	0	-	-	-	-
Ovary	Cyst, corpus luteum (+)	-	-	-	-	0	-	-	1
	Cyst, paraovarian (+)	-	-	-	-	1	-	-	0
Uterus	Dilatation, luminal (+)	-	-	-	-	1	-	-	2
Salivary gland	Ectopic tissue, parotid gland	1	-	-	0	0	-	-	0
Nasal cavity	Inflammation, nasolacrymal duct (+)	0	-	-	0	0	-	-	1
Glandular stomach	Cyst (±)	1	-	-	0	0	-	-	0
	Erosion (+)	0	-	-	2	0	-	-	0
Ileum	Diverticulum	1	-	-	0	0	-	-	0
Colon	Lymphocyte rich lymph vessels (±)	1	-	-	0	0	-	-	0
Pancreas	Autophagic vacuoles, acinar cell (±)	0	-	-	1	1	-	-	0
	Fibrosis, pancreatic islet (±)	0	-	-	1	0	-	-	0
	Infiltrate, mononuclear cell (±)	0	-	-	1	0	-	-	1

-, Not evaluated; ±, minimal; +, mild; ++, moderate. * and **: significantly different from the control at $p<0.05$ and $p<0.01$, respectively. [a]The number of effective animals was reduced to nine due to failure of tissue sampling.

Table 7. Accumulation Levels of α_{2u}-globulin in the Kidney of Male Crl:CD(SD) Rats

Dose (mg/kg/day)	0	80	250	800
No. of animals examined	10	10	10	10
Accumulation of α_{2u}-globurin (±,+,++)	10 (9, 1, 0)	10 (0, 7, 3)**	10 (1, 6, 3)**	10 (1, 5, 4)**

±, minimal; +, mild; ++, moderate. **; significantly different from the control at $p<0.01$.

In conclusion, a 13-week subchronic toxicity study demonstrated that oral administration of linalool oxide showed adverse clinical signs and histopathological and functional changes in the liver and kidneys of male and female Crl:CD(SD) rats. In addition to the clinically observed reduction of body weight gain, abnormal gait, and decreased locomotor activity, the following findings were considered adverse effects of linalool oxide; changes in serum γ-GTP, T-CHO, PL, TP, and GLU, absolute and/or relative liver weights, centrilobular hepatocellular hypertrophy, and periportal microvesicular fatty changes related to liver function; and increased serum Mg levels and increased kidney

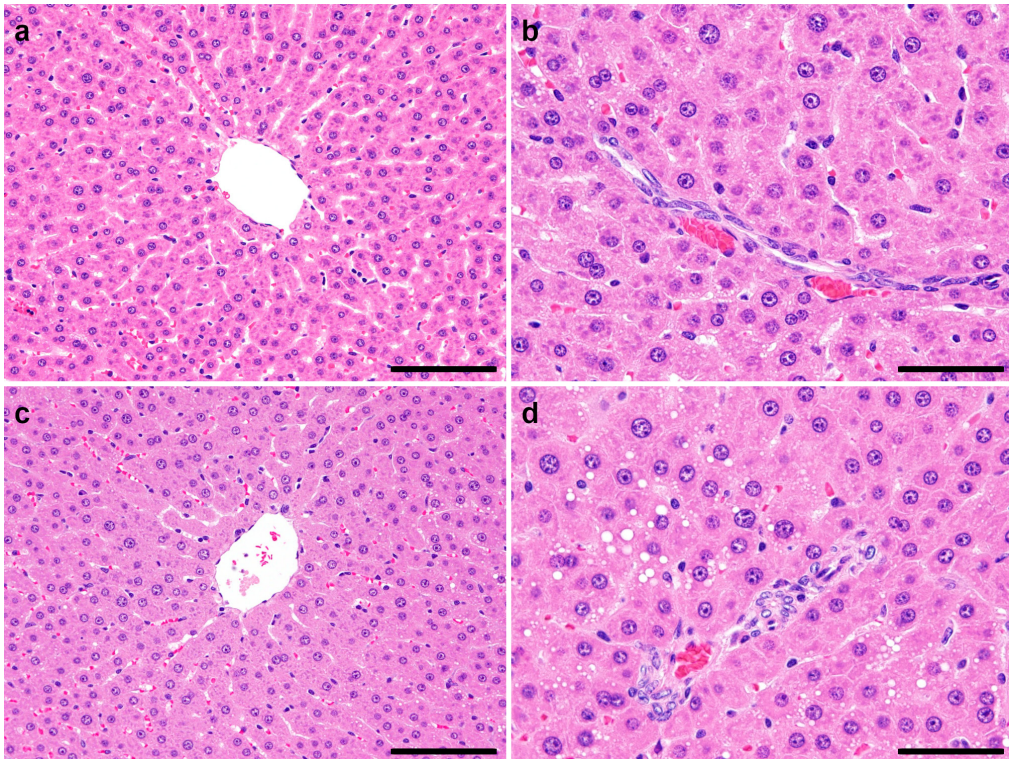

Fig. 2. Representative histopathological findings in the livers of female Crl:CD(SD) rats treated with linalool oxide for 13 weeks. (a and b) Normal liver from the control group. (c) Centrilobular hepatocellular hypertrophy in the 800 mg/kg group. (d) Periportal microvesicular fatty changes in the 800 mg/kg group. Hematoxylin and eosin staining. Scale bars=100 µm (a and c) or 50 µm (b and d).

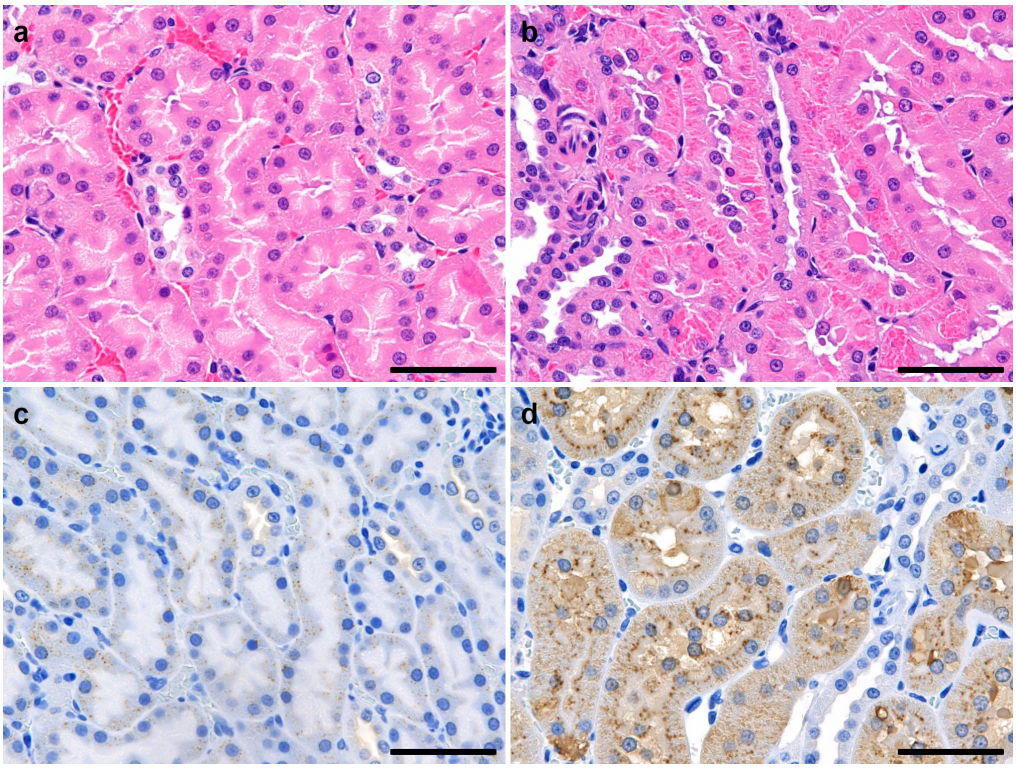

Fig. 3. Representative histopathological and immunohistochemical findings in the kidneys of male Crl:CD(SD) rats treated with linalool oxide for 13 weeks. (a) Normal kidneys in the control group. (b) Accumulation of hyaline droplets in epithelial cells of the proximal tubules in the 800 mg/kg group. Hematoxylin and eosin staining. (c and d) Immunoreactivity of α_{2u}-globulin in the control (c) and 800 mg/kg (d) groups. Scale bars=50 µm.

weights related to renal function. Based on the reduction in body weight gain in the male 250 mg/kg group and the decrease in serum GLU levels in the female 250 mg/kg group, the NOAEL of linalool oxide (furanoid) was set at 80 mg/kg bw/day for both sexes.

Disclosure of Potential Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgment: This study was supported by the Ministry of Health, Labour and Welfare, Japan.

References

1. JECFA. Safety Evaluation of Certain Food Additives. WHO Food Additives Series: 54. Geneva. 487–524. 2006.
2. Honma M, Yamada M, Yasui M, Horibata K, Sugiyama KI, and Masumura K. Genotoxicity assessment of food-flavoring chemicals used in Japan. Toxicol Rep. **9**: 1008–1012. 2022. [Medline] [CrossRef]
3. Toyoda T, Cho YM, Matsushita K, Tachibana S, Senuma M, Akagi J, and Ogawa K. A 13-week subchronic toxicity study of hexyl acetate in SD rats. J Toxicol Pathol. **32**: 205–212. 2019. [Medline] [CrossRef]
4. Matsushita K, Toyoda T, Morikawa T, and Ogawa K. A 13-week subchronic toxicity study of vanillin propylene glycol acetal in F344 rats. Food Chem Toxicol. **132**: 110643. 2019. [Medline] [CrossRef]
5. Ide T, Mizuta Y, Akagi J, Masumoto N, Sugimoto N, Sato K, Ogawa K, and Cho YM. A 90-day repeated oral dose toxicity study of four stereoisomers of 2,4-dimethyl-4-phenyltetrahydrofuran, a synthetic flavoring substance, in F344 rats. Regul Toxicol Pharmacol. **114**: 104664. 2020. [Medline] [CrossRef]
6. Nakamura K, Ishii Y, Takasu S, and Ogawa K. A 90-day subchronic toxicity study of 5-methyl-2-phenyl-2-hexenal in F344 rats. Food Chem Toxicol. **150**: 112041. 2021. [Medline] [CrossRef]
7. Toyoda T, Matsushita K, Akane H, Morikawa T, and Ogawa K. A 13-week subchronic toxicity study of 2-(*l*-menthoxy) ethanol in F344 rats. J Toxicol Pathol. **34**: 309–317. 2021. [Medline] [CrossRef]
8. Mitsumoto T, Ishii Y, Namiki M, Nakamura K, Takasu S, and Ogawa K. A 90-day subchronic toxicity study of Myrrh in F344 rats. Regul Toxicol Pharmacol. **127**: 105076. 2021. [Medline] [CrossRef]
9. Matsushita K, Toyoda T, Akane H, Morikawa T, and Ogawa K. A 13-week subchronic toxicity study of heme iron in SD rats. Food Chem Toxicol. **175**: 113702. 2023. [Medline] [CrossRef]
10. Moroki T, Matsuo S, Hatakeyama H, Hayashi S, Matsumoto I, Suzuki S, Kotera T, Kumagai K, and Ozaki K. Databases for technical aspects of immunohistochemistry: 2021 update. J Toxicol Pathol. **34**: 161–180. 2021. [Medline] [CrossRef]
11. Aprotosoaie AC, Hăncianu M, Costache II, and Miron A. Linalool: a review on a key odorant molecule with valuable biological properties. Flavour Fragrance J. **29**: 193–219. 2014. [CrossRef]
12. Gerb SA, Cook JE, Gochenauer AE, Young CS, Fulton LK, Grady AW, and Freeman KB. Ketamine tolerance in Sprague-Dawley rats after chronic administration of ketamine, morphine, or cocaine. Comp Med. **69**: 29–34. 2019. [Medline] [CrossRef]
13. Yoshida M, Umemura T, Kojima H, Inoue K, Takahashi M, Uramaru N, Kitamura S, Abe K, Tohkin M, Ozawa S, and Yoshinari K. [Basic principles of interpretation of hepatocellular hypertrophy in risk assessment in Japan]. Shokuhin Eiseigaku Zasshi. **56**: 42–48. 2015 (in Japanese). [Medline] [CrossRef]
14. Swenberg JA. α_{2u}-globulin nephropathy: review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Environ Health Perspect. **101**(Suppl 6): 39–44. 1993. [Medline] [CrossRef]
15. Seo JW, and Park TJ. Magnesium metabolism. Electrolyte Blood Press. **6**: 86–95. 2008. [Medline] [CrossRef]