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A 90-Day Inhalation Toxicity Study of Ethyl Formate in Rats

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Ethyl formate, a volatile solvent, has insecticidal and fungicidal properties and is suggested as a potential fumigant for stored crop and fruit. Its primary contact route is through the respiratory tract; however, reliable repeated toxicological studies focusing on the inhalation route have not been published to date. Therefore, the present study was conducted to investigate the safety of a 90-day repeated inhalation exposure in rats. Forty male and 40 female rats were exposed to ethyl formate vapor via inhalation at concentrations of 0, 66, 330, and 1,320 ppm for 6 hr/day, 5 days a week for 13 weeks. Clinical signs, body weights, food consumption, urinalysis, hematologic parameters, serum chemistry measurements, organ weights, necropsy, and histopathological findings were compared between the control and ethyl formate-exposed groups. Locomotor activity decreased during exposure and recovered afterward in male and female rats exposed to 1,320 ppm ethyl formate. Body weight and food consumption continuously decreased in both sexes exposed to 1,320 ppm ethyl formate from week 1 or 3 compared with the control values. The increases in adrenal weight and decreases in thymus weight were noted in both sexes exposed to ethyl formate at 1,320 ppm. Degeneration, squamous metaplasia of olfactory epithelium in the nasopharyngeal tissue, or both were noted in the male and female rats at 1,320 ppm and female rats at 330 ppm ethyl formate. Taken together, our results indicate that ethyl formate-induced changes were not observed in male and female rats at 330 and 66 ppm, respectively. This indicates that exposure to ethyl formate at concentrations below 66 ppm for 90 days is relatively safe in rats. This is the first report of a full-scale repeated inhalation toxicity assessment in rats and could contribute to controlling occupational environmental hazards related to ethyl formate.

***Key words*:** Ethyl formate, 90-day repeated inhalation toxicity, Occupational health

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Abbreviations: Alb, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Crea, creatinine; HCT, hematocrit; HGB, hemoglobin; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; OEL, occupational exposure limit; PLT, platelet; PT, prothrombin time; RBC, red blood cell; Reti, reticulocyte; T-Bili, total bilirubin; T-Chol, total cholesterol; TG, triglyceride; TP, total protein; WBC, white blood cell.

INTRODUCTION

Many fruits and vegetables produce ethyl formate, which is also found in most plant and animal-based product, such as beer, wine, tuna, cheese, and bread (1,2). Ethyl formate is an important flavoring and aroma component of fruits and vegetables and has been revealed to have insecticidal and fungicidal properties (3). Based on these characteristics, it has been used as a fumigant for grains, but is currently restricted to dried fruit and processed cereal products (4). However, it has been suggested as a potential fumigant for stored crops and fruit (5-7), and commercial products have been developed as a postharvest treatment for horticulture and stored grains (2). Ethyl formate is a nonchlorinated solvent, which has been used to prepare poly-D,L-lactide-co-glycolide (PLGA) microspheres using spray drying (8). It is also used as a flavoring for lemonade and essences, a solvent for nitrocellulose, and in organic

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synthesis (9).

Occupational exposure limits (OELs) are the maximum acceptable air concentrations of a hazardous substance above which it is not safe for a worker to be exposed. The current OEL of ethyl formate in the Republic of Korea, the US, England, Germany, Denmark, and the Netherlands is 100 ppm (300 mg/m^3), 8-hr time-weighted average (TWA) (9-11). Setting OELs is complicated and requires considerable animal and human toxicological data (12,13). The most important information for setting an OEL is human toxicity data. Exposure to ≥ 330 ppm ethyl formate in humans was reported to be slightly irritating to the eyes and nose, but the significance of this finding could not be assessed because of the limited reporting (9). The animal toxicity of ethyl formate has been revealed in acute toxicity studies by exposure via inhalation, dermal, and oral routes, and repeated toxicity studies using feeding and intraperitoneal routes (9,14,15). Considering the volatility of ethyl formate, administration through dermal and oral routes as well as feeding would not be appropriate methods. No reliable repeated toxicological studies focusing on the inhalation route have been published to date, although inhalation is the primary route of exposure to ethyl formate in contact workers.

Long-term and low-level exposure to a chemical in an industrial field could cause conflicts between workers and employers in cases of unpredicted health problems and deficient scientific evidence. Anxiety persists in workers who endure long-term and low-level exposure. OEL and reported toxicity results are insufficient for resolving this problem. Therefore, in this study, the safety of a 90-day repeated inhalation exposure in rats was investigated.

MATERIALS AND METHODS

Animal husbandry and maintenance. Forty-eight 6-week-old Sprague-Dawley rats of each sex were obtained from a specific pathogen-free colony at Japan SLC., Inc. (Hamamatsu, Japan) via Joongang Experimental Animal Co., Ltd. (Seoul, Korea) and used after 1-week quarantine and acclimatization. The rats were housed in a room maintained at a temperature of 22 ± 3°C under a relative humidity of 50 ± 20% with artificial lighting from 08:00 to 20:00 and 12~15 air changes/hr. They were housed individually in wire-bottomed stainless-steel mesh cages placed in exposure chambers and provided sterilized tap water and commercial rodent chow (PicoLab Rodent Diet 5053, LabDiet, USA) *ad libitum*. The Institutional Animal Care and Use Committee of the Inhalation Toxicity Research Center approved the protocols used in this study.

Test chemical and exposure. Ethyl formate was purchased from Acros Organic (Geel, Belgium). Whole-body exposure chambers (WITC-00-M, HCT Co., Korea) including a liquid vapor generator (LVg-04-A, HCT Co.) were used to expose the rats to ethyl formate. The test rats were exposed to either ethyl formate or fresh air for 6 hr/day, 5 days a week for 90 days. The inhalation exposure was carried out from 10:00 to 16:00 in the stainless-steel cage of the exposure chamber. The experimental design was established based on the usual schedule of workers, as well as the major exposure route of the test chemical.

Chamber condition. The temperature, relative humidity, pressure, and air ventilation in the chambers were recorded using an environmental controller (ITC manager, HCT Co.). The concentrations of ethyl formate in the chambers were calibrated to 49.6, 298, and 1,792 ppm ethyl formate standard gas (RI-GAS, Korea). The ethyl formate levels were detected using gas chromatography (TRACE1310, Thermo Scientific, China) with the following apparatus: detector, flame ionization detector; column, TraceGold TG-5MS 5% diphenyl-95% dimethyl polysiloxane capillary column (30 m length, 0.25 mm i.d., 0.25 mm film thickness). The conditions used were: detector, oven, and injector temperatures, 250, 100, and 130°C, respectively and formate to generate ethyl formate vapor-air mixture. Following airflow containing saturated ethyl formate was conditioned at 23°C by passing it through a thermostat-controlled condenser, and then diluted with a large volume of fresh air. Finally, the diluted vapor-air mixture was supplied to the inhalation exposure chambers. The ethyl formate vapor concentrations in the chambers were measured every 20 min during exposure and controlled within ± 10.7, 0.3, and 2% of the target concentration in the low-, mid-, high-dose groups, respectively. The mean concentration measured every 20 min for 6 hr was considered as the concentration value for each day. This was then averaged over the 90-day exposure period to obtain the mean and standard deviations. The daily vapor concentrations in the three chambers were 66.4 ± 2.05, 331.1 ± 6.19, and 1345.8 ± 24.3 ppm, respectively.

Experimental groups. Forty male and 40 female rats were randomly assigned to the following four groups and treated with the indicated doses: control (0 mg/L), low-dose (0.2 mg/L, 66 ppm), mid-dose (1 mg/L, 330 ppm), and high-dose (4 mg/L, 1,320 ppm) groups. The control group was exposed to fresh air only. Concentrations of 66, 330, and 1,320 ppm were selected based on a prior study (9,14,15) and the OEL.

Animal observation. All the rats were weighed individually on the first exposure day and once a week after that. The body weight data from the necropsy day were not included in the evaluation of body weight because these data reflected the fasting body weight of the rats. All rats were observed before, during, and after the exposure period

for mortality, general condition, and clinical signs. Daily food consumption was measured before the initiation of exposure and once a week after that. Food consumption was calculated by subtracting the amount of leftover feed from the total feed provided.

Ophthalmoscopy. All rats underwent an ophthalmic examination on week 12. The ophthalmic examination was performed after the administration of mydriatic ocutropic ophthalmic drops (Mydriacyl® eye drops 1%, Alcon Korea, Seoul, Korea), using a nonmydriatic eye fundus camera (VeraCamTM DS-10, Nidek, Gamagori, Japan) and slit-lamp biomicroscopy (KOWA® SL-15, Kowa Co. Ltd., Tokyo, Japan).

Urinalysis. The urinalysis was performed for five rats in each group on week 13 of exposure. Fresh urine (sampled within approximately 3 hr of urine excretion) was collected and the pH, protein, glucose, ketone body, bilirubin, occult blood, leukocyte, nitrite, urobilinogen, and specific gravity were determined using a urine analyzer (Clinitek Advantus, Siemens, Germany) or test kits (Multistix 10 SG, Siemens).

Hematology and serum chemistry. On day 90, the rats were fasted overnight and anesthetized the following day with isoflurane prior to being euthanized. Blood (10 mL) was collected via the abdominal aorta. Blood samples (approximately 2 mL) for hematology were placed in a vacutainer containing EDTA. Then, approximately 2.7 mL of blood mixed with 3.2% sodium citrate was centrifuged at 3,000 rpm for 10 min to measure prothrombin time (PT) and activated partial thromboplastin time (APTT). Blood samples in a vacutainer for blood chemistry were centrifuged at 3,000 rpm for 10 min to obtain serum within 1 hr after collection.

Hematology measurements performed were the total red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet (PLT) count, total white blood cell (WBC) count, differential WBC count (neutrophil, lymphocyte, monocyte, eosinophil, and basophil), reticulocyte (Reti) count, PT, and APTT using the ADVIA 2120i (Siemens) or ACL ELITE systems (Instrumentation Laboratory, USA).

The serum biochemistry parameters analyzed were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Crea), total bilirubin (T-Bili), total protein (TP), albumin (Alb), A/G ratio, total cholesterol (T-Chol), triglyceride (TG), glucose (Glu), potassium (K), calcium (Ca), chloride (Cl), inorganic phosphorus (P), and sodium (Na) using the TBA-120FR automated clinical analyzer (Toshiba Co., Japan).

Necropsy, organ weight, and histopathological examination. Following blood sample collection, all rats underwent a complete necropsy, which consisted of examinations of the external body surfaces and all orifices, as well as the cranial, thoracic, and abdominal cavities and their contents. The following organs were trimmed and weighed: the adrenals, brain, heart, kidneys, liver, spleen, testes, thymus, epididymides, lung, thyroids, ovaries, and uterus. Organ-to-body weight ratios were calculated for each of these organs. The following organ/tissues were preserved in 10% neutral buffered formalin: the adrenals, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur, Harderian glands, heart, ileum, jejunum, kidneys, larynx, liver, lung, lymph nodes (hilar and mesenteric), mammary gland, nasopharyngeal tissue, nerves (optic and sciatic), pancreas, parathyroids, pituitary, prostate, rectum, salivary glands (submandibular, sublingual, and parotid), seminal vesicles, skeletal muscle, skin, spinal cord (cervical, lumbar, and thoracic), spleen, sternum, stifle joint, stomach, testes, teeth, thymus, thyroids, trachea, urinary bladder, ovaries, and uterus. The eyes/optic nerve and testes were preserved in Davidson's solution. All the preserved tissues from rats exposed to 0 and 1,320 ppm ethyl formate were paraffin-embedded, sectioned, stained with hematoxylin and eosin, and then examined microscopically. In addition, nasopharyngeal tissue from rats exposed to 66 and 330 ppm was histopathologically evaluated because ethyl formate-related change was noted at 1,320 ppm.

Statistical analysis. Statistical analysis was performed for body weight, food consumption, hematology and serum biochemistry parameters, organ weights, and organ/body weight. For the control and ethyl formate-exposed groups, the homogeneity of the variance of numerical data was determined using Levene's test. Groups with homogenous and heterogeneous data were compared using one-way analysis of variance (ANOVA) and non-parametric Kruskal-Wallis test, respectively. If statistical significance was observed ($p < 0.05$), Dunnett's test (for ANOVA) or the Steel test (for Kruskal-Wallis) was used for multiple comparisons of the control group with each dose group.

RESULTS

Clinical signs, body weight and food consumption. No animal mortality occurred during this study. The locomotor activity of the male and female rats decreased during exposure to 1,320 ppm ethyl formate but recovered after the exposure ended. Food consumption significantly decreased in male and female rats exposed to 1,320 ppm ethyl formate from week 1 and week 3, respectively, compared with that of the control rats (Fig. 1). Body weight gain significantly decreased in male and female rats exposed to 1,320 ppm ethyl formate from week 1 compared with that of the

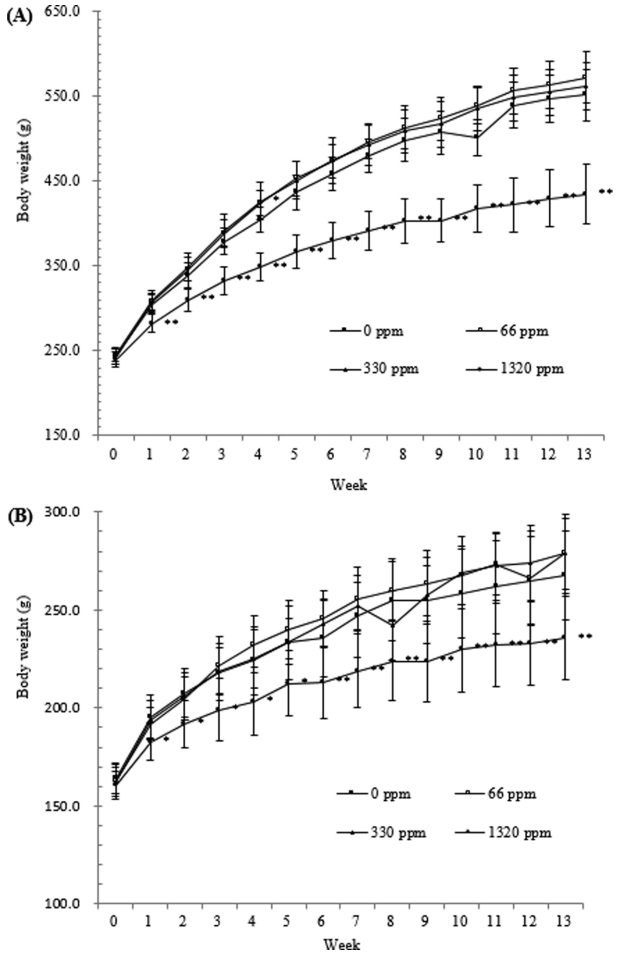

Fig. 1. Changes in body weight in (A) male and (B) female rats exposed to ethyl formate over time. Body weight was measured weekly for 13 weeks. Black squares, blank squares, triangles, and circles indicated mean values of 0, 66, 330, and 1,320 ppm ethyl formate groups, respectively. $^{*}p < 0.05$ and $^{**}p < 0.01$.

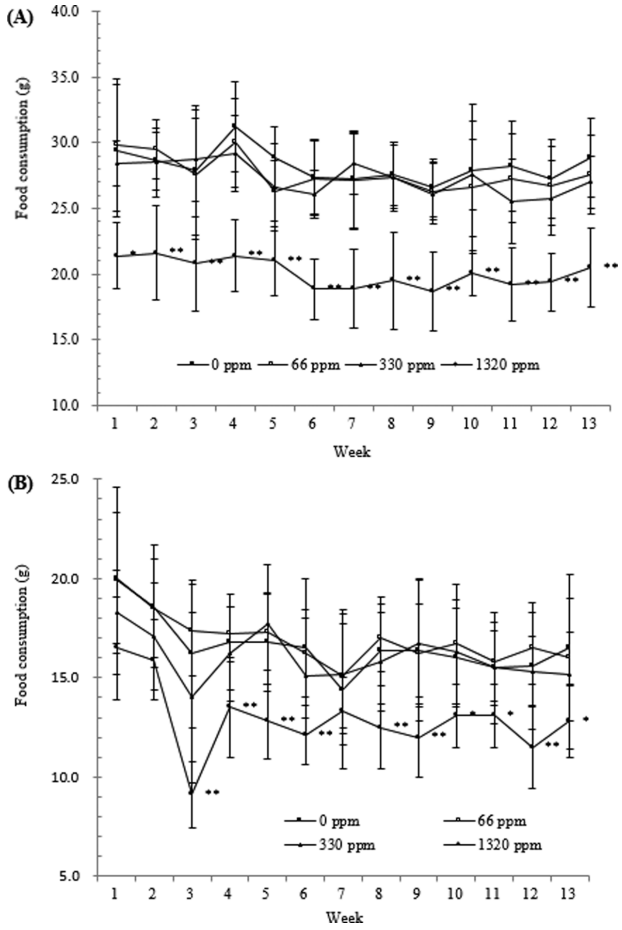

Fig. 2. Changes in food consumption of (A) male and (B) female rats exposed to ethyl formate over time. Food consumption was calculated weekly for 13 weeks. Black squares, blank squares, triangles, and circles indicated the mean values of 0, 66, 330, and 1,320 ppm ethyl formate groups, respectively. $^{*}p < 0.05$ and $^{**}p < 0.01$.

control rats (Fig. 1). Male rats exposed to 330 ppm ethyl formate showed increased body weight gain on week 4 compared with that of the control group (Fig. 2).

Urinalysis. Ketone body (15 mg/dL) levels above control values were detected in rats of both sexes exposed to 1,320 ppm and in female rats exposed to 330 ppm ethyl formate. Urobilinogen (1 mg/dL) levels above control values were detected in male rats exposed to 1,320 ppm ethyl formate.

Hematology. The hematological parameters analyzed are shown in Table 1. HGB and HCT increased in male rats that inhaled 1,320 ppm ethyl formate ($p < 0.01$). Reti decreased in female rats that inhaled 1,320 ppm ethyl formate ($p < 0.01$).

Serum chemistry. The serum biochemistry parameters are shown in Table 2. Ca and TG levels decreased in male and female rats exposed to ethyl formate at 1,320 ppm, respectively ($p < 0.01$).

Organ weight. The organ weights are shown in Table 3. The absolute and relative adrenal weight increased in male and female rats exposed to ethyl formate at 1,320 ppm compared with the value in the control group (absolute of male: $p < 0.05$, others: $p < 0.01$). The absolute or relative thymus weight or both decreased in male and female rats exposed to ethyl formate at 1,320 ppm compared with values in the control rats (absolute of male: $p < 0.01$, absolute and relative of female: $p < 0.01$).

The absolute heart, kidney, liver, lung, and spleen weights decreased in male and female rats exposed to 1,320 ppm ethyl formate. The relative brain, kidney, and lung weights increased in male and female rats exposed to 1,320 ppm ethyl formate.

Table 1. Hematology results of rats exposed to ethyl formate

Parameter (units of measure)	Concentration (ppm)			
	0	66	330	1320
Male				
No. of animals	10	10	10	10
WBC (10^3/μL)	6.55 ± 1.26	7.41 ± 1.37	7.02 ± 1.55	6.33 ± 1.38
WBC differential count				
Neutrophil (10^3/μL)	1.64 ± 0.36	2.12 ± 1.38	1.64 ± 0.25	1.55 ± 0.35
Lymphocyte (10^3/μL)	4.51 ± 1.01	4.82 ± 0.96	4.93 ± 1.25	4.44 ± 1.16
Monocyte (10^3/μL)	0.15 ± 0.03	0.19 ± 0.06	0.19 ± 0.06	0.13 ± 0.02
Eosinophil (10^3/μL)	0.12 ± 0.03	0.14 ± 0.03	0.13 ± 0.04	0.13 ± 0.03
Basophil (10^3/μL)	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02
Neutrophil (%)	25.33 ± 6.03	27.40 ± 12.18	24.04 ± 3.69	24.74 ± 4.29
Lymphocyte (%)	68.44 ± 6.20	66.20 ± 12.04	69.48 ± 3.75	69.72 ± 4.38
Monocyte (%)	2.40 ± 0.44	2.51 ± 0.66	2.65 ± 0.67	2.00 ± 0.40
Eosinophil (%)	1.81 ± 0.37	1.92 ± 0.52	2.83 ± 2.93	2.07 ± 0.61
Basophil (%)	0.68 ± 0.18	0.70 ± 0.18	0.77 ± 0.23	0.76 ± 0.33
RBC (10^6/μL)	8.44 ± 0.27	8.45 ± 0.31	8.52 ± 0.23	8.78 ± 0.37
HGB (g/dL)	15.15 ± 0.53	15.36 ± 0.47	15.18 ± 0.53	16.16 ± 0.48**
HCT (%)	48.91 ± 2.44	50.06 ± 1.85	50.05 ± 1.75	52.26 ± 2.32**
MCV (fL)	57.91 ± 1.70	59.36 ± 3.11	58.80 ± 2.21	59.58 ± 2.13
MCH (pg)	17.96 ± 0.40	18.21 ± 0.83	17.83 ± 0.51	18.42 ± 0.72
MCHC (g/dL)	31.05 ± 1.19	30.69 ± 0.88	30.34 ± 0.49	30.95 ± 1.01
PLT (10^3/μL)	807.1 ± 97.7	823.1 ± 80.2	868.5 ± 101.6	745.7 ± 92.8
Reti (10^3/μL)	188.9 ± 21.2	217.5 ± 34.2	209.2 ± 32.0	165.1 ± 40.0
Reti (%)	2.24 ± 0.26	2.57 ± 0.37	2.43 ± 0.43	1.88 ± 0.46
PT (sec)	10.89 ± 0.54	11.44 ± 1.34	11.06 ± 1.03	10.70 ± 0.62
APTT (sec)	18.66 ± 3.91	17.18 ± 1.48	17.02 ± 1.68	17.50 ± 1.99
Female				
No. of animals	10	10	10	10
WBC (10^3/μL)	5.04 ± 1.38	4.82 ± 1.21	5.08 ± 1.14	4.66 ± 1.20
WBC differential count				
Neutrophil (10^3/μL)	1.15 ± 0.32	1.21 ± 0.36	1.25 ± 0.53	1.04 ± 0.23
Lymphocyte (10^3/μL)	3.62 ± 1.11	3.35 ± 0.92	3.58 ± 0.76	3.39 ± 0.97
Monocyte (10^3/μL)	0.12 ± 0.05	0.12 ± 0.05	0.11 ± 0.06	0.08 ± 0.03
Eosinophil (10^3/μL)	0.09 ± 0.04	0.09 ± 0.03	0.10 ± 0.05	0.10 ± 0.03
Basophil (10^3/μL)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Neutrophil (%)	23.03 ± 5.15	25.01 ± 5.47	24.14 ± 6.91	22.85 ± 4.06
Lymphocyte (%)	71.51 ± 5.65	69.59 ± 6.24	71.08 ± 7.41	72.12 ± 4.20
Monocyte (%)	5.59 ± 10.70	2.52 ± 0.82	1.98 ± 0.69	1.78 ± 0.35
Eosinophil (%)	1.82 ± 0.43	1.89 ± 0.42	1.84 ± 0.69	2.26 ± 0.61
Basophil (%)	0.17 ± 0.09	0.13 ± 0.07	0.21 ± 0.09	0.13 ± 0.08
RBC (10^6/μL)	8.15 ± 0.56	8.02 ± 0.31	8.23 ± 0.26	8.36 ± 0.26
HGB (g/dL)	15.52 ± 0.69	15.48 ± 0.55	15.86 ± 0.42	16.08 ± 0.51
HCT (%)	43.57 ± 1.86	43.46 ± 1.45	44.48 ± 0.93	44.76 ± 1.41
MCV (fL)	53.52 ± 1.60	54.21 ± 0.96	54.09 ± 0.95	53.58 ± 1.49
MCH (pg)	19.09 ± 0.64	19.30 ± 0.51	19.29 ± 0.40	19.25 ± 0.59
MCHC (g/dL)	35.67 ± 0.62	35.59 ± 0.55	35.68 ± 0.58	35.90 ± 0.59
PLT (10^3/μL)	847.4 ± 300.6	880.5 ± 310.1	1014.6 ± 88.7	909.7 ± 116.2
Reti (10^3/μL)	163.0 ± 26.9	165.0 ± 21.6	163.7 ± 20.4	135.3 ± 25.7
Reti (%)	2.05 ± 0.41	2.06 ± 0.30	1.99 ± 0.27	1.62 ± 0.31**
PT (sec)	9.91 ± 0.70	9.68 ± 0.90	9.87 ± 0.67	10.35 ± 0.61
APTT (sec)	16.28 ± 0.62	17.24 ± 1.49	16.40 ± 1.29	16.50 ± 0.58

**Significantly different from the control group at $p < 0.01$.
Values indicate mean ± standard deviation.

Table 2. Serum biochemistry results in rats exposed ethyl formate

Parameter (units of measure)	Concentration (ppm)			
	0	66	330	1320
Male				
No. of animals	10	10	10	10
AST (U/L)	99.3 ± 38.4	85.9 ± 13.8	95.2 ± 28.5	82.4 ± 11.7
ALT (U/L)	43.8 ± 10.1	42.6 ± 6.3	46.9 ± 17.3	45.1 ± 6.0
GGT (U/L)	0.7 ± 0.5	0.7 ± 0.3	0.9 ± 0.7	0.7 ± 0.7
ALP (U/L)	342.2 ± 74.4	392.2 ± 82.1	322.0 ± 30.9	390.4 ± 103.2
T-Bili (mg/dL)	0.09 ± 0.11	0.13 ± 0.04	0.14 ± 0.03	0.13 ± 0.02
Glu (mg/dL)	140.2 ± 17.6	135.4 ± 9.9	138.0 ± 8.3	145.8 ± 16.9
TP (g/dL)	6.7 ± 0.3	6.8 ± 0.2	6.7 ± 0.2	6.6 ± 0.2
Alb (g/dL)	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	4.3 ± 0.2
A/G ratio	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
TG (mg/dL)	43.8 ± 20.7	78.6 ± 17.8	64.5 ± 18.4	35.7 ± 13.5
T-Chol (mg/dL)	77.2 ± 12.0	84.9 ± 13.7	80.8 ± 12.2	68.2 ± 8.9
BUN (mg/dL)	14.8 ± 2.2	14.2 ± 1.6	14.6 ± 1.6	15.5 ± 2..3
Crea (mg/dL)	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.00	0.47 ± 0.00
Ca (mg/dL)	10.2 ± 0.2	10.2 ± 0.3	10.2 ± 0.2	9.9 ± 0.2**
P (mg/dL)	6.4 ± 0.8	6.0 ± 0.7	6.3 ± 0.8	6.3 ± 0.6
Na (mmol/dL)	142.4 ± 0.9	143.4 ± 0.5	143.0 ± 0.6	143.4 ± 1.1
K (mmol/dL)	4.77 ± 0.57	4.74 ± 0.59	4.74 ± 0.27	4.65 ± 0.34
Cl (mmol/dL)	103.9 ± 0.8	104.1 ± 1.2	104.3 ± 0.9	104.7 ± 1.1
Female				
No. of animals	10	10	10	10
AST (U/L)	104.1 ± 43.3	197.4 ± 201.8	98.6 ± 25.5	84.1 ± 12.6
ALT (U/L)	54.6 ± 21.4	109.8 ± 116.6	46.3 ± 14.0	46.7 ± 6.4
GGT (U/L)	1.4 ± 0.8	2.2 ± 2.1	1.6 ± 0.7	1.4 ± 0.8
ALP (U/L)	192.2 ± 30.7	250.2 ± 108.2	219.5 ± 119.5	257.7 ± 73.3
T-Bili (mg/dL)	0.13 ± 0.03	0.11 ± 0.07	0.12 ± 0.02	0.12 ± 0.02
Glu (mg/dL)	113.1 ± 13.8	121.2 ± 8.3	124.3 ± 10.7	115.0 ± 14.2
TP (g/dL)	6.8 ± 0.5	7.3 ± 0.4	6.9 ± 0.6	6.4 ± 0.3
Alb (g/dL)	4.4 ± 0.3	4.6 ± 0.2	4.4 ± 0.4	4.2 ± 0.2
A/G ratio	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.1
TG (mg/dL)	28.0 ± 10.8	30.7 ± 8.6	20.0 ± 10.0	14.2 ± 7.0**
T-Chol (mg/dL)	102.3 ± 11.2	118.7 ± 20.2	94.3 ± 22.3	83.9 ± 17.6
BUN (mg/dL)	17.6 ± 3.6	17.7 ± 3.9	17.2 ± 1.9	16.7 ± 2.5
Crea (mg/dL)	0.44 ± 0.05	0.45 ± 0.05	0.46 ± 0.05	0.43 ± 0.05
Ca (mg/dL)	9.9 ± 0.7	10.2 ± 0.4	9.9 ± 0.5	9.6 ± 0.3
P (mg/dL)	4.9 ± 1.2	5.2 ± 1.2	5.1 ± 1.1	5.8 ± 1.0
Na (mmol/dL)	140.4 ± 0.7	139.5 ± 1.8	141.7 ± 1.2	142.1 ± 1.3
K (mmol/dL)	4.44 ± 0.16	4.75 ± 0.62	4.57 ± 0.46	4.57 ± 0.32
Cl (mmol/dL)	104.8 ± 1.7	103.7 ± 2.1	105.2 ± 1.5	106.3 ± 1.5

**Significantly different from the control group at $p < 0.01$.
Values indicate mean ± standard deviation.

Necropsy and histopathological examination. All the macroscopic findings of the eye, liver, and ovaries were considered to be incidental and of no toxicological significance.

Degeneration was observed in 9/10 male and 10/10 female rats exposed to 1,320 ppm and in 2/10 females at 330 ppm (Fig. 3). Squamous metaplasia was observed in 2/10 male and 1/10 female rats at 1,320 ppm (Fig. 3). Morphologically, degeneration was characterized by disarrangement of the cellular organization, cilia loss, epithelial alteration, apoptosis, mitosis, and atrophy of the olfactory and supporting cells of the olfactory epithelium. Lesions were mainly noted in the dorsal meatus and occasionally in the septum and turbinate. All other microscopic findings were consistent with the normal background lesions in SD rats of the same age group as the rats included in the study and, therefore, were considered to be spontaneous and incidental.

Table 3. Organ weights of rats exposed to ethyl formate

Organ	Concentration (ppm)			
	0	66	330	1320
Male				
No. of animals	10	10	10	10
Body weight[a)] (g)	525.6 ± 43.0	543.7 ± 28.3	529.4 ± 28.3	405.1 ± 35.7**
Brain (g)	2.18 ± 0.09	2.17 ± 0.10	2.17 ± 0.09	2.08 ± 0.11
% to body weight	0.42 ± 0.03	0.40 ± 0.02	0.41 ± 0.02	0.52 ± 0.05**
Adrenals (g)	0.064 ± 0.006	0.063 ± 0.008	0.068 ± 0.011	0.074 ± 0.008*
% to body weight	0.012 ± 0.001	0.012 ± 0.001	0.013 ± 0.002	0.018 ± 0.003**
Thyroid glands (g)	0.024 ± 0.00	0.026 ± 0.00	0.025 ± 0.01	0.021 ± 0.00
% to body weight	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Heart (g)	1.44 ± 0.12	1.47 ± 0.12	1.45 ± 0.11	1.18 ± 0.10**
% to body weight	0.28 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.29 ± 0.02
Lung (g)	1.64 ± 0.10	1.73 ± 0.15	1.81 ± 0.27	1.49 ± 0.11*
% to body weight	0.31 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.37 ± 0.03**
Liver (g)	12.59 ± 1.13	13.87 ± 1.35	13.44 ± 1.04	9.98 ± 1.19**
% to body weight	2.40 ± 0.13	2.55 ± 0.15	2.54 ± 0.14	2.46 ± 0.14
Spleen (g)	0.785 ± 0.080	0.835 ± 0.088	0.844 ± 0.077	0.601 ± 0.072**
% to body weight	0.150 ± 0.012	0.154 ± 0.013	0.159 ± 0.011	0.148 ± 0.009
Thymus (g)	0.391 ± 0.082	0.379 ± 0.116	0.399 ± 0.110	0.241 ± 0.050**
% to body weight	0.074 ± 0.013	0.070 ± 0.021	0.075 ± 0.020	0.059 ± 0.009
Kidneys (g)	3.12 ± 0.38	3.22 ± 0.46	3.03 ± 0.27	2.57 ± 0.23**
% to body weight	0.59 ± 0.05	0.59 ± 0.07	0.57 ± 0.03	0.63 ± 0.02*
Testes (g)	3.81 ± 0.23	3.78 ± 0.38	3.73 ± 0.24	3.61 ± 0.18
% to body weight	0.73 ± 0.06	0.70 ± 0.08	0.71 ± 0.06	0.89 ± 0.06**
Epididymides (g)	1.41 ± 0.15	1.39 ± 0.11	1.37 ± 0.10	1.34 ± 0.08
% to body weight	0.27 ± 0.03	0.26 ± 0.02	0.26 ± 0.02	0.33 ± 0.03
Female				
No. of animals				
Body weight[a)] (g)	252.2 ± 21.6	264.6 ± 17.0	259.1 ± 23.1	219.1 ± 20.1**
Brain (g)	1.98 ± 0.08	1.96 ± 0.06	1.96 ± 0.06	1.93 ± 0.08
% to body weight	0.79 ± 0.05	0.74 ± 0.06	0.076 ± 0.06	0.89 ± 0.07**
Adrenals (g)	0.060 ± 0.011	0.064 ± 0.009	0.066 ± 0.006	0.074 ± 0.007**
% to body weight	0.024 ± 0.005	0.024 ± 0.004	0.026 ± 0.003	0.034 ± 0.004**
Thyroid glands (g)	0.016 ± 0.003	0.015 ± 0.005	0.015 ± 0.005	0.013 ± 0.005
% to body weight	0.006 ± 0.001	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002
Heart (g)	0.81 ± 0.07	0.82 ± 0.07	0.81 ± 0.07	0.70 ± 0.07**
% to body weight	0.32 ± 0.02	0.31 ± 0.02	0.31 ± 0.03	0.32 ± 0.02
Lung (g)	1.12 ± 0.09	1.16 ± 0.11	1.14 ± 0.10	1.04 ± 0.12
% to body weight	0.45 ± 0.02	0.44 ± 0.03	0.44 ± 0.04	0.47 ± 0.04
Liver (g)	6.35 ± 0.75	6.91 ± 0.56	6.39 ± 0.60	5.31 ± 0.70**
% to body weight	2.51 ± 0.16	2.61 ± 0.16	2.47 ± 0.13	2.42 ± 0.11
Spleen (g)	0.528 ± 0.108	0.527 ± 0.070	0.507 ± 0.072	0.393 ± 0.061**
% to body weight	0.209 ± 0.035	0.199 ± 0.024	0.195 ± 0.016	0.179 ± 0.018
Thymus (g)	0.281 ± 0.041	0.278 ± 0.032	0.303 ± 0.047	0.197 ± 0.034**
% to body weight	0.112 ± 0.021	0.105 ± 0.012	0.117 ± 0.016	0.090 ± 0.011**
Kidneys (g)	1.70 ± 0.20	1.86 ± 0.20	1.72 ± 0.16	1.52 ± 0.15
% to body weight	0.67 ± 0.04	0.70 ± 0.05	0.66 ± 0.05	0.69 ± 0.03
Ovaries (g)	0.081 ± 0.014	0.116 ± 0.142	0.076 ± 0.006	0.063 ± 0.009
% to body weight	0.032 ± 0.005	0.044 ± 0.055	0.030 ± 0.003	0.029 ± 0.004
Uterus (g)	0.608 ± 0.164	0.724 ± 0.230	0.690 ± 0.248	0.531 ± 0.219
% to body weight	0.241 ± 0.059	0.276 ± 0.098	0.265 ± 0.088	0.243 ± 0.100

[a)]The body weights were measured right before necropsy after overnight fasting.
Significantly different from vehicle control: *$p < 0.05$, **$p < 0.01$. Values are mean ± SD.

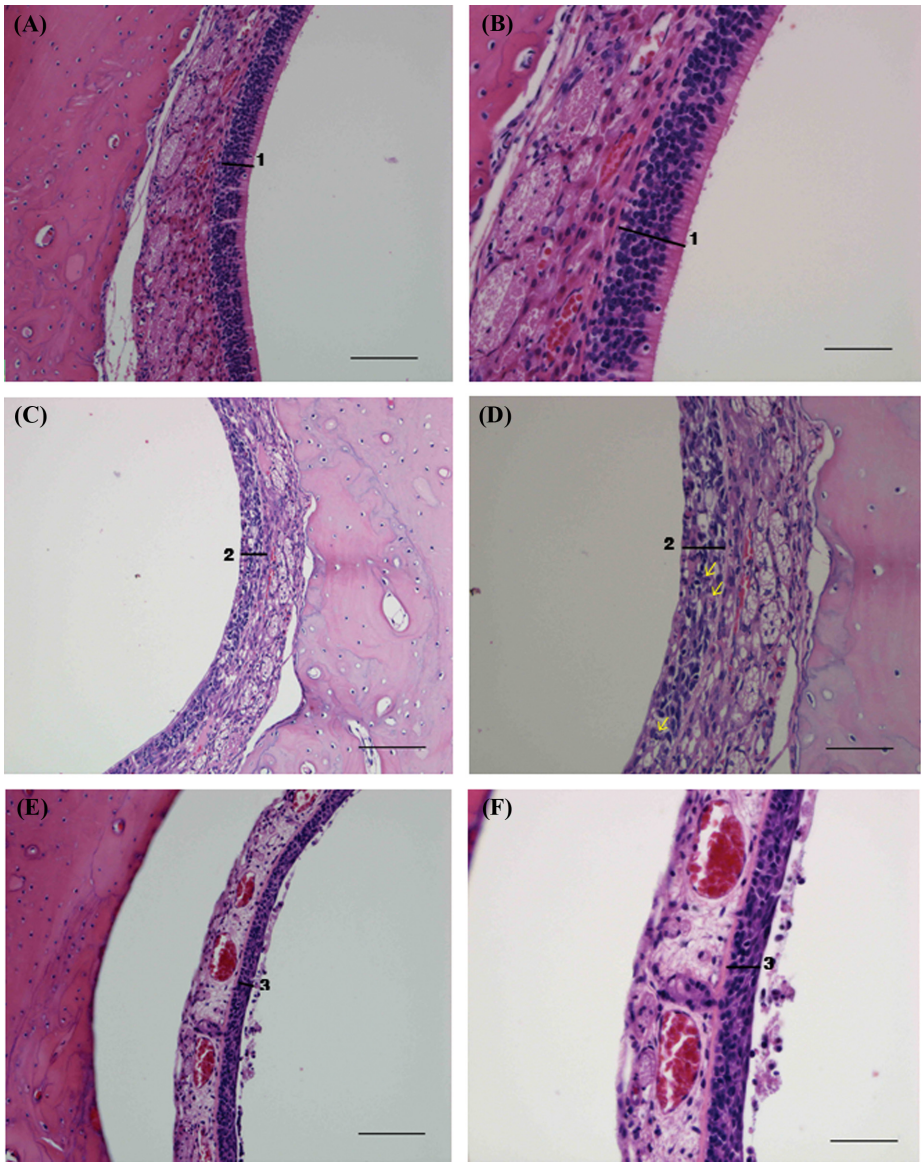

Fig. 3. Histopathological alterations in the nasopharyngeal tissue of rats exposed to (C)-(F) 1320 ethyl formate and (A), (B) control. (A), (B) Olfactory epithelium of rats exposed to fresh air was intact (for line 1) while that of rats exposed to 1,320 ppm ethyl formate showed (B), (C) degeneration characterized by cilia loss (arrow), disarrangement of the cellular organization (line 2), atrophy (line 2), and apoptosis (arrow) and (D), (F) squamous metaplasia (line 3). Magnification: (A), (C), (E) × 200, (B), (D), (F) × 400. Scale Bar (A), (C), (E) 100 μm, (B), (D), (F) 50 μm. Hematoxylin and eosin (H&E) staining.

DISCUSSION

We evaluated the potential toxicity of ethyl formate in rats to determine its safety for long-term and low-level exposure.

Locomotor activity decreased during exposure and recovered afterward in males and females exposed to 1,320 ppm ethyl formate, and this effect was considered an ethyl formate-related change. However, it was not considered adverse because the change was transient and noted only during exposure.

Body weight and food consumption decreased continuously in both sexes exposed to 1,320 ppm ethyl formate from week 1 or 3 compared with the control values. The hallmarks of stress response include decreased body weight and food consumption in toxicity study (16). We hypothesized that these changes were in responses to stress. The decreased body weight and food consumption were considered to be ethyl formate-related adverse effects since the pattern was continuous and significant. The increased body weight of the male rats exposed to 330 ppm at week 4 was not considered to be ethyl formate-related change because

they were isolated changes.

Ketone body (15 mg/dL) levels above the control values were detected in both sexes and females exposed to 1,320 and 330 ppm ethyl formate, respectively. Reduced body weight and food consumption are occasionally accompanied by increase in urinary ketone levels, because energy metabolism under those conditions shifts from gluconeogenesis to incomplete oxidation of fatty acids (17,18). The body weight and food consumption decreased in both sexes exposed to 1,320 ppm ethyl formate. However, it was unclear whether the effect on ketone body levels was ethyl formate-related because of the small variance and uncertain dose-dependency.

Urobilinogen (1 mg/dL) levels above control values were detected in males exposed to 1,320 ppm ethyl formate. However, this effect was not considered ethyl formate-related because 1 mg/dL urobilinogen is regarded as the normal range according to the kit product insert.

Following the inhalation of ethyl formate 1,320 ppm, the HGB and HCT levels increased in male rats while Reti count decreased in female rats. However, these effects were not considered ethyl formate-related because no other RBC-related changes occurred (18).

Furthermore, Ca and TG levels decreased in male and female rats exposed to ethyl formate 1,320 ppm, respectively. These were not considered ethyl formate-related changes owing to the small magnitude of change and absence of related alterations.

Absolute and relative increases in the adrenal weight, as well as absolute or relative decreases in the thymus weight or both were noted in rats of both sexes exposed to ethyl formate 1,320 ppm. Increased adrenal and decreased thymus weights could be regarded as a stress response based on a weight-of-evidence approach (16). These changes were accompanied by decrease in body weights and food consumption considered hallmarks of stress response. The histopathological examination did not reveal any evidence to explain the weight changes observed in the adrenal gland and thymus. Therefore, these were also considered to be ethyl formate-related and secondary to stress. The other organ weight changes were considered secondary to body weight alteration and not adverse effects.

Degeneration or squamous metaplasia of the olfactory epithelium or both in the nasopharyngeal tissue was noted in male and female rats exposed to 1,320 ppm ethyl formate and in female rats exposed to 330 ppm. The severity of the degeneration and squamous metaplasia was minimal to mild and the affected cells were mainly supporting and olfactory cells. Inhaled and deposited material in nasal cavity could have potential toxicity in the nasal cavity (19). These were considered to be reactive and reparative responses to repeated irritation.

Taken together, our results indicate that ethyl formate-induced changes were not observed in male and female rats exposed to 330 and 66 ppm, respectively. This observation indicates that the exposure of rats to < 66 ppm ethyl formate for 13 weeks is relatively safe. However, the reversibility of nasopharyngeal tissue injury and the effects of more prolonged exposure to ethyl formate were not confirmed in the present study. Further study is needed to confirm reversibility and chronic toxicity. Although this study has certain limitation, to the best of our knowledge, this is the first study to comprehensively repeated inhalation toxicity of ethyl formate in rats, and these results could contribute to the development of strategies for the control of occupational environmental hazards related to this substance.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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