



Subchronic Inhalation Toxicity of Trichloroacetonitrile on the Sprague Dawley Rats

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Trichloroacetonitrile is used as an intermediate in insecticides, pesticides, and dyes. In Korea alone, over 10 tons are used annually. Its oral and dermal toxicity is classified as category 3 according to the globally harmonized system of classification and labelling of chemicals, and it is designated a toxic substance by the Ministry of Environment in Korea. There are no available inhalation toxicity data on trichloroacetonitrile. Thus, the present study performed inhalation tests to provide data for hazard and risk assessments. Sprague-Dawley rats were exposed to trichloroacetonitrile at concentrations of 4, 16, or 64 ppm for 6 hour per day 5 days per week for 13 weeks in a repeated study. As a result, salivation, shortness of breath, and wheezing were observed, and their body weights decreased significantly ($p < 0.05$) in the 16 and 64 ppm groups. All the rats in 64 ppm group were dead or moribund within 4 weeks of the exposure. Some significant changes were observed in blood hematology and serum biochemistry (e.g., prothrombin time, ratio of albumin and globulin, blood urea nitrogen, and triglycerides), but the values were within normal physiological ranges. The major target organs of trichloroacetonitrile were the nasal cavity, trachea, and lungs. The rats exposed to 16 ppm showed moderate histopathological changes in the transitional epithelium and olfactory epithelium of the nasal cavity. Nasal-associated lymphoid tissue (NALT) and respiratory epithelium were also changed. Respiratory lesions were common in the dead rats that had been exposed to the 64 ppm concentration. The dead animals also showed loss of cilia in the trachea, pneumonitis in the lung, and epithelial hyperplasia in the bronchi and bronchioles. In conclusion, the no-observed-adverse-effect level (NOAEL) was estimated to be 4 ppm. The main target organs of trichloroacetonitrile were the nasal cavity, trachea, and lungs.

Key words: Acute toxicity, Inhalation, Repeated toxicity, Trichloroacetonitrile, No-observed-adverse-effect level

INTRODUCTION

Increasing numbers of chemicals are used in industries today, putting workers at greater risk of exposure.

There is limited information to assess these risks (1). To protect workers in the workplace, it is necessary to control the exposure to a chemical substance below an occupational exposure limit value, which is the level that a worker can be exposed to for a working lifetime without the chemical exerting adverse health effects. In the workplace, thresh-

old limit values (TLVs) are set for some chemicals by specific authorities, such as the Ministry of Employment and Labor. However, TLVs are not available for 700 TLVs of the 40,000 chemicals used in the workplace in Korea (2). In 2006, the European Union adopted a new integrated regulation on the registration, evaluation, authorization, and restriction of chemicals (REACH) to protect human health and the environment from the risks posed by chemicals (3). According to REACH, manufacturers or importers must perform chemical safety assessments for all substances manufactured or imported in quantities of 10 tons or more per year (3). The derived no-effect level (DNEL), which is the level of exposure to a substance above which humans should not be exposed, is calculated during the assessment. Both DNELs and TLVs have a similar purpose: both are intended to prevent health impairments by controlling exposure levels to certain chemicals (4). Over 10 tons of trichloroacetonitrile is reportedly used in Korea, and it is designated a toxic chemical (5,6). However, no TLV has

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been set for trichloroacetonitrile. With regards to the potential hazard posed by trichloroacetonitrile, its LD₅₀ for acute oral toxicity in rats was 250 mg/kg, and its LD₅₀ for acute dermal toxicity was 1,300 mg/kg in rabbits (7). Studies reported that trichloroacetonitrile may cause drowsiness and breathing difficulties when acutely inhaled (7,8). However, acute inhalation toxicity studies and repeated inhalation toxicity studies, which are needed for classifying hazards and assessing DNEL, have not been performed. Thus, acute inhalation toxicity and 13-week repeated inhalation toxicity studies were performed in the present study to provide information for risk assessments of this chemical.

MATERIALS AND METHODS

Animals. Six-week old male and female specific pathogen-free Sprague-Dawley rats obtained from Japan SLC Inc. (Shizuoka, Japan) were acclimated for one week prior to trichloroacetonitrile exposure. The rats were housed individually in stainless steel wire mesh cages in whole-body exposure chambers during the exposure period. The animal rooms and inhalation chambers were maintained at a temperature of 22 ± 3°C and relative humidity of 50 ± 20%, with 12~15 air changes per hour. The lighting in the animal rooms was controlled, with the lights on from 08:00 to 20:00 hr. The rats had access to sterilized tap water and commercial rodent chow (5053-Picolab Rodent 20; PMI Nutrition, St. Louis, MO, USA) *ad libitum*. Prior to obtaining the rats for the study was approved by the laboratory's Animal Ethics Committee, and all the experiments were conducted in accordance with established animal care protocols.

Exposure to trichloroacetonitrile vapor. Trichloroacetonitrile was purchased from Qingdao On-Billion Industrial Co., Ltd., Quando, China). The rats were exposed to the chemical in whole-body exposure chambers (Sibata Co., SIS-20RG, Niigata, Japan), which included a gas generator (Sibata Co., VG-4R). The animals were exposed to trichloroacetonitrile at concentrations of 4, 16, or 64 ppm or fresh air for 6 hour per day 5 days per week for 13 weeks. The concentrations of trichloroacetonitrile vapor in the chambers were measured by gas chromatography (Shimadzu Co., GCS -14FFS, Kyoto, Japan) every 15 min during the exposure period, and they were controlled to be within ± 5% of each target concentration. The equipment used in the experiments included a flame ionization detector and a Silicon DC-200 15% chromosorb (AW-DMCS) column. The test conditions were as follows: a detector temperature of 120°C, an oven temperature of 100°C, an injector temperature of 120°C, and an injection volume of 1 mL.

Assessment of adverse effects. The rats were observed twice daily (before and after the chemical exposure)

throughout the study period to check for any clinical signs of toxicity, morbidity, and/or mortality. The animals' body weights were measured at the beginning of the exposure to trichloroacetonitrile and once a week during the exposure period. The activities of the rats were measured for 3 days at the end of the exposure period with an activity monitoring system (Sibata Co., MCMEA-8). All rats were fasted overnight prior to necropsy, and blood samples were drawn from the abdominal artery after they were anesthetized with isoflurane (Hana Pharm, Seoul, Korea). The blood samples were collected into blood count bottles containing EDTA-3K (Beckton Dickinson, USA), and the total leucocytes, differential leucocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and erythrocytes were counted within 20 min using an automatic hematology analyzer (Advia 120E, Bayer, Boston, MA, USA). The remaining blood samples were centrifuged at 3,000 rpm for 10 min within one hour after collection to obtain sera. The sera were stored at -80°C in a freezer prior to the analysis. The following serum biochemistry parameters were evaluated using an automatic biochemical analyzer (HITACHI 7060, Tokyo, Japan): total protein, albumin, albumin/globulin ratio, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ -glutamyl transferase, creatinine, blood urea nitrogen, total cholesterol, triglycerides, and glucose. The tissues were routinely processed, embedded in paraffin, and sectioned to 3~5 μ m. The sections were stained with hematoxylin-eosin stain for microscopic examination and examined microscopically. All gross lesions, as defined by the study pathologist, were included in the examination (9).

Statistical analysis. The means and standard deviations were calculated for all the experimental groups. The data were processed with a one-way analysis of variance, followed by Dunnett's test to identify differences from the control. Statistical analyses were performed using SPSS (version 19.0; IBM, NY, USA) and SigmaStat software (version 3.5; Systat Software, San Jose, CA, USA). In all cases, a *p* value of < 0.05 was accepted as statistically significant.

RESULTS

In an earlier study, five male Sprague-Dawley were exposed to 85, 340, and 1,700 ppm of trichloroacetonitrile at each concentration for 4 hr. In that study, all the rats exposed to 85 ppm survived, whereas all those exposed to 340 ppm died (data not shown). This helped to determine the test concentrations for the present 13-week repeated inhalation toxicity as 4, 16, and 60 ppm. The concentrations of trichloroacetonitrile during the experimental period were 4.06 ± 0.17, 16.11 ± 0.59, and 62.72 ± 3.64 ppm for the low-, medium-, and high-exposure groups, respectively, which were within 10% of the preset concentrations (Fig. 1).

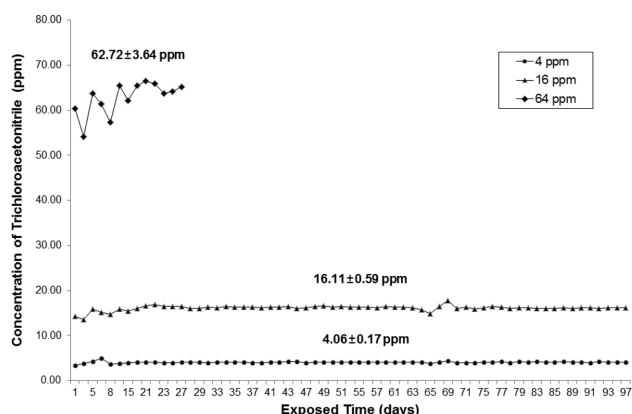


Fig. 1. Concentrations of trichloroacetonitrile in the inhalation chamber during the experimental period.

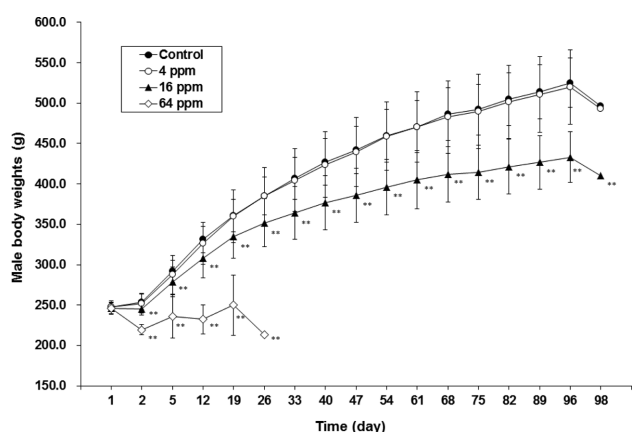


Fig. 2. Changes in the body weights of the male rats exposed to trichloroacetonitrile for 13 weeks. Significant differences as compared with a control, ** $p < 0.01$.

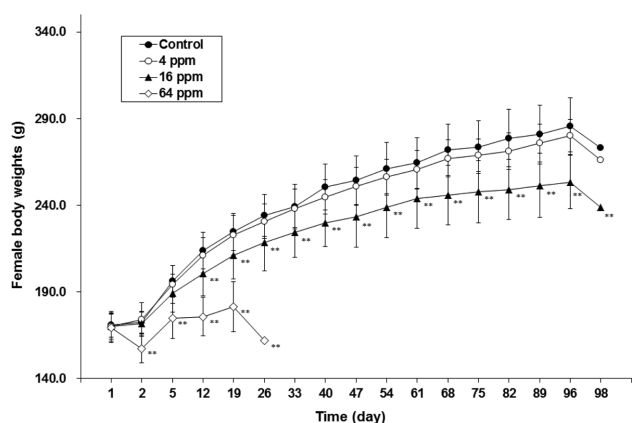


Fig. 3. Changes in the body weights of female rats exposed to trichloroacetonitrile for 13 weeks. Significant differences as compared with a control, ** $p < 0.01$.

Salivation, shortness of breath, and wheezing were observed in the medium- and high-exposure groups. There

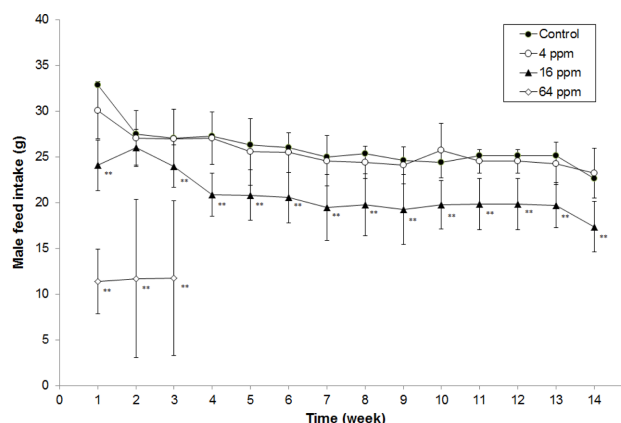


Fig. 4. Daily mean food consumption of male rats exposed to trichloroacetonitrile for 13 weeks. Significant differences as compared with a control, ** $p < 0.01$.

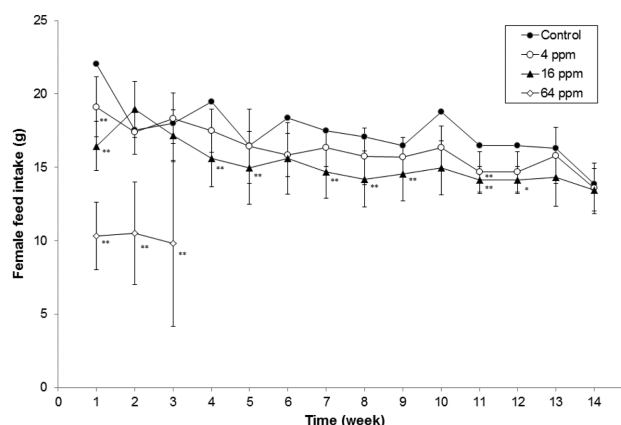


Fig. 5. Daily mean food consumption of male rats exposed to trichloroacetonitrile for 13 weeks. Significant differences as compared with a control, * $p < 0.05$, ** $p < 0.01$.

were no treatment-related toxic signs observed in any of the rats in the low-exposure group (data not shown). The average body weights of the rats in the high-exposure group decreased on the first day of the exposure and then increased slowly for the next 2 weeks. However, all were dead or moribund within 4 weeks of the exposure. The body weight gains of both male and female rats were significantly slow ($p < 0.01$) in the medium-exposure group (Fig. 2 and 3). The food consumption of the males and females in the medium-exposure group decreased significantly ($p < 0.05$ or $p < 0.01$) compared to that of a normal control group (Fig. 4 and 5). Trichloroacetonitrile exposure did not affect the physical activity level of the rats (Fig. 6).

The hematological findings showed a significantly increased prothrombin time in the female rats in the medium-exposure group ($p < 0.01$), but none of the other parameters differed significantly in either sex in the trichloroacetonitrile-exposed group compared to those of the normal control

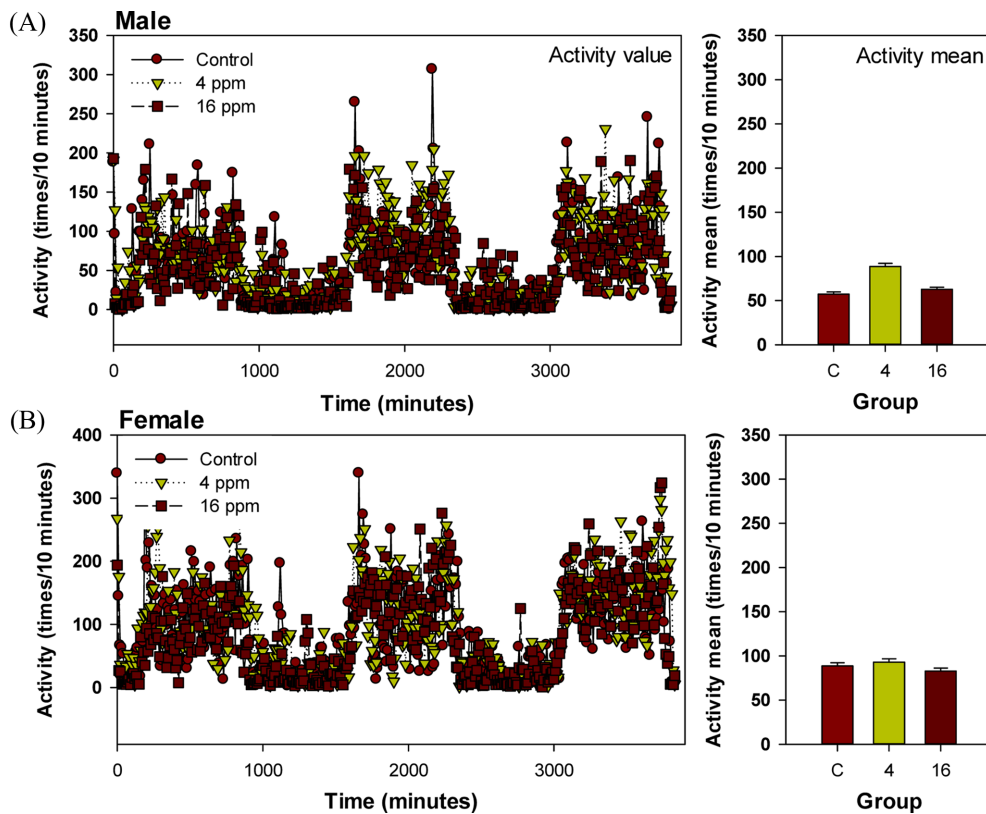


Fig. 6. Activities of the male (A) and/or female (B) rats exposed to trichloroacetonitrile for 13 weeks.

Table 1. Effects of trichloroacetonitrile on blood hematology

Tests	Units	Male (groups/ppm)			Female (groups/ppm)		
		Control (0)	Low (4)	Medium (16)	Control (0)	Low (4)	Medium (16)
WBC	$10^3/\mu\text{L}$	4.62 ± 0.99	5.38 ± 1.58	4.32 ± 1.04	3.74 ± 0.91	3.99 ± 1.01	3.05 ± 1.09
NE	%	22.9 ± 6.0	24.3 ± 13.7	26.0 ± 5.5	20.1 ± 3.9	21.0 ± 5.9	22.5 ± 5.8
LYM	%	73.5 ± 6.6	72.3 ± 13.9	70.3 ± 5.3	75.5 ± 3.7	74.3 ± 6.4	73.3 ± 5.7
MONO	%	1.9 ± 0.4	1.7 ± 0.6	1.7 ± 0.4	2.1 ± 0.4	2.5 ± 0.5	2.1 ± 0.7
EOS	%	1.2 ± 0.5	1.2 ± 0.5	1.6 ± 0.7	1.8 ± 0.6	1.7 ± 0.5	1.6 ± 1.0
BASO	%	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
RBC	$10^6/\mu\text{L}$	9.09 ± 0.46	8.83 ± 0.39	9.1 ± 0.18	7.98 ± 0.25	7.98 ± 0.23	8.12 ± 0.32
HB	g/dL	15.0 ± 0.7	14.6 ± 0.5	15.3 ± 0.5	14.7 ± 0.5	14.6 ± 0.5	15 ± 0.4
HCT	%	45.1 ± 1.5	43.8 ± 1.4	45.5 ± 1.1	42.9 ± 1.4	43.1 ± 1.2	43.9 ± 1.0
MCV	fL	49.6 ± 2.2	49.6 ± 1.3	50.1 ± 1.1	53.8 ± 0.7	54.0 ± 0.9	54.1 ± 1.3
MCH	pg	16.5 ± 0.9	16.5 ± 0.4	16.8 ± 0.5	18.4 ± 0.3	18.3 ± 0.4	18.5 ± 0.5
MCHC	g/dL	33.2 ± 0.5	33.4 ± 0.4	33.6 ± 0.4	34.2 ± 0.2	34.0 ± 0.4	34.2 ± 0.5
RDW	%	13.5 ± 1.2	14.3 ± 1.1	12.5 ± 0.6	11.3 ± 0.3	11.4 ± 0.3	11.2 ± 0.3
PDW	%	53.7 ± 2.5	51.4 ± 4.4	51.4 ± 4.8	52.2 ± 2.0	53.1 ± 2.5	54.3 ± 2.7
RETI	%	2.34 ± 0.43	2.69 ± 0.9	1.96 ± 0.36	1.88 ± 0.18	1.90 ± 0.34	1.81 ± 0.25
PLT	$10^3/\mu\text{L}$	912 ± 117	971 ± 115	825 ± 75	942 ± 79	896 ± 70	878 ± 96
MPV	fL	6.7 ± 0.3	6.6 ± 0.4	6.7 ± 0.5	7.0 ± 0.3	7.2 ± 0.2	7.0 ± 0.3
PT	sec	8.8 ± 0.3	9.0 ± 0.4	9.1 ± 0.3	8.1 ± 0.3	8.2 ± 0.4	$8.6 \pm 0.4^{**}$
APTT	sec	22.5 ± 1.1	21.5 ± 1.4	22.4 ± 0.7	20.6 ± 0.9	20.9 ± 0.8	22.8 ± 4.2

The values are presented as the mean \pm standard deviation. Significant differences were compared with a control, * $p < 0.05$; ** $p < 0.01$. WBC, white blood cell count; NE, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, RBC distribution width; PDW, platelet distribution width; RETI, reticulocyte; PLT, platelets; MPV, mean platelet volume; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 2. Effects of trichloroacetonitrile on the serum biochemistry

Tests	Units	Male (groups/ppm)			Female (groups/ppm)		
		Control (0)	Low (4)	Medium (16)	Control (0)	Low (4)	Medium (16)
TP	g/dL	6.4 ± 0.2	6.2 ± 0.2	6.2 ± 0.3	6.9 ± 0.4	6.8 ± 0.4	6.4 ± 0.4*
ALB	g/dL	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	3.1 ± 0.2	3.1 ± 0.3	2.8 ± 0.2
A/G	-	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0*	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0
BUN	mg/dL	15.3 ± 1.1	16.0 ± 1.3	13.8 ± 1.1*	15.5 ± 2.9	17.7 ± 2.7	15.0 ± 1.7
CREA	mg/dL	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
TBIL	mg/dL	0.0 ± 0.0	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01**
ALT	IU/L	52 ± 17	55 ± 13	57 ± 25	70 ± 16	88 ± 50	59 ± 11
AST	IU/L	109 ± 37	107 ± 37	94 ± 25	123 ± 32	169 ± 85	101 ± 17
LDH	IU/L	910 ± 473	852 ± 320	836 ± 244	684 ± 297	721 ± 324	652 ± 267
ALP	IU/L	443 ± 101	451 ± 117	424 ± 80	296 ± 133	288 ± 103	320 ± 136
GLU	mg/dL	147 ± 14	147 ± 16	141 ± 10	157 ± 10	150 ± 19	144 ± 13
TCHO	mg/dL	92 ± 11	92 ± 15	87 ± 16	120 ± 14	113 ± 16	101 ± 12*
TG	mg/dL	99 ± 33	83 ± 27	57 ± 21**	49 ± 26	32 ± 10	25 ± 7*
γ-GT	IU/L	10.3 ± 0.4	10.1 ± 0.3	10.0 ± 0.3	10.6 ± 0.2	10.6 ± 0.4	10.3 ± 0.3
CPK	IU/L	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Ca ²⁺	IU/L	539 ± 252	520 ± 190	446 ± 125	382 ± 179	394 ± 165	378 ± 158
IP	mg/dL	5.6 ± 1.0	5.8 ± 1.4	5.2 ± 1.0	5.3 ± 0.4	5.5 ± 0.5	5.1 ± 0.3
Na ⁺	mmol/L	142 ± 1.1	141.6 ± 0.9	141.6 ± 1.2	140.8 ± 0.9	140.8 ± 1.3	140.7 ± 1.4
K ⁺	mmol/L	4.71 ± 0.2	4.74 ± 0.19	4.46 ± 0.21*	4.61 ± 0.22	4.52 ± 0.32	4.42 ± 0.16
Cl ⁻	mmol/L	107.8 ± 1.1	108.2 ± 1.1	108.8 ± 1.5	107.9 ± 1.8	108.6 ± 0.7	108.5 ± 1.4

The values presented as the mean ± standard deviation. Significant differences were compared with a control, * $p < 0.05$, ** $p < 0.01$.

TP, total protein; ALB, albumin; BUN, blood urea nitrogen; CREA, creatinine; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GLU, glucose; TCHO, total cholesterol; TG, triglycerides; γ-GT, γ-glutamyl transpeptidase; CPK, creatine phosphokinase; IP, inorganic phosphorus.

Table 3. Absolute organ weights of the Sprague-Dawley rats

Organ weights (g)	Male (groups/ppm)			Female (groups/ppm)		
	Control (0)	Low (4)	Medium (16)	Control (0)	Low (4)	Medium (16)
Thymus	0.42 ± 0.06	0.40 ± 0.07	0.31 ± 0.06**	0.30 ± 0.04	0.31 ± 0.04	0.25 ± 0.04*
Heart	1.44 ± 0.13	1.45 ± 0.13	1.30 ± 0.08	0.94 ± 0.06	0.91 ± 0.06	0.89 ± 0.05
Adrenal L	0.035 ± 0.004	0.036 ± 0.006	0.032 ± 0.004	0.04 ± 0.008	0.034 ± 0.009	0.038 ± 0.007
Adrenal R	0.032 ± 0.008	0.033 ± 0.006	0.03 ± 0.004	0.035 ± 0.004	0.032 ± 0.005	0.037 ± 0.006
Testis L	1.87 ± 0.13	1.93 ± 0.08	1.81 ± 0.25	0.043 ± 0.008	0.045 ± 0.009	0.044 ± 0.007
Testis R	1.88 ± 0.11	1.89 ± 0.13	1.80 ± 0.20	0.041 ± 0.009	0.044 ± 0.005	0.040 ± 0.007
Lung	1.63 ± 0.10	1.65 ± 0.12	1.56 ± 0.10	1.16 ± 0.12	1.14 ± 0.05	1.18 ± 0.05
Kidney L	1.47 ± 0.21	1.50 ± 0.16	1.33 ± 0.08	0.88 ± 0.06	0.82 ± 0.07	0.82 ± 0.07
Kidney R	1.46 ± 0.15	1.49 ± 0.1	1.33 ± 0.09	0.90 ± 0.07	0.84 ± 0.06	0.84 ± 0.07
Spleen	0.80 ± 0.10	0.83 ± 0.15	0.65 ± 0.07**	0.51 ± 0.06	0.47 ± 0.02	0.44 ± 0.04*
Liver	13.72 ± 1.06	14.2 ± 1.97	11.90 ± 0.71**	7.80 ± 0.79	7.15 ± 0.63	6.60 ± 0.38**
Brain	2.23 ± 0.08	2.23 ± 0.07	2.12 ± 0.07**	2.05 ± 0.10	2.03 ± 0.07	1.96 ± 0.08

The values are presented as the mean ± standard deviation. Significant differences are compared with a control: * $p < 0.05$, ** $p < 0.01$.

group (Table 1). Table 2 summarizes the effects of trichloroacetonitrile on the serum biochemistry. The ratio of albumin to globulin significantly increased ($p < 0.05$) and blood urea nitrogen, triglycerides, and potassium significantly decreased in the male rats in the medium-exposure group compared to those of the normal control group ($p < 0.05$ or $p < 0.01$). Total bilirubin significantly increased ($p < 0.01$) and total protein, total cholesterol, and triglycerides significantly decreased in the female rats of the medium-exposure

group compared to that of the normal control ($p < 0.05$).

In terms of organ weights, the absolute weights of the liver, spleen, brain, and thymus significantly decreased ($p < 0.01$), whereas the relative weights of the kidney, testis, brain, lung, and heart significantly increased in the male rats in the medium-exposure group compared to those of the normal control group ($p < 0.05$ or $p < 0.01$). The absolute weights of the liver, spleen, and thymus significantly decreased ($p < 0.05$ or $p < 0.01$) and the relative weights of

Table 4. Relative organ weights of the Sprague-Dawley rats

Organ weights (%)	Male (groups/ppm)			Female (groups/ppm)		
	Control (0)	Low (4)	Medium (16)	Control (0)	Low (4)	Medium (16)
Thymus	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.11 ± 0.02	0.12 ± 0.01	0.10 ± 0.02
Heart	0.29 ± 0.02	0.29 ± 0.03	0.32 ± 0.02*	0.34 ± 0.02	0.34 ± 0.02	0.37 ± 0.03*
Adrenal L	0.007 ± 0.001	0.007 ± 0.001	0.008 ± 0.001	0.015 ± 0.003	0.013 ± 0.004	0.016 ± 0.003
Adrenal R	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.013 ± 0.001	0.012 ± 0.002	0.015 ± 0.002*
Testis L	0.38 ± 0.03	0.39 ± 0.30	0.44 ± 0.04**	0.016 ± 0.003	0.017 ± 0.003	0.018 ± 0.003
Testis R	0.38 ± 0.03	0.39 ± 0.20	0.44 ± 0.03**	0.015 ± 0.004	0.016 ± 0.002	0.017 ± 0.003
Lung	0.33 ± 0.02	0.33 ± 0.20	0.38 ± 0.04**	0.43 ± 0.04	0.43 ± 0.02	0.49 ± 0.03**
Kidney L	0.30 ± 0.04	0.31 ± 0.30	0.32 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.34 ± 0.02
Kidney R	0.29 ± 0.02	0.30 ± 0.02	0.32 ± 0.02**	0.33 ± 0.02	0.32 ± 0.02	0.35 ± 0.03
Spleen	0.16 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.19 ± 0.02
Liver	2.76 ± 0.17	2.87 ± 0.21	2.91 ± 0.12	2.86 ± 0.28	2.68 ± 0.19	2.77 ± 0.18
Brain	0.45 ± 0.03	0.45 ± 0.03	0.52 ± 0.05**	0.75 ± 0.04	0.76 ± 0.04	0.82 ± 0.04**

The values are presented as the mean ± standard deviation. Significant differences are compared with a control: * $p < 0.05$, ** $p < 0.01$.

the adrenal gland, brain, lung, and heart significantly increased in the female rats (Table 3 and 4).

In the necropsy, discolorations were found in the lobes of the lung, which did not seem to be correlated with the exposure concentrations of trichloroacetonitrile (data not shown). In the histopathological findings, the main changes were observed in the nasal cavities of the trichloroacetonitrile-exposed rats. For the nasal cavity, decreased cellularity was observed in the nasal-associated lymphoid tissue (NALT) (Fig. 7B), in addition to hyperplasia in the respiratory epithelium (Fig. 7D), epithelial hyperplasia (Fig. 7F) in the transitional epithelium and epithelial atrophy, nerve fascicle atrophy, and Bowman's gland atrophy in the olfactory epithelium (Fig. 7H). The histopathological symptoms were graded and scored as minimal, 1; slight, 2, and moderate, 3. The effects of trichloroacetonitrile on the nasal cavity are summarized in the Table 5. The cellularity in the NALT decreased slightly in the medium-exposure group and decreased minimally in the low-exposure group. In the respiratory epithelium, minimal goblet cell hyperplasia was present in the 4 ppm- and 16 ppm-exposed rats. The transitional epithelium was affected more in male than in female rats. Slight squamous metaplasia was present in the 16 ppm-exposed male rats. Overall, the most prominent target organ was the olfactory epithelium. Moderate atrophy and nerve fascicle atrophy and slight Bowman's gland atrophy were found in the 16 ppm-exposed rats. In the 4 ppm-exposed rats, only the transitional epithelium in the male rats was slightly affected. The levels of decreased cellularity of NALT in both the male and female rats, goblet cell hyperplasia of the respiratory epithelium in male rats, and atrophy of the olfactory epithelium were minimal.

DISCUSSION

The present study was performed to identify the toxicity

of trichloroacetonitrile by inhalation exposure. The exposure concentrations in a previous acute toxicity study were 85 ppm (0.5 mg/L), 340 ppm (2 mg/L), and 1,700 ppm (10 mg/L) according to acute inhalation toxicity test guideline 403 (10) of the OECD (Organization for Economic Cooperation and Development), which is a test method suggested by the United Nations for a globally harmonized system of classification and labeling (11). In that study, all the rats died after exposure to 340 ppm for 4 hr, and all the rats exposed to 85 ppm survived. This means that the acute inhalation toxicity of trichloroacetonitrile should be classified as category 2 and that trichloroacetonitrile is more toxic when inhaled than when administered orally or dermally. In the present repeated toxicity study, the highest concentration used was 64 ppm because all the rats were alive at 85 ppm in the previous study. The lowest concentration was 4 ppm, which is not likely to cause any toxic effects, and the medium concentration was 16 ppm.

In the present study, the body weights of the male rats in the high-exposure group decreased by 10.8% and that of the female rats decreased by 7.1%. Rats of both sexes did not recover and were dead or moribund within 4 weeks of exposure. The body weight gains and food consumption were significantly lower in the medium-exposure group than in the normal control group during the exposure period. In contrast to the body weights, the trichloroacetonitrile exposure did not affect the animals' physical activities, blood hematology, serum biochemistry, or histopathological findings, except for the nasal cavity. Although the absolute weights of the liver, spleen, and thymus significantly decreased and the relative weights of the brain, lung, and heart significantly increased in the medium-exposure group, these were not associated with changes in the histopathological studies. Therefore, it seems that the weight differences were not related to the trichloroacetonitrile exposure. Statistically significant differences in the blood hematology

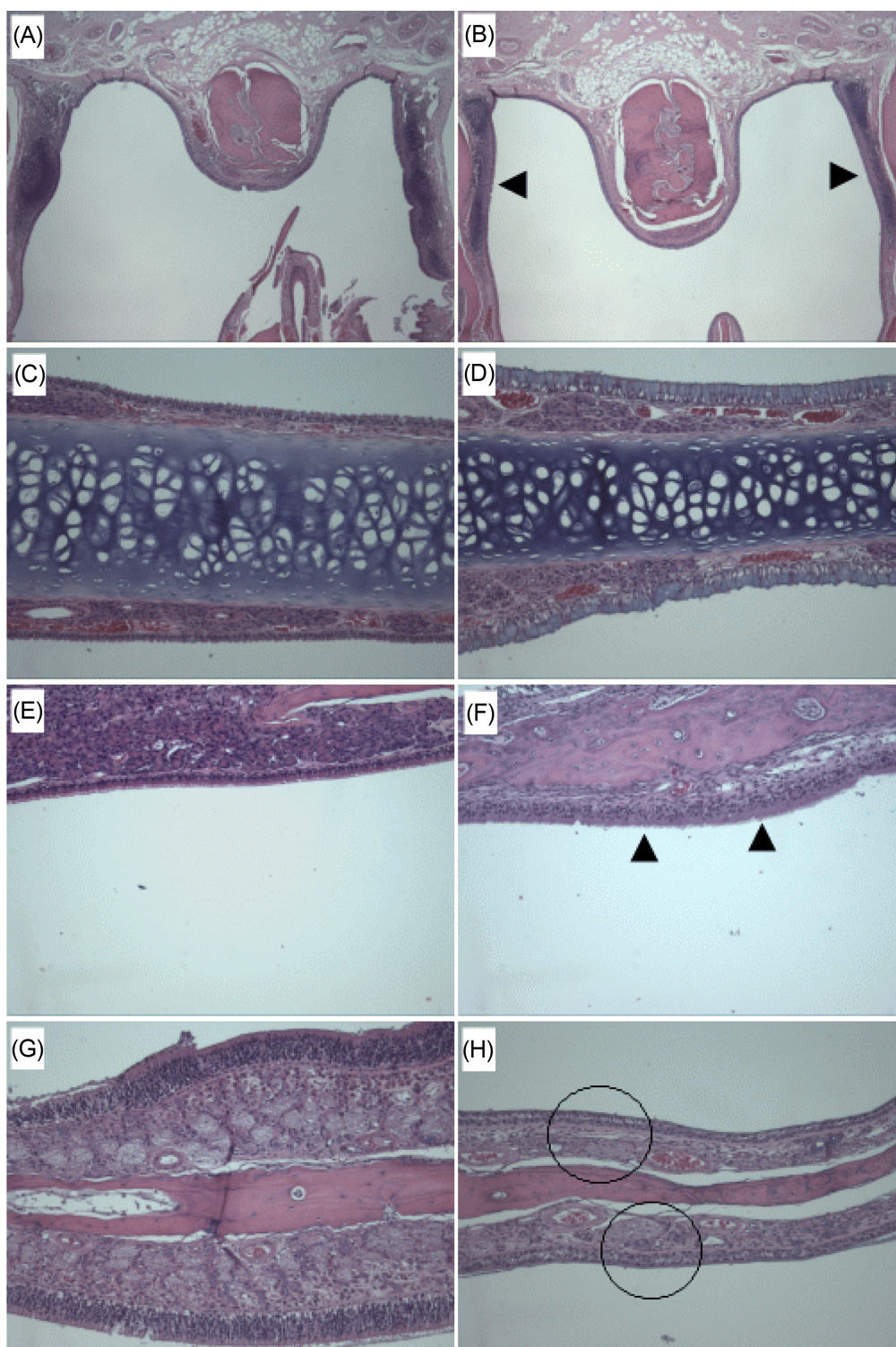


Fig. 7. Histological sections of trichloroacetonitrile-treated Sprague-Dawley rats. Transitional epithelium of the control group (A) and 16 ppm-exposed group (B), showing epithelial hyperplasia (arrow heads) in the 16 ppm-exposed group (B); Olfactory epithelium in the control group (C) and 16 ppm-exposed group (D), showing epithelial atrophy, nerve fascicle atrophy and Bowman's gland atrophy (circles) in the 16 ppm group (D); Nasal cavity in the control group (E) and 16 ppm-exposed group (F), showing decreased cellularity of nasal-associated lymphoid tissue (arrow heads) in the 16 ppm group (F); Respiratory epithelium in the control group (G) and 16 ppm-exposed group (H), showing epithelial hyperplasia and goblet cell hyperplasia (arrow heads) in the 16 ppm group (H). Tissues stained with hematoxylin and eosin and imaged at 50 \times magnification (A and B) or 200 \times magnification (C-H).

Table 5. Summary of the histopathological findings in the nasal cavity

Organs	Findings	Male (ppm)			Female (ppm)		
		0	4	16	0	4	16
Nasal associated lymphoid tissue	Decreased cellularity	0	1	2.1	0	1.4	2.1
	Minimal	0	2	1	0	3	0
	Slight	0	4	4	0	4	3
	Moderate	0	0	4	0	1	5
Respiratory epithelium	Goblet cell hyperplasia	0	1.3	1.4	0	0.6	0.7
	Minimal	0	5	6	0	6	5
	Slight	0	4	4	0	0	1
	Hyperplasia	0	0.3	0.5	0	0	0.7
	Minimal	0	1	3	0	0	2
	Slight	0	1	1	0	0	1
	Moderate	0	0	0	0	0	1
Transitional epithelium	Hyperplasia	0	1.6	2.5	0	0.3	1.8
	Minimal	0	7	5	0	3	1
	Slight	0	0	5	0	0	7
	Moderate	0	0	5	0	0	1
	Squamous metaplasia	0	0	2.2	0	0	0.3
	Minimal	0	0	0	0	0	3
	Slight	0	0	5	0	0	0
	Moderate	0	0	4	0	0	0
	Subepithelial inflammatory cell infiltration	0	0	0.1	0	0	0.6
	Minimal	0	0	1	0	0	4
Slight	0	0	0	0	0	1	
Olfactory epithelium	Atrophy	0	1.2	2.9	0	0.3	3.0
	Minimal	0	1	0	0	1	0
	Slight	0	4	1	0	1	0
	Moderate	0	1	9	0	0	10
	Nerve fascicle atrophy	0	0.4	2.6	0	0	3.0
	Minimal	0	2	0	0	0	0
	Slight	0	1	1	0	0	0
	Moderate	0	0	8	0	0	10
	Bowman's gland atrophy	0	0.4	1.7	0	0	2.4
	Minimal	0	2	2	0	0	2
	Slight	0	1	3	0	0	2
	Moderate	0	0	3	0	0	6
	Squamous metaplasia	0	0	0.8	0	0	0.8
Minimal	0	0	0	0	0	1	
Slight	0	0	4	0	0	2	
Moderate	0	0	0	0	0	1	

The histopathological findings were graded as follows: 1 for minimal, 2 for slight, and 3 for moderate.

and serum biochemistry were also not associated with histopathological changes. Therefore, we concluded that these differences were not the result of trichloroacetonitrile exposure.

In the histopathological findings, the major target organ of trichloroacetonitrile was the nasal cavity. Decreased cellularity was observed in NALT, in addition to hyperplasia,

goblet cell hyperplasia, subepithelial inflammatory cell infiltration, atrophy in the respiratory epithelium, epithelial hyperplasia, squamous metaplasia, subepithelial inflammatory cell infiltration in the transitional epithelium, and epithelial atrophy, nerve fascicle atrophy, Bowman's gland atrophy, and squamous metaplasia in the olfactory epithe-

lium. Moderate hyperplasia in transitional epithelium, in addition to atrophy and nervous fascicle atrophy in the olfactory epithelium, was observed in the male and female rats in the medium-exposure group. Moderate atrophy and nerve fascicle atrophy was also observed in the olfactory epithelium of the female rats in the aforementioned group.

In addition, the histopathological studies of the dead rats commonly revealed respiratory lesions in the high-exposure group. The lesions found in the high-exposure group were severe relative to those in the low- and medium-exposure groups. Loss of cilia in the trachea, pneumonitis in the lung, and epithelial hyperplasia in the bronchi and bronchioles were found only in the dead rats in the high-exposure group. Therefore, it seemed that the target organs of trichloroacetonitrile are the nasal cavity, trachea, and lungs.

In conclusion, the no-observed-adverse-effect level of trichloroacetonitrile obtained in this 13-week repeated inhalation study with rats was calculated as 4 ppm.

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