ORIGINAL ARTICLE

Thirteen-week inhalation toxicity study of 1-methylnaphthalene in F344 rats

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Abstract

1-Methylnaphthalene is generally utilized in solvents, as an intermediate in organic synthesis, a dye carrier, in resins, and others. There are some toxicological studies of 1-methylnaphthalene; however, inhalation toxicity studies are rare. Each of 10 male and female F344 rats was exposed to vapors of 1-methylnaphthalene for 13 weeks (6 h a day, 5 days per week) at concentrations of 0, 0.5, 4, and 30 ppm in a whole-body inhalation chamber system. The exposure concentrations were 0.52 ± 0.05 , 4.08 ± 0.25 , and 30.83 ± 1.28 ppm for the low-, middle-, and high-dose group, respectively. Body weight changes were not affected by exposure to 1-methylnaphthalene. Blood prothrombin time was delayed at 30 ppm in male and female groups, and activated partial thromboplastin time was also delayed at 30 ppm in the male group. Values of alanine aminotransferase in the serum were decreased and those of albumin were increased at 30 ppm in the male group. Differential cell counts and levels of lactate dehydrogenase in the bronchoalveolar lavage fluid were not affected. However, mucous cell hyperplasia in the nasopharyngeal tissues was found and the severity was correlated to exposure concentrations. In conclusion, 1-methylnaphthalene mainly affects the upper respiratory system and the no-observed-adverse-effect level is suggested to be 4 ppm on the basis of histopathological findings.

Keywords Inhalation toxicity · 1-Methylnaphthalene · No-observed-adverse-effect level · Upper respiratory system

Introduction

1-methylnaphthalene is generally utilized in solvents, as an intermediate of organic synthesis, as a dye carrier, in resins, and others. The American conference of governmental industrial hygienists (ACGIH) has suggested an 8 h time-weighted average threshold limit value (TLV) of 0.5 ppm since 2007 [1]. The amount of usage for 1-methylnaphthalene was surveyed to be more than 5 thousand tons per year in Korea [2] but the limit value for the workplace is not determined. In a previous report, the oral lethal dose 50 (LD50) was 1840 mg/kg in rats and the dermal LD50 was over 2000 mg/kg in rats [2, 3]. Genotoxicity was negative in Ames's test, the chromosomal aberration test, and/or the micronucleus test [3]. Murata et al. reported that orally fed 1-methylnaphthalene in B6C3F1 for 81 weeks increased the incidence of pulmonary alveolar proteinosis both in male

and female groups and increased the incidence of lung adenomas only in the male group [4, 5]. They also suggested the lowest observed adverse effect level was 71.6 mg/kg/day [4, 6]. Pulmonary alveolar proteinosis was also noted in female B6C3F1 mice, which were dermally administrated 119 mg/kg twice a week for 30 weeks and/or 61 weeks [5–7]. Inhalation toxicity studies were also reported [8, 9]; however, existing data are not sufficient to evaluate the hazards of 1-methylnaphthalene, also the inhalation lethal concentration 50 (LC50) has not been determined. Korsak et al. reported neurotoxic and sensory respiratory irritation effects in an acute inhalation study, and the results showed that sensitivity to pain decreased in the male Wistar rats after exposure to 44 ppm or 70 ppm for 4 h, but not after exposure to 26 ppm. The respiratory rates were decreased in the male Balb/C mice after 6 min starting vapor exposure and the concentration that decreased the respiratory rate by 50% was 22.1 ppm [8]. ACGIH determined the TLV of 1-methylnaphthalene mainly based on this acute respiratory irritation [1, 8]. Alveolar proteinosis found in chronic feed studies in mice and chronic dermal studies in mice were also conducted [4, 5, 7]. However, the results of the acute inhalation toxicity

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Fig. 1 Concentrations of 1-methylnaphthalene in the inhalation chambers during the 13-week exposure period

study were not sufficient to evaluate worker safety for those who are exposed to 1-methylnaphthalene daily for relatively long periods. Therefore, a 13-week repeated inhalation study was conducted to better elucidate the repeated toxicity of 1-methylnaphthalene.

Materials and methods

Test material and animals

1-Methylnaphthalene (purity was 97.3%) was purchased from Hangzhou Think Chemicals Co., Ltd. (Hangzhou, China). Six-week-old specific pathogen free Fischer 344 (F344) rats were purchased from Japan SLC Inc (Tokyo, Japan). Rats were housed in an animal room and inhalation chambers, which were maintained at 22 ± 3 °C, relative humidity of $50 \pm 20\%$, and a 12 h light/dark cycle. The chambers were vented 12 times per h and the oxygen concentrations were maintained at least at 19% during the exposure period. Rats were fed animal diet (18% protein Rodent Diet 2918C, Envigo RMS Inc., IN, USA) and water ad libitum. During the exposure period, rats were individually housed in multi-compartment stainless steel wire mesh cages ($W230 \times L1200 \times H200$) in the whole-body inhalation chambers (WITC-14M, HCT Co., Icheon, Korea). Groups of 10 rats of each sex were exposed to 0 ppm, 0.5 ppm, 4 ppm, and 30 ppm 6 h a day, 5 days per week, for 13 weeks. The study was approved by the Laboratory's Animal Ethics Committee prior to obtaining the rats and all the experiments were conducted in accordance with the established animal care protocols.

Male

Female

Fig. 2 Effects on the mean body weight changes in 1-methylnaphthalene exposed rats during the exposure period. *Significantly different when compared to the control group, $p < 0.05$. **Significantly different when compared to the control group, $p < 0.01$. Top panels male. Bottom panels female

Test material generation and analysis

The test material was generated in the form of vapor by a liquid vapor generator (LVG-04-A, HCT, Icheon, Korea) and gas chromatography (Trace 1310, Thermo Scientific, Waltham, MA, USA) was used for the analysis of the concentrations in the inhalation chambers sequentially, approximately every 40 min. During the exposure period, the test material in the liquid reservoir was replaced every week.

Clinical observations and body weight

Rats were observed daily during the exposure period for mortality and development of clinical signs. Body weights

Table 1 Effects of 1-methylnaphthalene on differential cell counts and the lactate dehydrogenase in the bronchoalveolar lavage (BAL) fluids (unit: $10^3/\mu\text{L}$)

	0	0.5	4	30
Male				
Total cells	1.25 ± 0.44	1.35 ± 0.14	1.29 ± 0.38	1.37 ± 0.38
Macrophages	1.23 ± 0.44	1.33 ± 0.16	1.27 ± 0.36	1.35 ± 0.39
PMNs	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.00 ± 0.01
Lymphocyte	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
LDH	13.54 ± 2.68	17.64 ± 18.70	9.66 ± 2.54	10.60 ± 3.32
Female				
Total cells	0.63 ± 0.10	0.80 ± 0.21	0.66 ± 0.20	0.71 ± 0.19
Macrophages	0.62 ± 0.10	0.78 ± 0.21	0.64 ± 0.19	0.70 ± 0.19
PMNs	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Lymphocyte	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
LDH	4.38 ± 2.64	4.38 ± 3.60	5.62 ± 2.20	3.42 ± 1.37

Values are expressed as mean $\pm$ standard deviation. No statistically differences were found according to one-way Analysis of variance

PMN polymorphonuclear leukocyte, LDH lactate dehydrogenase

were measured twice a week for the first 4 weeks and once a week for the remaining exposure period.

Hematology and blood biochemistry

The rats were fasted overnight and were anesthetized using isoflurane (Il-sung Pharm, Seoul, Korea) and necropsied after the 13 weeks of exposure. Hematological parameters were measured with a blood cell analyzer (ADIVA 2120i, Siemens Diagnostics, Tarrytown, NY, USA; ACL ELITE systems, Instrumentation Laboratory, Massachusetts, USA). Hematology measurements performed were the total red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count, total white blood cell (WBC) count, differential WBC count (neutrophil, lymphocyte, monocyte, eosinophil, and basophil), reticulocyte (RET) count, prothrombin time (PT), and activated partial thromboplastin time (APTT). Blood biochemical parameters were measured with a blood chemistry analyzer (TBA-120FR, Toshiba Co., Tokyo, Japan). The blood biochemistry parameters analyzed were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CREA), creatinine phosphokinase (CPK), total bilirubin (TBIL), total protein (TP), albumin (ALB), total cholesterol (TCHO), triglyceride (TG), and sodium (Na).

Bronchoalveolar lavage (BAL) fluid analysis

After collecting the blood, 5 rats of each sex were cannulated in the trachea with a polyethylene tube (PE-90, Clay Adams, NJ, USA) and lavaged 5 times with 3 mL of 0.9% saline solution. Lactate dehydrogenase was measured in the supernatant

of the first BAL fluid. Cells for differential cell counts were collected from the first and other BAL fluids. Total cells were counted with a blood cell analyzer (ADIVA 2120i, Siemens Diagnostics, Tarrytown, NY, USA) and numbers of leukocytes were counted by microscopic observation after the cells were centrifuged (Hanil Cellspin, Incheon, Korea) and Diff-Quick stained (Sysmex, Kobe, Japan).

Necropsy, organ weight, and histopathological examination

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: adrenal glands, brain, heart, kidneys, liver, spleen, testes, thymus, epididymides, lung, ovaries, and uterus.

The following organ/tissues were preserved in 10% neutral buffered formalin: adrenal glands, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, esophagus, femur, Harderian glands, heart, ileum, jejunum, kidneys, larynx, liver, lung, lymph nodes (tracheobronchial and mesenteric), mammary gland, nasopharyngeal tissue, nerve (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, teeth, thymus, thyroids, tongue, 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 the control and high concentrations (30 ppm) were paraffin-embedded, sectioned, stained with hematoxylin and eosin, and then examined microscopically. In addition, nasopharyngeal tissue from rats exposed to low

Table 2 Effects of 1-methylnaphthalene on the hematological parameters

	0	0.5	4	30
Male				
RBC ($\times 10^6/\mu\text{L}$)	8.91 ± 0.20	8.84 ± 0.26	8.94 ± 0.23	8.90 ± 0.17
HCT (%)	44.7 ± 1.0	44.6 ± 1.1	45.0 ± 1.2	44.7 ± 0.8
HGB (g/dL)	15.1 ± 0.3	15.0 ± 0.4	15.2 ± 0.3	15.0 ± 0.2
MCV (fL)	50.2 ± 0.6	50.4 ± 0.7	50.3 ± 0.5	50.2 ± 0.6
MCH (g/dL)	16.9 ± 0.3	17.0 ± 0.2	17.0 ± 0.1	16.8 ± 0.2
MCHC (g/dL)	33.7 ± 0.7	33.7 ± 0.3	33.8 ± 0.5	33.5 ± 0.3
PLT ($\times 10^3/\mu\text{L}$)	688 ± 37	640 ± 190	671 ± 45	654 ± 157
RET ($\times 10^3/\mu\text{L}$)	226.2 ± 14.9	246.4 ± 26.9	228.3 ± 22.4	241.8 ± 14.8
WBC ($\times 10^3/\mu\text{L}$)	5.36 ± 1.69	4.66 ± 1.58	5.17 ± 0.78	5.15 ± 0.92
NEUA ($\times 10^3/\mu\text{L}$)	1.40 ± 0.60	1.22 ± 0.48	1.15 ± 0.14	1.11 ± 0.14
LYMA ($\times 10^3/\mu\text{L}$)	3.71 ± 1.12	3.21 ± 1.12	3.79 ± 0.67	3.81 ± 0.80
MONA ($\times 10^3/\mu\text{L}$)	0.13 ± 0.05	0.12 ± 0.05	0.12 ± 0.05	0.12 ± 0.02
EOSA ($\times 10^3/\mu\text{L}$)	0.07 ± 0.02	0.07 ± 0.03	0.07 ± 0.01	0.07 ± 0.02
BASA ($\times 10^3/\mu\text{L}$)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
APTT (s)	17.5 ± 1.7	18.0 ± 0.8	18.6 ± 0.8	$18.9 \pm 0.5^*$
PT (s)	10.2 ± 0.3	10.5 ± 0.4	10.6 ± 0.3	$11.2 \pm 0.6^{**}$
Female				
RBC ($\times 10^6/\mu\text{L}$)	8.35 ± 0.21	8.44 ± 0.18	8.32 ± 0.11	8.38 ± 0.19
HCT (%)	43.8 ± 1.0	44.3 ± 1.1	43.6 ± 1.0	43.9 ± 1.2
HGB (g/dL)	15.0 ± 0.3	15.2 ± 0.3	15.0 ± 0.3	15.1 ± 0.4
MCV (fL)	52.5 ± 0.7	52.5 ± 0.9	52.4 ± 0.8	52.4 ± 0.7
MCH (g/dL)	18.0 ± 0.1	18.1 ± 0.2	18.0 ± 0.1	18.0 ± 0.2
MCHC (g/dL)	34.4 ± 0.5	34.4 ± 0.6	34.5 ± 0.5	34.4 ± 0.4
PLT ($\times 10^3/\mu\text{L}$)	751 ± 20	705 ± 54	722 ± 35	768 ± 55
RET ($\times 10^3/\mu\text{L}$)	182.5 ± 24.1	172.2 ± 9.7	186.8 ± 25.6	199.5 ± 29.3
WBC ($\times 10^3/\mu\text{L}$)	3.12 ± 0.61	3.27 ± 0.62	3.35 ± 0.89	3.38 ± 0.62
NEUA ($\times 10^3/\mu\text{L}$)	0.70 ± 0.16	0.73 ± 0.08	0.75 ± 0.27	0.74 ± 0.15
LYMA ($\times 10^3/\mu\text{L}$)	2.30 ± 0.59	2.40 ± 0.59	2.45 ± 0.63	2.49 ± 0.56
MONA ($\times 10^3/\mu\text{L}$)	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.03	0.07 ± 0.03
EOSA ($\times 10^3/\mu\text{L}$)	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
BASA ($\times 10^3/\mu\text{L}$)	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
APTT (s)	17.9 ± 0.8	18.5 ± 1.4	18.2 ± 1.0	19.1 ± 0.6
PT (s)	9.9 ± 0.4	10.0 ± 0.6	10.1 ± 0.2	$10.7 \pm 0.7^{**}$

All values are expressed as mean $\pm$ standard deviation. Significantly different from control by Dunnett's test (* $p < 0.05$; ** $p < 0.01$)

RBC red blood cell count, HCT hematocrit, HGB hemoglobin, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, PLT platelet, RET reticulocyte, WBC white blood cell count, NEU neutrophil, BAS basophil, EOS eosinophil, LYM lymphocyte, MON monocyte, APTT activated partial thromboplastin time, PT prothrombin time

(0.5 ppm) and middle (4 ppm) doses was histopathologically evaluated because a 1-methylnaphthalene-related change was noted at 30 ppm.

Statistical analysis

The data obtained during the study period are presented as means and standard deviation values. The data were statistically analyzed using the PASW Statistics 18 program (IBM

SPSS Statics, Armonk, NY, USA) or SigmaPlot 12 program (Systat Software, San Jose, CA, USA).

Results

Exposure and analysis of test material

Vapors were generated by regulating air flow through the liquid reservoir container of the test materials in the generating

Table 3 Effects of 1-methylnaphthalene on the serum chemistry parameters

	0	0.5	4	30
Male				
ALT (IU/L)	50.8 ± 5.6	51.2 ± 8.8	45.2 ± 4.6	42.9 ± 5.8*
AST (IU/L)	91.5 ± 13.1	95.7 ± 12.8	88.7 ± 10.6	89.5 ± 36.0
ALP (IU/L)	456.0 ± 27.5	445.6 ± 35.2	455.9 ± 43.0	443.0 ± 44.9
TP (g/dL)	7.7 ± 0.3	7.7 ± 0.2	7.8 ± 0.2	8.7 ± 2.4
ALB (g/dL)	4.1 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.3 ± 0.2**
TG (mg/dL)	122.2 ± 41.9	98.1 ± 29.2	102.1 ± 34.2	89.9 ± 32.1
TCHO (mg/dL)	84.7 ± 7.6	78.5 ± 4.9	78.9 ± 6.8	82.5 ± 8.7
TBIL (mg/dL)	0.16 ± 0.04	0.15 ± 0.03	0.14 ± 0.02	0.12 ± 0.05
BUN (mg/dL)	20.2 ± 2.3	18.7 ± 2.0	19.9 ± 2.7	21.6 ± 3.5
CREA (mg/dL)	0.43 ± 0.04	0.43 ± 0.03	0.44 ± 0.03	0.44 ± 0.03
CPK (U/L)	268.4 ± 157.3	292.3 ± 83.8	305.9 ± 75.6	374.7 ± 221.0
Na (mmol/L)	144.3 ± 0.8	144.6 ± 0.8	145.1 ± 1.0	145.7 ± 1.8*
Female				
ALT (IU/L)	38.4 ± 7.2	44.7 ± 11.3	40.9 ± 11.0	38.8 ± 5.2
AST (IU/L)	81.6 ± 14.6	94.5 ± 18.4	86.5 ± 12.6	81.9 ± 7.3
ALP (IU/L)	362.5 ± 33.3	365.6 ± 50.9	367.0 ± 32.0	365.3 ± 51.8
TP (g/dL)	8.0 ± 0.4	8.2 ± 0.4	8.0 ± 0.4	7.7 ± 0.4
ALB (g/dL)	4.3 ± 0.2	4.4 ± 0.2	4.3 ± 0.2	4.2 ± 0.2
TG (mg/dL)	14.3 ± 5.5	19.7 ± 7.0	16.2 ± 5.5	18.3 ± 6.3
TCHO (mg/dL)	86.5 ± 15.3	96.8 ± 13.0	93.3 ± 9.1	93.3 ± 12.0
TBIL (mg/dL)	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.01
BUN (mg/dL)	21.0 ± 2.9	22.3 ± 2.8	19.7 ± 3.1	21.1 ± 2.6
CREA (mg/dL)	0.43 ± 0.05	0.42 ± 0.02	0.41 ± 0.04	0.39 ± 0.03
CPK (U/L)	265.1 ± 96.2	266.7 ± 89.5	207.2 ± 81.3	205.8 ± 95.3
Na (mmol/L)	146.3 ± 1.3	147.2 ± 2.4	146.1 ± 0.6	146.4 ± 0.9

All values are expressed as mean ± standard deviation. Significantly different from control by Dunnett's test (* $p < 0.05$; ** $p < 0.01$)

ALT alanine aminotransferase, AST aspartate aminotransferase, ALP alkaline phosphatase, TP total protein, ALB albumin, TG triglyceride, TCHO total cholesterol, TBIL total bilirubin, BUN blood urea nitrogen, CREA creatinine, CPK creatine phosphokinase, Na Sodium

system. The concentrations of 1-methylnaphthalene during the exposure period for low (0.5 ppm), middle (4 ppm), and high (30 ppm) dose groups were 0.52 ± 0.05 , 4.08 ± 0.25 , 30.83 ± 1.28 ppm, respectively (Fig. 1).

Clinical signs and mean body weight changes

No animal showed exposure-related clinical signs (data not shown). Mean body weight of the males and females in the high dose (30 ppm) group were significantly decreased 2 days after the exposure; however they soon recovered to their normal level. Although mean body weight of the female high (30 ppm) dose group was not significantly different from that of the control, except at 16 days after exposure, they were lower than that of the control and the trend was persistent (Fig. 2).

Analysis of bronchoalveolar lavage (BAL) fluid

1-Methylnaphthalene did not affect the inflammatory indicators in the BAL fluid. Namely, the cell differentiation counts were not changed by exposure of 1-methylnaphthalene. The levels of lactate dehydrogenase were also not changed (Table 1).

Hematology and blood biochemistry

No significant changes were noted in hematology results between the control and the dose groups except for APTT and PT. APTT was increased ($p < 0.05$) for the male high (30 ppm) dose group and PT was increased ($p < 0.01$) for both sexes in the high (30 ppm) dose groups (Table 2). In the blood biochemistry results, decreased ALT ($p < 0.05$), increased ALB ($p < 0.01$) and Na ($p < 0.05$) were observed for the male high (30 ppm) dose group (Table 3).

Table 4 Effects of 1-methylnaphthalene on the absolute organ weight (unit: g)

	0	0.5	4	30
Male				
Adrenal glands	0.048 ± 0.002	0.046 ± 0.005	0.044 ± 0.002	0.049 ± 0.005
Brain	1.88 ± 0.06	1.86 ± 0.05	1.85 ± 0.10	1.85 ± 0.05
Epididymides	0.88 ± 0.06	0.89 ± 0.06	0.88 ± 0.03	0.91 ± 0.06
Heart	0.83 ± 0.03	0.81 ± 0.05	0.80 ± 0.04	0.83 ± 0.05
Kidneys	1.80 ± 0.09	1.85 ± 0.26	1.63 ± 0.27	1.86 ± 0.06
Liver	8.79 ± 0.79	8.37 ± 0.62	8.28 ± 0.50	9.11 ± 0.51
Lung	1.35 ± 0.25	1.32 ± 0.28	1.34 ± 0.27	1.35 ± 0.28
Spleen	0.67 ± 0.04	0.69 ± 0.05	0.66 ± 0.04	0.67 ± 0.04
Testes	2.86 ± 0.07	2.91 ± 0.26	2.82 ± 0.11	2.87 ± 0.09
Thymus	0.20 ± 0.01	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02
Female				
Adrenal glands	0.046 ± 0.004	0.045 ± 0.006	0.045 ± 0.006	0.047 ± 0.003
Brain	1.70 ± 0.13	1.75 ± 0.05	1.75 ± 0.05	1.73 ± 0.04
Heart	0.52 ± 0.03	0.52 ± 0.03	0.51 ± 0.04	0.51 ± 0.03
Kidneys	1.13 ± 0.06	1.11 ± 0.06	1.11 ± 0.07	1.14 ± 0.04
Liver	4.27 ± 0.25	4.32 ± 0.36	4.18 ± 0.27	4.17 ± 0.27
Lung	1.01 ± 0.21	0.96 ± 0.21	0.98 ± 0.20	0.97 ± 0.21
Ovaries	0.051 ± 0.004	0.048 ± 0.009	0.050 ± 0.009	0.050 ± 0.005
Spleen	0.38 ± 0.02	0.37 ± 0.02	0.39 ± 0.03	0.37 ± 0.02
Thymus	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.19 ± 0.13
Uterus	0.49 ± 0.14	0.42 ± 0.10	0.53 ± 0.20	0.43 ± 0.09

Values are expressed as mean ± standard deviation. No statistically differences were found according to one-way analysis of variance

Organ weight and histopathological examination

Organ weights including lungs were not affected by the exposure of 1-methylnaphthalene (Table 4).

Mucous cell hyperplasia in the nasopharyngeal tissue was noted for the male low (0.5 ppm), middle (4 ppm), and high (30 ppm) dose groups and for the female middle (4 ppm) and high (30 ppm) dose groups. Transitional cell hyperplasia in the nasopharyngeal tissue was noted for the male middle (4 ppm) and high (30 ppm) dose groups (Table 5, Fig. 3). All other microscopic findings observed in examined organs were consistent with the normal background lesions in rats of the same age group; therefore, were considered to be spontaneous and incidental.

Discussion

More than 5 thousand tons of methylnaphthalene is used per year in Korea for organic solvents, organic synthesis, and other uses [2]. The American Conference of Governmental Industrial In 2007, hygienists (ACGIH) recommended the 8 h time weighted average Threshold Limit Value (TLVs) of 1-methylnaphthalene to be 0.5 ppm on the basis of respiratory irritation [1]. There are some toxicological studies of

1-methylnaphthalene, but most are oral and dermal routes, and there is little study of the inhalation route. Inhalation

Table 5 Effects of 1-methylnaphthalene on the Histopathological finding of the nasopharyngeal tissues

Histopathological findings	0	0.5	4	30
Male				
Hyperplasia, mucous cell				
Total	10	10	10	10
Minimal	0	4	4	0
Mild	0	0	6	0
Moderate	0	0	0	10
Hyperplasia, transitional epithelial cell				
Total	10	10	10	10
Minimal	0	0	5	5
Mild	0	0	0	0
Moderate	0	0	0	0
Female				
Hyperplasia, mucous cell				
Total	10	10	10	10
Minimal	0	0	3	2
Mild	0	0	0	6
Moderate	0	0	0	2

Fig. 3 Histopathological changes in the nasopharyngeal tissue of the rats at **a, c** control and **b, d** high (30 ppm) dose 1-methylnaphthalene. **a** Respiratory and **c** transitional cells are intact at control rats. Rats

exposed to high (30 ppm) dose showed hyperplasia (arrow) in **b** respiratory and **d** transitional cells. Magnification, $\times 100$

is the route by which workers are most easily exposed to chemicals in the workplace. Therefore, inhalation toxicity studies are required for the safety assessment of workers. For inhalation toxicity studies, 1-methylnaphthalene could be generated and inhaled in the form of vapors and/or aerosols in our inhalation system. In this study, vapor was selected as the exposure form because the major probable exposure form in the workplace is vapor. Thus, we decided that the low-dose group concentration would be 0.5 ppm, which was the same concentration that ACGIH recommended as TLV and the high-dose group concentration would be approximately 30 ppm. In this study, vapors of 1-methylnaphthalene were exposed to F344 rats 6 h a day, 5 days per week, for 13 weeks. No significant changes were found in body weight, food consumption (data not shown) during the 13-week exposure period. Prothrombin times in the high (30 ppm) dose group, for both sexes were increased, and activated partial thromboplastin time for the males in the high (30 ppm) dose group was increased. However, it was not

considered adverse owing to the small magnitude of change and no evidence of hemorrhage or coagulopathy. Although alanine aminotransferase and albumin in blood biochemistry of the 30 ppm-exposed males were statistically different from that of the control, these differences were slight. These systemic and hematological findings were similar to those from a previous report, although the route of administration was different [4]. On the other hand, in the previous study, 2-methylnaphthalene, which is an analogues material with 1-methylnaphthalene, showed a higher number of goblet cells in lobar bronchi, and hyperplasia of hepatic bile ducts in the study of a 4-week inhalation toxicity study [10]. It appeared that the hepatotoxic effects of 1-methylnaphthalene needed further study. No critical changes in the lung weights or the histopathological findings were identified in the lung and total cells and neutrophils in the bronchoalveolar lavage (BAL) fluid were not increased. In addition, lactate dehydrogenase in the BAL was not increased. Thus, it appeared that the lung is not the major target organ in this

study. However, pulmonary alveolar proteinosis in the lung has been observed in mice of both sexes exposed to 1-methylnaphthalene in the diet for 81 weeks in a previous report [5]. This difference was apparently related to the duration of exposure to the test material. The 13-week range-finding study that exposed B6C3F1 mice to dietary doses as high as 2500 mg/kg/day suggests that the development of alveolar proteinosis in the lung requires chronic-duration exposure [5]. Therefore, chronic exposure of 1-methylnaphthalene by route of inhalation may cause lesions of the lung. In contrast to the result in the lungs, nasopharyngeal tissues were affected by exposure of 1-methylnaphthalene in this study. Mucous cell hyperplasia noted in all 1-methylnaphthalene exposed males and in 4 ppm and 30 ppm exposed females. Transitional epithelial cell hyperplasia was also observed in 4 ppm and 30 ppm exposed males. These changes showed dose-dependent patterns in incidence and severity, which were considered to be adaptive responses to irritation by 1-methylnaphthalene [11, 12]. However, a moderate and over graded change should not be concluded merely an adaptive response caused by its severity and industrial use. In conclusion, we suggest that the lack of observed adverse effects level of 1-methylnaphthalene is 4 ppm based on the nasopharyngeal tissue effects.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

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