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Original Article

Subchronic Inhalation Toxicity Study of *n*-pentane in Rats

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Objectives: This study was conducted in order to obtain information concerning the health hazards that may result from a 13 week inhalation exposure of *n*-pentane in Sprague-Dawley rats.
Methods: This study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals No. 413 'Subchronic inhalation toxicity: 90-day study (as revised in 2009)'. The rats were divided into 4 groups (10 male and 10 female rats in each group), and were exposed to 0, 340, 1,530, and 6,885 ppm *n*-pentane in each exposure chamber for 6 hour/day, 5 days/week, for 13 weeks. All of the rats were sacrificed at the end of the treatment period. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, locomotion activity, urinalysis, hematology, serum biochemistry, gross findings, organ weights, and histopathology were assessed.
Results: During the period of testing, there were no treatment related effects on the clinical findings, body weight, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross findings, relative organ weight, and histopathological findings.
Conclusion: The no-observable-adverse-effect level (NOAEL) of *n*-pentane is evaluated as being more than 6,885 ppm (20.3 mg/L) in both male and female rats. *n*-pentane was not a classified specific target organ toxicity in the globally harmonized classification system (GHS).

Key Words: *n*-Pentane, Subchronic inhalation toxicity, Sprague-Dawley rats, Globally harmonized classification system

Introduction

n-Pentane (CAS No. 109-66-0) is derived from petroleum, such as raw materials, natural gas, and crude oil. *n*-Pentane is a hydrocarbon solvent and a flammable liquid with an estimated production of 100,000-500,000 tons [1]. It is used as a component of aerosol propellant, as a raw material for the production of chlorinated pentanes and pentanols, additive in liquid fumigants, additive in automotive fuels, and as a blowing agent for plastics. Further, it is employed in the production of olefin, hydrogen and ammonia, artificial ice manufacturing, low temperature thermometers, and cosmetics in diverse applications [2].

In Korea, four workplaces with 128 workers produce approximately 584,000 tons of *n*-pentane per year. Additionally, more than 480 workers in 65 workplaces are involved in various manufacturing industries that use approximately 3,710,000 tons of *n*-pentane as the raw material [3].

As a volatile material, *n*-pentane can disperse in ambient conditions and workers can be readily exposed via inhalation at the workplaces. Therefore, the respiratory system serves as the primary target of *n*-pentane exposure.

In human exposure studies, pentane is a central nerve

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system depressant, and can produce chemical pneumonitis or pulmonary edema [4]. In addition, it may cause narcosis, hemorrhage, anorexia, dizziness, depression, confusion, polyneuropathies, seizure, and respiratory arrest [2]. Chronic exposure has resulted in anoxia. Human volunteers, who had an intake of 5,000 ppm pentane vapor for 10 minutes, showed no mucous membrane irritation or other symptoms. The dermal effects of pentane vapor applied to the skin of 5 volunteers were studied. Erythema, hyperemia, swelling, and pigmentation were observed after dermal exposure [5].

There were some *n*-pentane inhalation toxicity tests; yet, all of them are non-good laboratory practice tests and further, exposure concentrations of *n*-pentane were low. The aim of this study was to determine the potential subchronic inhalation toxicity of *n*-pentane via whole-body exposure in Sprague-Dawley (SD) rats. Inhalation exposure to *n*-pentane is the main route for humans because this chemical is volatile and can permeate through the skin. We carried out subchronic toxicity tests with *n*-pentane using SD rats through the Organization for Economic Co-operation and Development (OECD) guidelines in order to provide the exact toxicological information as well as to sort out its globally harmonized classification system (GHS) category.

Materials and Methods

Animals

Eighty 6-week-old SD rats (40 males and 40 females) were obtained from a specific pathogen-free colony from the Central Lab Animal Inc. (Seoul, Korea); the rats were used after 6 days of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20% with artificial lighting from 08:00 to 20:00 along with 12-15 air changes per hour. The animals were housed individually in wire-bottomed stainless steel wire mesh cages that were placed in exposure chambers. They were allowed sterilized tap water and commercial rodent chow (5053-PICOLAB RODENT 20; PMI Nutrition, St. Louis, MO, USA) *ad libitum*. Before rats were obtained for research, the rat studies were approved by an Animal Ethics Committee (IACUC-11-3) in order to ensure appropriate animal care.

Test chemical and exposure

n-Pentane was purchased from Sigma-Aldrich (Lot No. 83396PM; St. Louis, MO, USA). Whole-body exposure chambers (Shibata Co., Niigata, Japan), including a gas generator (Shibata Co.), were used to expose rats to *n*-pentane. The test animals were exposed to 340, 1,530, or 6,885 ppm *n*-pentane or fresh air for 6 hours per day, 5 days per week, for 13 weeks. The inhalation exposure was carried out from 10:00 to 16:00 in a stainless steel chamber (1,000 L). The experimental design was based on the usual working schedule for workers as well as on the major exposure route for the test chemical.

Experimental design

Prior to testing, rats were evaluated by clinical observations and body weight determinations over a course of a 6 day quarantine period in order to assure freedom from potential confounding variables. Forty males and 40 females were randomly assigned to four experimental groups: three treatment groups receiving 340, 1,530, and 6,885 ppm *n*-pentane, and a vehicle control group. Each group consisted of 20 rats (10 males and 10 females). All of the rats were sacrificed after treatment for 13 weeks. The experimental concentrations were selected based on the results of an acute inhalation toxicity study. Considering the classification and safety of GHS, we selected 340 ppm as a low dose. One thousand five hundred thirty and 6,885 ppm were selected as the medium and high dose, respectively, using a scaling factor of 4.5.

Temperature, relative humidity, pressure, and air ventilation in the chambers were recorded using an environmental controller (Shibata Co.). The temperature and relative humidity were maintained at 23.6-25.4°C and 45.4-53.9%, respectively. The concentrations of *n*-pentane in the chambers were calibrated with a standard gas (RIGAS, Daejeon, Korea). The conditions used for detecting *n*-pentane by gas chromatography (Shimadzu Co., Kyoto, Japan) were as follows: detector temperature, 120°C; oven temperature, 100°C; injector temperature, 120°C; and injection volume, 1 mL of gas sample. *n*-Pentane vapor concentrations in the chambers were measured every 15 minutes during exposure and were controlled to be within ± 5% of the target concentration using a computer. The mean concentration, which was measured every 30 minutes for 6 hours, was taken as the value on a given day. This was then averaged over the 13-week exposure period in order to obtain the mean and standard deviations; the daily gas concentrations in the three chambers were measured at 339.8 ± 4.52, 1,540.6 ± 25.66, and 6,917 ± 91.64 ppm, respectively.

Clinical examination

All animals were observed twice daily (before and after exposure) throughout the study period for any clinical signs of toxicity, morbidity, and mortality.

Body weights of each rat were measured at the beginning of exposure and once a week during the exposure period. Food consumption was measured at the beginning of exposure

and once a week during the exposure period. The amounts of food were calculated before they were supplied to each cage, and their remnants were measured on the next day in order to calculate the difference, which was regarded as the daily food consumption (g/rat/day).

External eye examination on all males and females was carried out shortly before the beginning of the experiments and in the last week of the exposure period. The ocular fundus was examined during the last week of the exposure period using an indirect binocular ophthalmoscope (IO-H; Neitz Instruments Co., Tokyo, Japan). The conjunctiva, sclera, cornea, lens, and iris of each eye were also examined.

During the last week of exposure, urinalysis was carried out with fresh urine in order to determine the blood, bilirubin, urobilinogen, ketone body, protein, nitrite, glucose, pH, specific gravity, and leukocyte contents by using a Uriscan S-300 urine chemistry analyzer (Yeongdong Electronic Co., Seoul, Korea).

The animals were fasted overnight prior to necropsy and blood collection. Blood samples were drawn from the abdominal artery by using a syringe with a 24-gauge needle under isoflurane anesthesia. The blood samples were collected into complete blood count (CBC) bottles containing EDTA-3K (Green Cross Medical Industry, Yongin, Korea), and analyzed within 20 minutes using an automatic hematology analyzer (Advia 120E, Bayer, Boston, MA, USA). The following parameters were determined: total leucocyte count (WBC), differential leucocyte count, neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophile (EO), basophils (BA), total erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), MCH concentration (MCHC), reticulocyte (RETIC), RBC distribution width (RDW), platelet distribution width (PDW), platelet (PLT), prothrombin time (PT), and activated partial thromboplastin time (APTT).

Blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour after collection. The sera were stored at -80°C in a freezer prior to analysis. The following serum biochemistry parameters were evaluated using an autoanalyzer (HITACHI 7060, Hitachi, Tokyo, Japan; EasyLyte, Werfen Medical, Newtonville, IL, USA): total protein (TP), albumin (ALB), A/G ratio (A/G), total bilirubin (T-BIL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), γ-glutamyl transferase (γ-GTP), creatinine (CREA), blood urea nitrogen (BUN), total cholesterol (CHOL), triglycerides (TG), glucose (GLU), calcium (Ca), inorganic phosphorus (IP), creatine kinase (CK), sodium (Na^+), and potassium (K^+), chloride (Cl^-).

At the end of the experiments, all surviving animals were anesthetized by isoflurane. The rats were then sacrificed by exsanguinations from the abdominal artery. Complete gross postmortem examinations were performed on all terminated animals.

The absolute and relative (organ-to-body weight ratios) weights of the following organs were measured: liver, kidney, spleen, adrenal gland, testis, ovary, brain, lung, heart, and thymus.

The following tissues were obtained from all animals: liver, kidney, adrenal gland, heart, lung, cerebrum, cerebellum, olfactory bulb, pituitary, spleen, seminal vesicle, prostate, testis, epididymis, ovary, uterus, vagina, tongue, trachea, esophagus, thymus, thyroid, stomach, duodenum, urinary bladder, small/large intestine, eye/harderian gland, skeletal muscle, sciatic nerve, pancreas, intestinal lymph node, femur, larynx, and nasal cavity.

The tissues were routinely processed, embedded in paraffin, and sectioned at 3-5 μm. The sections were stained with hematoxylin-eosin stain for microscopic examination. All organs and tissues taken from all of the animals in the vehicle control and the high dose groups were examined microscopically. All gross lesions, as defined by the study pathologist, were also included in the examination.

Statistical analysis

The means and standard deviations were calculated for all experiment groups. The data were subjected to a one way analysis of variance, followed by Dunnett's test, in order to determine the significant difference from the control. Statistical analyses

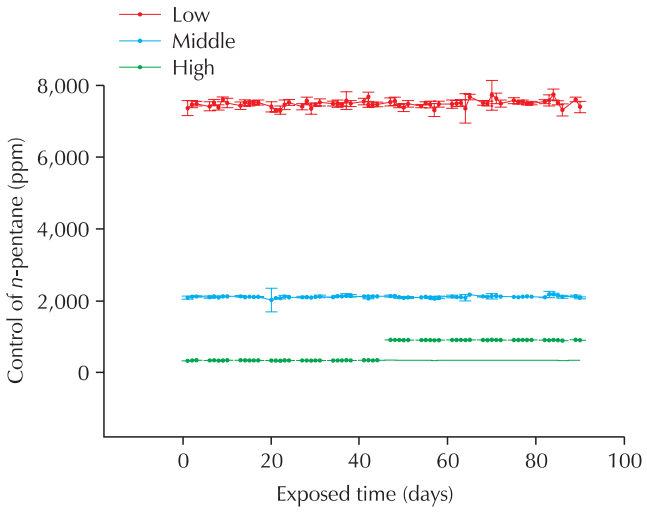

Fig. 1. Changes of concentration in inhalation chamber during the experiment.

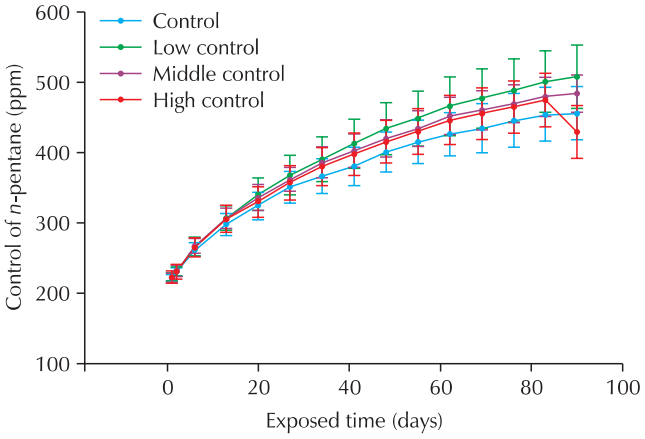

Fig. 2. Changes of body weight in male rats exposed to *n*-pentane for 13 weeks.

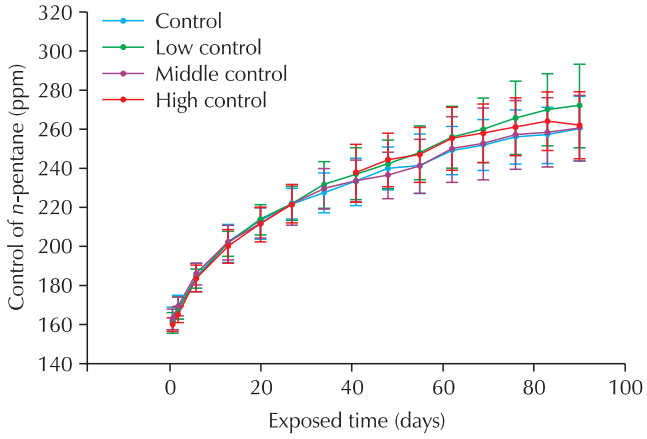

Fig. 3. Changes of body weight in female rats exposed to *n*-pentane for 13 weeks.

were performed using the SigmaStat software (version 3.5; Systat Software, San Jose, CA, USA). In all cases, p-value of < 0.05 was used to determine the significance.

Results

n-Pentane concentration in inhalation chamber during the experiment

The concentrations of *n*-pentane were 339.8, 1,540.6, and 6,917.1 ppm for low, middle, and high levels of exposure, respectively, which were within 10% of the target concentrations (Fig. 1).

Clinical signs observed

No treatment-related toxic signs or mortality were observed in any of the animals treated with *n*-pentane during the study period (data not shown).

Body weight changes and food consumption

As shown in Fig. 2, the body weight gain of male rats statistically and significantly increased in the 340 ppm group on 83-90 days compared with that of the control group; yet, there was no dose-response relationship. As presented in Fig. 3, the body weight gain of female rats was not significantly different between the groups. On the 13th and 48th days, food consumption in the male exposed groups statistically and significantly increased than the control group; however, there was no dose-response relationship (data not shown).

Clinical examination

Ophthalmologic examinations did not show any treatment-related ocular lesions in any of the animals (data not shown).

The results of urinalysis are presented in Table 1. When compared with the control group, an increase of urine protein in a *n*-pentane exposed male was observed; however, it was not statistically significant. The other urinary parameters tested in both sexes were not significantly different between the treatment groups and controls.

Tables 2, 3 summarize the hematological findings for male and female rats that inhaled *n*-pentane for 13 weeks. In males, WBC significantly increased in the low dose group (4.60 ± 0.85 K/μg) and in the middle dose group (4.59 ± 0.7 K/μg) when compared with the control group (3.39 ± 1.03 K/μg). NE significantly decreased in the middle dose group (21.5 ± 4.4%), whereas LY increased significantly (74.1 ± 4.7%). In female rats, WBC significantly decreased in the middle dose group (2.69 ± 0.58 K/μg) compared to the control group (3.7 ± 1.22 K/μg). The other hematological parameters tested in both sexes were not significantly different between the treatment groups and the control group.

Table 4 shows the serum biochemical findings for male and female rats that inhaled *n*-pentane for 13 weeks. In male rats, ALP tended to decrease with concentration, but it was not statistically significant. In female rats, a statistically significant increase in CHOL was observed in the lose dose group (116 ± 20 mg/dL) compared to those of the control group (92 ± 21 mg/dL). Moreover, CHOL, in the middle dose and high dose group, increased more than the control group. A statistically significant decrease in the CK was observed in the middle dose group (418 ± 160 IU/L). The other serum biochemical parameters tested in both sexes were not significantly different between the test groups and the control group.

Table 1. Results of urinary analysis in male and female rats after inhalation of *n*-pentane for 13 weeks

Parameters	Unit	Grade	Male				Female			
			G1	G2	G3	G4	G1	G2	G3	G4
Blood	RBC/μL	-	9	9	8	9	10	9	8	10
		±	1	1	1	1	0	0	2	0
		+	0	0	1	0	0	0	0	0
		++	0	0	0	0	0	1	0	0
Bilirubin	mg/dL	-	10	9	10	10	9	10	10	10
		±	0	0	0	0	0	0	0	0
		+	0	1	0	0	1	0	0	0
Urobilinogen	mg/dL									
		±	10	10	10	10	10	10	10	10
Keton	mg/dL	-	1	2	1	1	2	4	6	6
		±	2	3	3	2	7	6	4	4
		+	7	5	6	7	1	0	0	0
Protein	mg/dL	-	2	1	1	1	5	5	6	5
		±	3	0	2	0	0	5	1	2
		+	2	2	0	1	3	0	1	2
		++	3	2	6	0	2	0	1	1
		+++	0	3	0	2	0	0	1	0
		++++	0	2	1	6	0	0	0	0
Nitrite	-	-	9	8	10	8	9	10	8	8
		+	1	2	0	2	1	0	2	2
Glucose	mg/dL	-	10	10	10	10	10	10	9	10
		±	0	0	0	0	0	0	1	0
pH	-	5.0	0	0	0	0	1	0	0	0
		6.0	2	2	0	0	3	2	1	3
		6.5	3	7	5	3	3	2	2	3
		7.0	3	1	1	1	2	4	5	1
		7.5	2	0	3	2	1	1	2	2
		8.0	0	0	1	1	0	1	0	0
		8.5	0	0	0	1	0	0	0	1
		9.0	0	0	0	2	0	0	0	0
Specific gravity	mg/dL									
		1.005	1	0	2	0	0	2	2	2
		1.010	2	2	1	4	1	4	3	3
		1.015	2	1	1	2	4	3	4	1
		1.020	4	4	4	2	2	1	0	2
		1.025	1	3	2	2	3	0	1	2
Leukocyte	WBC/μL	-	0	1	0	1	5	5	8	5
		±	1	0	2	1	5	3	1	5
		+	3	2	0	3	0	2	1	0
		++	6	5	7	4	0	0	0	0
		+++	0	2	1	1	0	0	0	0

G1: control group, G2: 340 ppm group, G3: 1,530 ppm group, G4: 6,885 ppm group.
The animal numbers of each group is 10.
RBC: red blood cell count, WBC, white blood cell count.

Table 2. Hematological results of male rats exposed to *n*-pentane for 13 weeks

Parameter	Unit	Control	Low	Middle	High
WBC	K/μg	3.39 ± 1.03	4.60 ± 0.85*	4.59 ± 0.70*	4.15 ± 0.80
NE	%	29.5 ± 7.9	25.7 ± 5.5	21.5 ± 4.4*	28.6 ± 4.9
LY	%	65.7 ± 8.5	69.7 ± 5.4	74.1 ± 4.7*	67.2 ± 4.9
MO	%	2.2 ± 0.8	2.3 ± 0.4	1.8 ± 0.4	1.8 ± 0.5
EO	%	2.1 ± 1.0	1.7 ± 0.4	1.9 ± 0.5	1.8 ± 0.4
BA	%	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
RBC	M/μg	9.31 ± 0.26	9.40 ± 0.39	9.35 ± 0.39	9.28 ± 0.44
Hb	g/dL	14.9 ± 0.4	14.8 ± 0.4	14.7 ± 0.7	14.7 ± 0.3
Hct	%	45.9 ± 1.2	46.0 ± 1.6	45.7 ± 1.8	45.3 ± 1.4
MCV	fL	49.3 ± 0.9	49.0 ± 1.1	48.9 ± 1.3	48.9 ± 0.9
MCH	pg	16.0 ± 0.3	15.8 ± 0.5	15.7 ± 0.4	15.9 ± 0.5
MCHC	g/dL	32.4 ± 0.3	32.2 ± 0.3	32.1 ± 0.5	32.5 ± 0.6
RDW	%	12.9 ± 0.8	13.5 ± 1.0	13.7 ± 1.1	12.9 ± 1.0
PDW	g/dL	2.86 ± 0.21	3.07 ± 0.24	3.06 ± 0.27	2.94 ± 0.20
RETIC	%	2.00 ± 0.34	2.22 ± 0.34	2.41 ± 0.62	1.79 ± 0.46
PLT	K/μL	822 ± 65	915 ± 127	884 ± 90	853 ± 109
MPV	fL	8.7 ± 0.9	8.5 ± 1.0	8.8 ± 0.8	8.9 ± 1.0
PT	Second	14.4 ± 1.0	13.9 ± 0.7	14.0 ± 0.6	14.4 ± 0.8
APTT	Second	19.5 ± 1.1	19.7 ± 1.6	19.1 ± 1.2	19.7 ± 1.6

All values are expressed as mean ± standard deviation.
*Significant differences as compared with the control group ($p < 0.05$).
WBC: white blood cell count, NE: neutrophil, LY: lymphocyte, MO: monocyte, EO: eosinophile, BA: basophil, RBC: red blood cell count, Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: RBC distribution width, PDW: platelet distribution width, RETIC: reticulocyte, PLT: platelet, PT: prothrombin time, MPV: mean platelet volume, PT: prothrombin time, APTT: activated partial thromboplastin time.

Organ weight and histopathological analyses

At the scheduled necropsy, there were no treatment-related gross findings in any of the treated animals.

In male rats, the brain relative weight in the low dose group (0.44 ± 0.03%) significantly decreased compared to those of the control group (0.51 ± 0.08%). The other organs tested in both sexes were not significantly different between the test groups and the control group (Tables 5, 6).

The results of histopathological examination are presented in Table 7. In high dose exposed male rats, 1 case of periarteriolar inflammatory cells, 2 cases of hepatocyte necrosis, 1 case of inflammatory cells, 1 case of periductular inflammatory cells, 1 case of periportal inflammatory cells, and 1 case of centrilobular inflammatory cells were observed in the livers. Seven cases of tubular basophilia and 2 cases of interstitial inflammatory cells were observed in the kidney. One case of extra medullary hematopoiesis was observed in the spleen. One case of concretion was observed in the prostate. Three cases of vacuolation, 1 case of ainar cell atrophy, 1 case of interstitial inflammatory cells, and 1 case of lobular atrophy were observed in the pancreas. One case of extra periarteriolar inflammatory cells was observed in the lung. In high dose exposed female rats, 4 cases of microgranuloma and 1 case of inflammatory cells were observed in the liver. Six cases of cortical mineralization were observed in the kidney. One case of nasal-associated lymphoid tissue hyperplasia, 1 case of olfactory epithelium disorganization, and 1 case of subepithelial vascular dilatation were observed in the nasal cavity. The other histopathological findings observed in both genders of the treatment groups were also found in the control group or were determined to be acci-

Table 3. Hematological results of female rats exposed to *n*-pentane for 13 weeks

Parameter	Unit	Control	Low	Middle	High
WBC	K/μg	3.70 ± 1.22	3.45 ± 0.85	2.69 ± 0.58*	3.36 ± 0.90
NE	%	21.6 ± 4.1	26.5 ± 9.4	25.9 ± 5.9	25.9 ± 6.8
LY	%	74.8 ± 3.9	70.0 ± 9.8	70.7 ± 6.3	70.5 ± 6.7
MO	%	1.3 ± 0.3	1.3 ± 0.4	1.2 ± 0.4	1.4 ± 0.5
EO	%	1.6 ± 0.6	1.6 ± 0.8	1.5 ± 0.3	1.7 ± 0.7
BA	%	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
RBC	M/μg	8.58 ± 0.37	8.50 ± 0.36	8.73 ± 0.19	8.62 ± 0.30
Hb	g/dL	15.3 ± 0.5	15.1 ± 0.5	15.4 ± 0.4	15.2 ± 0.4
Hct	%	46.3 ± 1.6	45.3 ± 1.8	46.5 ± 0.8	45.9 ± 1.5
MCV	fL	54.0 ± 1.1	53.3 ± 0.5	53.3 ± 0.7	53.3 ± 0.6
MCH	pg	17.8 ± 0.6	17.8 ± 0.4	17.6 ± 0.4	17.7 ± 0.3
MCHC	g/dL	33.0 ± 0.7	33.3 ± 0.7	33.1 ± 0.6	33.1 ± 0.4
RDW	%	10.9 ± 0.3	11.0 ± 0.4	10.8 ± 0.3	11.0 ± 0.3
PDW	g/dL	2.51 ± 0.06	2.53 ± 0.10	2.54 ± 0.08	2.56 ± 0.10
RETIC	%	1.68 ± 0.27	1.44 ± 0.38	1.45 ± 0.24	1.63 ± 0.21
PLT	K/μL	977 ± 80	985 ± 128	974 ± 121	989 ± 90
MPV	fL	7.9 ± 0.4	8.1 ± 1.1	7.8 ± 0.6	7.8 ± 0.4
PT	Second	14.5 ± 0.7	14.5 ± 0.7	14.5 ± 0.8	14.7 ± 0.7
APTT	Second	17.6 ± 0.8	17.9 ± 1.1	17.1 ± 0.9	17.6 ± 1.2

All values are expressed as mean ± standard deviation.
*Significant differences as compared with the control group ($p < 0.05$).
WBC: white blood cell count, NE: neutrophil, LY: lymphocyte, MO: monocyte, EO: eosinophile, BA: basophil, RBC: red blood cell count, Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: RBC distribution width, PDW: platelet distribution width, RETIC: reticulocyte, PLT: platelet, PT: prothrombin time, MPV: mean platelet volume, PT: prothrombin time, APTT: activated partial thromboplastin time.

dental changes without any dose-response relationship.

Discussion

The present study was conducted in order to investigate the potential subchronic toxicity of *n*-pentane by a 13-week repeated inhalation exposure to SD rats at concentrations of 0, 340, 1,530, and 6,885 ppm, respectively. The concentration of the high dose group was considered as the highest level to test safely.

Inhalation exposure of *n*-pentane did not engender prominent signs of toxicity based on clinical examination, urinalysis, blood analysis, and histopathological examination.

Since *n*-pentane is a chemical that acts as a respiratory irritant, some adverse clinical signs of respiratory systems were expected. However, under these experimental conditions, *n*-pentane at concentrations up to 6,885 ppm did not induce toxic symptoms in any of the animals tested.

McKee et al. [6] reported that there were some slight but statistically significant increase in food consumption and body weight gain in the treated group; however, these changes were considered to be incidental. There were no changes in organ weight, hematology parameters, or serum chemistry values that were considered to be treatment related in rats exposed to 5,000, 10,000, 20,000 mg/m^3 of pentane. In this study, the body weight of male rats statistically increased in the low dose group on the 83th-90th day compared with that of the control group; yet, there was no dose response relationship. This finding is consistent with the study of McKee et al. Insufficient data are available in this study in order to determine the mechanism for

Table 4. Results of serum biochemical indices in male and female rats after inhalation of *n*-pentane for 13 weeks

Parameters	Unit	Dose (ppm), male				Dose (ppm), female			
		Control (0)	Low (340)	Middle (1,530)	High (6,885)	Control (0)	Low (340)	Middle (1,530)	High (6,885)
TP	g/dL	6.6 ± 0.2	6.4 ± 0.2	6.5 ± 0.2	6.4 ± 0.2	6.8 ± 0.3	7.1 ± 0.3	6.8 ± 0.3	6.8 ± 0.4
ALB	g/dL	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.2 ± 0.2	3.3 ± 0.2	3.1 ± 0.2	3.1 ± 0.2
A/G	g/dL	0.8 ± 0	0.8 ± 0	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
T-BIL	mg/dL	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
ALP	U/L	410 ± 102	402 ± 86	393 ± 70	387 ± 54	199 ± 56	251 ± 91	242 ± 71	237 ± 65
AST	U/L	122 ± 48	129 ± 34	117 ± 30	109 ± 20	117 ± 29	215 ± 189	155 ± 70	118 ± 34
ALT	U/L	62 ± 23	60 ± 17	62 ± 14	57 ± 13	52 ± 19	88 ± 76	75 ± 46	58 ± 18
CREA	mg/dL	0.6 ± 0	0.6 ± 0	0.7 ± 0.1	0.6 ± 0	0.6 ± 0	0.7 ± 0.1	0.6 ± 0	0.6 ± 0
BUN	mg/dL	16 ± 2.2	16.1 ± 1.2	16.9 ± 1.2	14.8 ± 3.4	18.5 ± 1.3	18.1 ± 2.3	17.5 ± 2.7	17.8 ± 2.8
CHOL	mg/dL	66 ± 18	80 ± 15	82 ± 19	68 ± 21	92 ± 21	116 ± 20*	105 ± 14	105 ± 14
TG	mg/dL	66 ± 32	91 ± 32	82 ± 23	79 ± 49	27 ± 13	32 ± 13	17 ± 7	30 ± 18
GLU	mg/dL	164 ± 20	335 ± 533	168 ± 20	150 ± 10	145 ± 16	142 ± 12	143 ± 11	150 ± 17
Ca	mg/dL	9.8 ± 0.3	10.1 ± 0.2	10.0 ± 0.2	9.9 ± 0.2	10.3 ± 0.4	10.5 ± 0.4	10.2 ± 0.4	10.2 ± 0.3
IP	mg/dL	5.1 ± 0.4	5.5 ± 0.3	5.4 ± 0.6	5.3 ± 0.7	6.2 ± 0.6	6.0 ± 0.6	6.1 ± 0.5	6.2 ± 0.3
GGT	U/L	0.5 ± 0.4	0.3 ± 0.6	0.5 ± 0.4	0.4 ± 0.3	1.0 ± 0.5	1.7 ± 1.1	1.8 ± 1.2	1.2 ± 0.4
CK	IU/L	589 ± 296	681 ± 423	743 ± 438	642 ± 364	569 ± 199	621 ± 253	418 ± 160*	365 ± 201
Na	mmol/L	143.5 ± 1.6	142.8 ± 1.3	143.0 ± 1.3	143.6 ± 1.6	143.2 ± 1.1	143.3 ± 1.1	144.2 ± 1.7	143.7 ± 1.2
K	mmol/L	4.68 ± 0.28	4.91 ± 0.21	4.82 ± 0.25	4.65 ± 0.17	4.65 ± 0.19	4.83 ± 0.27	4.76 ± 0.25	4.64 ± 0.23
Cl	mmol/L	109.9 ± 1.9	108.4 ± 1.1	108.4 ± 1.5	109.2 ± 1.6	109.7 ± 1.4	110.4 ± 1.5	111.7 ± 1.1	110.4 ± 1.6

TP: total protein, ALB: albumin, A/G: albumin/globulin ratio, T-BIL: total bilirubin, ALP: alkaline phosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CREA: creatinine, BUN: blood urea nitrogen, CHOL: total cholesterol, TG: triglycerides, GLU: glucose, Ca: calcium, IP: inorganic phosphorus, GGT: gamma (γ)-glutamyl transferase, CK: creatine kinase, Na: sodium, K: potassium, Cl: chloride.
*Significant differences as compared with the control group ($p < 0.05$).

Table 5. Relative organ weights (%) of male rats exposed to *n*-pentane for 13 weeks

Organ	Control	Low (340 ppm)	Middle (1,530 ppm)	High (6,885 ppm)
Liver	2.43 ± 0.27	2.48 ± 0.17	2.55 ± 0.13	2.27 ± 0.17
Kidney L	0.29 ± 0.05	0.28 ± 0.01	0.29 ± 0.02	0.29 ± 0.03
Kidney R	0.28 ± 0.06	0.28 ± 0.02	0.29 ± 0.02	0.29 ± 0.03
Spleen	0.14 ± 0.05	0.16 ± 0.02	0.17 ± 0.02	0.15 ± 0.01
Adrenal gland L	0.006 ± 0.001	0.0057 ± 0.0009	0.0060 ± 0.0009	0.0059 ± 0.0010
Adrenal gland R	0.006 ± 0.003	0.0056 ± 0.0003	0.0056 ± 0.0009	0.0057 ± 0.0009
Testis L	0.43 ± 0.09	0.35 ± 0.07	0.37 ± 0.10	0.41 ± 0.05
Testis R	0.42 ± 0.09	0.34 ± 0.07	0.36 ± 0.11	0.41 ± 0.05
Brain	0.51 ± 0.08	0.44 ± 0.03*	0.46 ± 0.03	0.49 ± 0.05
Lung	0.35 ± 0.05	0.33 ± 0.02	0.33 ± 0.03	0.35 ± 0.03
Heart	0.28 ± 0.03	0.27 ± 0.02	0.27 ± 0.03	0.27 ± 0.02
Thymus	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.02

The animal numbers of each group is 10.
*Significant differences as compared with the control group ($p < 0.05$).
L: left, R: right.

Table 6. Relative organ weights (%) of female rats exposed to *n*-pentane for 13 weeks

Organ	Control	Low (340 ppm)	Middle (1,530 ppm)	High (6,885 ppm)
Liver	2.25 ± 0.12	2.3 ± 0.13	2.24 ± 0.13	2.28 ± 0.16
Kidney L	0.31 ± 0.02	0.3 ± 0.03	0.32 ± 0.02	0.31 ± 0.02
Kidney R	0.31 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.31 ± 0.02
Spleen	0.18 ± 0.04	0.17 ± 0.02	0.19 ± 0.01	0.18 ± 0.02
Adrenal gland L	0.012 ± 0.002	0.011 ± 0.002	0.014 ± 0.003	0.013 ± 0.002
Adrenal gland R	0.016 ± 0.011	0.010 ± 0.002	0.012 ± 0.003	0.012 ± 0.001
Ovary L	0.014 ± 0.004	0.013 ± 0.004	0.013 ± 0.001	0.013 ± 0.004
Ovary R	0.02 ± 0.015	0.014 ± 0.002	0.015 ± 0.004	0.014 ± 0.002
Brain	0.83 ± 0.07	0.75 ± 0.08	0.82 ± 0.07	0.77 ± 0.09
Lung	0.45 ± 0.04	0.44 ± 0.04	0.44 ± 0.03	0.43 ± 0.03
Heart	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.03	0.34 ± 0.03

The animal numbers of each group is 10.
*Significant differences as compared with the control group ($p < 0.05$).
L: left, R: right.

the increase in body weight.

Stadler et al. [7] observed that increases in serum calcium and phosphorus concentrations were seen in rats exposed to either 3,000 or 10,000 ppm after inhalation of pentane, 6 hour/day, 5 days/week, for 2 weeks to either 0 (control), 1,000, 3,000, or 10,000 ppm. However, they could not see the unusual clinical signs of toxicity, functional behavior, body weights, clinical pathology, and gross and microscopic pathology, including organ weights. In this study, increases in serum calcium and phosphorus concentrations were not observed.

Table 7. Result of histopathological findings in male and female rats after inhalation of *n*-pentane for 13 weeks

Histopathological findings		Male		Female	
		Control	High dose	Control	High dose
Liver	Microgranuloma	1	0	3	4
	Extra medullaryhematopoiesis	1	0	0	0
	Periarteriolar inflammatory cells	0	1	0	0
	hepatocyte necrosis	0	2	0	0
	Inflammatory cells	0	1	1	1
	Periductular inflammatory cells	0	1	0	0
	Periportal inflammatory cells	0	1	1	0
	Centrilobular inflammatory cells	0	1	1	0
Kidney	Tubular basophilia	9	7	0	0
	Cortical scar	1	0	1	0
	Interstitial inflammatory cells	3	2	1	0
	Cortical mineralization	0	0	3	6
Adrenal gland	Cortical vacuolation	7	6	0	0
Heart	Myocardial degeneration	1	0	0	0
	Cardiomyopathy	3	0	0	0
Spleen	Extra medullaryhematopoiesis	0	1	0	0
Pituitary	Cyst in pars distalis present	1	0	0	0
Prostate	Concretion	0	1	0	0
Thyroid	Cyst	0	0	1	0
Pancreas	Vacuolation	3	3	0	0
	Acinar cell atrophy	0	1	0	0
	Interstitial inflammatory cells	0	1	0	0
	lobular atrophy	0	1	0	0
Nasal cavity	NALT hyperplasia	0	0	0	1
	Olfactory epithelium disorganization	0	0	0	1
	Subepithelial vascular dilatation	0	0	0	1
Lung	Periarteriolar inflammatory cells	0	1	0	0

The animal numbers of each group is 10.
NALT: nasal-associated lymphoid tissue.

In a 500, 2,000 mg/kg gavage administered study, the absolute kidney weights were significantly lower than those of the control, but the histopathological effects were not noted in the kidney, and there was no evidence of hydrocarbon induced nephropathy [8,9]. We could not find microscopic pathological changes in the kidney. An increase of urine protein in *n*-pentane exposed male rats was not considered to be treatment related.

The increase of WBC in male rats in the low dose and middle dose groups, decrease of NE, and increase of LY in the middle dose group were considered not to be treatment related in the comparison of 13-week-old SD male rats hematologi-

cal reference values: WBC (4.8-20.1 K/μg), NE (8.1-44.6), and LY (44.8-86.5). These values were in the normal range. In comparison with the hematological reference values of female rats (WBC 3.0-14.3 K/μg), the decrease of WBC in the middle dose group was considered to be incidental. The value of the CK in the middle dose female group, CHOL in the low dose female group, and the brain relative weights in the low dose male group were in the normal ranges: CK (305-790 IU/L), CHOL (51.0-155.0 mg/dL), and brain relative weight (0.3979-0.8360%) [10].

In this study, the neurotoxicity of *n*-pentane was not found unlike the *n*-hexane. *n*-Hexane is metabolized to a 2,5-hexanedione, which decreases the phosphorylation of neurofilaments, destroys the normal cytoskeletal matrix, and produces the giant axonal swelling and neurotoxicity [11]. In a study by Takeuchi et al. [12], *n*-hexane disturbed the conduction velocity of the motor nerve and the mixed nerve; however, *n*-pentane did not.

The solubility of *n*-pentane in the blood was low, and the blood/air partition coefficients was low as well [13]. *n*-Pentane was metabolized into a 2-pentanol by the liver [14]. Glucuronidation of 2-pentanol are typically much more water-soluble [15], and allows for 2-pentanol elimination from the body through urine or feces. For this reason, the toxicity of *n*-pentane seems to be lower than other chemicals.

In conclusion, the 13-week repeated inhalation exposure of rats to *n*-pentane did not engender prominent signs of toxicity based on clinical examination, urinalysis, blood analysis, and histopathological examination.

The no-observable-adverse-effect level was considered to be more than 6,885 ppm for 6 hours per day, 5 days per week, for 13 weeks in rats. The present results are expected to provide some information on the general toxic effects and target organ toxicity of *n*-pentane via repeated inhalation exposure, which can aid in the process of risk assessment.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. IUCLID Dataset (PENTANE). Bethesda (MD): National Library of Medicine. 2011 [cited 2011 Nov 1]. Available from: http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp.
2. Hazardous Substances Data Bank (PENTANE) [Internet]. Bethesda (MD): National Library of Medicine. 2011 [cited 2011 Nov 1]. Available from http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.
3. Korea Occupational Safety and Health Agency (KOSHA). Surveys on Work Environment in Korea, 2009. Incheon (Korea): KOSHA, Ministry of Employment and Labor; 2009.
4. Prager JC. Environmental contaminant reference databook, volume 2. New York (NY): Van Nostrand Reinhold; 1996. p. 685.
5. Bingham E, Cohrssen B, Powell CH. Patty's toxicology, volume 4. 5th ed. New York (NY): Wiley-Interscience, 2001. p. 23.
6. McKee R, Frank E, Heath J, Owen D, Przygoda R, Trimmer G, Whitman F. Toxicology of n-pentane (CAS no. 109-66-0). J Appl Toxicol 1998;18:431-42.
7. Stadler JC, O'Neill AJ, Elliott GS, Kennedy GL Jr. Repeated exposure inhalation study of pentane in rats. Drug Chem Toxicol 2001;24:75-86.
8. American Petroleum Institute. Four week oral nephrotoxicity screening study in male F344 rats. Washington, DC: American Petroleum Institute; 1985. Report No.: API 32-30966.
9. Aranyi C, O'Shea WJ, Halder CA, Holdsworth CE, Cockrell BY. Absence of hydrocarbon-induced nephropathy in rats exposed subchronically to volatile hydrocarbon mixtures pertinent to gasoline. Toxicol Ind Health 1986;2:85-98.
10. Han ZZ, Xu HD, Kim TH, Ahn TH, Bae JS, Lee JY, Gil KH, Lee JY, Woo SJ, Yoo HJ, Lee HK, Kim KH, Park CK, Zhang HS, Song SH. Reference data of the main physiological parameters in control sprague-dawley rats from pre-clinical toxicity studies. Lab Anim Res 2010;26;153-64.
11. IUCLID Dataset (n-Hexane) [Internet]. Bethesda (MD): National Library of Medicine. 2011 [cited 2011 Nov 1]. Available from http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp.
12. Takeuchi Y, Ono Y, Hisanaga N, Kitoh J, Sugiura Y. A comparative study on the neurotoxicity of n-pentane, n-hexane, and n-heptane in the rat. Br J Ind Med 1980;37:241-7.
13. Perbellini L, Brugnone F, Caretta D, Maranelli G. Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br J Ind Med 1985;42:162-7.
14. Frommer U, Ullrich V, Staudinger H. Hydroxylation of aliphatic compounds by liver microsomes. I. The distribution pattern of isomeric alcohols. Hoppe Seylers Z Physiol Chem 1970;351:903-12.
15. Notten WR, Henderson PT. Action of n-alkanes on drug-metabolizing enzymes from guinea-pig liver. Biochem Pharmacol 1975;24:1093-7.