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Original Article

Acute and Subchronic Inhalation Toxicity of n-Octane in Rats

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Objectives: We have investigated the toxic effects of the inhalation of subchronic and acute levels of n-octane.
Methods: The rats were exposed to n-octane of 0, 2.34, 11.68 and 23.36 mg/L (n = 5 rats/group/gender) in an acute inhalation test (Organization for Economic Co-operation and Development (OECD) TG 403), or to 0, 0.93, 2.62 and 7.48 mg/L (n = 10 rats/group/gender) for a subchronic inhalation test (OECE TG 413), to establish a national chemical management system consistent with the Globally Harmonized Classification System (GHS).
Results: Acutely-exposed rats became lethargic but recovered following discontinuation of inhalation. Other clinical symptoms such as change of body weight and autopsy finds were absent. The LC50 for the acute inhalation toxicity of n-octane was determined to exceed 23.36 mg/L and the GHS category was 'not grouping'. Subchronically-treated rats displayed no significant clinical and histopathological differences from untreated controls; also, target organs were affected hematologically, biochemically and pathologically. Therefore, the no observable adverse effect level was indicated as exceeding 7.48 mg/L and the GHS category was 'not grouping' for the specific target organ toxicity upon repeated exposure.
Conclusion: However, n-octane exposure should be controlled to be below the American Conference of Industrial Hygienists recommendation (300 ppm) to prevent inhalation-related adverse health effects of workers.

Key Words: n-octane, Acute, Subchronic, Inhalation toxicity, GHS

Introduction

n-Octane (CAS 111-65-9) is an aliphatic hydrocarbon that, along with other such compounds including decane, hexane and nonane, is used as a solvent in organic synthesis and azeotropic distillations. n-In Korea as elsewhere, n-octane is a particularly important chemical in the petroleum, rubber and paper processing industries [1]. According to the Korea Ministry of Environment, 6.687 tons of n-octane was manufactured in Korea, 1.126 tons were used and 8.547 tons were distributed in 2006 [2].

n-Octane produces narcosis in mice and rats after acute exposure to high concentrations [1], with the effects being evident within 30-90 min after exposure to air laden with 6,600-13,700 ppm n-octane [3]. Acute inhalation exposure can be rapidly lethal due to cardiac arrest, respiratory paralysis and asphyxiation [4].The response of mice to high concentration of isooctane can differ from the responses to n-heptane, hexane and pentane exposure, with mucous membrane irritation and respiratory arrest occurring at lower concentrations in the apparent absence of anesthesia [5]. For n-octane and related

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paraffins, narcosis and mucous membrane irritation are common and progressively increase in intensity with increasing molecular weight of the irritant [6]. In a study in which rats received daily intraperitoneal (IP) injection of n-octane (1.0 ml/kg) for 7 days, decreased body weight and liver enlargement were evident [7]. In another study in which albino rats received the same quantity of n-octane by the same IP route for 7 days, the activities of hepatic and serum enzymes, which reflect abnormal liver function, and the concentration of lipid and nucleic acid in the serum and liver as an indication of cell destruction were monitored after 2 and 7 days, decreased activities of serum acetylcholine esterase and carboxyl esterase were observed, together with an increase in fructose 1,6-diphosphate aldolase activity [7]. Significant decrease in the concentrations of serum albumin, total protein, and total and esterified cholesterol have been noted after n-octane administration for 7 days [8]. In yet another study [9], the distribution of n-octane in blood, liver, kidney, and brain of mice was studied at different inspired air concentrations (10-10,000 ppm) and different exposure times (0.5-24 h). There was a linear dependence between the inspired air concentration and the tissue concentration at fixed exposure times. A correlation between blood and organ concentration was observed in animals exposed to different inhaled concentrations but not in animals exposed to a fixed concentration [9].

Documented symptoms of short-term exposure include headache, dizziness, lightheadedness, confusion and vertigo [10], and direct aspiration into the lungs of C6-C16 paraffins may cause chemical pneumonitis, pulmonary edema and hemorrhaging [11]. However, little is known concerning the inhalation toxicity about n-octane, and it has not been categorized in the Globally Harmonized Classification System (GHS).

Accordingly, the present study sought to determine the acute and subchronic toxicity of n-octane, using inhalation tests since inhalation is the major route of absorption [12]. A rat model system was used in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines to provide toxicological information, to establish a GHS category, and to inform hazard prediction and management system proposals for the establishment of public health and safety exposure guidelines.

Materials and Methods

Test substances

n-Octane (97% pure; CAS No. 111-65-9; Lot No. 254102009) was obtained from Daejung Chemicals & Metals (Daejung, Korea) and was kept at room temperature. Theimpurities in n-octane (< 0.01% v/v) include aromatic hydrocarbons, benzene, olefin, oxygen and methanol. For the inhalation toxicity study, HEPA-filtered air was used as the vehicle.

Generation and monitoring and analysis of n-octane in inhalation chamber

To ensure the accuracy of the n-octane concentration in the inhalation chambers, the chambers were evaluated 2 weeks prior to the beginning of the study using a model 4912 n-octane reservoir (HCT, Seoul, Korea) consisting of a liquid pump and a liquid flow-meter. The concentration in chambers was adjusted using the flow control of the flow-meter to ensure target concentration. n-Octane concentration determinations in the chambers was done as described in the National Institute for Occupational Safety and Health manual of analytical methods (NMAM; No. 1500; "Hydrocarbons, BP 36-216°C") [13]. The n-octane sampling was done using a model 497701 Escot Elf pump personal air sampler (MSA, Pittsburgh, PA, U.S.A) connected to a Gemini twin port sampler (MSA) and a charcoal tube (50/100 mg). The sampler flow rate was 0.2 L/min. The n-octane in the charcoal tube was measured by gas chromatography (GC) using a model 6890N apparatus (Agilent Technologies, Santa Clara, CA, U.S.A.).

Animals and conditions

Five to six-week-old male and 6-week-old female, specific-pathogen-free (SPF) Fisher 344 (F344) rats were purchased from SLC (Tokyo) for the acute inhalation test (n = 40; 20 males and 20 females) and subchronic inhalation toxicity test (n = 80; 40 males and 40 females). All animals were acclimated for 5 days before starting each experiments. During the acclimation and experimental periods, the rats were housed in polycarbonate cages (n = 5 per cage) in a room with controlled temperature (23 ± 2°C) and humidity (55 ± 7%) with a 12 h light/dark cycle. The temperature and humidity ranges conformed to the OECD test guideline for inhalation exposure chambers. The rats were fed a rodent diet (Harlan Teklab; Plaster International, Seoul, Korea) and filtered water ad libitum. The acute inhalation toxicity test was performed as dictated by OECD guideline TG 403 [14]. The rats (approximate weight of 111 g and 130 g for males and females, respectively) were divided into four groups (n = 10 per group): fresh-air control, low-dose group (target dose: 2.34 mg/L, 500 ppm), middle-dose group (target dose: 11.68 mg/L, 2,500 ppm), and high-dose group (target dose: 23.36 mg/L, 5,000 ppm) according to the material safety data sheet and GHS classification of the test substance. The animals were exposed to n-octane once for 4 h. The subchronic inhalation toxicity test was performed according to

OECD guideline TG 413 [15]. The rats (approximate weight of 127 g and 110 g for males and females, respectively) were divided into four groups (n = 10 per group): fresh-air control, low-dose group (target dose: 0.93 mg/L, 200 ppm), middle-dose group (target dose: 2.62 mg/L, 560 ppm), and high-dose group (target dose: 7.48 mg/L, 1,600 ppm) according to the GHS classification for the specific target organ toxicity (STOT) (repeated exposure) and considering the results of acute inhalation toxicity test, ACGIH threshold limit value (TLV) and exposure periods. The animals were exposed to n-octane for 6 h each day, 5 days/week for 13 weeks. The animals were examined daily for any evidence of exposure-related effects, including respiratory, dermal, behavioral, nasal, or genitourinary changes that were suggestive of irritancy. The body weights were recorded at the time of purchase, grouping and on days 1, 3, 7, and termination (15 days after exposure; exposure was day 0). Surviving animals were euthanatized, necropsies were performed and any gross observations were recorded in the acute inhalation toxicity test. The animals were examined daily for any evidence of exposure-related effects, including respiratory, nasal and any toxicity-related organ and behavioral changes throughout the experiment. The body weights were evaluated at the time of purchase, grouping, once a week during the inhalation exposure and before necropsy in the subchronic inhalation toxicity test. Acclimation and all animal experiments were approved by the animal care and use committee of the Korea Environment and Merchandise Testing Institute.

Ophthalmoscopy

In the subchronic inhalation test, all animals were subjected to ophthalmologic examination before the start of the exposure and the end of study with a model RC-2 slit lamp (Kowa Optimed, Torrence, CA, U.S.A.).

Urinalysis

Urine samples were collected during a 3 h period of food and water deprivation once at the end of the exposure. Urine samples were analyzed for bilirubin, blood pigments, glucose, ketones, leukocytes, nitrites, pH, protein and urobilinogen using multistix (Bayer Diagnostics, Jena, Germany) and, in the subchronic inhalation test, a CliniTek Atlas urine auto-analyzer (Bayer Diagnostics).

Hematology and blood biochemistry

Before necropsy, food was withheld for 24 h and the rats were anesthetized with pentobarbital. Blood was then drawn from the abdominal aorta, collected in heparinized vacutainers and analyzed for albumin (ALB), alkaline phosphatase (ALP), calcium (Ca), cholesterol (CHO), creatinine (CRE), gamma-glutamyl transpeptidase (gamma-GT), glucose (GLU), glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), inorganic phosphorus (IP), lactate dehydrogenase (LDH), magnesium (Mg), total protein (TP), uric acid (UA), blood urea nitrogen (BUN), total bilirubin (TBIL), creatine phosphokinase (CK), sodium (Na), potassium (K), chloride (Cl), triglyceride (TG) and to determine the ratio of ALB to globulin (A/G) using a model 7180 biochemical blood analyzer (Hitachi, Tokyo, Japan). Blood was also analyzed for the white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red-cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), number of neutrophils (NE#), percent of neutrophils (NE%), number of lymphocytes (LY#), percent of lymphocytes (LY%), number of monocytes (MO#), percent of monocytes (MO%), number of eosinophils (EO#), percent of eosinophils (EO%), number of basophils (BA#) and percent of basophils (BA%) using a Hemavet 0950 blood cell counter (CDC Technology, Irvine, CA, U.S.A.) for the subchronic inhalation test.

Coagulation time

Blood samples were individually collected in vacutainers coated with 3.2% sodium citrate, and were analyzed to determine activated partial thromboplastin time (APTT) and prothrombin time (PT) using an ACL 7,000 blood coagulation analyzer (Instrumentation Laboratory, Orangeburg, NY, U.S.A.) for the subchronic inhalation test.

Organ weights and histopathology

After blood collection, the animals were sacrificed by cervical dislocation. The adrenal glands, bladder, testes, ovaries, uterus, epididymis, seminal vesicle, heart, thymus, thyroid gland, trachea, esophagus, tongue, prostate, lungs, nasal cavity, kidneys, spleen, liver, pancreas and brain were all carefully removed. Each organ was weighed and fixed in 10% formalin containing neutral pH phosphate-buffered saline. The organs were embedded in paraffin and stained with hematoxylin and eosin. All organs from all animals were examined by light microscopy for the subchronic inhalation test.

Statistical analysis

The differences between the groups were examined using the standard one-way analysis of variance (ANOVA). If these test showed statistical significance, the data was analyzed using the

multiple comparison procedure. Duncan's or Dunnett's multiple range test was used to compare the body weights, organ weights and results of the blood biochemistry and hematology for the three experimental groups with those for control animals. SPSS for Windows version 12.0 software (SPSS, Chicago, IL, U.S.A.) was used for analyses. A p-value < 0.05 indicated statistical significance.

Results

Exposure chamber environment and n-octane concentrations in the inhalation toxicity test

The temperature and humidity were within the range of the pertinent OECD test guidelines. The concentrations of the test substance in the acute inhalation toxicity test were 2.34, 11.68 and 23.36 mg/L for the low, middle and high dose, respectively (Fig. 1A), and 0.93, 2.62 and 7.48 mg/L for the same respective doses in the subchronic inhalation toxicity test (Fig. 1B). The n-octane concentrations of each exposure chamber were within the range of target concentrations, and were consistently maintained for each inhalation toxicity test.

Animal observation, food consumption, and effects on body and organ weights

No significant toxic signs or mortality were observed in the test rats after exposure during the observation period in the subchronic inhalation toxicity test (data not shown). However, in acute inhalation toxicity test, male and female rats became lethargic after 4 h exposure to 23.36 mg/L n-octane; all animals were clinically normal by the following day. None of the rats died. No significant differences were observed in food consumption between the treated animals and the control group,

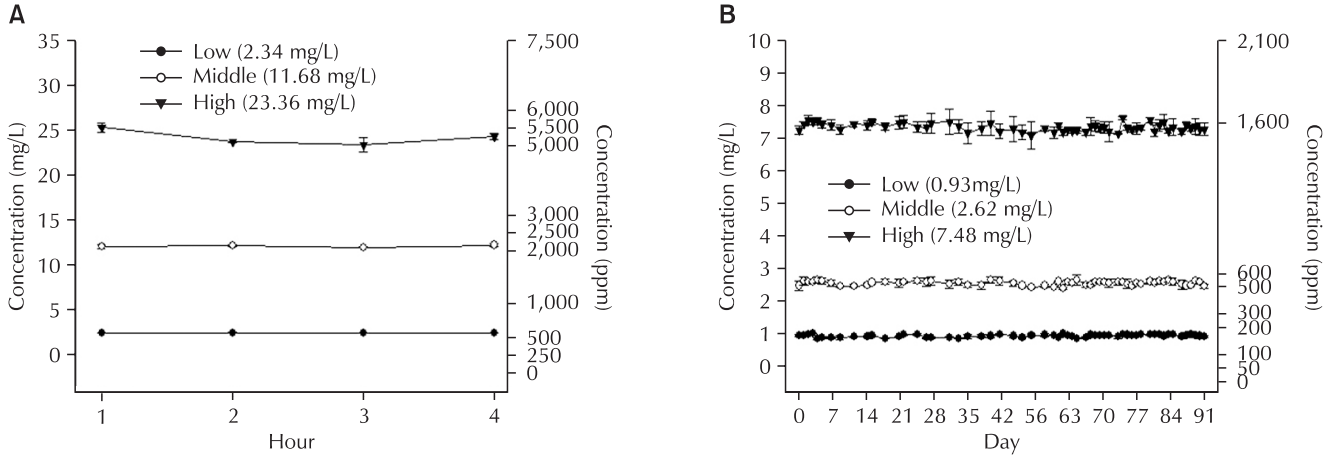

Fig. 1. Distribution of n-octane concentration. (A) Acute inhalation toxicity test. (B) Subchronic inhalation toxicity test.

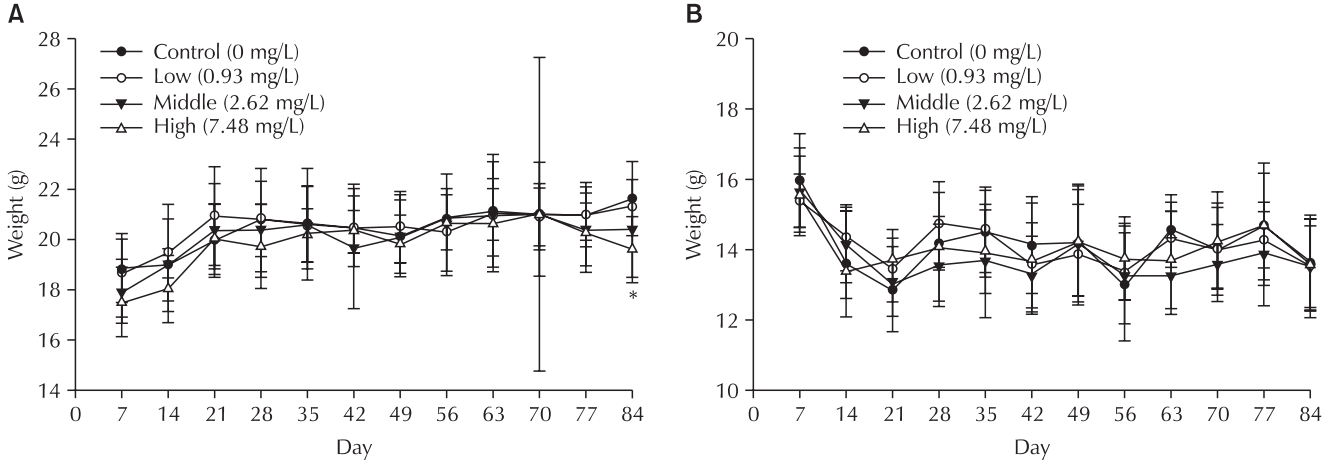

Fig. 2. Changes of food consumption in the subacute inhalation toxicity study. Panel A present data on male rats and panel B contains data on female rats. *p < 0.05, high group vs. control and low groups.

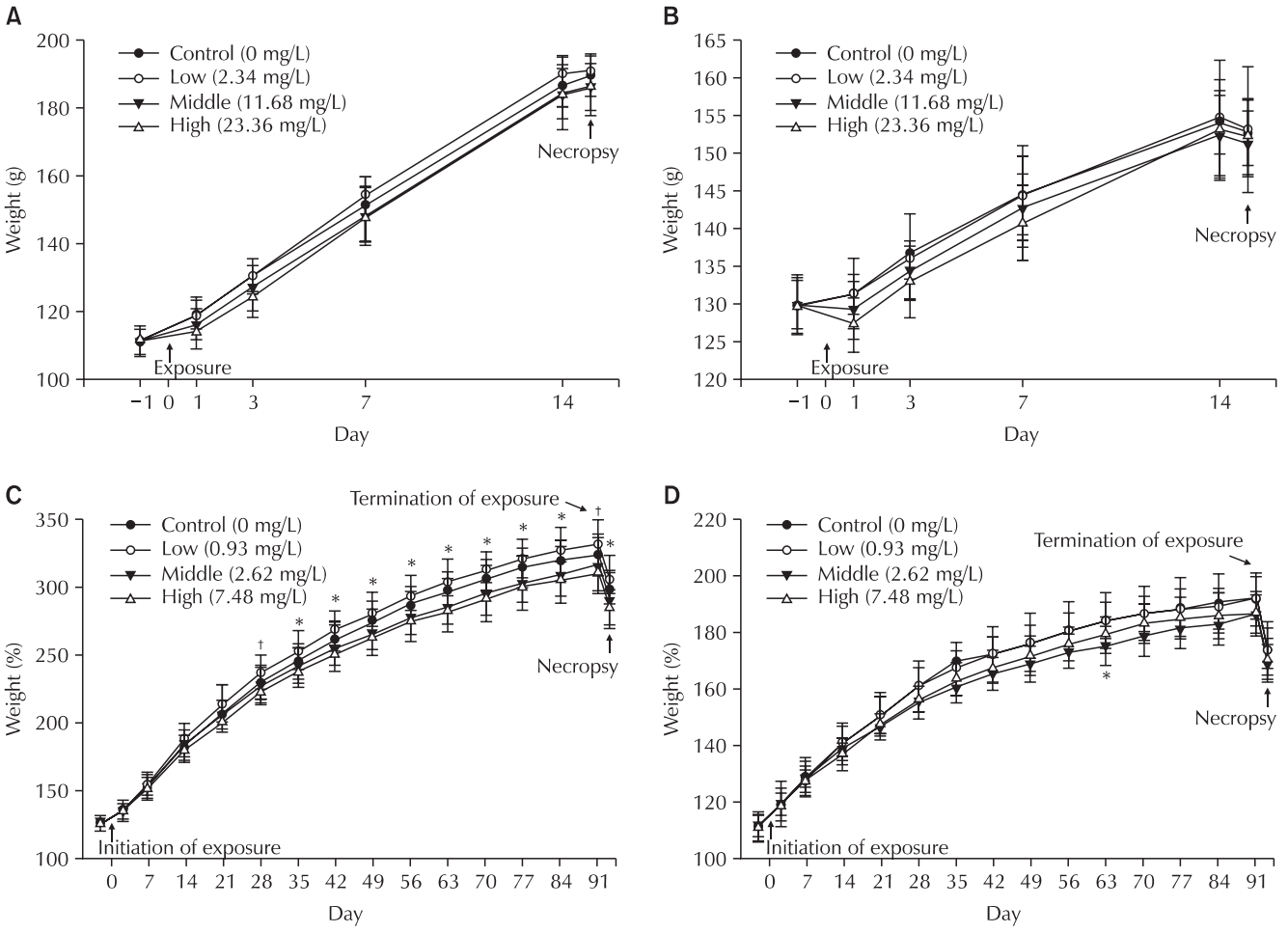

Fig. 3. Changes of body weights in the tested animals. (A) Acute inhalation toxicity test (male). (B) Acute inhalation toxicity test (female). (C) Subchronic inhalation toxicity test (male). *$p < 0.05$, low group vs. high group, †$p < 0.01$, low group vs. middle and high groups. (D) Subchronic inhalation toxicity test (female). *$p < 0.05$, middle group vs. control and low groups.

except between rats exposed to 7.48 mg/L n-octane and control animals 12 weeks after exposure (Fig. 2). There were no significant changes in body weight of male and female animals in the acute inhalation toxicity test (Fig. 3A, B). Although male and female animals displayed a significant body weight difference between the low and high dose groups (male animals) and the low and middle dose groups (female animals), there were no significant dose-related changes in the subchronic inhalation toxicity test (Fig. 3C, D). No significant dose-dependent organ weight changes were observed in male and female animals after 13 weeks of n-octane exposure (data not shown).

Ophthalmoscopy and urinalysis

Ophthalmologic examination was unremarkable in all rats (data not shown) and there were no statistically significant changes in male and female animals in the subchronic inhalation toxicity test (data not shown).

Hematology and blood biochemistry

There were no significant dose-related changes in the hematology values among the groups for both male and female animals (data not shown). Also, were no significant dose-related changes in the blood biochemical parameters among the groups except for ALB and CHO in male animals (Table 1, 2).

Coagulation time

No significant differences between treated and control animals were evident for PT and APTT in male animals (Fig. 4A). The APTT of rats exposed to 2.62 and 7.48 mg/L was significantly increased compared with control and 0.93 mg/L exposure females (Fig. 4B).

Histopathological examination

Microscopic examination did not reveal any exposure or dose-related changes. Non-specific histopathological changes of

Table 1. Serum biochemical values of male rats in the subchronic inhalation toxicity groups

Group (mean ± S.D)	Control (0 mg/l)		Low (0.93 mg/l)		Middle (2.62 mg/l)		High (7.48 mg/l)	
ALB (g/dL)	2.67 ± 0.07	[10]	2.62 ± 0.09	[10]	2.68 ± 0.08	[10]	2.79* ± 0.16	[10]
ALP (IU/L)	322.30 ± 45.51	[10]	311.50 ± 34.93	[10]	338.80 ± 73.43	[10]	314.00 ± 30.70	[10]
CA (mg/dL)	9.50 ± 0.19	[10]	9.46 ± 0.16	[10]	9.50 ± 0.24	[10]	9.58 ± 0.23	[10]
CHO (mg/dL)	64.70 ± 7.60	[10]	71.60† ± 6.50	[10]	74.40† ± 6.60	[10]	89.60† ± 6.98	[10]
CRE (mg/dL)	0.66 ± 0.10	[10]	0.79 ± 0.15	[10]	0.83 ± 0.17	[10]	0.72 ± 0.09	[10]
γ-GT (IU/L)	0.50 ± 0.53	[10]	0.60 ± 0.52	[10]	0.50 ± 0.53	[10]	1.10 ± 0.57	[10]
GLU (mg/dL)	166.30 ± 16.55	[10]	171.80 ± 20.15	[10]	166.60 ± 26.58	[10]	176.10 ± 14.42	[10]
GOT (IU/L)	124.20 ± 18.03	[10]	123.60 ± 14.06	[10]	120.60 ± 13.33	[10]	144.00 ± 63.10	[10]
GPT (IU/L)	77.30 ± 8.25	[10]	75.00 ± 6.25	[10]	74.40 ± 9.43	[10]	90.10 ± 44.62	[10]
IP (mg/dL)	6.46 ± 0.97	[10]	7.10 ± 0.49	[10]	6.84 ± 0.69	[10]	6.83 ± 0.49	[10]
LDH (IU/L)	229.60 ± 95.24	[10]	302.00 ± 181.22	[10]	283.50 ± 139.33	[10]	248.00 ± 83.10	[10]
MG (mg/dL)	1.68 ± 0.08	[10]	1.84 ± 0.15	[10]	1.93 ± 0.19	[10]	1.85 ± 0.13	[10]
TP (g/dL)	6.03 ± 0.14	[10]	5.96 ± 0.20	[10]	6.08 ± 0.17	[10]	6.24 ± 0.29	[10]
UA (mg/dL)	0.89 ± 0.22	[10]	1.22 ± 0.43	[10]	1.32 ± 0.36	[10]	0.99 ± 0.17	[10]
BUN (mg/dL)	17.37 ± 1.24	[10]	17.95 ± 1.42	[10]	18.00 ± 1.28	[10]	18.94 ± 2.21	[10]
TBIL (mg/dL)	0.007 ± 0.011	[10]	0.003 ± 0.005	[10]	0.000 ± 0.000	[10]	0.011 ± 0.019	[10]
TG (mg/dL)	87.30 ± 34.27	[10]	63.80 ± 24.95	[10]	57.50 ± 26.90	[10]	67.30 ± 23.34	[10]
CK (IU/L)	303.20 ± 77.58	[10]	458.90 ± 228.94	[10]	562.70 ± 230.70	[10]	288.00 ± 38.29	[10]
Na (mmol/L)	144.70 ± 0.67	[10]	144.30 ± 0.95	[10]	144.70 ± 0.82	[10]	145.60 ± 3.53	[10]
K (mmol/L)	3.94 ± 0.17	[10]	4.10 ± 0.73	[10]	4.13 ± 0.46	[10]	4.21 ± 0.38	[10]
Cl (mmol/L)	103.10 ± 1.60	[10]	102.00 ± 1.41	[10]	102.20 ± 1.03	[10]	103.30 ± 2.79	[10]

[ ]: number of animals.
ALB: albumin, ALP: alkaline phosphatase, CA: calcium, CHO: cholesterol, CRE: creatinine, γ-GT: gamma glutamyl transpeptidase, GLU: glucose, GOT: glutamic oxalacetic transaminase, GPT: glutamic pyruvic transaminase, IP: inorganic phosphorus, LDH: lactate dehydrogenase, MG: magnesium, TP: total protein, UA: uric acid, BUN: blood urea nitrogen, TBIL: total bilirubin, TG: triglyceride, CK: creatine kinase, Na: sodium, K: potassium, Cl: chloride.
*$p < 0.01$: high group vs. other groups.
†$p < 0.01$: low, middle and high groups vs. control group.

a slight to mild grade inflammatory cell infiltration in liver, kidneys, lung and epididymis, and thymus degranulation were evident in some animals in all groups. Focal mineralization in the kidney were present in all female animals, but those in female treated with 2.62 mg/L were slightly increased in size and number compared to the control group. All changes were similarly distributed between the control and treated groups (data not shown).

Discussion

The present study has demonstrated that acute and subchronic 13 week inhalation toxicity of n-octane, in experiments conducted according to the pertinent OECD guidelines. No major toxicology-related abnormal changes were evident, with the exception of a nervous system-related lethargy after 4 h in rats expose to a dose of 23.36 mg/L. However, recovery was evidently complete after 18 h, consistent with the reversible response noted previously [4]. There were no significant differences of body weight change between the control and exposure groups. At the end of study, all animals were subjected to necropsy, and no abnormal gross findings were observed that could be related to n-octane. The LC50 was considered to be ≥ 23.36 mg/L in male and female animals. The GHS category

Table 2. Serum biochemical values of female rats in the subchronic inhalation toxicity groups

Group (mean ± S.D)	Control (0 mg/l)		Low (0.93 mg/l)		Middle (2.62 mg/l)		High (7.48 mg/l)	
ALB (g/dL)	2.66 ± 0.08	[10]	2.68 ± 0.08	[10]	2.59 ± 0.09	[10]	2.66 ± 0.08	[10]
ALP (IU/L)	257.00 ± 34.81	[10]	247.80 ± 29.45	[10]	259.60 ± 23.95	[10]	244.40 ± 23.07	[10]
CA (mg/dL)	9.32 ± 0.23	[10]	9.21 ± 0.11	[10]	8.99 ± 0.13	[10]	9.14 ± 0.16	[10]
CHO (mg/dL)	77.30 ± 6.50	[10]	81.10 ± 7.43	[10]	77.80 ± 7.61	[10]	94.60 ± 10.39	[10]
CRE (mg/dL)	0.61 ± 0.10	[10]	0.68 ± 0.13	[10]	0.77 ± 0.14	[10]	0.66 ± 0.10	[10]
γ-GT (IU/L)	1.40 ± 0.70	[10]	1.90 ± 0.57	[10]	1.40 ± 0.52	[10]	1.40 ± 0.52	[10]
GLU (mg/dL)	135.70 ± 11.94	[10]	136.10 ± 11.53	[10]	137.50 ± 12.99	[10]	138.50 ± 14.92	[10]
GOT (IU/L)	126.50 ± 37.92	[10]	129.80 ± 38.59	[10]	118.20 ± 40.08	[10]	96.40 ± 10.29	[10]
GPT (IU/L)	68.50 ± 19.14	[10]	71.70 ± 15.46	[10]	60.50 ± 17.08	[10]	54.80 ± 8.47	[10]
IP (mg/dL)	6.72 ± 0.67	[10]	6.62 ± 0.82	[10]	6.91 ± 0.59	[10]	7.11 ± 0.47	[10]
LDH (IU/L)	164.80 ± 50.52	[10]	155.60 ± 36.96	[10]	167.20 ± 56.07	[10]	118.60 ± 34.24	[10]
MG (mg/dL)	1.82 ± 0.13	[10]	1.80 ± 0.11	[10]	1.93 ± 0.12	[10]	1.89 ± 0.09	[10]
TP (g/dL)	5.86 ± 0.15	[10]	5.89 ± 0.18	[10]	5.73 ± 0.18	[10]	5.73 ± 0.14	[10]
UA (mg/dL)	0.86 ± 0.12	[10]	0.86 ± 0.21	[10]	1.11 ± 0.14	[10]	0.86 ± 0.15	[10]
BUN (mg/dL)	18.92 ± 2.04	[10]	18.22 ± 0.89	[10]	18.79 ± 2.23	[10]	18.06 ± 1.92	[10]
TBIL (mg/dL)	0.015 ± 0.016	[10]	0.014 ± 0.013	[10]	0.006 ± 0.007	[10]	0.009 ± 0.009	[10]
TG (mg/dL)	14.00 ± 4.24	[10]	14.70 ± 6.50	[10]	11.40 ± 6.47	[10]	11.50 ± 2.46	[10]
CK (IU/L)	259.80 ± 131.67	[10]	236.10 ± 55.10	[10]	357.40 ± 114.86	[10]	198.70 ± 54.03	[10]
Na (mmol/L)	144.50 ± 1.27	[10]	145.00 ± 1.41	[10]	147.20 ± 3.29	[10]	145.10 ± 1.45	[10]
K (mmol/L)	3.81 ± 0.24	[10]	3.68 ± 0.21	[10]	3.65 ± 0.30	[10]	3.88 ± 0.29	[10]
Cl (mmol/L)	105.90 ± 1.20	[10]	105.90 ± 1.20	[10]	107.00 ± 2.26	[10]	106.00 ± 0.94	[10]

[ ]: number of animals.
ALB: albumin, ALP: alkaline phosphatase, CA: calcium, CHO: cholesterol, CRE: creatinine, γ-GT: gamma glutamyl transpeptidase, GLU: glucose, GOT: glutamic oxalacetic transaminase, GPT: glutamic pyruvic transaminase, IP: inorganic phosphorus, LDH: lactate dehydrogenase, MG: magnesium, TP: total protein, UA: uric acid, BUN: blood urea nitrogen, TBIL: total bilirubin, TG: triglyceride, CK: creatine kinase, Na: sodium, K: potassium, Cl: chloride.

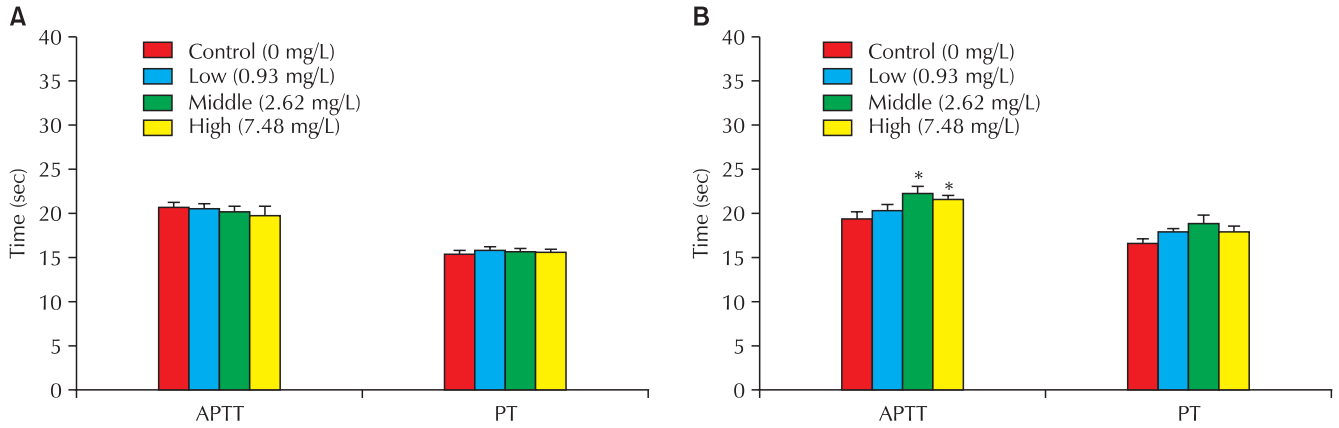

Fig. 4. Blood coagulation time of animals exposed to n-octane. Panels A and B display results from male and female rats, respectively. The * symbol indicates a significant difference ($p < 0.05$) between the middle and high dose groups vs. control group.
APTT: activated partial thromboplastin time, PT: prothrombin time.

[16] for n-octane was 'not grouping' for acute inhalation toxicity in this condition. In the subchronic inhalation toxicity test, n-octane exposure at doses up to 7.48 mg/L was not lethal and prominent signs of toxicity based on clinical examination, urinalysis, ophthmoscopy, blood analysis and histopathological examination. There were significant changes in body weight, food consumption, organ weight, blood chemistry parameters of ALB and CHO, inflammatory cell infiltration and focal mineralization. However, these changes were not related to n-octane dose and were within normal ranges [17-19]. In the kidney, a similar and statistically insignificant incidence of focal mineralization was noted in all control and treated rats. This indicatedthat the changes were unrelated to test substance exposure, but were rather normal age-related changes. Therefore, the no observed adverse effect level values for the subchronic test was determined to be ≥ 7.48 mg/L and the GHS category [16] for n-octane was 'not grouping' for the STOT (repeated exposure) in this condition. According to the results of the inhalation toxicity study, n-octane should not be considered to be a noxious material. However, this conclusion remains equivocal pending further acute oral, genetic, and other toxicity test-related evaluations of n-octane toxicity.

Most n-octane aliphatic hydrocarbons can cause lung toxicity if accidently inhaled, as occurs in an estimated 3% of all poisoning incidents in the United States [20]. The current occupational exposure level time weighted average is 500 ppm (2,350 mg/m^3) according to Occupational Safety and Health Association standards [21], 75 ppm (350 mg/m^3) according to the National Institute for Occupational Safety and Health standards time weighted average [22] and 300 mg/m^3 according to Belgium government standards and 1,400 mg/m^3 according to national standards in Italy [23]. The American Conference of Governmental Industrial Hygienists has recommended 300 ppm as the occupational exposure standard of n-octane [24,25]. In seeking a balance between the various recommended levels and striving for safety, we suggest 300 ppm as a recommended concentration to prevent adverse health effect of workers.

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