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LC_{50} Determination of *tert*-Butyl Acetate using a Nose Only Inhalation Exposure in Rats

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tert-Butyl acetate (TBAc) is an organic solvent, which is commonly used in architectural coatings and industrial solvents. It has recently been exempted from the definition of a volatile organic compound (VOC) by the Air Resources Board (ARB). Since the use of TBAc as a substitute for other VOCs has increased, thus its potential risk in humans has also increased. However, its inhalation toxicity data in the literature are very limited. Hence, inhalation exposure to TBAc was carried out to investigate its toxic effects in this study. Adult male rats were exposed to TBAc for 4 h for 1 day by using a nose-only inhalation exposure chamber (low dose, 2370 mg/m^3 (500 ppm); high dose, 9482 mg/m^3 (2000 ppm)). Sham-treated control rats were exposed to clean air in the inhalation chamber for the same period. The animals were killed at 2, 7, and 15 days after exposure. At each time point, body weight measurement, bronchoalveolar lavage fluid (BALF) analysis, histopathological examination, and biochemical assay were performed. No treatment-related abnormal effects were observed in any group according to time course. Based on those findings, the median lethal concentration (LC_{50}) of TBAc was over 9482 mg/m^3 in this study. According to the MSDS, the 4 h LC_{50} for TBAc for rats is over 2230 mg/m^3. We suggested that this value is changed and these findings may be applied in the risk assessment of TBAc which could be beneficial in a sub-acute study.

Key words: Inhalation toxicity, *tert*-Butyl acetate, Nose-only inhalation exposure chamber, Bronchoalveolar lavage fluid analysis, Rats

INTRODUCTION

tert-Butyl acetate (TBAc; Chemical Abstracts Service (CAS) No. 540-88-5) is a colorless flammable liquid with a fruity odor and an effective viscosity reducer. It has an intermediate flash point (15.5°C) and vapor pressure (47 mmHg at 25°C). TBAc is present in various natural and food products and also produced chemically. It has excellent solvency for a variety of substances. Therefore, TBAc is mainly used for architectural coatings, industrial cleaning, and as a solvent in paints and lacquers (Groth and Freundt, 1994; Budroe *et al*., 2004; Yang *et al*., 2007). Moreover, TBAc has recently been exempted from the definition of a volatile organic compound (VOC) by both the Air Resources Board (ARB) and the local air pollution control and air quality management agencies. This is because it forms lesser tropospheric ozone than other VOCs (Budroe *et al*., 2004). Since the use of TBAc as a substitute for other VOCs has increased, its potential risk in humans has also increased. Severe health impacts may be caused by TBAc inhalation, ingestion, and eye or skin contact in homes and workplaces (Hazardous Substances Data Bank (HSDB), 2007). However, TBAc toxicity data are limited. In an acute inhalation toxicity study conducted by Industrial Bio-Test Laboratories Inc. (1958), inhalation exposure to TBAc at above 500 mg/m^3 (105 ppm) caused alterations in the central nervous system (CNS) and pulmonary congestion/hemorrhage in rats; the inhalation median lethal concentration (LC_{50}) of TBAc was 1330 mg/m^3 (281 ppm). The results of an acute toxicity study conducted by Stillmeadow Inc. (1997) indicated that inhalation exposure to TBAc did not exert any adverse effect even at 2230 mg/m^3 (470 ppm) in

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rats and that the no-observed-adverse-effect exposure concentration was above 2230 mg/m^3. In another study, a 6-h exposure of Sprague-Dawley rats to TBAc at 9000, 17000, or 24000 mg/m^3 (1898, 3586 or 5062 ppm) caused exaggerated breathing immediately after exposure, periodic shaking of the head and thorax, lethargy and immobility, sensation of cold to touch, unconsciousness, and death; the LC_{50} of TBAc was 20000 mg/m^3 (4218 ppm). Postmortem examination showed evidence of pulmonary congestion in the decedents and no compound-related pathology was seen in the survivors (observation time, 14 days) (Kenney, 1999).

However, the studies cited above did not include a control group, and a time-course toxicity study has not been conducted thus far. The aim of this study was to provide basic data on acute TBAc inhalation toxicity in Sprague-Dawley rats according to time course.

MATERIALS AND METHODS

Animals and treatment. Specific pathogen-free (SPF) male Sprague-Dawley rats (n = 54; age, 7 weeks) were purchased from Orient Bio Inc. (Gyeonggi-do, Republic of Korea). The rats were acclimated for 1 week and then randomly divided into the following 3 groups on the basis of exposure concentrations: unexposed or control (0 ppm) group, 2370 mg/m^3 (500 ppm) or low-dose group, and 9482 mg/m^3 (2000 ppm) or high-dose group. After exposure, 3 individual necropsies were performed at 2, 7, and 15 days. The rats were fed Lab Diet 5053, given filtered tap water, and housed in a room maintained at a temperature of 23 ± 3°C and a relative humidity of 50 ± 10% with artificial lighting from 08:00 h to 20:00 h and 13~18 air changes per hour. All the animal facilities provided in this study were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. This study was conducted with reference to the Oraganization for Economic Cooperation and Development (OECD) Guideline for the testing of chemicals 403 "Acute Inhalation Toxicity" (OECD, 2009).

Nose-only inhalation exposure to TBAc. TBAc was purchased from Sigma-Aldrich Co. (Milwaukee, WI, USA). Its chemical purity was determined to be > 99% by gas chromatography. We exposed the rats to TBAc for 4 h for 1 day by using a nose-only inhalation exposure chamber (low dose, 500 ppm; high dose, 2000 ppm). Sham-treated control rats were exposed to filtered air in the inhalation chamber for the same period. TBAc doses were selected from the experiments conducted in previous studies (Kay, 1953; Bennick, 1997).

TBAc vapor-air mixture generation. Airflow containing TBAc vapor at a target concentration of 500 or 2000 ppm was prepared by a vaporization technique. The saturated vapor-air mixture was generated by bubbling clean air through TBAc liquid in a temperature-regulated glass flask (25°C) and then cooling it by passing through a chiller at 20°C (Organic solvent gas generator, VG-24S; Yotsubishi Corporation, Tokyo, Japan). The airflow containing the saturated vapor was diluted with clean air and then supplied to an inhalation exposure chamber. The flow rate of the vapor-air mixture was regulated with a flow meter (Kasai *et al*., 2002). The concentration of TBAc in the chamber during exposure was measured every 7 min by gas chromatography (GC-2014, Shimadzu, Japan) and controlled using a flow meter.

Collection of bronchoalveolar lavage fluid (BALF) and blood. At selected time intervals after exposure to TBAc, the animals were sacrificed using isoflurane. Blood was collected from the caudal vena cava for serum biochemistry measurements. BALF was collected by cannulating the trachea and lavaging the lungs. The left lung was clamped off and the right lung was washed 3 times with 3 m*l* sterilized saline. The lavage fluid was centrifuged (500 *g*, 10 min, 4°C) to obtain the cell pellet. The cell pellets obtained from the lavage fluids recovered from all the rats were combined and resuspended in 1 m*l* of saline in order to evaluate the cellular parameters.

Evaluation of BALF cells. The cells recovered from BALF were counted and identified. The total cells in BALF were quantified using a cell viability analyzer (Beckman Coulter, Miami, FL, USA). BALF cells were spun at 800 rpm for 5 min, pelleted onto a slide by using a cytospin centrifuge (Thermo Shandon, Pittsburgh, PA, USA), and stained with Wright-Giemsa Sure stain (Muto Pure Chemicals, Tokyo, Japan). Alveolar macrophages (AM), polymorphonuclear neutrophils (PMN), and lymphocytes were identified by their characteristic shapes.

Biochemical assay. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), and alkaline phosphatase (ALP) levels in the serum were measured. Biochemical assay was performed using DRI-CHEM 3500s (Fuji Film, Tokyo, Japan). The measurements were performed according to the manufacturer's instructions.

Histological examination. After collection of BALF, the lung tissue of each rat was fixed in a 10% formaldehyde solution at room temperature for 2 days and then embedded in paraffin. The paraffin-embedded tissues were sectioned at a thickness of 4 μm for histological examination. The sections were deparaffinized with xylene and then stained with hematoxylin and eosin (H&E). All the stained tissue sections were analyzed under a bright-field microscope. For the assessment of lung injury, the airways, termi-

nal bronchioles, and lung parenchyma were examined microscopically to evaluate cellular changes and inflammation. Pathological scores 0 (no histopathological change) to 5+ (severe abnormalities) were assigned to each lung tissue on the basis of the degree of alveolitis, bronchiolitis, bronchitis, fibrosis, and the extent of involvement, as described previously (Pryhuber *et al*., 2003).

Statistical analysis. All results are calculated as mean ± standard deviation (SD). Statistical analyses were carried out using the Statistical Analysis System (SAS) (SAS Institute Incorporated, Cary, NC, USA, 1997). For all the parameters, Bartlett's test was performed to determine the existence of a significant interaction, while Dunn's rank sum and analysis of variance (ANOVA) tests were used to compare the experimental groups with the control group. The significance between individual groups at each time point was analyzed using a *t*-test. Statistical difference was considered significant at $p < 0.05$ and $p < 0.01$.

RESULTS

Chamber concentrations of TBAc. Target chamber concentrations of TBAc were 500 and 2000 ppm, and the actual concentration in each chamber was monitored using gas chromatography. The concentrations over the exposure period were as follows: 502.5 ± 5.67 ppm and 2001.0 ± 22.13 ppm (mean ± SD). For each group, the concentrations in the exposure period are presented in Fig. 1.

Clinical signs and mortality. No treatment-related abnormal clinical signs were observed, and there were no unscheduled deaths during the study period (data not shown).

Body weight and gross findings. The body weight of each rat was measured just before beginning exposure (day 1) and on days 2, 7, and 15 after the exposure. As shown in Table 1, decreased body weight gains were observed on day 2 in all the groups. At the scheduled necropsy, there were no treatment-related gross findings in any animal.

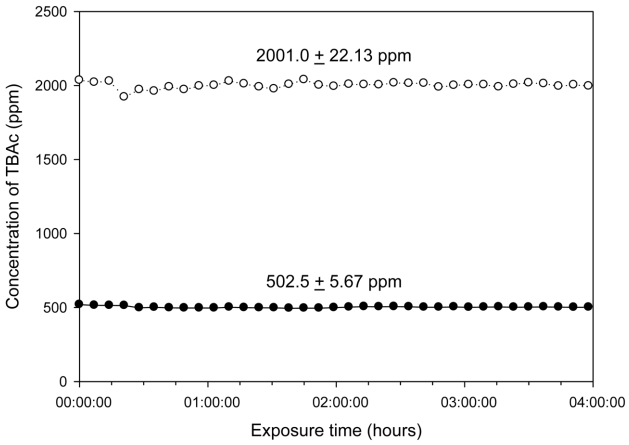

Fig. 1. Inhalation chamber concentrations of TBAc for 4 h. The concentrations in each inhalation chamber were controlled by mixing TBAc vapor and high-efficiency particulate air (HEPA)-filtered air. They were monitored by gas chromatography, using a flame ionization detector-equipped chromatograph and silicone DC-200 column with 20% Chromosorb.

Table 1. Body weights of rats exposed to TBAc vapor by inhalation. Three groups of 18 rats (each group, 6 rats) were exposed to TBAc at concentrations of 0, 500, and 2000 ppm for 4 h

	Control (g)	500 ppm (g)	2000 ppm (g)
Pre-exposure (Day 1)	284.0 ± 9.96	283.3 ± 7.63	283.5 ± 7.59
Day 2	279.7 ± 14.11	281.1 ± 7.42	279.9 ± 8.50
Day 7	318.4 ± 19.96	320.1 ± 18.66	320.1 ± 17.56
Day 15	366.1 ± 14.35	371.7 ± 17.93	373.1 ± 19.68

Values are presented as mean ± SD.

Clinical chemistry and cytology. Serum AST level was significantly increased in the 2000-ppm group, compared with the control group, on day 2 (Table 2). The number and types of cells recovered from BALF were used to indicate the extent of pulmonary inflammation. The mean number of cells recovered from BALF was similar in all the groups (Table 3). Further, the cytological profile was simi-

Table 2. Biochemical blood analysis of rats exposed to TBAc vapor by inhalation

	Day 2			Day 7			Day 15		
	Control	500 ppm	2000 ppm	Control	500 ppm	2000 ppm	Control	500 ppm	2000 ppm
n	6	6	6	6	6	6	6	6	6
AST	73 ± 11.8	87 ± 26.0	109 ± 23.6*	68 ± 14.5	75 ± 10.0	62 ± 8.0	60 ± 10.1	67 ± 7.9	66 ± 12.9
ALT	30 ± 1.4	27 ± 3.1	34 ± 5.2	34 ± 7.5	30 ± 5.8	29 ± 2.8	28 ± 3.7	32 ± 4.0	32 ± 2.6
TBIL	0.43 ± 0.052	0.47 ± 0.052	0.40 ± 0.000	0.47 ± 0.082	0.45 ± 0.055	0.48 ± 0.041	0.45 ± 0.055	0.37 ± 0.058	0.47 ± 0.103
ALP	1572 ± 188	1532 ± 226	1350 ± 265	1474 ± 394	1599 ± 306	1481 ± 292	1362 ± 226	1223 ± 245	1429 ± 449

Values are presented as mean ± SD.
n, number of animals.
*P < 0.05, significant difference as compared with the control.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, total bilirubin; ALP, alkaline phosphatase.

Table 3. BALF analysis of rats exposed to TBAc vapor by inhalation

	Total number of cells in BALF	Percentage of cells in BALF (%)		
	(Total number of cells/m*l*) × 10^5	Macrophages	Neutrophils	Lymphocytes
Control				
Day 2	5.5 ± 1.4[a]	99.22	0.33	0.44
Day 7	4.6 ± 1.6	99.50	0.25	0.25
Day 15	4.7 ± 1.3	99.22	0.39	0.39
500 ppm				
Day 2	3.8 ± 1.0	97.87	0.93	1.20
Day 7	4.8 ± 2.5	98.00	1.13	0.87
Day 15	3.1 ± 0.9	99.20	0.13	0.67
2000 ppm				
Day 2	3.8 ± 1.4	98.17	1.11	0.72
Day 7	5.2 ± 3.4	99.47	0.00	0.53
Day 15	4.4 ± 1.4	99.67	0.17	0.17

[a]Values are presented as mean ± SD.
BALF, bronchoalveolar lavage fluid.

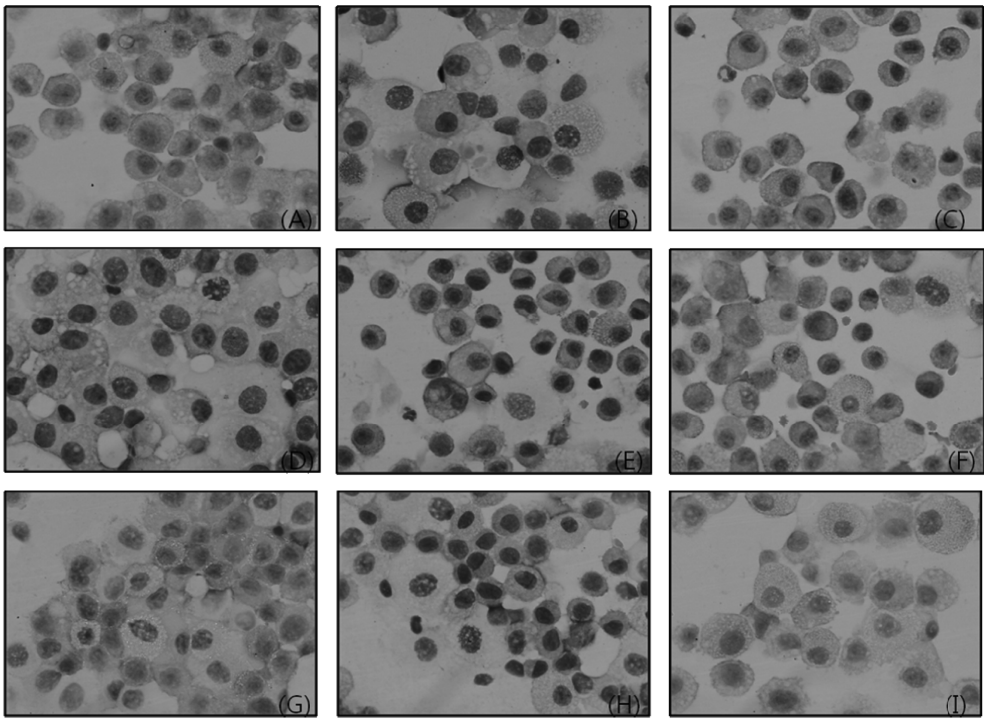

Fig. 2. Cytospin images of alveolar cells in the bronchoalveolar lavage fluid (BALF) of rats exposed to TBAc vapor by inhalation. (A) Control - Day 2, (B) 500 ppm - Day 2, (C) 2000 ppm - Day 2, (D) Control - Day 7, (E) 500 ppm - Day 7, (F) 2000 ppm - Day 7, (G) Control - Day 15, (H) 500 ppm - Day 15, (I) 2000 ppm - Day 15.

lar in all the groups (Fig. 2).

Histopathological findings. We histologically analyzed the nasal cavity, lung, and liver sections obtained from the rats in all the groups after TBAc exposure by inhalation. The time course of histopathological changes in the lungs, liver, and nasal cavity are shown in Figs. 3, 4, and 5, respectively. The TBAc-induced histopathological changes were scored on days 2, 7, and 15, as shown in Table 4. An inflammatory cell focus was observed in the nasal cavity and lung on days 2 and 7, respectively, in the 500-ppm group. Foamy macrophages in the lung were observed in all the groups. However, no significant changes were found in the terminal bronchiole and alveolar space with thin alveolar septa. Hemopoiesis and inflammatory cell foci in the liver were observed in all the groups. One case of focal

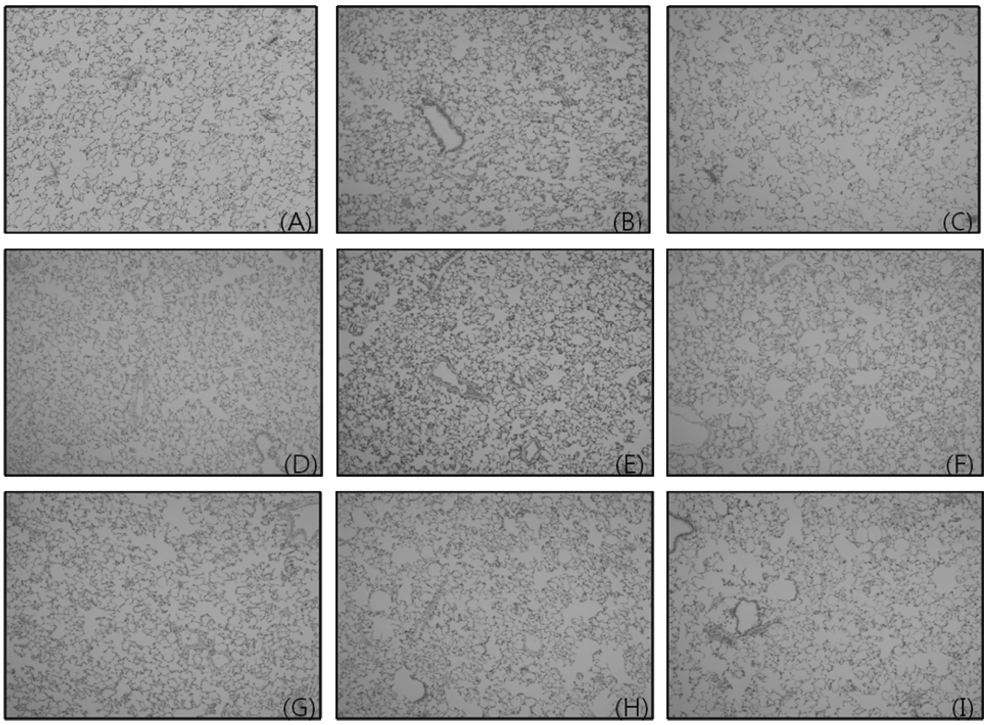

Fig. 3. Histopathologic response in the lungs of rats exposed to TBAc vapor by inhalation (hematoxylin and eosin stain). (A) Control - Day 2, (B) 500 ppm - Day 2, (C) 2000 ppm - Day 2, (D) Control - Day 7, (E) 500 ppm - Day 7, (F) 2000 ppm - Day 7, (G) Control - Day 15, (H) 500 ppm - Day 15, (I) 2000 ppm - Day 15.

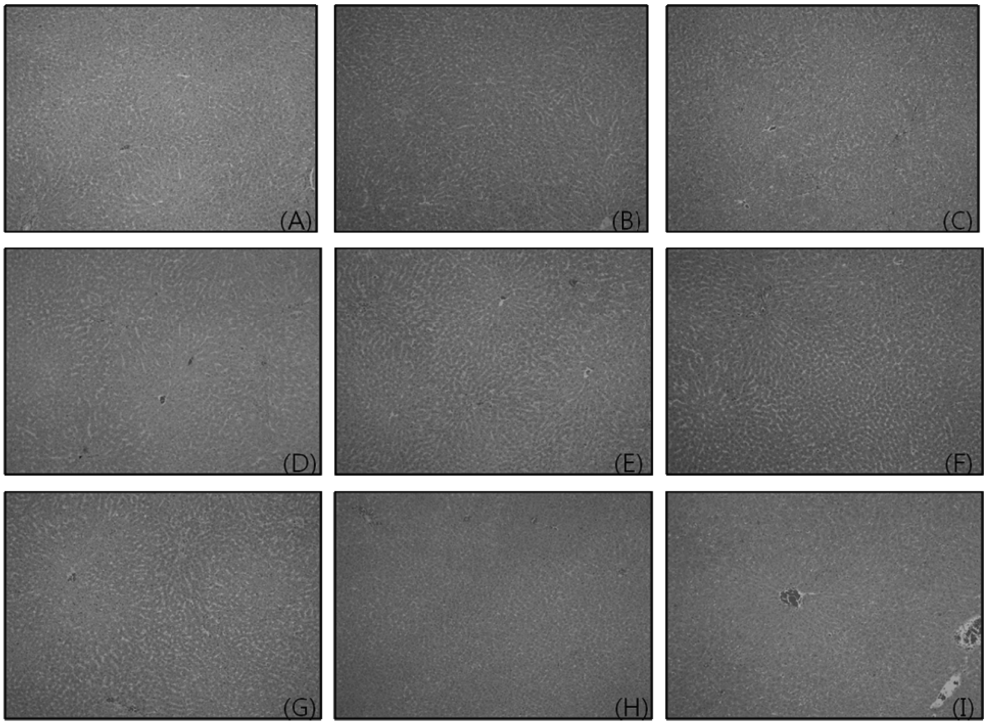

Fig. 4. Histopathologic response in the liver of rats exposed to TBAc vapor by inhalation (hematoxylin and eosin stain). (A) Control - Day 2, (B) 500 ppm - Day 2, (C) 2000 ppm - Day 2, (D) Control - Day 7, (E) 500 ppm - Day 7, (F) 2000 ppm - Day 7, (G) Control - Day 15, (H) 500 ppm - Day 15, (I) 2000 ppm - Day 15.

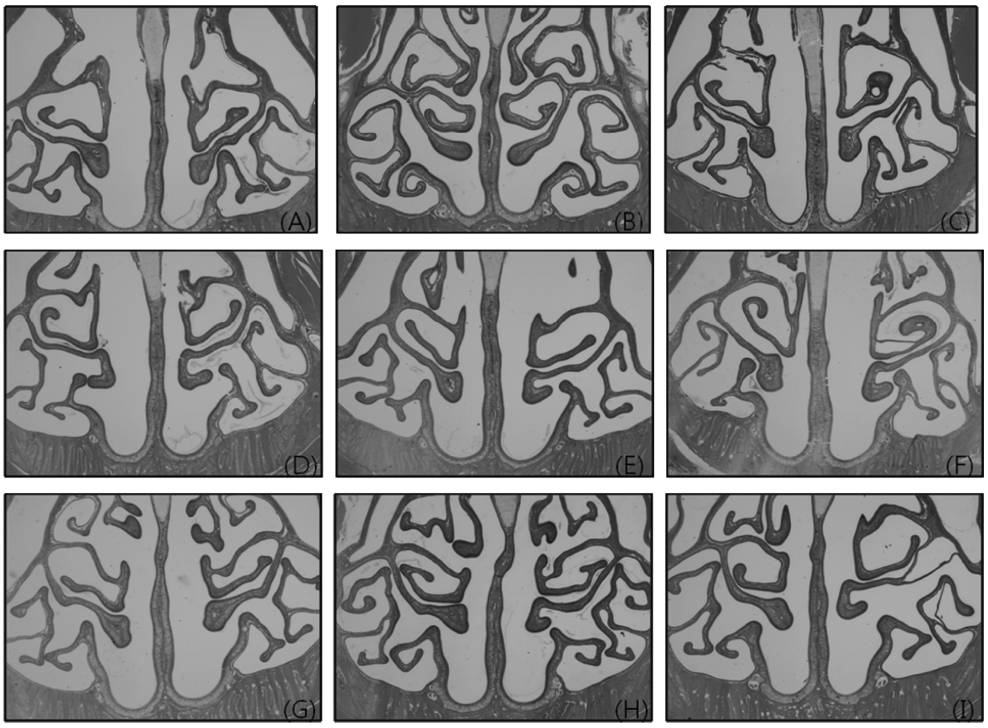

Fig. 5. Histopathologic response in the nasal cavity of rats exposed to TBAc vapor by inhalation (hematoxylin and eosin stain). (A) Control - Day 2, (B) 500 ppm - Day 2, (C) 2000 ppm - Day 2, (D) Control - Day 7, (E) 500 ppm - Day 7, (F) 2000 ppm - Day 7, (G) Control - Day 15, (H) 500 ppm - Day 15, (I) 2000 ppm - Day 15.

Table 4. Histopathological changes in rats exposed to TBAc vapor by inhalation

	Day 2			Day 7			Day 15		
	Con	T1	T2	Con	T1	T2	Con	T1	T2
Nasal cavity									
Inflammatory cell foci	–	–	–	–	+ (1)	–	–	–	–
Lung									
Inflammatory cell foci	–	+ (1)	–	–	–	–	–	–	–
Foamy macrophages	+ (1)	–	–	–	+ (1)	+ (1)	–	–	+ (1)
Liver									
Hemopoiesis	+ (3)	+ (3)	+ (4)	+ (4)	+ (4)	+ (3)	+ (1)	+ (3)	+ (4)
Inflammatory cell foci	+ (5)	+ (6)	+ (2)	+ (3)	+ (2)	+ (3)	+ (4)	+ (2)	+ (3)
					++ (1)				++ (1)
Focal necrosis	–	–	+ (1)			+ (1)	–	–	–

Histopathological changes were scored on the basis of the degree of abnormality in the tissues observed under a light microscope.
The grading criteria used are as follows: –, no remarkable findings; +, minimal abnormality; ++, slight abnormality; +++, moderate abnormality; ++++, marked abnormality; +++++, severe abnormality.
Con, control (0 ppm); T1, 500 ppm; T2, 2000 ppm.

necrosis in the liver was observed on days 2 and 7 at 2000 ppm. The other groups did not show any distinct histopathological changes.

DISCUSSION

The present study was conducted to investigate the potential acute toxicity of TBAc according to time course by inhalation exposure of Sprague-Dawley rats at concentrations of 0, 2370 mg/m^3 (500 ppm), and 9482 mg/m^3 (2000 ppm). The study was conducted because no guidelines or standards for the protection of the general population from the adverse health effects associated with TBAc exposure are currently available and limited data on TBAc toxicity have been published.

No treatment-related clinical signs were observed in any

of the dose groups. Some adverse clinical signs in the eye and respiratory system were expected in the animals because the Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) and American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for TBAc (8-h time-weighted) are based on irritation in the eye and respiratory system of exposed workers (International Labour Organization (ILO), 1983; ACGIH, 1986; Proctor *et al*., 1988; HSDB, 2007). However, under these experimental conditions, TBAc at concentration up to 2000 ppm did not cause any toxic symptoms in the animals tested. Decreased body weight gains were observed on day 2, but they did not show a dose-response relationship and normal body weight gain was restored on day 7. The difference was presumed to be related to the additional stress of handling and placement of rats in the exposure tubes and the additional time spent away from the feed containers (Rothenberg *et al*., 2000).

Girkin and Kirkpatrick (2000) reported a pharmacokinetic study of TBAc. They exposed rats to ^{14}C-TBAc at concentrations of 100 and 1000 ppm by inhalation for 6 h. Low-dose animals excreted most of the TBAc in the urine, while high-dose animals excreted most of it in the expired air. Therefore, we investigated the histopathological changes in the nasal cavity and lungs. In addition, we investigated the total cell count and differential count of inflammatory cells in BALF. In the tissues, inflammatory cell foci and foamy macrophages were observed. However, these changes were not considered as the toxicological effects of TBAc because their incidence was low without any dose-response relationship and they were similar to those sporadically or incidentally observed in normal control rats. Further, with regard to the total cell count and differential inflammatory cell count in BALF, there was no significant difference among the groups before and after exposure. Previous studies have shown that when the lung is exposed to a toxic material, inflammations generally occur after 2 days and manifest after 7 days of exposure (Yang *et al*., 2008; Park *et al*., 2010).

Serum AST level was significantly increased in the 2000-ppm group, compared with the control group, on day 2, and 1 case of focal necrosis was observed in the liver on days 2 and 15 at 2000 ppm. The increase in the serum AST level was within the limits of normal biological variations and was not observed on days 7 and 15 (Kang *et al*., 2004; Giknis and Clifford, 2006).

Previous acute TBAc inhalation toxicity studies were performed in rats, and the LC_{50} ranged from 1330 mg/m^3 (281 ppm) to 20000 mg/m^3 (4218 ppm) (Industrial Bio-Test Laboratories Inc., 1958; Stillmeadow Inc., 1997; Kenney, 1999). An oral LD_{50} ranged from 3420 mg/kg body weight to 4500 mg/kg body weight (males, 4100 mg/kg; females, 4750 mg/kg) (Kay, 1953; ACGIH, 1991; DeGeorge, 1997). A previous short-time study reported that centrilobular hepatocyte hypertrophy and an increased number of cortical tubules with hyaline droplets were observed on exposure to a concentration of 7900 mg/m^3 at 6 h/day and 5 days per week for 2 weeks (Kenney, 2000). A recent developmental study showed that TBAc was embryotoxic at a maternally toxic dose (1600 mg/kg/day) and minimally embryotoxic at a nonmaternally toxic dose (800 mg/kg/day) in Sprague-Dawley rats (Yang *et al*., 2007). In this study, TBAc alone exhibited no toxicity. However, further studies are needed because the toxicity may produce unexpected endpoints on co-exposure to other chemicals. In fact, exposure to a single environmental chemical is uncommon, and humans may be co-exposed to more than one chemical (Song *et al*., 2005).

In summary, it was found that inhalation exposure to TBAc vapor at 9482 mg/m^3 (2000 ppm) for 4 h induced no special changes with regard to clinical signs, body weight, blood chemistry, BALF, and pathology in Sprague-Dawley rats for 14 days after exposure. Under these experimental conditions, it was considered that the 4-h inhalation LC_{50} of TBAc was over 9482 mg/m^3 (2000 ppm).

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REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH). (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices (5th edition), ACGIH, Cincinnati, OH, pp. 74.

American Conference of Governmental Industrial Hygienists (ACGIH). (1991). *tert*-Butyl acetate in Documentation of the Threshold Limit Values and Biological Exposure Indices, ACGIH, Cincinnati, OH, pp. 167.

Bennick, J.E. (1997). Acute inhalation toxicity study in rats. Study No. 3639-97, Stillmeadow Inc., Sugar Land, TX (available from the National Technical Information Service (NTIS), Springfield, VA; Order No. OTSO0573684-1).

Budroe, J.D., Brown, J.P., Salmon, A.G. and Marty M.A. (2004). Acute toxicity and cancer risk assessment values for *tert*-butyl acetate. *Regul. Toxicol. Pharmacol.*, **40**, 168-176.

DeGeorge, G. (1997). Single dose oral toxicity in rats/LD50 in rats. Research Project No. MB 97-6119.01, MB Research Laboratories Inc., Spinnerstown, PA (available from the National Technical Information Service (NTIS), Springfield, VA; Order No. OTSO0573684-1).

Giknis, M.L.A. and Clifford, C.B. (2006). Clinical laboratory parameters for Crl:CD(SD) rats, Charles River Laboratories, pp. 10.

Girkin R, Kirkpatrick D. (2000). ^{14}C-[*tertiary*-butyl-acetate] metabolism and pharmacokinetic study in the rat after inhalation. Report to Lyondell Chemicals Worldwide, Newtown Square,

PA. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, pp. 1-90.

Groth, G. and Freundt, K.J. (1994). Inhaled *tert*-butyl acetate and its metabolite *tert*-butyl alcohol accumulate in the blood during exposure. *Hum. Exp. Toxicol.*, **13**, 478-480.

Hazardous Substances Data Bank (HSDB). (2007). National Library of Medicine, Bethesda, MD. Available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Industrial Bio-Test Laboratories Inc. (1958). Toxicity studies on TFA-168. *tert*-Butyl acetate. Report to Albemarle Corporation, Baton Rouge, LA. Industrial Bio-Test Laboratories Inc., Northbrook, IL. EPA/OTS Doc x 86940000229, NTIS/OTS0556824.

International Labour Organization (ILO). (1983). Encyclopedia of Occupational Health and Safety (Vols. I and II), Geneva, Switzerland, pp. 783.

Kang, B.H., Kim, Y.B., Lee, H.S., Kim, Y.H., Im, W.J. and Ha, C.S. (2004). Background data on hematology, blood biochemistry and organ weights for 2 weeks and 4 weeks repeated-dose toxicity studies using Sprague-Dawley (SD) rats. *Kor. J. Lab. Anim. Sci.*, **20**, 134-140.

Kasai, T., Nishizawa, T., Arito, H., Nagano, K., Yamamoto, S., Matsushima, T. and Kawamoto, T. (2002). Acute and subchronic inhalation toxicity of chloroform in rats and mice. *J. Occup. Health*, **44**, 193-202.

Kay, I.H. (1953). Toxicity studies on TFA-168. Report to The Texas Company. Industrial Bio-Test Laboratories Inc., Northbrook, IL (available from the National Technical Information Service (NTIS), Springfield, VA; Order No. OTS00556824).

Kenney, T.J. (1999). *tertiary*-Butyl acetate acute (six-hour) inhalation study in rats. Report to Lyondell Chemicals Worldwide, Newtown Square, PA. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, pp. 1-50.

Kenney, T.J. (2000). *tertiary*-Butyl acetate: 14 day repeat dose snout only inhalation toxicity range finding study in rats. Report to Lyondell Chemicals Worldwide, Newtown Square, PA. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, pp. 1-107.

OECD. (2009). Acute Inhalation Toxicity Testing. OECD guideline for testing of chemicals No. 403, OECD, Paris. Available at http://www.oecd.org/env/testguidelines.

Park, H.J., Yang, M.J., Oh, J.H., Yang, Y.S., Kwon, M.S., Song, C.W. and Yoon, S. (2010). Genome-wide transcriptional response during the development of bleomycin-induced pulmonary fibrosis in Sprague-Dawley rats. *Toxicol. Res.*, **26**, 137-147.

Proctor, N.H., Hughes, J.P. and Fischman, M.L. (1988). Chemical Hazards of the Workplace (2nd edition), J.B. Lippincott & Co., Philadelphia, PA, pp. 105.

Pryhuber, G.S., Huyck, H.L., Baggs, R., Oberdorster, G. and Finkelstein, J.N. (2003). Induction of chemokines by low-dose intratracheal silica is reduced in TNFR I (p55) null mice. *Toxicol. Sci.*, **72**, 150-157.

Rothenberg, S.J., Parker, R.M., York, R.G., Dearlove, G.E., Martin, M.M., Denny, K.H., Lief, S.D., Hoberman, A.M. and Christian, M.S. (2000). Lack of effects of nose-only inhalation exposure on testicular toxicity in male rats. *Toxicol. Sci.*, **53**, 127-134.

Song, K.S., Park, K.H., Kim, J.H., Han, D.U., Chae, C.H., Park, S.J., Kim, H.W., Kim, J.S., Park, J.H., Eu, G.J., Hua, J., Cho, H.S., Hwang, S.K., Chang, S.H., Yu, K.N., Kim, W.J., Kwon, J.T., Bhandari, D., Tehrani, A.M. and Cho, M.H. (2005). 90-day inhalation toxicity of dimethylamine in F344 rats. *Toxicol. Res.*, **21**, 179-186.

Stillmeadow Inc. (1997). *tert*-Butyl acetate. Vol. V. Acute inhalation toxicity study in rats. Sponsored by MB Research Laboratories Inc., Spinnerstown, PA, for Arco Chemical Company, Newtown Square, PA. Submitted to US Environmental Protection Agency (EPA), November 4, 1997. (EPA/Office of Technical Service (OTS), Doc. #87980000002) (available from the National Technical Information Service (NTIS), Springfield, VA; Order No. OTS0573684-1).

Yang, M.J., Yang, Y.S., Kim, Y.B., Cho, K.H., Heo, J.D., Lee, K. and Song, C.W. (2008). Noninvasive monitoring of bleomycin-induced lung injury in rats using pulmonary function test. *Toxicol. Res.*, **24**, 273-280.

Yang, Y.S., Ahn, T.H., Lee, J.C., Moon, C.J., Kim, S.H., Park, S.C., Chung, Y.H., Kim, H.Y. and Kim, J.C. (2007). Effects of *tert*-butyl acetate on maternal toxicity and embryo-fetal development in Sprague-Dawley rats. *Birth Defects Res. B. Dev. Reprod. Toxicol.*, **80**, 374-382.