A study on formation of hemoglobin adduct due to inhalation exposure with 1,3-butadiene in female mice

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Abstract

The purpose of this study is the identification of (N-2-hydroxy-3-butenyl)valine(HBVal adduct) and (N-2,3,4-trihydroxy-butyl)valine(THBVal adduct) with mice inhalation exposure with 1,3-butadiene for 3 weeks(6 hr/day×5 days/week).

Body weights were significantly lower from 4 or 9 exposure post-day in 1000 or 500ppm inhalation group than in control. The levels of HBVal adducts are 1.8, 3.7 and 6.2 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 500 ppm 1,3-butadiene(BD), and 5.7, 7.4 and 16.0 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 1000 ppm BD inhalation exposure. The levels of THBVal adducts are 32.0, 42.0 and 55.0 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 500 ppm BD, and 67.8, 72.7 and 83.5 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 1000 ppm BD inhalation exposure. Their ratios of THBVal and HBVal adducts are higher at earlier exposure and lower concentration. They are17.8, 11.4 and 8.87 in 1^{st}, 2^{nd} , and 3^{rd} week for 500 ppm BD, and 11.9, 9.8 and 5.2 in 1^{st}, 2^{nd} , and 3^{rd} week for 1000 ppm BD inhalation exposure.

In conclusion, THBVal and HBVal adducts are a important hemoglobin adduct for monitoring of BD exposure, and the latter is more biomarker than the other.

Key words : 1,3-butaiene(BD), (N-2-hydroxy-3-butenyl)valine(HBVal adduct), (N-2,3,4-trihydroxy-butyl)valine(THBVal adduct), GC/MS

Introduction

Sources

1,3-butadiene(BD) is an important petrochemical products. It can be produced by petrol and widely used for chemical synthesis, particularly in the preparation of polymers, i.e., styrene/butadiene synthetic rubber(SBR), acrylonitrile /butadiene/styrene

thermoplastic(ABS) and styrene/butadiene/styrene block copolymer(SBS). And it also occurs as an environmental contaminant. It has been estimated that most BD emissions derive from mobile sources, although leaks and waste emissions from manufacturing facilities may be locally important. All burning of organic material produces emissions containing minor amounts of BD, and cigarette smoke contains small amount of BD. Low exposure to BD is thus a common characteristics of whole human population, although the exposure levels in industry might be several orders of magnitude higher.

Carcinogenicity

BD is a well-established rodent carcinogen. Although both sexes of B6C3F1 mice and Sprague-Dawley rats developed tumors in 2 years inhalation bioassay(Huff et al., 1985; Melnick et al., 1990; Owen et al., 1987; Osterman-Golkar et al., 1998). The most striking aspect of butadiene-induced carcinogenicity in rodent is the high sensitivity of mice compared with rats. Rats developed tumors from exposure to butadiene concentrations(1,000～8,000 ppm) that were as much as three orders of magnitude higher than those that caused cancer in mice(6.25～1,250 ppm)(Osterman-Golkar et al., 1998)..

Based on sufficient evidence of carcinogenicity in laboratory and partial evidence of carcinogenicity in epidemiological studies in humans, the International Agency for Research on Cancer(IARC), in 1999, classified BD as a probable carcinogen to humans(group 2A)(IARC, 1999). The European Union also labels BD as a potential carcinogen(R45: " may cause cancer"). In 2000, the ninth report on carcinogens edited by the Department of Health and Human Services, US, described BD as "known to be a human carcinogen"(Department of Health and Human Services, 2000). In 2002, the US Environmental Protection Agency classified BD as a human carcinogen by inhalation(EPA, 2002)

To prevent health risks in humans arising from exposure to BD, occupational exposure limits were established or recommended in different countries. A concentration of 2 ppm (4.4 mg/m^3) as a threshold limit value time-weighted average(TLV-TWA) during an 8 h shift is recommended by the American Conference of Governmental Industrial Hygienists(ACGIH, 2002). Concentration of 15 ppm (34 mg/m^3) and 5 ppm (11 mg/m^3) are established as technical exposure limit(TRK) by the German Federal Ministry for Employment and Social Affairs(AGS Committee for hazardous substances) for processing after polymerization or loading and other applications, respectively(Deutsche Forschungsgemeinschaft, 2002). At the present time, BD is under evaluation by the Scientific Committee for Occupational Exposure

Limits(SCOEL) of the European Union(Fustinoni et al., 2004). A concentration of 10 ppm(22 mg/m^3) as a permission limit value time-weighted average during 8h shift is established by the ministry of labor in Korea.

Metabolism

Once inhaled and absorbed through the respiratory tract, BD is distributed through the blood to various organs. Its elimination may occur either via exhaled air, for the un-metabolized compound, or via urinary excretion, after transformation in hydrophilic metabolites.

The metabolism of BD has been studied in rats and mice by several different investigators and reviewed by Himmelstein et al.(1997), Albertini et al.(2001) and Swenberg et al.(2001). The general metabolic scheme is shown in Figure 1.

It is generally accepted that BD is first metabolized to 1,2-eopxy-3-butene(EB), a process that is primary associated with CYP2E1 and CYP2A6(Albertini et al., 2001). This electrophilic metabolite can be detoxified by congjugation with glutathione and subsequent excretion in the urine as 1-hydroxy-2-(N-acetylcysteinyl)-3-butene(M-2) and 1-(N-acetylcysteinyl)-2-hydroxy-3-butene. It can also undergo hydrosis by epoxide hydrolase(EH) to form 1,2-dihydroxy-3-butene(BD-diol). BD-diol is a major metabolite of BD in livers. It can be conjugated with glutathione and subsequently excreted in the urine as 1,2-dihyroxy-4-(N-acetylcysteinyl)-butane(M-1). It can be further oxidized by CYP to the 1,2-dihydroxy-3,4-epoxybutane(epoxybutane diol, EBD). Alternative pathway for the metabolism of EB is its further oxidation to 1,2,3,4-diepoxybutane (DEB), which can be further hydrolyzed to EBD or conjugated by glutathione and excreted in the urine(Henderson et al., 1993). This series of epoxidation and detoxification steps generates three electrophilic metabolites: EB, DEB and EBD.

< Figure 1 >

Biological monitoring

The main goal of biological monitoring of exposure is to ensure that the current or past exposure of workers and citizens is safe, i.e., does not entail an unacceptable health risk. Biological monitoring of exposure is clearly distinguished from health surveillance. Whereas it attempts to detect unhealthy exposure condition(health risk), health surveillance evaluates the health status and aims at identifying individuals with early signs of adverse health effects, i.e., effects which are likely to be reversible or which do

not progress to significant functional impairments when the exposure conditions are improved.

Biological monitoring of exposure can be classified in three categories, but of different importance(Bernard and Lauwerys, 1986; Lauwerys and Hoet, 1993): 1) determination of the chemical or its metabolites in biological fluid; 2) quantification of non-adverse biological effects related to the internal dose; and 3) direct measurement of the amount of active chemical interacting with the target molecules. Biological responses include biomarkers of exposure(urinary metabolite concentrations, hemoglobin adducts and DNA adducts), biomarkers of effects(in vivo somatic gene mutations and chromosome changes) and biomarkers of susceptibility(metabolic genotypes)(Albertini et al, 2001). Figure 2 is the scheme used to validate biomarkers of 1,3-butadiene exposure in epidemiological studies(Committee of biological Markers of the National Research Council, 1987).

< Figure 2 >

Purpose

The purposes of this study were the identification of metabolites in hemoglobin adducts as biomarkers, and dose response of adduct formation during BD inhalation exposure by GC/MS. Metabolites of BE formed hemoglobin adducts. They are (N-2-hydroxy-3-butenyl)valine(HBVal) and (N-2,3,4-trihydroxy-butyl)valine(THBVal) which are produced by the EB and EBD metabolites of BD, respectively.

Materials and Methods

Chemicals

1,3-butadiene(BD) was obtained from Sigma(St. Louis, MO, USA). Analytical hydroxide, potassium bishydrogen phosphate, sodium sulfate, acetyl chloride and acetic ethanol, acetone and ethyl acetate(E. Merck, Darmstadt, Germany) were used as solvents. All other chemicals were of the highest purity available from Sigma and Merck.

Synthesis Metabolites

N-(2-hydroxy-3-butenyl)valine(HBVal) was synthesized by incubation of butadiene monoxide with L-valine in 3 ml of water, pH 10 for overnight at 37℃. N-(2,3,4-

trihydroxybutyl)valine(THBVal) was synthesized by incubation of butadiene dioxide with L-valine in 3 ml of water, pH 10 for overnight at 37℃. They were dry with N_2 stream, dissolved in acetone and identified with GC/MS.

Animals and treatment

Forty five female ICR mice with a body weight of about 20g, were obtained from Haehanbiolink(Chongju, South Korea). They were acclimatized for one week in Maecrlone cages(temperature, 20℃; humidity, 30~70%; illumination time, 06:00h to 18:00h), and had free access to tap water and food. Fifteen mice in each group were randomized with respect to body weight before onset of exposure and then weighted three times weekly. The animals were exposed for 1, 2 or 3 weeks, 5h/day, 5day/week, to nominal butadiene concentration of 0, 500 or 1,000ppm. The animals were exposed in inhalation chamber with 1,3-butadiene dilution systems. Butadiene was generated directly from a liquid-gas storage tank and passed through a calibrated flow control device prior to mixing with clean air(99.999%). Air flow through the inhalation chamber was maintained at 5±2 L/min to ensure 5 to 7 air changes per hour. The animals had free access to water and food during non-exposure periods, but were deprived of water and food during the 5h exposures. The animals were exsanguinated by cardiac puncture. Blood samples were collected in heparinized syringes from groups of five mice at the end of each week during the 3 week inhalation exposure period. The erythrocytes were isolated by centrifugation, washed three times with 0.9% NaCl solution, and stored at -20℃ before isolation of globin.

Quantitative determination of hemoglobin adducts

Erythrocytes were separated from the plasma by centrifugation at 1,000×g for 10min, and were then washed twice with saline. The cells were lysed by adding one volume of distilled water for each volume of packed saline.

Samples of 50 mg of globin were dissolved in 1ml of formamide. 0.1M NaOH and 10 ul MSTA were added and the samples were left on a rocking mixer at room temperature over night and then 45℃ for 1.5 h. 2 ml of water was added to the formamid phase and MSTA derivatives were extracted with ethyl ether(3 times with 3 ml). The ether extract was evaporated to dryness, dissolved in 2 ml toluene and washed with 0.1 ml of water. The toluene extracts were pooled in a new test tube, and analyzed with GC/MS(Osterman-Golkar et al., 1996).

Conditions of GC/MS

All mass spectra were obtained with 6890/5973 GC/MS(Agilent Techologies; Palo Alto, CA, USA). The ion source was operated in the electron ionization mode(EI: 70 eV, 230℃). Full-scan mass spectra(m/z 30~800) were recorded for identification of analysts. Column was HP-5MS(30m×0.25mm×0.25um F.T.). Samples were injected in the pulsed split ratio(1/10). The flow rate of helium was 1.0 ml/min. The GC operating temperatures were: injector temperature, 280℃; transfer line temperature, 280℃; oven temperature, programmed from 50℃ at 10℃/min to 300℃(hold for 5 min)(Lee et al., 2002).

Results

Body weight changes

Table 1 is the changes of body weights in mice inhaled with BD for 3 weeks(5 hr/day, 5days/week). Body weights were significantly lower from 9 exposure post-day in 500ppm inhalation group, and from 4 exposure post-day in 1000ppm inhalation group than in control

<Table 1 >

Hemoglobin adducts

Table 2 is the levels of N-(2-hydroxy-3-butenyl)valine(HBVal) and N-(2,3,4-trihydroxybutyl)valine(THBVal), which extracted from hemoglobin adducts of mice inhaled with BD for 3 weeks(6 hr/day×5 days/week).

The levels of HBVal adducts are 1.8, 3.7 and 6.2 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 500 ppm BD, and 5.7, 7.4 and 16.0 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 1000 ppm BD inhalation exposure. The levels of THBVal adducts are 32.0, 42.0 and 55.0 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 500 ppm BD, and 67.8, 72.7 and 83.5 pmol/mg globin in 1^{st}, 2^{nd} , and 3^{rd} week for 1000 ppm BD inhalation exposure

<Table 2 >

Ratio of THBVal and HBVal adducts

Table 3 is the ratios of THBVal and HBVal extracted from hemoglobin for BD inhalation exposure(6 hr/day×5 days/week).

Their ratios are higher at earlier exposure and lower concentration. They are17.8,

11.4 and 8.87 in 1st, 2nd , and 3rd week for 500 ppm BD, and 11.9, 9.8 and 5.2 in 1st, 2nd , and 3rd week for 1000 ppm BD inhalation exposure.

<Table 3 >

Discussion

Hemoglobin adducts are offer an effective measure of exposure to reactive intermediates of chemicals. They have several advantages for molecular epidemiology studies including that they accumulate over the life of the red cell, which is ~43, 63 and 120 days in mice, rats and humans, respectively(Van Putten, 1958).

1,2-epoxy-3-butene(EB), the primary metabolite of BD may react with the N-terminal valine of hemoglobin, such as N-(2-hydroxy-3-butenyl)valine (HBVal adduct) and N-(2,3,4-trihydroxybutyl)valine (THBVal adduct)(Swenberg, et al., 2001).

For HBVal adduct, Boogaad, et al.(2001) reported it was a sensitive method for monitoring cumulative exposure to BD at or above 0.35ppm(0.771.1 mg/m^3), its concentration was 0.2 pmol/g globin in workers exposed with 1.1 mg/m^3 BD. Albrecht et al.(1993) reported the adduct was 17 nmol/g globin in mice exposed at 500ppm BD, 6 h/day, 5days/week, and it was five times higher in rats(3.5 nmol/g globin). This is similar to our results, of which HBVal adduct are 1.8~6.2 pmol/mg globin at 1st, 2nd and 3rd week for 500ppm BD, and 5.7~16.0 pmol/mg globin at 1st, 2nd and 3rd week for 1000ppm BD inhalation exposure(5hr/day, 5day/week). Mean HBVal adduct concentration, determined in Amsterdam, were 0.47, 2,23, 0.22 pmol/g globin for the monomer production, polymerization and administration workers, respectively(Albertini et al., 2001).

THBVal adduct are potentially produced by either DEB or EB-diol, although current evidence indicates that they derive almost entirely from the latter(Perez et al., 1997). Mean THBVal adduct concentrations, determined in Chapel Hill, were 178.73, 716.7, 94.77 pmol/g globin for the monomer production, polymerization and administration workers, respectively(Albertini et al., 2001). This is similar to our results, of which THBVal adducts are 32.0~55.0 pmol/mg globin at 1st, 2nd and 3rd week for 500ppm BD, and 67.8~83.5 pmol/mg globin at 1st, 2nd and 3rd week for 1000ppm BD inhalation exposure(5hr/day, 5day/week).

After exposure to 1000ppm(2210.0 mg/m^3) BD by inhalation, the average male and female concentrations of THBVal adducts were 4.3 fold greater in mice than in rats(Swenberg et al., 2000). The ratio of THBVal to total HBVal adduct concentration

was 6.3 for male mice and 3.4 for female mice. At high exposure of BD(1000~1250ppm, for 90 or 10 days, respectively), the ratio of THBVal : HBVal adducts was 2~6 in mice and rats. The exposure response ratio of THBVal : HBVal adducts was 39:1 at 62.5 ppm BD exposure, while at 1250ppm BD, it was 5.7:1(Swenberg et al., 2001). This is similar to our results, of which ratios are 17.8:1 at 1st week for 500 ppm BD, and 5.2:1 at 3rd week for 1000ppm BD inhalation exposure. When mice was exposed by inhalation

Conclusion

This study investigates (N-2-hydroxy-3-butenyl)valine(HBVal adduct) and (N-2,3,4-trihydroxy-butyl)valine (THBVal adduct) with ICR mice inhalation exopsure with 1,3-butadiene for 3 weeks(6 hr/day×5 days/week).

Body weights were significantly lower from 4 or 9 exposure post-day in 1000 or 500ppm inhalation group than in control The levels of HBVal adducts are 1.8, 3.7 and 6.2 pmol/mg globin in 1st, 2nd , and 3rd week for 500 ppm BD, and 5.7, 7.4 and 16.0 pmol/mg globin in 1st, 2nd , and 3rd week for 1000 ppm BD inhalation exposure. The levels of THBVal adducts are 32.0, 42.0 and 55.0 pmol/mg globin in 1st, 2nd , and 3rd week for 500 ppm BD, and 67.8, 72.7 and 83.5 pmol/mg globin in 1st, 2nd , and 3rd week for 1000 ppm BD inhalation exposure Their ratios are higher at earlier exposure and lower concentration. They are17.8, 11.4 and 8.87 in 1st, 2nd , and 3rd week for 500 ppm BD, and 11.9, 9.8 and 5.2 in 1st, 2nd , and 3rd week for 1000 ppm BD inhalation exposure

In conclusion, THBVal and HBVal adducts are a important hemoglobin adduct for monitoring of BD exposure, but the latter is more biomarker than the other.

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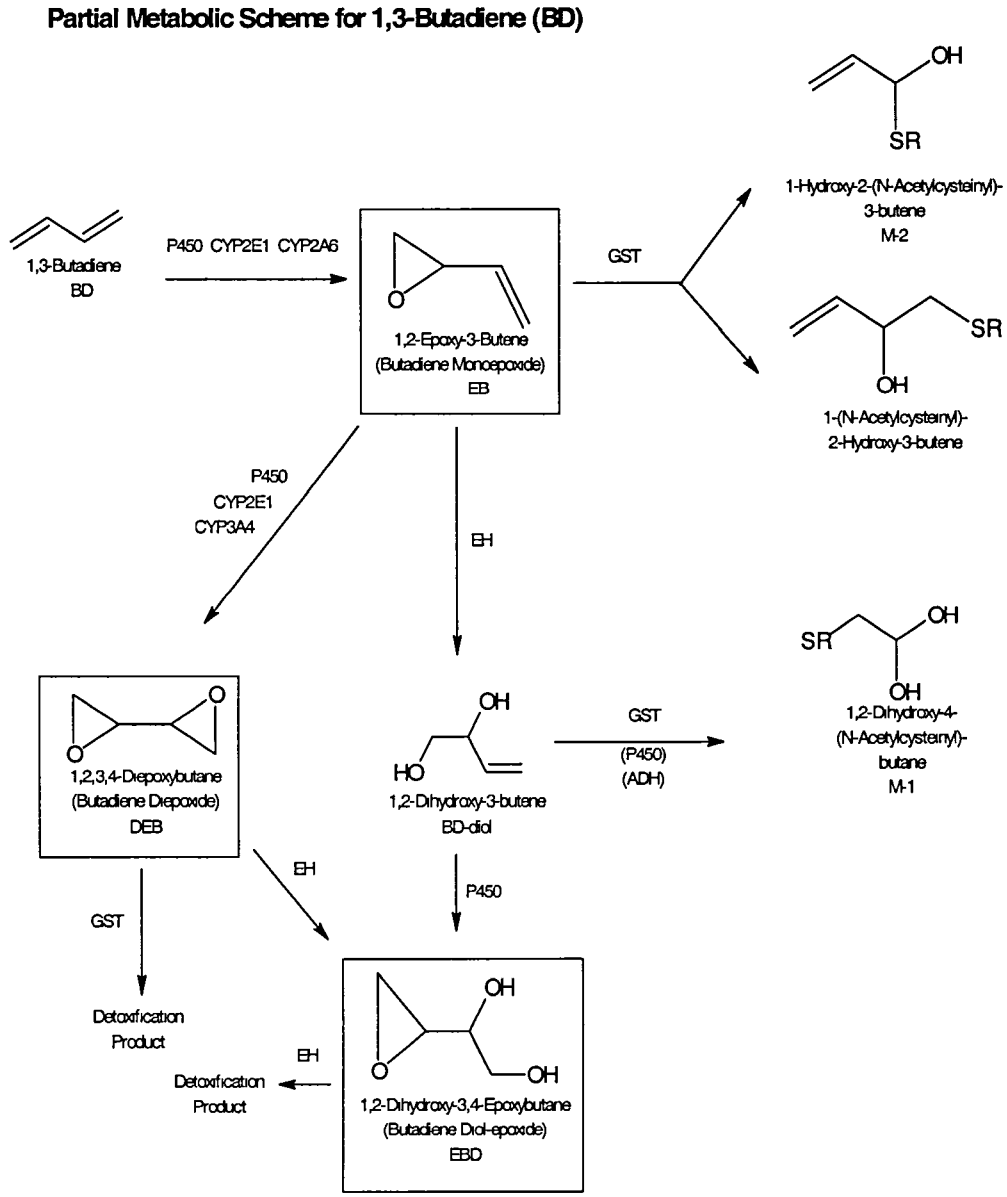

Figure 1. General scheme of the metabolism of 1,3-butadiene Abbreviations over arrows indicate enzymes that mediate the reactions. CYP2E1, CYP2P6, CYP3A4 are cytochrome P450-dependent monooxygenases. GST, EH and ADH are the detoxifying enzymes glutathione-S-transferase, epoxide hydrolase and alcohol dehydrogenase, respectively

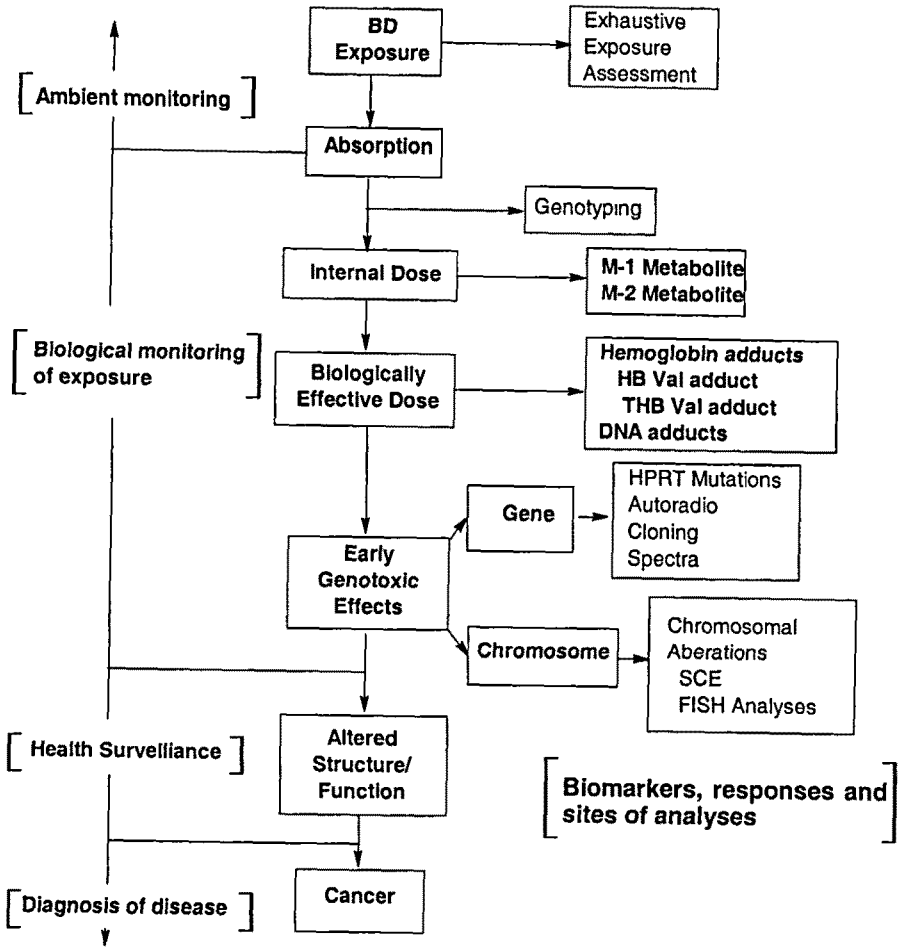

Figure 2. Biomarkers as surrogate measures of 1,3-butadiene exposure

Table 1. Changes of body weight in mice inhalation exposure with 1,3-butadiene(6 hr/day×5 days/week)

Mean±S.D, g

Exposure Post-days	Control	Inhalation exposure groups		F-value	p-value
		500ppm BD	1,000ppm BD		
0	26.35±1.4661	26.12±1.7135	25.55±2.1371	2.588	0.120
2	26.82±1.3058	25.82±1.6246	24.37±1.6193	2.253	0.108
4	27.03±2.1240	25.69±1.8317	24.54±2.1781*	3.317	0.058
7	27.61±1.7860	25 47±1.1870	24.81±2.2521*	4.720	0.019
9	27.83±1.7247	25.34±1.4811*	24.83±2.3838*	4.849	0.017
11	28.06±2.2483	25.29±1.2974*	24.26±2.3552*	7.112	0.009
14	28.15±3.2005	25.16±1.8215*	23.92±1.2988*	7.292	0.005
16	28.30±2.2913	25.24±1.6502*	24.36±1.4258*	7.136	0.003
18	28.66±2.6312	26.30±1.5379*	24.66±1.6906*	7.807	0.001

Table 2. The levels of N-(2-hydroxy-3-butenyl)valine(HBVal adduct) and N-(2,3,4-trihydroxybutyl)valine(THBVal adduct) extracted from hemoglobin of mice inhalation exposure with 1,3-butadiene(6 hr/day×5 days/week)

Unit : pmol/mg globin

	Inhalation	1st week	2nd week	3rd week
HBVal adduct	500ppm	1.8±0.6	3.7±0.3	6.2±0.4
	1,000ppm	5.7±0.9	7.4±1.5	16.0±1.5
THBVal adduct	500ppm	32.0±2.3	42.0±4.1	55.0±2.1
	1,000ppm	67.8±3.4	72.7±5.3	83.5±3.9

Table 3. The ratios of N-(2-hydroxy-3-butenyl)valine(HBVal adduct) and N-(2,3,4-trihydroxybutyl)valine(THBVal adduct) extracted from hemoglobin of mice inhalation exposure with 1,3-butadiene(6 hr/day×5 days/week)

Unit : pmol/mg globin

	Inhalation Exposure	1st week	2nd week	3rd week
Ratio of THBVal and HBVal adduct	500ppm	17.78	11.35	8.87
	1,000ppm	11.89	9.82	5.22