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Acute Effects of 2-Bromopropane and 1,2-Dibromopropane on Hepatotoxic and Immunotoxic Parameters in Female BALB/c Mice

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In the present studies, the acute toxic effects of 2-bromopropane (2-BP) and its analog, 1,2-dibromopropane (1,2-DBP), were investigated in female BALB/c mice. The mice were treated orally with either 2-BP at 2000 and 4000 mg/kg or 1,2-DBP at 300 and 600 mg/kg. Four days before necropsy, the mice were immunized intraperitoneally with sheep red blood cells (SRBCs). 1,2-DBP reduced the weights of the spleen and thymus weights and decreased the number of splenic cells. In addition, treatment with 1,2-DBP suppressed the antibody response to SRBCs. Meanwhile, only the antibody response was significantly suppressed by treatment with 2-BP. In the subsequent studies, the time course effects of 2-BP and 1,2-DBP on the hepatotoxic parameters were compared in female BALB/c mice. When mice were treated orally with either one of these chemicals for 6, 12, 24 and 48 h, the activities of serum alanine aminotransferase and aspartate aminotransferase elevated significantly only with 1,2-DBP 24 h after the treatment. The hepatic content of glutathione was reduced by 1,2-DBP. Meanwhile, these parameters were increased by 2-BP. The present results suggest that 1,2-DBP in the Solvent 5200 also contributes to the immnunotoxicity, although 2-BP is a major component.

Key words: 2-Bromopropane, 1,2-Dibromopropane, Hepatotoxicity, Antibody response, Glutathione

NTRODUCTION

Halogenated alkanes including 2-BP have been used industrially as chemical intermediates, extraction solvents, degreasing compounds, copolymer cross-linking agents, and pesticides. Several of the compounds have been reported to be mutagenic and carcinogenic, and to cause acute toxic effects in the kidney, testis, and/or liver (Lag *et al.,* 1991). Many countries regulate the production and use of chlorofluorocarbons, which destroy the ozone layer and cause global warming.

2-BP was developed and used as an alternative chemical to chlorofluorocarbons in the work place. 2-BP is a major component of the mixture of SPG-6AR and Solvent 5200, which is a substitute for chlorofluorocarbons in the electronic industry. Although 2-BP was used as a degreaser, occupational hazards due to 2-BP had been unknown. In 1995, a possible health problem caused by an occupational exposure to the 2-BP occurred in Korea, characterized by disorders in hematopoietic and reproductive functions in workers exposed to cleaning solvents containing 2-BP (Kim *et al.,* 1996; Park *et al.,* 1997). Ovarian dysfunction induced by 2-BP has been described in female factory workers and experimental animals (Lim *et al.,* 1997). In *Salmonella* Ames tests, 2-BP induced mutagenicity in TA100 and TA1535 strains (Maeng and Yu, 1997). The chromosomal aberration analysis did not reveal positive results in Chinese hamster lung cells *in vitro* or in the micronucleus test in the bone marrow of rats (Maeng and Yu, 1997). Since reduced reproductive function was found at high frequencies in female and male workers following the occupational exposure to 2-BP, the toxic effects of 2-BP on gonads drew attention in the field of industrial health, especially in Asia.

Since the first report on 2-BP intoxication, the reproductive toxicity of 2-BP has been studied using female mice (Sekiguchi and Honma, 1998), female rats (Kamijima *et*

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al., 1997; Lim *et al.,* 1997; Yu *et al.,* 1999b), and male rats (Ichihara *et al.,* 1997; Yu *et al.,* 1997; Omura *et al.,* 1999; Wu *et al.,* 1999). The findings obtained in these serial studies clearly indicated the reproductive toxicity of 2-BP in experimental animals. In 1998, the occupational exposure level for 2-BP was established at 1 ppm for the security and occupational health of workers (Yu *et al.,* 1999a). The Japanese Society for Occupation Health also decided recently to recommend 1 ppm as the occupational exposure limit for 2-BP to prevent adverse health effects on workers (Japanese Society for Occupational Health, 1999). Recently, a possible immunotoxic potential of 2-BP was reported, when male Sprague-Dawley rats were treated with 2-BP for 28 consecutive days (Jeong *et al.,* 2002). In addition, it was reported that 2-BP might form a DNA adduct on N^7 position of 2-deoxyguanosine at a physiological condition (Zhao *et al.,* 2002).

1,2-Dibromopropane (1,2-DBP) is an analog chemical of 2-BP. This compound has been used as an alternative to 2-BP after the toxicity of 2-BP was elucidated. Mutagenicity experiments with *Drosophila* and *Salmonella typhimurium* have shown 1,2-DBP to be weakly mutagenic (Volgel and Chandler, 1974; Stolzenberg and Hine, 1980).

In the present studies, the acute immunotoxic and hepatotoxic effects of 2-BP and an analog, 1,2-DBP, were investigated in female BALB/c mice. In addition, time course effects of 2-BP and 1,2-DBP on the hepatotoxic parameters were studied in mice.

MATERIALS AND METHODS

Animals

Specific pathogen-free female BALB/c mice (18 to 20 g) were obtained from Daehan Biolink (Eumsong, Korea). The animals were received at 5-6 weeks of age and were acclimated for at least 1 week. Upon arrival, the animals were randomized and housed five per cage. The animal quarters were strictly maintained at 23±3°C and 50±10% relative humidity. A 12 h light and dark cycle was used with an intensity of 150-300 Lux.

Materials

2-BP, 1,2-DBP, agar, Alserver's solution, DEAE-dextran, the kits for aspartate aminotrasferase (AST) and alanine aminotransferase (ALT) assay and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Earle's balanced salt solution (EBSS) and guinea pig complement were purchased from GIBCO (Grand Island, NY, USA). SRBCs were obtained from the College of Natural Resources at Yeungnam University. All other chemicals of reagent grade were commercially available.

Animal treatments

2-BP in corn oil was orally administered to the animals at 2000 and 4000 mg/kg in 10 mL corn oil once. 1,2-DBP in corn oil was orally administered to the animals at 300 and 600 mg/kg in 10 mL corn oil once. The animals in the vehicle control group received 10 mL/kg of corn oil.

For time course studies, 2-BP at 4000 mg/kg or 1,2-DBP at 600 mg/kg were treated orally. All animals were subjected to necropsy at 6, 12, 24 and 48 h after the treatment.

Hepatotoxicity parameters

For assaying the activities of AST and ALT, the serum was prepared by a centrifugation of the blood at 2,500×g for 10 min. The activities were determined according to an instruction manual prepared by the manufacturer.

Content of reduced glutathione (GSH)

Livers were removed and homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4. Aliquots of liver homogenates were stored at -80 °C until use. The content of the liver homogenate protein was determined according to the methods of Lowry *et al.* (1951) using bovine serum albumin as a standard. Liver GSH levels were determined by the Ellman's method (Ellman, 1959).

Antibody response to SRBCs

Mice were sensitized intraperitoneally 4 days prior to the assay with 5×10^8 SRBCs per mouse in 0.5 mL of EBSS. Single cell suspensions of splenocytes were prepared in 3 mL of EBSS, washed and resuspended in 3 mL of EBSS. Spleen cells were then diluted 30-fold by resuspending a 100 μL aliquot of each suspension in 2.9 mL of EBSS. The number of antibody-forming cells (AFCs) was determined using a modified Jerne plaque assay, as described previously (Kaminski *et al.,* 1990). Briefly, 0.05% DEAE-dextran was added into melted 0.5% agar in EBSS and maintained at 47 °C throughout the assay. Then 400 μL of melted agar was dispensed into 12×75 mm heated glass tubes (Corning), followed by the addition of 25 μL of guinea pig complement, 25 μL of SRBCs, and 50 μL of spleen cell suspension. The SRBCs were washed at least three times with EBSS before use. A 200 μL aliquot from the tube was then immediately pipetted onto a 100×15 mm Petri dish and the agar solution was covered with a 24×40 mm microscopic cover glass. The Petri dishes were placed at room temperature for several minutes to allow the agar to solidify and then, were incubated at 37 °C for 3 h to form hemolytic plaques in a humidified 37°C incubator. Following the incubation, the AFCs were counted. The cell number of each spleen was determined using a Coulter counter. The results were expressed as

AFCs/10^6 spleen cells or AFCs/spleen.

Statistics

The mean value±standard error (S.E.) was determined for each treatment group of a given experiment. Dunnett's *t*-test was used to compare statistical significance of data. The significant values at $P<0.05$ (*) or $P<0.01$ (**) were represented as asterisks.

RESULTS

Acute effects of 2-BP on the hepatotoxicity and immunotoxicity parameters

To investigate the effect of 2-BP on general toxicity, changes in body and organ weights were determined after treatment with 2-BP in corn oil orally (Table I). The body and organ weights did not change with 2-BP.

Fig. 1 shows the acute effect of 2-BP on AST and ALT activities. 2-BP did not affect the activities of AST and ALT.

In Fig. 2, the effects of 2000 and 4000 mg/kg of 2-BP on the antibody response to SRBCs were studied. 30 min after treatment of female BALB/c mice with 2-BP, the mice were immunized with SRBCs. The antibody response to SRBCs was enumerated 4 days later. Treatment with 2-

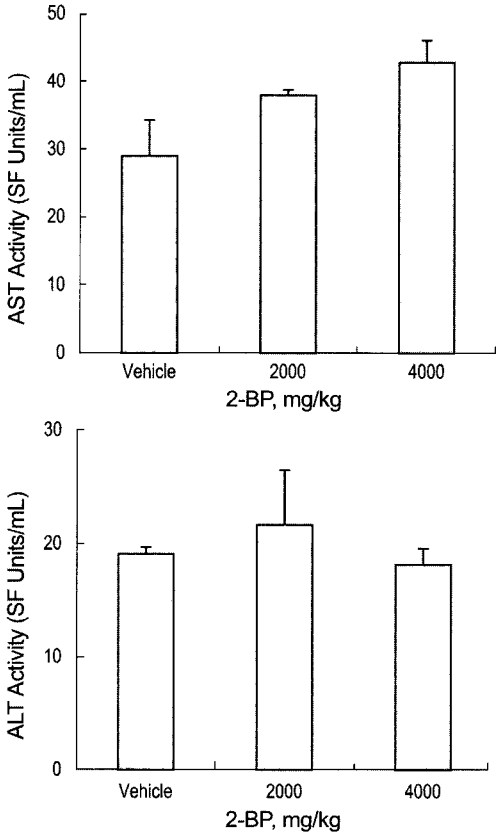

Fig. 1. Acute effects of 2-BP on serum AST and ALT activities in mice. Female BALB/c mice were treated orally with 2000 and 4000 mg/kg of 2-BP in corn oil once. The blood was collected 4 days after the treatment. Each bar represents the mean±S.E. of five animals.

Table I. Acute effects of 2-BP on body and organ weights in female BALB/c mice

2-BP, mg/kg	Body (g)	Liver (g)	Thymus (mg)	Spleen (mg)
Vehicle	19.7±0.7	1.037±0.032	68±6	135±15
2000	19.4±0.2	0.976±0.043	60±11	128±1
4000	18.9±0.4	0.898±0.032	58±6	123±6

Female BALB/c mice were treated orally with 2000 and 4000 mg/kg of 2-BP in corn oil once. All animals were subjected to necropsy 4 days after the treatment. Each value represents the mean±S.E. of five animals.

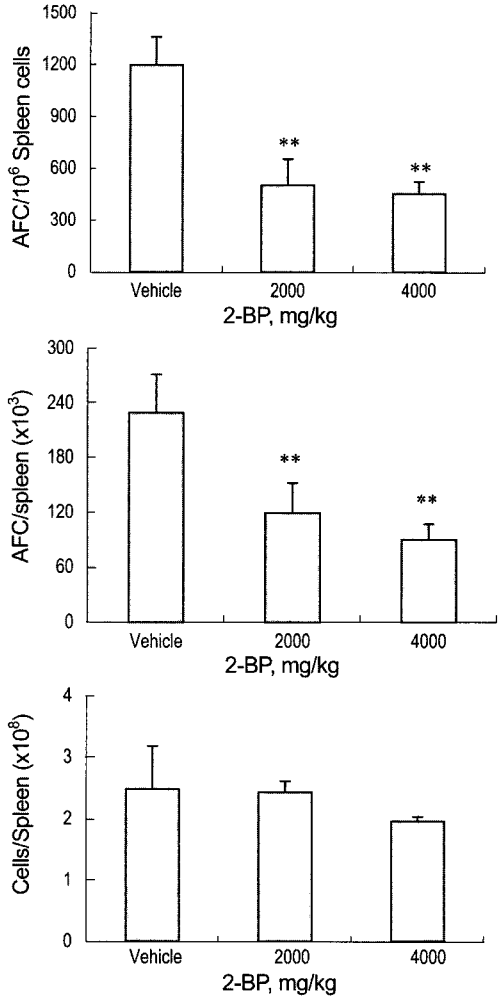

Fig. 2. Acute effects of 2-BP on antibody response to SRBCs. Female BALB/c mice were treated orally with 2000 and 4000 mg/kg of 2-BP once. Thirty minutes later, mice were immunized with SRBCs. Four days later, the number of antibody-forming cells (AFCs) was enumerated. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the vehicle control at $P<0.01$ (**).

BP significantly suppressed the antibody response to SRBCs.

Acute effects of 1,2-DBP on the hepatotoxicity and immunotoxicity parameters

Table II. Acute effects of 1,2-DBP on body and organ weights in female BALB/c mice

1,2-DBP, mg/kg	Body (g)	Liver (g)	Thymus (mg)	Spleen (mg)
Vehicle	19.7±0.7	1.037±0.032	68±6	135±15
300	17.9±0.9	0.860±0.042	41±7*	110±11
600	15.8±0.7*	0.903±0.033	26±4**	86±4*

Female BALB/c mice were treated orally with 300 and 600 mg/kg of 1,2-DBP in corn oil once. All animals were subjected to necropsy 4 days after the treatment. Each value represents the mean±S.E. of five animals. Values significantly different from the vehicle control are indicated with asterisks at either P<0.05 (*) or P<0.01 (**).

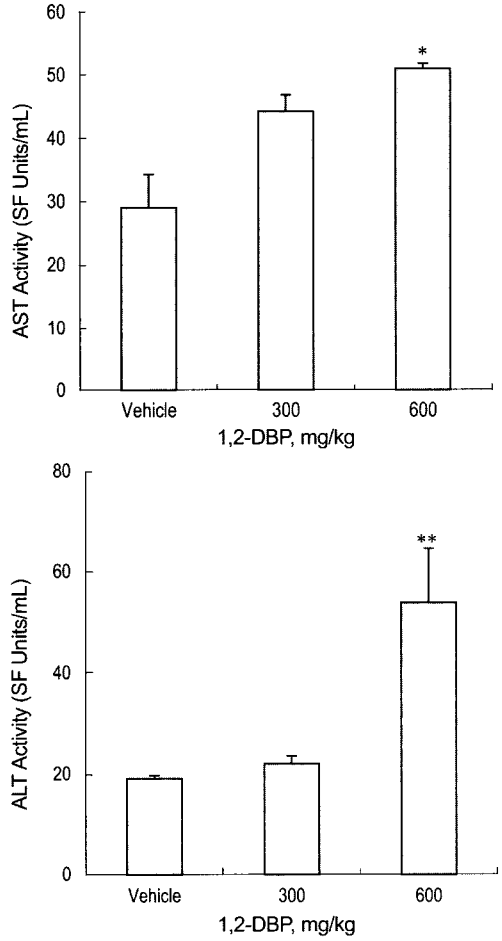

Fig. 3. Acute effects of 1,2-DBP on serum AST and ALT activities in female BALB/c mice. Female BALB/c mice were treated orally with 300 and 600 mg/kg of 1,2-DBP in corn oil once. The blood was collected 4 days after the treatment. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the vehicle control at either P<0.05 (*) or P<0.01 (**).

To study the effect of 1,2-DBP on general toxicity, changes in body and organ weights were determined after treatment with 1,2-DBP in corn oil orally (Table II). The body and organ weights decreased from the treatment with 1,2-DBP.

Fig. 3 shows the acute effects of 1,2-DBP on serum AST and ALT activities. 1,2-DBP elevated the activities of AST and ALT.

In Fig. 4, the effect of 300 and 600 mg/kg of 1,2-DBP on the antibody response to SRBCs was studied. 30 min

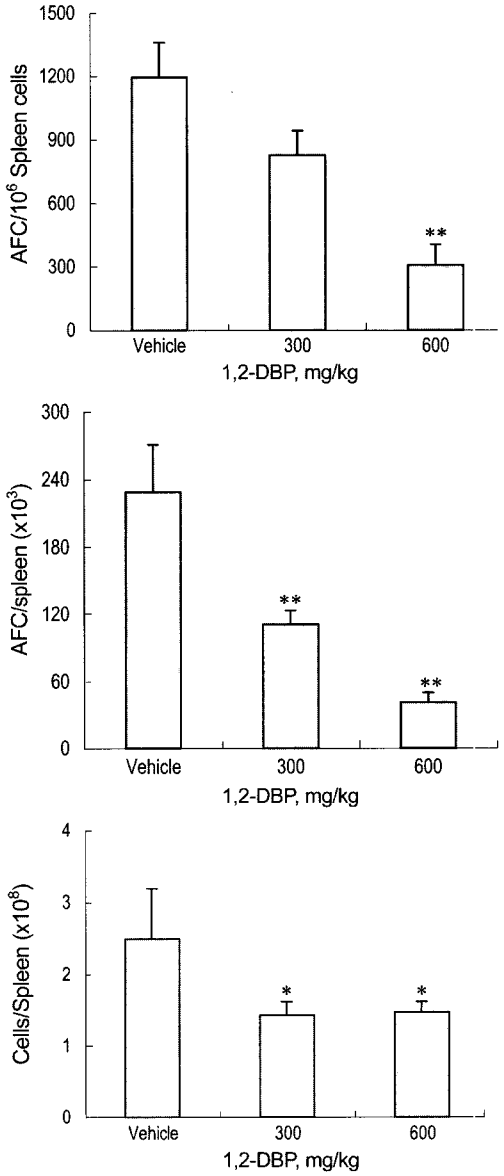

Fig. 4. Acute effects of 1,2-DBP on antibody response to SRBCs. Female BALB/c mice were treated orally with 300 and 600 mg/kg of 1,2-DBP once. Thirty minutes later, mice were immunized with SRBCs. Four days later, the number of antibody-forming cells (AFCs) was enumerated. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the vehicle control at either P<0.05 (*) or P<0.01 (**).

after treatment of female BALB/c mice with 1,2-DBP, the mice were immunized with SRBCs. The antibody response to SRBCs was enumerated 4 days later. Treatment of mice with 1,2-DBP significantly suppressed the antibody response to SRBCs, accompanied by a reduction in spleen cell number.

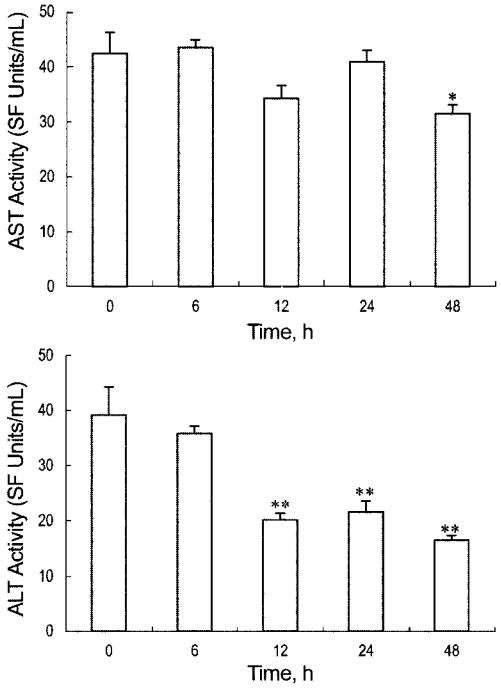

Fig. 5. Time course effects of 2-BP on serum AST and ALT activities in mice. Female BALB/c mice were treated orally with 4000 mg/kg of 2-BP in corn oil once. The blood was collected 6, 12, 24 and 48 h after the treatment. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the 0-h control at either $P<0.05$ (*) or $P<0.01$ (**).

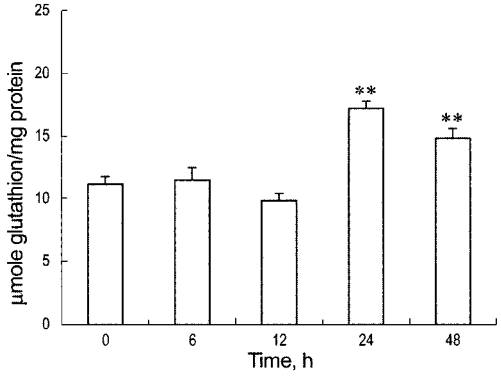

Fig. 6. Time course effects of 2-BP on glutathione contents in mice. Female BALB/c mice were treated orally with 4000 mg/kg of 2-BP in corn oil once. 6, 12, 24 and 48 h after treatment, animals were subjected to necropsy. The liver was removed to prepare the homogenate from each animal. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the 0-h control at either $P<0.05$ (*) or $P<0.01$ (**).

Time course effects of 2-BP and 1,2-DBP on serum enzymes and liver GSH contents

When mice were treated orally with 400 mg/kg of 2-BP once for 6, 12, 24 and 48 h, the activities of serum AST and ALT did not elevate (Fig. 5). In fact, the activities of these hepatotoxic parameters were significantly reduced. The hepatic GSH level increased 24 h after a single dose of 2-BP (Fig. 6). Meanwhile, the activities of serum AST and ALT increased significantly with 1,2-DBP 24 h after treatment (Fig. 7). The hepatic content of GSH was reduced by 1,2-DBP (Fig 8). Time course effects of 1,2-DBP on body and organ weights were more severe than those of 2-BP (Tables III and IV). These results indicated that 2-BP might not be hepatotoxic at the immunosuppressive doses, and that 1,2-DBP might be hepatotoxic at the immunosuppressive doses.

DISCUSSION

As mentioned before, reproductive toxicity of the alternatives for ozone-depleting solvents has become an important issue since the outbreak of reproductive and hematopoietic disorders in workers exposed to 2-BP in Korea. In the accident, 33 workers (i.e., 25 women and 8 men) were exposed to solvents containing 2-BP as a major

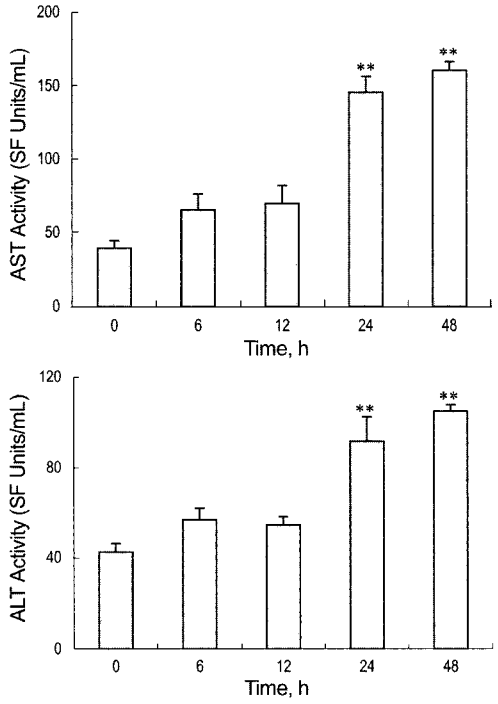

Fig. 7. Time course effects of 1,2-DBP on serum AST and ALT activities in mice. Female BALB/c mice were treated orally with 600 mg/kg of 1,2-DBP in corn oil once. The blood was collected 6, 12, 24 and 48 h after the treatment. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the 0-h control at $P<0.01$ (**).

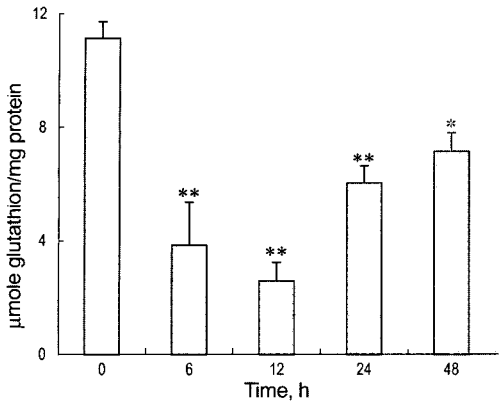

Fig. 8. Time course effects of 1,2-DBP on glutathione contents in mice. Female BALB/c mice were treated orally with 600 mg/kg of 1,2-DBP in corn oil once. 6, 12, 24 and 48 h after the treatment, the animals were subjected to necropsy. The liver was removed to prepare the homogenate from each animal. Each bar represents the mean±S.E. of five animals. The asterisks indicate values significantly different from the 0-h control at either P<0.05 (*) or P<0.01 (**).

Table III. Time course effects of 2-BP on body and organ weights in female BALB/c mice

Time, h	Body (g)	Liver (g)	Thymus (mg)	Spleen (mg)
0	19.4±0.3	1.115±0.014	75±13	99±5
6	19.3±0.2	1.166±0.032	76±4	90±5
12	20.2±0.3	1.096±0.033	81±9	110±10
24	19.2±0.2	1.145±0.022	84±2	86±5
48	19.0±0.3	1.022±0.022*	54±4*	87±4

Female BALB/c mice were treated orally with 4000 mg/kg of 2-BP in corn oil once the given time. All animals were subjected to necropsy at 6, 12, 24 and 48 h after the treatment. Each value represents the mean±S.E. of five animals. Values significantly different from the 0-hr control are indicated with an asterisk at P<0.05 (*).

Table IV. Time course effects of 1,2-DBP on body and organ weights in female BALB/c mice

Time, h	Body (g)	Liver (g)	Thymus (mg)	Spleen (mg)
0	19.4±0.3	1.115±0.014	75±13	99±5
6	19.1±0.1	1.119±0.061	73±7	88±4
12	19.2±0.1	0.959±0.053	61±2	80±1*
24	18.3±0.3	0.953±0.023	71±3	74±4**
48	16.7±0.5**	1.113±0.112	44±2*	77±5**

Female BALB/c mice were treated orally with 600 mg/kg of 1,2-DBP in corn oil once for the given time. All animals were subjected to necropsy at 6, 12, 24 and 48 h after the treatment. Each value represents the mean±S.E. of five animals. Values significantly different from the 0-h control are indicated with asterisks at either P<0.05 (*) or P<0.01 (**).

ingredient, resulting in amenorrhea in 16 females and azoospermia or oligospermia in 6 males. No exposure data were available, but the geometric mean concentration of 2-BP was estimated to be 12.4 ppm ranging from 9.2 to 19.6 ppm with a short-term level of 4360 ppm, based on a simulation study after the incident (Yu *et al.*, 1999b). Since the toxicity of 2-BP was unknown then, several efforts have been made to investigate whether such toxicities occur in experimental animals.

The reproductive and neuronal systems have been recognized as target organs for 2-BP. The effects of 2-BP on reproductive systems of males and females appear to be a major concern, as evidenced by the reproductive dysfunction of male and female workers in an electronic company in Korea (Kim *et al.*, 1996; Ichihara *et al.*, 1997; Kamijima *et al.*, 1997; Lim *et al.*, 1997; Yu *et al.*, 1997). In addition to reproductive toxicity, long-term exposure to 2-BP results in peripheral neurotoxicity in rats (Yu *et al.*, 1999b; 2001). Studies have also implicated a toxic potential of 2-BP on the immune system, since the number of white blood cells decreased significantly when rats were exposed to 2-BP (Yu *et al.*, 1997). In fact, from our recent studies on a possible immunotoxic potential of 2-BP, we found that a 28-day exposure of male Sprague-Dawley rats to 2-BP caused a significant suppression of antibody response to SRBCs (Jeong *et al.*, 2002). The immunophenotyping of splenic and thymic cells exposed to 2-BP indicated that 2-BP might have a nonspecific immunotoxic potential, because most subpopulations of cells in the spleen and thymus were nonspecifically reduced at a dose of 1000 mg/kg, with a significant drop in body weight.

Regarding the mechanism of immunotoxicity induced by 2-BP, studies on the DNA adduct formation were investigated in our laboratory in response to reports indicating that 2-BP might induce DNA damage. In literatures, 2-BP induced mutagenicity in TA100 and TA1535 strains of *Salmonella typhimurium* (Maeng and Yu, 1997). Although neither chromosomal aberration analysis in Chinese hamster lung cells nor the micronucleus test in the bone marrow of rats showed positive results, a recent report showed an induction of micronuclei formation in preimplantation mouse embryos after maternal treatment with 2-BP, accompanied by a decrease in embryo cell number (Ishikawa *et al.*, 2001). Most recently, our studies suggested that 2-BP might form a DNA adduct in N^7 position of 2'-deoxyguanosine at a physiological condition (Zhao *et al.*, 2002). In these studies, an incubation of 2-deoxyguanosine with 2-BP in phosphate-buffered saline at pH 7.4 and 37°C, produced a detectable amount of N^7-isopropyl guanine by an HPLC and UV analyses. We also found that N^7-isopropyl guanine adduct could not only be produced in an *in vitro* incubation of calf thymus DNA with 2-BP, but could also be detected in liver tissues isolated from 2-BP-treated mice *in vivo* (unpublished data, manuscript in preparation). Therefore, it is clear that 2-BP might be able to form DNA adduct(s). Although the possible role of adduct formation in 2-BP-

induced immunotoxicity remains to be examined further, this approach would be useful for understanding the molecular mechanism of the toxic effect induced by 2-BP. Likewise, a possible formation of DNA adduct by 1,2-DBP is under investigation in our laboratory.

From the present studies, both 2-BP and 1,2-DBP suppressed the antibody response to SRBCs with different potentials. 2-BP at the doses showing immunosuppression did not cause hepatotoxicity. However, 1,2-DBP caused the elevation of serum AST and ALT activities, as well as the depletion of hepatic GSH, at the doses for immunosuppression. These results indicated that 1,2-DBP might be able to interact with hepatic GSH. From these results, a possible formation of GSH conjugate with 1,2-DBP is currently under investigation. In addition, the results suggest that 1,2-DBP contained in the Solvent 5200 might contribute to the Solvent 5200-induced toxicity, although 2-BP is a major component.

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