Arch Pharm Res Vol 28, No 10, 1177-1182, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Hepatotoxic Effect of 1-Bromopropane and Its Conjugation with Glutathione in Male ICR Mice

Sang Kyu Lee, Sang Wook Jo, Tae Won Jeon, In Hye Jun, Chun Hua Jin, Ghee Hwan Kim, Dong Ju Lee, Tae-Oh Kim[1], Eung-Seok Lee, and Tae Cheon Jeong

College of Pharmacy, Yeungnam University, Gyeongsan, Korea and [1]School of Civil and Environmental Engineering, Kumoh National Institute of Technology, Gumi, Korea

(Received June 27, 2005)

The hepatotoxic effects of 1-bromopropane (1-BP) and its conjugation with glutathione were investigated in male ICR mice. A single dose (1000 mg/kg, po) of 1-BP in corn oil to mice significantly increased serum activities of alanine aminotransferase and aspartate aminotransferase. Glutathione (GSH) content was dose-dependently reduced in liver homogenates 12 h after 1-BP treatment. In addition, 1-BP treatment dose-dependently increased levels of *S*-propyl GSH conjugate at 12 h after treatment, as measured by liquid chromatography-electrospray ionization tandem mass spectrometry. The GSH conjugate was maximally increased in liver at 6 h after 1-BP treatment (1000 mg/kg), with a parallel depletion of hepatic GSH content. Finally, 1-BP induced the production of malondialdehyde in liver. The present results suggest that 1-BP might cause hepatotoxicity, including lipid peroxidation via the depletion of GSH, due to the formation of GSH conjugates in male ICR mice.

Key words: 1-Bromopropane, Hepatotoxicity, Glutathione conjugates, LC/ESI-MS

INTRODUCTION

1-Bromopropane (1-BP) is widely used as a substitute for chlorofluorocarbons, which destroy the ozone layer. It is used as a cleaning agent or adhesive solvent in workplaces because of its lower ozone-depleting potency, high volatility, and lack of flammability. In previous studies, 1-BP caused toxic effects on various body systems, such as the central nervous system and reproductive system (Ichihara *et al.*, 2000; Yu *et al.*, 1998). When male Wistar rats received the inhalation exposure to 200, 400, or 800 ppm of 1-BP for 12 weeks, γ-enolase, Hsp27, creatine, and total glutathione (GSH) contents were decreased in all brain regions studied (Wang *et al.*, 2003). Likewise neurospecific γ-enolase, GSH and nonprotein sulfhydryl groups in the cerebrum and cerebellum were decreased dose-dependently in male Wistar rats exposed to 200, 400 or 800 ppm of 1-BP for 7 days (Wang *et al.*, 2002).

Cytochrome P450 (CYP)-dependent oxidation and GSH-dependent conjugation are the primary metabolic routes of certain haloalkanes. For example, the metabolism of 1-BP has been studied in rats (Jones and Walsh, 1979). Two major routes of 1-BP metabolism are oxidation at C2 or C3 *via* the CYP-associated monooxygenase system, and the formation of GSH conjugates *via* GSH-*S*-alkyltransferase (B'Hymer and Cheever, 2004). 1,2-Dibromopropane (1,2-DBP) and 1,3-dibromopropane (1,3-DBP) could also form GSH conjugates (Lee *et al.*, 2005; Tornero-Velez *et al.*, 2003).

When male Wistar rats were exposed to 1-BP vapor, either for 6 h a day, 5 days a week, for 3 and 4 weeks at 1500 ppm or for 1 day, 4 or 12 weeks at 700 ppm, the activities of hepatic CYPs, serum alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST) were decreased significantly (Ishidao *et al.*, 2002). When mice were treated orally with 1,2-DBP, an analog of 1-BP, the activities of serum ALT and AST were elevated significantly 24 h after the treatment (Kim *et al.*, 2003). In addition, the hepatic content of GSH was reduced by haloalkanes (Kim *et al.*, 2003; Tornero-Velez *et al.*, 2004). All these reports support the possible involvement of GSH conjugation in haloalkane-induced toxicities.

In the present study, the dose-response and time-course effects of 1-BP on the hepatotoxicity parameters were studied in male ICR mice. In addition, GSH conjugate

Correspondence to: Tae Cheon Jeong, Associate Professor, College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Gyeongsan 712-749, Korea
Tel: 82-53-810-2819, Fax: 82-53-810-4654
E-mail: taecheon@yumail.ac.kr

levels were measured by LC/ESI-MS to determine the relationship between hepatotoxicity and GSH depletion.

MATERIALS AND METHODS

Materials

1-BP (purity, >99%) was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Standard *S*-propyl GSH, 5.5'-dithio-bis(2-nitrobenzoic acid), and 5-sulfosalicylic acid were purchased from Sigma Chemical Company (St. Louis, MO). The kits for ALT and AST assays were purchased from Asan Pharm., Co. (Hwasung, Korea). All other chemicals were of analytical grade.

Animals

Specific pathogen-free male ICR mice (28 to 33 g, 4 weeks old) were obtained from Orient (Seoul, Korea). The animals were acclimated for at least 2 weeks. Upon arrival, 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 at an intensity of 150-300 Lux. All animal procedures were based on guidelines recommended by the Society of Toxicology (U.S.A.) in 1989.

Animal treatments

1-BP in corn oil was treated once orally at 200, 500, and 1000 mg/kg, and animals were euthanized 12 h or 24 h later. For time course studies, 1-BP (1000 mg/kg) was treated orally and animals were subjected to necropsy at 6, 12, or 24 h after the treatment. Following the blood collection, the livers were removed and homogenized with 4 volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4. Aliquots of tissue homogenates and sera were stored at -80°C until use.

Hepatotoxicity parameters

For assaying ALT and AST activity, serum was prepared by centrifugation of the blood at 2,500 × g at 4°C for 15 min. The activities of enzymes were determined according to an instruction manual prepared by the manufacturer. Malondialdehyde levels were determined by the method of Ohkawa *et al.* (1979).

Content of reduced GSH

Liver GSH levels were determined by Ellman's method (Ellman, 1959). 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.

Sample analysis by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS)

The conjugates were extracted by adding 4 volumes of acetonitrile (ACN). After vortex mixing for 20 min and centrifugation to remove the proteins, the resulting supernatant was evaporated under a stream of nitrogen in a water bath maintained at 60°C. The resulting residue was reconstituted in a 50/50 mixture of 0.1% aqueous formic acid/ACN by vortexing, and subsequent centrifugation at 15,000×g at 15°C for 10 min. A 100 μL aliquot was used for the LC/ESI-MS analysis.

The HPLC consisted of a surveyor system (Thermo Finnigan, San Jose, CA, U.S.A.) with the LCQ advantage trap mass spectrometer (Thermo Finnigan, San Jose, CA, U.S.A.) equipped with an electrospray ionization source. The column used for the separation was an Xterra C18 (2.1×150 mm, 5 μm). The HPLC mobile phases consisted of 0.1% aqueous formic acid, pH 4.0 (A), and 50% ACN in 0.1% formic acid (B). A gradient program was used for the HPLC separation with a flow rate of 210 μL/min. The initial composition was 0% B and programmed linearly to 10% B after 13 min. Nitrogen was used both as the sheath gas at 1.05 L/min and as the auxiliary gas at 6 L/min, with a capillary temperature of 215°C and the spray voltage set to 4 kV. The mass spectrometer was operated in the positive ion mode in *m/z* range 100-400. Helium was used as the collision gas for tandem mass spectrometry, followed by the isolation of ions over a selected mass window of 1 Da.

Statistics

The mean ± standard error (SE) was determined for each treatment group. Dunnett's t-test was used to determine statistical significance. Significance is shown by asterisks, $P<0.05$ (*) or $P<0.01$ (**).

RESULTS

Acute effects of 1-BP on hepatotoxicity

To investigate the effect of 1-BP on general toxicity, changes in body and liver weights were determined after a single oral treatment with 1-BP in corn oil. Body and liver weights were not changed by 1-BP (Table I). Meanwhile,

Table I. Dose-dependent effects of 1-BP on body and liver weights and serum enzymes in male ICR mice

Dose, mg/kg	Body weight (g)	% of Liver	ALT (Karmen unit/mL)	AST (Karmen unit/mL)
Vehicle	35.6±1.7	5.61±0.21	46 ±10	58 ± 6
200	30.6±0.5*	4.87±0.21*	33 ± 6	70 ± 9
500	33.1±0.5	4.98±0.09	34 ± 2	76 ± 7
1000	32.9±0.4	5.64±0.24	1972 ± 1648**	653 ± 478**

Male ICR mice were treated once orally with 0, 200, 500, and 1000 mg/kg of 1-BP in corn oil. All animals were subjected to necropsy 24 h after the last treatment. Data represents the mean ± SE of five animals. $P < 0.05$ (*) or $P < 0.01$ (**) versus vehicle.

Table II. Time-dependent effects of 1-BP on body and liver weights and serum enzymes in male ICR mice

Time, h	Body weight (g)	% of Liver	ALT (Karmen unit/mL)	AST (Karmen unit/mL)
0	35.6 ± 1.7	5.61 ± 0.21	46 ± 10	58 ± 6
6	31.2 ± 0.4*	5.06 ± 0.08	38 ± 3	106 ± 12
12	29.2 ± 0.3**	5.40 ± 0.20	1031 ± 613	293 ± 120
24	32.9 ± 0.4	5.64 ± 0.24	1972 ± 1648**	653 ± 478**

Male ICR mice were treated once orally with 1000 mg/kg of 1-BP in corn oil. Animals were subjected to necropsy 0, 6, 12, and 24 h after the treatment. Data represents the mean ± SE of five animals. $P < 0.05$ (*) or $P < 0.01$ (**) versus 0-h group.

the activities of serum ALT and AST were increased by 43- and 11-fold compared with the vehicle control group, respectively, after a single treatment with 1000 mg/kg 1-BP.

Time course effects of 1-BP on serum enzymes

A single dose of 1-BP (1000 mg/kg) did not change liver weights at 6, 12, or 24 h, after treatment (Table II). Serum ALT and AST activity was increased at 12 and 24 h after 1-BP treatment.

Identification of 1-BP-GSH conjugates by LC/ESI-MS

The protonated molecular ion $[M+H]^+$ of *S*-propyl GSH was observed at *m/z* 350 in the positive ESI spectrum, with a retention time of 5.45 min (Fig. 1A). Losses of water were detected at *m/z* 332 (Fig. 1B). The product

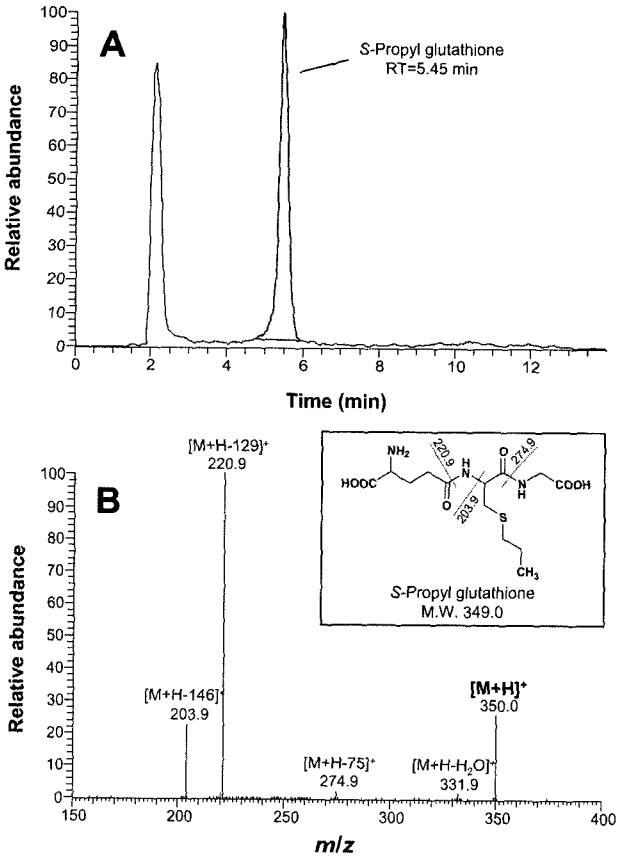

Fig. 1. A, Extracted ion chromatogram of *S*-propyl GSH, *m/z* 350; B, CID spectrum of protonated *S*-propyl GSH

ions at *m/z* 275 probably resulted from the elimination of the glycine moiety from the corresponding GSH conjugates

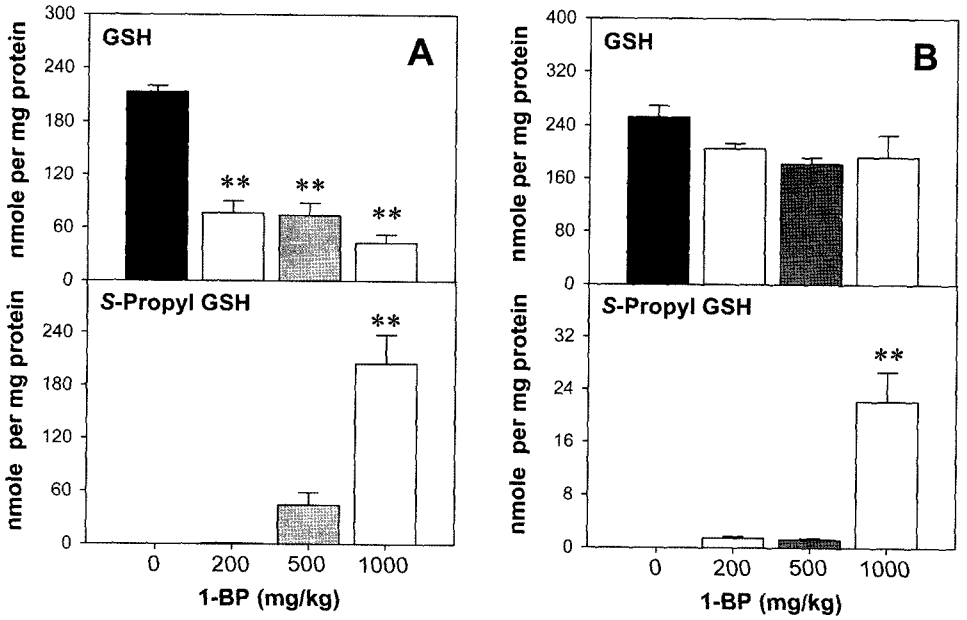

Fig. 2. Effects of 1-bromopropane (1-BP) on liver GSH content and formation of S-propyl GSH; Levels at 12 h (A) or 24 h (B) after 1-BP treatment; Each bar represents the mean ± SE of five animals. $P < 0.01$ (**) versus vehicle.

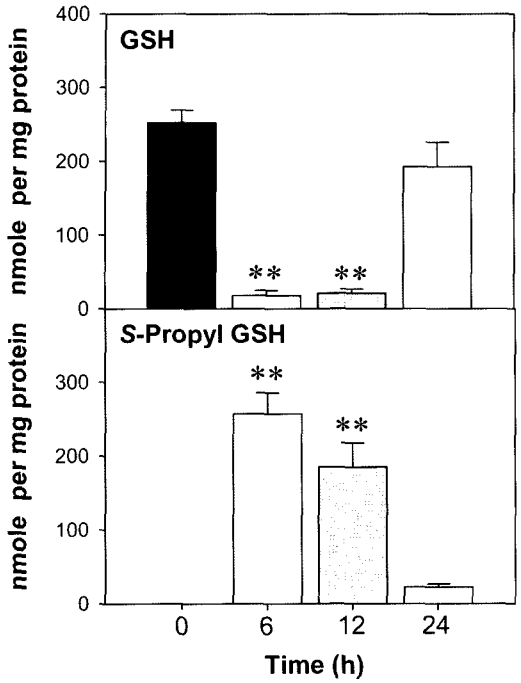

Fig. 3. Effects of 1-bromopropane (1-BP) on GSH content and formation of *S*-propyl GSH; Each bar represents the mean ± SE of five animals. P < 0.01 (**) versus 0 h.

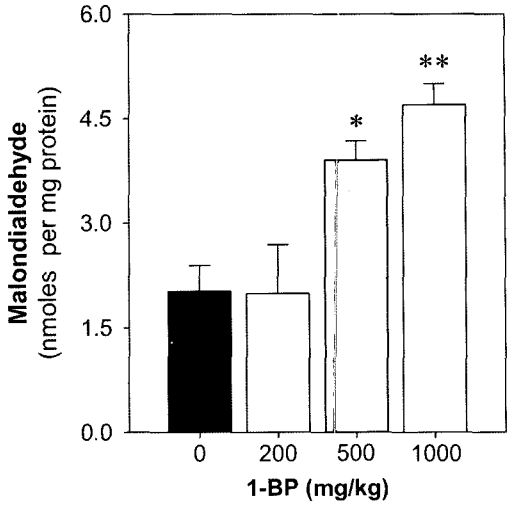

Fig. 4. Effects of 1-bromopropane (1-BP) on hepatic lipid peroxidation; Malondialdehyde levels were measured in liver homogenates12 h after 1-BP treatment. Each bar represents the mean ± SE of five animals. P < 0.05 (*) or P < 0.01 (**) versus vehicle control.

(Loughlin *et al.*, 2001). The fragment ions at *m/z* 221 were formed by the loss of the glutamate moiety (Prabhu *et al.*, 2002), and the ions at *m/z* 204 correspond to the loss of the amino-glutamyl moiety from GSH conjugates.

Dose- and time-dependent formation of the 1-BP-GSH conjugate

Following treatment of mice with 200, 500, and 1000 mg/kg of 1-BP once for 12 h or 24 h, the liver homogenates were analyzed for hepatic GSH contents and the presence of 1-BP-GSH conjugates (Fig. 2). The hepatic content of GSH was dose-dependently reduced 12 h after 1000 mg/kg treatment, but mostly recovered at 24 h. In parallel, *S*-propyl GSH levels were increased 12 h after treatment, but returned to normal by 24 h.

S-propyl GSH peaked 6 h after the treatment (Fig. 3), which was consistent with decreases in hepatic GSH levels at 6 and 12 h after treatment. GSH levels recovered 24 h after treatment (Fig. 3).

Malondialdehyde measurement provides a marker of hepatic lipid peroxidation. 1-BP caused a dose-dependent elevation of malondialdehyde (Fig. 4), suggesting that 1-BP-induced hepatotoxicity might be related to lipid peroxidation.

DISCUSSION

The neurotoxic effects of 1-BP, a compound widely used as an alternative to ozone-depleting solvents, have been described (Yu *et al.*, 1998; Ichihara *et al.*, 2000), but hepatotoxicity has not been studied. In our previous study, the hepatotoxic potential of 1,2-DBP, an analog of 1-BP, was investigated in mice. Oral 1,2-DBP (600 mg/kg) increased serum ALT and AST activity and decreased GSH content 24 h after treatment (Kim *et al.*, 2003). 1-BP had similar effects (Table I and II).

GSH is a ubiquitous tripeptide in mammalian systems, where its intracellular concentration can be as high as 10 mM (Kosower and Kosower, 1978). GSH plays an important role in the detoxification of electrophilic foreign compounds and chemically reactive intermediates which may arise during the biotransformation of xenobiotics (Dalton *et al.*, 2004). GSH depletion followed by exposure to a toxicant that exceeds the detoxification ability of GSH would lead to increased toxicity, as in case of acetaminophen-induced toxicity (Moldéus, 1978; Kyle *et al.*, 1990).

The metabolism of 1-BP has been studied (Jones and Walsh, 1979). GSH conjugate formation was shown to be important among many metabolic routes that can lead to the formation of mercapturic acids and DNA adducts (Zhao *et al.*, 2002; Lee *et al.*, 2003; B'Hymer and Cheever, 2004). Moreover, 1-BP incubation with calf thymus DNA under physiological conditions for 16 h produced N^7-guanine adducts, suggesting that the formation of DNA adducts might be responsible for 1-BP-induced toxicity (Lee *et al.*, 2003). However, we are the first to use LC/ESI-MS to directly show the formation of GSH conjugates of 1-BP in mouse liver (Fig. 1).

The hepatic content of GSH was greatly decreased 12 h after a single treatment with 1-BP, and GSH conjugate was formed dose-dependently (Fig. 2). GSH content was

reduced at 6 and 12 h, and the GSH conjugate peaked 6 h after the treatment (Fig. 3), indicating that the reduced GSH levels recovered within 24 h of 1-BP treatment.

In some cases, conjugation with GSH enhances the toxicity of xenobiotics (Kim *et al.*, 1990; Kim and Guengerich, 1990; Humphreys *et al.*, 1991). 1-BP inhalation increased the production of lipid peroxide (Kim *et al.*, 1999), suggesting that GSH conjugation could potentially enhance its hepatotoxicity. In addition, 1-BP dose-dependently increased malondialdehyde production (Fig. 4). In conclusion, 1-BP causes hepatotoxicity, including lipid peroxidation, *via* the conjugation with GSH and subsequent depletion of GSH levels.

ACKNOWLEDGEMENT

This study was supported by a grant from Yeungnam University.

REFERENCES

B'Hymer, C. and Cheever, K. L., Development of a gas chromatographic test for the quantification of the biomarker 3-bromopropionic acid in human urine. *J. Chromatogr., B*, 802, 361-366 (2004).

Dalton, T. P., Chen, Y., Schneider, S. N., Nebert, D. W., and Shertzer, H. G., Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radic. Biol. Med.*, 37, 1511-1526 (2004).

Ellman, G. L., Tissue sulfhydryl group. *Arch. Biochem. Biophys.*, 82, 70-77 (1959).

Humphreys, W. G., Kim, D. H., and Guengerich, F. P., Isolation and characterization of N^7-guanyl adducts derived from 1,2-dibromo-3-chloropropane. *Chem. Res. Toxicol.*, 4, 445-453 (1991).

Ichihara, G., Kitoh, J., Yu, X., Asaeda, N., Iwai, H., and Kumazawa, T., 1-Bromopropane, an alternative to ozone layer-depleting solvents, is dose-dependently neurotoxic to rats in long-term inhalation exposure. *Toxicol. Sci.*, 55, 116-123 (2000).

Ishidao, T., Kunugita, N., Fueta, Y., Arashidani, K., and Hori, H., Effects of inhaled 1-bromopropane vapor on rat metabolism. *Toxicol. Lett.*, 134, 237-243 (2002).

Jones, A. R. and Walsh, D. A., The oxidative metabolism of 1-bromopropane in the rat. *Xenobiotica*, 9, 763-772 (1979).

Kim, D. H. and Guengerich, F. P., Formation of the DNA adduct *S*-[2-(N^7-guanyl)ethyl]glutathione from ethylene dibromide: effects of modulation of glutathione and glutathione *S*-transferase levels and lack of a role for sulfation. *Carcinogenesis*, 11, 419-424 (1990).

Kim, D. H., Humphreys, W. G., and Guengerich, F. P., Characterization of *S*-[2-(N^1-adenyl)ethyl]glutathione as an adduct formed in RNA and DNA from 1,2-dibromoethane. *Chem. Res. Toxicol.*, 3, 587-594 (1990).

Kim, N. H., Hyun, S. H., Jin, C. H., Lee, S. K., Lee, D. W., Jeon, T. W., Choi, C. B., Lee, E. S., Chae, W., and Jeong, T. C., Acute effects of 2-bromopropane and 1,2-dibromopropane on hepatotoxic and immunotoxic parameters in female BALB/c mice. *Arch. Pharm. Res.*, 26, 943-950 (2003).

Kim, K. W., Kim, H. Y., Park, S. S., Jeong, H. S., Park, S. H., Lee, J. Y., Jeong, J. H., and Moon, Y. H., Gender differences in activity and induction of hepatic microsomal cytochrome P-450 by 1-bromopropane in Sprague-Dawley rats. *J. Biochem. Mol. Biol.*, 32, 232-238 (1999).

Kosower, N. S. and Kosower, E. M., The glutathione status of cells. *Int. Rev. Cytol.* 54, 109-160 (1978).

Kyle, M. E., Sakaida, I., Serroni, A., and Farber, J. L., Metabolism of acetaminophen by cultured rat hapatocytes. *Biochem. Pharmacol.*, 40, 1211-1218 (1990).

Lee, E. S., Moon, Y. S., Zhao, L. X., Kim, E., Lim, H. T., Basnet, A., Jeong, T. C., and Chae, W., Synthesis, characterization, *in vitro* and calf thymus DNA identification of N^7-guanine adducts of 1- and 2-bromopropane. *Toxicol, Sci.*, 72(S-1), 996 (2003).

Lee, S. K., Jin, C. H., Hyun, S. H., Lee, D. W., Kim, G. H., Jeon, T. W., Lee, J., Kim, D. H., Jeong, H., G., Lee, E. S., and Jeong, T. C., Identification of glutathione conjugates of 1,2-dibromopropane in female BALB/c mice by liquid chromatography-electrospray ionization tandem mass spectrometry. *Xenobiotica*, 35, 97-105 (2005).

Loughlin, A. F., Skiles, G. L., Alberts, D. W., and Schaefer, W. H., An ion exchange liquid chromatography/mass spectrometry method for the determination of reduced and oxidized glutathione and glutathione conjugates in hepatocytes. *J. Pharmaceut. Biomed.*, 26, 131-142 (2001).

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Moldéus, P., Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. *Biochem. Pharmacol.*, 27, 2859-2863 (1978).

Ohkawa, H., Ohishi, N., and Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95, 351-358 (1979).

Prabhu, S., Fackett, A., Lloyd, S. McClellan, H. A., Terrell, C. M., Silber, P. M., and Li, A. P., Identification of glutathione conjugates of troglitazone in human hepatocytes. *Chem. Res. Toxicol.*, 142, 83-97 (2002).

Tornero-Velez, R., Ross, M. K., Granville, C., Laskey, J., Jones, J. P., DeMarini, D. M., and Evans, M. V., Metabolism and mutagenicity of source water contaminants 1,3-dichloropropane and 2,2-dichloropropane. *Drug Metab. Dispos.*, 32, 123-131 (2004).

Wang, H., Ichihara, G., Ito, H., Kato, K., Kitoh, J., Yamada, T., Yu, X., Tsuboi, S., Moriyama, Y., Sakatani, R., Shibata, E., Kamijima, M., Itohara, S., and Takeuchi, Y., Biochemical

changes in the central nervous system of rats exposed to 1-bromopropane for seven days. *Toxicol. Sci.*, 67, 114-120 (2002).

Wang, H., Ichihara, G., Ito, H., Kato, K., Kitoh, J., Yamada, T., Yu, X., Tsuboi, S., Moriyama, Y., and Takeuchi, Y., Dose-dependent biochemical changes in rat central nervous system after 12-week exposure to 1-bromopropane. *Neurotoxicology*, 24, 199-206 (2003).

Yu, X., Ichihara, G., Kitoh, J., Xie, Z., Shibata, E., and Kamijima, M., Preliminary report on the neurotoxicity of 1-bromopropane, an alternative solvent for chlorofluorocarbons. *J. Occup. Health*, 40, 234-235 (1998).

Zhao, L. X., Kim, E., Lim, H. T., Moon, Y. S., Kim, N. H., Kim, T. H., Choi, H. S., Chae, W., Jeong, T. C., and Lee, E. S., Synthesis, characterization and *in vitro* identification of N^7-guanine adduct of 2-bromopropane. *Arch. Pharm. Res.*, 25, 39-44 (2002).