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Gender Differences in Activity and Induction of Hepatic Microsomal Cytochrome P-450 by 1-Bromopropane in Sprague-Dawley Rats

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Sex differences in the induction of microsomal cytochrome P-450 (CYP) and the activities of several related enzymes of Sprague-Dawley rats treated with 1-bromopropane (1-BrP) were investigated. Male and female rats were exposed to 50, 300, and 1800 ppm of 1-BrP per kg body weight (6 h a day, 5 days a week, 8 weeks) by inhalation. The mean body weight of 1-BrP treated groups increased according to the day elapsed, but four and five weeks respectively after the start of the exposure, the mean body weight of male and female rats had significantly reduced in the group treated with 1800 ppm 1-BrP compared with the control group ($p<0.01$). While the relative weights of liver increased in both sexes, statistical significance in both sexes was found only in the group receiving 1800 ppm/kg of 1-BrP ($p<0.01$). The total contents of CYP, b_5, NADPH-P-450 reductase, NADH b_5 reductase, ethoxyresorufin-O-deethylase (EROD), pentoxyresorufin-O-dealkylase (PROD), and *p*-nitrophenol hydroxylase (*p*NPH) activities were examined for the possible effects of 1-BrP. No significant changes in the CYP and b_5 contents, NADPH-P-450 reuctase, NADH b5 reductase, ethoxyresorufin-O-deethylase (EROD), and pentoxyresorufin-O-dealkylase (PROD) were observed between the control and treated groups. The activity of *p*NPH increased steadily with the increase in the concentration of 1-BrP in both sexes, but was significantly increased only in the 1800 ppm-treated group of male rats ($p<0.05$). When Western blottings were carried out with three monoclonal antibodies (MAb 1-7-1, MAb 2-66-3, and MAb 1-98-1) which were specific against CYP1A1/2, CYP2B1/2, and CYP2E1, respectively, a strong signal corresponding to CYP2E1 was observed in microsomes obtained from rats treated with 1-BrP. Glutathione S-transferase (GST) activity and the content of lipid peroxide significantly increased in the treated groups compared with the control group ($p<0.05$). These results suggest that 1-BrP can primarily induce CYP2E1 as the major form and that GST phase II enzymes play important roles in 1-BrP metabolism, showing sex-dependence in the metabolic mechanism of 1-BrP in the rat liver.

Keywords: 1-Bromopropane, Cytochrome P-4502E1, Cytochrome P-450 dependent monooxygenase, Glutathione S-transferase.

Introduction

1-Bromopropane (1-BrP; CAS No. 106-94-5) is the International Union of Pure and Applied Chemistry (IUPAC) name for propyl bromide and *n*-propyl bromide. It is a clear, colorless liquid, volatile (vapour pressure of 110.8 Torr at 20°C) and flammable at room temperature. 1-BrP can be produced from propanol by dehydration of propanol with bromine (Br_2) or hydrogen bromide (HBr) under the sulfur catalyst. 1-BrP is completely soluble in organic solvents such as ethanol, ether, and benzene, and has been widely used in the production of drugs, pesticides, and other chemicals (Tachizawa *et al.*, 1982). Accordingly, 1-BrP has been studied for its utility and effect on animals by many investigators (Barnsley, 1966; Jones and Walsh, 1979; Gschwend *et al.*, 1985). Many of the organohalide compounds (chloride, bromide, and iodide) are carcinogenic, and they are metabolized by the microsomal cytochrome P-450 (CYP) system (Van Dyke, 1977; Stevens and Anders, 1979; Guengerich *et al.*, 1980). In *in vitro* studies, Tachizawa *et al.* (1982) detected propene, 1,2-epoxypropane, 1,2-propanediol, propionic acid, and undefined species bound to protein when rat liver

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homogenates were incubated with organohalides (1-chloropropane, 1-bromopropane, and 1-iodopropane) in the presence of an NADPH-generating system. Jones and Walsh (1979) isolated the three metabolites, and confirmed them mercapturic acids as N-acetyl-S-propyl cystein, N-acetyl-S-propyl cysteine-S-oxide, and N-acetyl-S-(2-hydroxypropyl) cysteine in the urine of rats treated with 1-BrP. However, the inducibility and effect of 1-BrP on specific CYP are not known. In the present study, we examined the effects of 1-BrP on the induction of CYP isozymes and other related enzymes.

Materials and Methods

Materials Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), cumene hydroperoxide, cytochrome C, EDTA, ethoxyresorufin, glucose-6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PD), GSH, glutathione reductase, H_2O_2, NADH, NADPH, Na_2CO_3, NaOH, *p*-nitrophenol, 7-pentoxyresorufin, sucrose, and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St Louis, USA). Western immunoblotting kits employing enhanced chemiluminescence detection were purchased from TROPIX Inc. (Bedford, USA). All other chemicals were obtained commercially and were of analytical grade.

Animals and preparation of liver microsomes Male and female Sprague-Dawley rats (7 weeks old) were obtained from the animal breeding laboratory of the Korea Institute of Science & Technology (KIST, Taejon, Korea), and were kept in cages for 1 week prior to treatment. Animals were kept under controlled conditions with respect to temperature (23 ± 2°C), humidity (45–50%), and light (12 h light/dark cycle). They had free access to food (Mouse Pellets, Jeil Lab Chow, Taejon, Korea) and water. Groups of male and female rats were exposed to 50, 300, and 1800 ppm of 1-BrP per kg body weight (6 h a day, 5 days a week, 8 weeks) by inhalation and an electric fan mounted on the chamber ceiling recirculated the air thus providing homogenous exposure to solvent. The atmospheric concentration was monitored by gas chromatography (GCS-14PFFS, Shimadzu, Tokyo, Japan) every 15 min during exposure. Rats were anaesthetized with dry-ice at 10 a.m. and the livers were quickly removed, placed in ice-cold 0.9% NaCl, homogenized in 0.25 M sucrose, and subjected to centrifugation at 12,000 × *g* for 40 min. Microsomal fractions were obtained from the 12,000 × *g* supernatant by centrifugation for 60 min at 105,000 × *g* and resuspended in 0.25 M sucrose (Park and Kim, 1984).

Enzyme assays Protein concentration was determined by the Lowry procedure using BSA as a standard (Lowry *et al.*, 1951). The CYP content in the liver microsomal fraction was measured from the carbon monooxide-reduced difference spectrum according to Omura and Sato (1964), and the b_5 content was determined by the method of Werringloar and Estabrook (1975). NADPH P-450 reductase activity was determined by the method of Masters *et al.* (1967). The reaction mixture (3 ml) consisted of 0.3 nM potassium phosphate buffer (pH 7.7), 40 nM cytochrome C, and 250 μg of diluted microsomes, and was placed in a cuvette. The reaction was initiated by the addition of 100 μl of 0.1 nM NADPH and the rate of cytochrome C reduction was determined spectrophotometrically at 550 nm using an extinction coefficient of 21 $cm^{-1}mM^{-1}$. NADH b_5 reductase activity was determined by the method of Hultquist (1978). Ethoxyresorufin-O-deethylase (EROD) activity was determined by the method of Burke and Meyer (1975) with slight modification. The reaction mixture (2 ml) consisted of 0.1 M potassium phosphate buffer (pH 7.5) containing 2 mg/ml BSA, 10 μM dicumarol, 5 mM G-6-P, 5 units/ml G-6-PD, 5 μM NADPH, and 2.5 μM 7-ethoxyresorufin. The reaction was started by the addition of substrate into the reaction tube and was performed at 37°C for 20 min. The formation of resorufin was monitored fluorometrically at an excitation wavelength of 550 nm and an emission wavelength of 585 nm.

The results are presented as pM resorufin generated/min per mg protein. The activity of pentoxyresorufin-O-dealkylase (PROD) was determined by the method of Lubet *et al.* (1985). All reaction components and assay conditions were the same as for the EROD assay except for the substrate, 2.0 μM of pentoxyresorufin. The results are also presented as pM resorufin formed/min per mg protein. The activity of *p*-nitrophenol hydroxylase (*p*NPH) was measured by spectrophotometric determination of 4-nitrocatechol formed from the hydroxylation of *p*-nitrophenol (Koop, 1986). The content of lipid peroxide (LPO) was spectrophotometrically measured as described by Yagi (1987). Glutathione S-transferase (GST) activity was determined by the method of Habig *et al.* (1974), described briefly as follows: The reaction mixture contained 0.1 M potassium phosphate buffer (pH 6.5), 10 mM GSH, and 50 μg of microsomal proteins in a final volume of 1.0 ml. The reaction was started by addition of 20 mM CDNB. GST activity was determined spectro-photometrically at 340 nm using an extinction coefficient of 9.6 $cm^{-1}mM^{-1}$. Glutathione peroxidase (GSHPx) activity was spectrophotometrically measured as described by Tappel (1978)

Western immunoblot analysis Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 8.5%) was done by the method of Laemmli (1970) using a Bio-Rad Protein II Cell apparatus. Immunoblotting of CYP isozymes was carried out as described previously (Kim *et al.*, 1997) using monoclonal anti-mouse CYP1A1/2 (MAb 1-7-1), CYP2B1/2 (MAb 2-66-3), and CYP2E1 (MAb 1-98-1) antibodies. Liver microsomal proteins were separated on 8.5% SDS-PAGE and electrophoretically transferred to nitrocellulose membrane. The membranes were incubated with the primary antibodies (ascite, 500 μg/ml) overnight, followed by the incubation with biotinylated goat anti-mouse IgG (TROPIX) at a 1:5000 dilution for 1 h after washing. The membranes were incubated in the chemiluminescence substrate solution containing 1:20 Nitro-Block for 5 min and specific protein bands were visualized by exposing the membrane indirectly through a plastic cover to standard x-ray film.

Results

Body weight increased considerably as expected in the control rat, reaching 400 g in the male and 260 g in the female rats. The body weights of the 1-BrP treated groups were also increased as days elapsed. However, four and five weeks after the start of the exposure, body weights of

male and female rats in the group treated with 1800 ppm doses were significantly reduced compared with the control group ($p<0.01$) (Fig. 1.). The relative weights of liver had increased in both sexes, but the statistical significance in both sexes was found only in the group treated with 1800 ppm/kg of 1-BrP ($p<0.01$) (Table 1). However, there was no significant variation in food consumption (Fig. 2.). The effects of increasing doses of 1-BrP on the total microsomal protein, CYP, and b_5 contents are presented in Table 2. By repeated inhalation, the microsomal protein contents in male and female rats were significantly increased in the 300 and 1800 ppm-treated groups ($p<0.05$). No significant change in CYP and b_5 contents was observed between the control and 1-BrP treated groups. NADPH-P-450 reductase activity was not affected by 1-BrP in both sexes, but NADH b_5 reductase increased with the increase in 1-BrP inhalation exposure (Table 3). The effects of 1-BrP on the catalytic activities of EROD, PROD, and *p*NPH are shown in Table 4. Catalytic

Table 1. Average body and liver weight in the male and female Sprague-Dawley rats inhaling 1-bromopropane after 8 weeks exposure to 1-bromopropane.

Treatment (ppm/kg)	Sex	Body weight (g)	Liver weight (g)	Liver weight (% of total body weight)
Control	M	405.2 ± 37.2	10.4 ± 1.2	2.57 ± 0.11
	F	259.6 ± 9.74	6.2 ± 0.4	2.39 ± 0.05
50	M	419.3 ± 22.1	11.1 ± 1.4	2.64 ± 0.20
	F	254.0 ± 8.56	6.0 ± 0.3	2.37 ± 0.07
300	M	397.8 ± 25.6	11.0 ± 1.4	2.74 ± 0.19
	F	254.2 ± 4.76	6.3 ± 0.5	2.44 ± 0.15
1800	M	372.2 ± 23.8*	11.2 ± 1.0	3.01 ± 0.13*
	F	243.4 ± 12.2*	7.5 ± 0.5	3.06 ± 0.12*

Values represent the mean ± SD of 10 rats.
* $p<0.01$

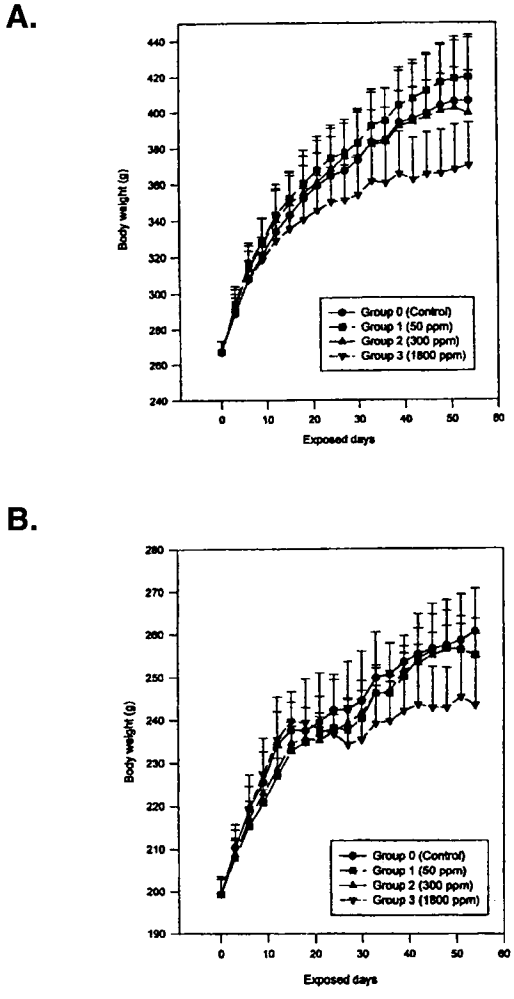

Fig. 1. Changes of body weight in male and female Spague-Dawley rats exposed to 1-bromopropane during the experiment. A. Male; B. Female.

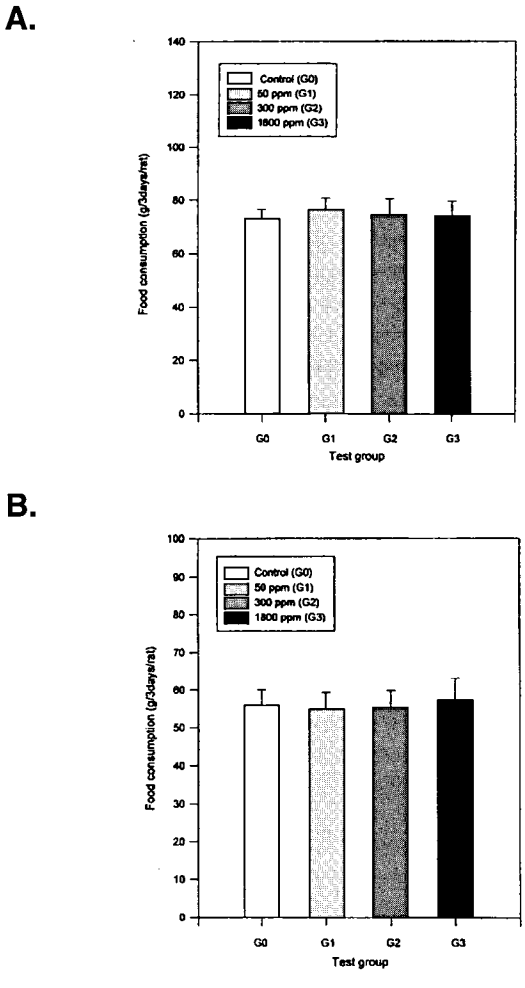

Fig. 2. Changes of total food consumption of male and female Spague-Dawley rats exposed to 1-bromopropane during the experiment. A. Male; B. Female.

Table 2. The contents of hepatic proteins in male and female Sprague-Dawley rats after 8 weeks exposure to 1-bromopropane.

Treatment (ppm/kg)	Sex	Protein (mg/ml)	CYP (nmol/mg protein)	b_5 (nmol/mg protein)
Control	M	37.64 ± 0.56	0.42 ± 0.04	0.26 ± 0.03
	F	32.78 ± 1.39	0.26 ± 0.01	0.22 ± 0.07
50	M	36.39 ± 0.61	0.39 ± 0.01	0.28 ± 0.01
	F	32.74 ± 1.56	0.24 ± 0.04	0.22 ± 0.02
300	M	42.54 ± 1.77*	0.40 ± 0.07	0.28 ± 0.02
	F	38.68 ± 0.73*	0.26 ± 0.05	0.23 ± 0.01
1800	M	42.93 ± 1.83*	0.35 ± 0.06	0.29 ± 0.02
	F	38.90 ± 2.10*	0.25 ± 0.01	0.23 ± 0.02

Values represent the mean ± SD of 10 rats.
* $p<0.05$

Table 3. The activities of NADPH-Cytochrome P-450 reductase and NADH-Cytochrome b_5 reductase in liver microsomes from rats exposed to 1-bromopropane for 8 weeks.

Treatment (ppm/kg)	Sex	NADPH P-450 reductase (μmol/min/mg protein)	NADH b_5 reductase (μmol/min/mg protein)
Control	M	0.022 ± 0.0012	0.065 ± 0.0062
	F	0.016 ± 0.0011	0.059 ± 0.0027
50	M	0.025 ± 0.0049	0.067 ± 0.0034
	F	0.015 ± 0.0024	0.065 ± 0.0007
300	M	0.023 ± 0.0018	0.083 ± 0.0095
	F	0.016 ± 0.0019	0.064 ± 0.0062
1800	M	0.024 ± 0.0017	0.088 ± 0.0036*
	F	0.014 ± 0.0015	0.070 ± 0.0011*

Values represent the mean ± SD of 10 rats.
* $p<0.05$

Table 4. The activities of catalytic enzymes in liver microsomes from rats exposed to 1-bromopropane for 8 weeks.

Treatment (ppm/kg)	Sex	EROD (pmol/min/mg protein)	PROD (pmol/min/mg protein)	pNPH (pmol/min/mg protein)
Control	M	0.28 ± 0.060	1.10 ± 0.113	792.58 ± 43.699
	F	0.34 ± 0.037	1.40 ± 0.085	594.45 ± 73.468
50	M	0.24 ± 0.017	1.06 ± 0.045	828.00 ± 41.066
	F	0.33 ± 0.075	1.50 ± 0.048	686.78 ± 89.781
300	M	0.30 ± 0.059	1.09 ± 0.039	850.29 ± 83.500
	F	0.32 ± 0.030	1.43 ± 0.078	784.89 ± 121.611
1800	M	0.32 ± 0.048	1.12 ± 0.112	1011.89 ± 265.809*
	F	0.35 ± 0.052	1.47 ± 0.069	857.99 ± 56.937*

Values represent the mean ± SD of 10 rats. EROD, ethoxyresorufin-O-deethylase; PROD, pentoxyresorufin-O-dealkylase; *p*NPH, *p*-nitrophenol hydroxylase.
* $p<0.05$

activities associated with CYP1A1/2 and CYP2B1/2, EROD (CYP1A1/2) and PROD (CYP2B1/2) were not changed by 1-BrP treatment. *p*NPH activity associated with CYP2E1 increased steadily with the increase in the concentration of 1-BrP in both sexes, but it was significant only in the 1800 ppm-treated groups in male and female rats ($p<0.05$). Figure 3 shows the results of immunoblot analyses which were carried out with microsomes from control rats and rats treated with 1-BrP, using MAb specific to CYP1A1/2, CYP2B1/2, and CYP2E1. The densities for CYP2E1 were considerably enhanced according to the dosages of 1-BrP which were inhaled by rats. GST, GSHPx activities and the content of LPO were significantly increased in the treated groups compared with the control group (Table 5) ($p<0.05$).

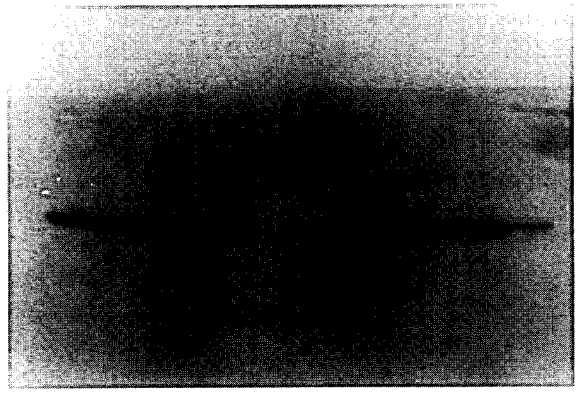

Fig. 3. Western immunoblot for hepatic microsomes of rats treated with 1-bromopropane (1-BrP) utilizing mouse monoclonal anti-rat CYP2E1 antibodies. Liver microsomes (10 μg) were loaded for the groups. MW, molecular weight standard; C, control; G1, 1-BrP 50 ppm/kg; G2, 1-BrP 300 ppm/kg; G3, 1-BrP 1800 ppm/kg; and E, ethanol (0.5 μg loaded)

Table 5. The activities of GST, GSHPx, SOD and generation contents of LPO in liver microsomes from rats exposed to 1-bromopropane for 8 weeks.

Treatment (mg/kg)	Sex	GST (nmol/min/mg protein)	GSHPx (nmol/min/mg protein)	LPO (nmol/mg protein)
Control	M	71.88 ± 2.977	0.324 ± 0.0302	1.002 ± 0.0989
	F	62.01 ± 4.959	0.325 ± 0.0127	1.678 ± 0.0810
50	M	85.91 ± 0.147*	0.383 ± 0.0144*	1.162 ± 0.0423
	F	67.24 ± 3.080	0.354 ± 0.0099*	1.848 ± 0.0706
300	M	80.86 ± 3.763*	0.397 ± 0.0022*	1.279 ± 0.0377
	F	76.10 ± 4.590*	0.361 ± 0.0113*	1.878 ± 0.0094*
1800	M	81.96 ± 2.456*	0.399 ± 0.0044*	1.295 ± 0.0766*
	F	103.74 ± 6.661*	0.38 ± 0.0101*	2.101 ± 0.0424*

Values represent the mean ± SD of 10 rats.
* $p<0.05$

Discussion

When a living organisms is exposed to environmental xenobiotics, it is able to produce specific enzymes, and metabolize and excrete the metabolites from their bodies by detoxification processes. Accordingly, monocyclic and polycyclic aromatic hydrocarbons (MAH and PAH), cyclic, aliphatic, hydroxyl, and halogenated compounds have been studied by many investigators (Okay, 1990; Gonzalez and Gelboin, 1992; Niwa *et al.*, 1995). In a previous study, Kim *et al.* (1966) treated rats with a series of MAH (benzene, toluene, and m-xylene) and examined the effect of MAH on the induction of CYP and the activities of several other related enzymes and their metabolites excreted in urine. CYP2E1 isozyme might be responsible for the metabolism of benzene and toluene, and CYP2B1 isozyme is responsible for xylene metabolism. Nakajima *et al.* (1994) reported that at least four CYPs, CYP2C11/6, CYP2E1, CYP2B1/2, and CYP1A1/2, contribute to the formation of styrene glycol from styrene. Guengrich *et al.* (1980) reported that the organohalide compounds are metabolized by microsomal CYP. Trichloroethylene, another halogenated compound, is metabolized by CYP2E1 (Guengrich *et al.*, 1991; Nakajima *et al.*, 1992; Chang *et al.*, 1997). The halogenoalkanes are transformed in animals to a general type of sulfur-containing metabolite, alkylmercapturic acid. Ethylmercapturic acid is formed from bromoalkane and iodo-ethane (Barnsley *et al.*, 1966). Previous studies of *in vivo* and *in vitro* metabolism have led to postulate of several mechanisms for the CYP-mediated metabolism of organohalides (R1R2CHX), including the oxidation of the halogen-bound carbon via carbon-hydrogen bond oxygen insertion (R1R2COHX) (Stevens and Anders, 1979; Ullrich, 1979), reduction of the carbon-halogen bond generating a halide ion (X-) and a carbon centered free radical (R1R2HC•) (Sipes *et al.*, 1977), and direct halogen oxidation to a hypervalent organohalogen species (R1R2CHX=O) (Macdonald *et al.*, 1980). However, the ability of 1-BrP to induce specific CYP isozymes for its metabolism was not clearly demonstrated. Thus, we studied the effect of 1-BrP on the induction of microsomal CYP using male and female Sprague-Dawley rats. By repeated inhalation, the body weights of male and female rats increased at all dosages of 1-BrP during the weeks of treatment except at dosages of 1800 ppm of 1-BrP. Since the effects of xenobiotics on living bodies are dose-dependent, 1-BrP at low dosages might stimulate growth, but high doses of 1-BrP might have toxic effects and reduce the growth as we observed with the 1800 ppm doses. Liver weight increased steadily with the increased concentration of 1-BrP in both sexes without significant changes in the total CYP and b_5 contents. In the case of propyl chloride and propyl iodide, the formation of S-propyl glutathione was NADPH-dependent, but no dependence on NADPH could be demonstrated consistently for formation of S-propyl glutathione from 1-BrP (Tachizawa *et al*, 1982). In this study, we also found that the activity of NADPH-P-450 reductase was not affected by 1-BrP. The activity of NADH b_5 reductase increased with the increase in 1-BrP treatment dosage, but statistical significance was found only in the 1800 ppm-treated group of male and female rats ($p<0.05$). The microsomal activity of *p*NPH treated groups was higher than in the control group but the increase was significant only in the 1800 ppm treated male rat group ($p<0.05$). There were no changes in the activities of EROD and PROD by the 1-BrP treatment. When Western blottings were carried out utilizing three monoclonal antibodies (MAb) specific against CYP1A1/2, CYP2B1/2, and CYP2E1, a strong band corresponding to CYP2E1 was observed in microsomes obtained from rats treated with 1-BrP but the signals of immunoblot corresponding to CYP1A1/2 and CYP2B1/2 isozymes were not enhanced by the treatment with 1-BrP.

Reactive oxygen species (ROS) are produced endogenously by normal metabolic processes, but their levels are markedly increased by environment pollutants such as chemicals, heavy metals, cigarette smoking, and ionizing radiation (Chance *et al.*, 1979; Church and Pryor, 1985; Wurzel *et al.*, 1995). ROS possesses the potential to damage either cellular or organelle membranes and macromolecules, causing lipid peroxidation and nucleic acid and protein alteration. Living organisms are protected from ROS and oxidative damage by several defence mechanism, such as SOD, catalase, and GSHPx (Chance *et al.*, 1979). The main function of GST is to conjugate electrophilic and hydrophobic compounds with cellular glutathione, increasing their solubility and facilitating excretion of the compounds. These reactions result in the detoxification of many endogeneous electrophiles as well as environmental chemicals (Schecter *et al.*, 1991; Tew, 1994). In this study, the activities of GST, GSHPx, and LPO concentration increased steadily with the increase in the concentration of 1-BrP in both sexes ($p<0.05$). With respect to sex differences, the contents of total CYP, b_5, and LPO, the activities of NADPH P-450 and NADH b_5 reductase, and the activities of *p*NPH and GST of liver microsomes derived from male rats were higher than those from female rats except for the activities of EROD and PROD.

Our results from this study suggest the following: (1) a clear presence of sex-dependent activity in the metabolic mechanism of 1-BrP in the rat livers, (2) that the CYP2E1 isozyme is possibly responsible for the metabolism of 1-BrP, (3) that free radicals are produced by the activated intermediates as halide radicals during the metabolic processing of 1-BrP, and (4) GST is associated with the detoxification and protection of tissues against oxidative damage by halide radicals.

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