reviewed[1], and the Japan Society for Occupational Health recommends an occupation exposure limit of 0.5 ppm[2]. Previously, we studied the effects of inhaled 1-BP vapor in male rats on the nervous[3-8] and immune systems[9, 10].

We also studied the effects of inhaled 1-BP vapor on metabolism in male rats and reported that 1-BP rapidly decomposes and releases bromine ion in the blood[11], indicating that bromine ion is a major index of 1-BP exposure. Recently, because of the health effects reported in female workers exposed to 1-BP[12-14], there is concern regarding the health effects of 1-BP exposure on the next generation. Some researchers have reported results of experiments in female animals[15-17]; however, the kinetics of bromine ion distribution to the next generation has not been elucidated. In this study, pregnant rats were exposed to 700 ppm of 1-BP vapor, and the concentration of bromine ion in the rat brain was measured. The distribution of bromine in fetuses and cross-fostered pups was investigated. A one-compartment model was employed to analyze the behavior of bromine ion in rats.

Methods

Animals

Female (9-week-old) and male (10-week-old) Wistar rats were purchased from Kyudo Co., Ltd. (Saga, Japan). After acclimation in polycarbonate cages with dry chips, they were housed in pairs in animal rooms under 12-h light–dark cycle conditions at 22 ± 1°C and 55 ± 5% relative humidity, with free access to food and water. The presence of sperm in the vaginal smear was defined as day 0 of gestation (GD0; female rats were 11 weeks old). In the inhalation study, the female rats were divided into three groups: 1-BP-exposed virgin female group (n = 5), 1-BP-exposed mother group (n = 11), and the control mother group (n = 5). After the final exposure of mother rats on GD20, they

were housed in an animal room for the onset of birth. Postnatal day (PND) i.e., the day after birth, was defined as day 0 (PND0 = GD21). On PND1, a litter size of eight pups was assembled and cross-fostering[17, 18] of pups was performed between mother exposure groups (n = 3) and mother control groups (n = 3). The pups were subdivided into four groups: (1) exposure group (1-BP-exposed pups were raised by their birth mother exposed to 1-BP), (2) postnatal exposure group (control pups were raised by 1-BP-exposed mother), (3) gestation exposure group (1-BP-exposed pups were raised by control mother), and (4) control group (control pups were raised by their control mother). The experimental groups are summarized in Table 1. Body weight was measured periodically. The experiments were conducted per the guidance of the Ethics Committee of Animal Care and Experimentation in accordance with The Guiding Principle for Animal Care Experimentation, University of Occupational and Environmental Health, Japan (AE03-065), which conforms to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Japanese Law for Animal Welfare and Care.

Exposure

Reagent-grade 1-BP was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). 1-BP vapor was introduced into a 400-*l* stainless-steel exposure chamber. Details of this apparatus and procedure have been given elsewhere[11]. In order to study change in bromine ion in blood and brain when the condition of dysfunction of feedback inhibition (i.e., disinhibition) was confirmed, exposure concentration was designed to be 700 ppm, which was higher than LOAEL (400 ppm) for disinhibition[7]. The actual concentration of 1-BP vapor in the chamber was 701.3 ± 5.2 ppm. In the control group, only clean air was introduced into the chamber. The exposure period was 6 h per day

between 9 a.m. and 3 p.m. throughout gestation or GD1-20 (virgin female group was exposed until GD21). Table 1 displays the age of the rats on sampling day. They were deeply anesthetized with diethyl ether and then decapitated. The brains and the stomachs with milk from only the exposure group on PND1 were gently removed and stored in a freezer.

Measurement of bromine ion concentration

The brains (cerebrum and diencephalon) and stomachs (0.25 g) were homogenized with water (1.5 m*l*) at 0°C. The sample (1 m*l*) was dispensed into a vial, and 0.1 m*l* of dimethyl sulfate was added to convert bromine ion to methyl bromide. Then, 0.1 m*l* of an aqueous solution of isopropyl alcohol (0.5 volume percent) was added as an internal standard. The vial was heated at 50°C for 1 h. The bromine ion concentration was determined by measuring peak area of methyl bromide vapor in the headspace by using a gas chromatograph mass spectrometer (GC/MS, QP-5050; Shimadzu, Kyoto, Japan)[11].

Estimation method of bromine ion concentration

As previously described, inhaled 1-BP was metabolized and bromine ions were released. In this study, the behavior of released bromine ion concentration in the brain was analyzed by using a one-compartment model[19]. We assumed the bromine ion uptake rate, i.e., the generation rate of bromine ion, is equal to the 1-BP uptake rate because 1-BP is decomposed quickly[11] and releases bromine ion. Under this assumption, mass balance equations of bromine ion during exposure and clearance periods respectively were as follows:

$$\frac{dx}{dt} = R - kx \tag{1}$$

$$\frac{dx}{dt} = -kx \tag{2}$$

where x is the amount of bromine ion (μg); t is time (h); R is the generation rate of bromine ion (μg/h), which corresponds to the 1-BP uptake rate; and k is the excretion rate constant (1/h). From equations (1) and (2), the bromine ion concentrations C (μg/g) during exposure and clearance respectively were obtained as follows:

$$C = \frac{R}{\rho V k}\left(1 - e^{-kt}\right) \tag{3}$$

$$C = C_0 e^{-kt} \tag{4}$$

where V is the volume of the compartment (ml), ρ is the density of the compartment (g/ml), and C_0 is the initial concentration during clearance (μg/g). The excretion rate constant k is given by the biological half-life, $t_{1/2}$ (h) or $T_{1/2}$ (days).

$$k = \frac{\ln 2}{t_{1/2}(\mathrm{h})} = \frac{0.693}{T_{1/2}(\mathrm{days}) \times 24} \tag{5}$$

Experimental Results

Fig. 1 shows the change in the average body weight of mother rats exposed to 700 ppm of 1-BP up to GD20 and that of the pups after the exposure. The time, T (on the

horizontal axis), includes the GDs and PNDs. Litter sizes of exposed mothers and control mothers were 15.0 ± 2.8 and 14.9 ± 2.5 pups, respectively. The body weight of both mothers and pups increased rapidly. This tendency was also observed in the control group, and there was no significant difference between the exposure group and the control group. For the virgin female group, body weight did not change significantly (271.1 ± 17.0 g) during GD1-20.

Bromine ion concentration in the rat brain (μg/g-brain) exposed to 700 ppm of 1-BP on GDs is presented as symbols in Fig. 2. The bromine ion concentration in mother rats was lower than that in virgin rats, and the concentration in fetuses was higher than that in mothers. Fig. 3 shows changes in bromine ion concentration in pup brain for PNDs. The concentration in the gestation exposure group decreased between PND4 and PND8, whereas that in the postnatal exposure group increased from PND2 to PND4 and then decreased. This tendency was also observed in the exposure group, although the concentration on PND1 was lower than that on GD20 (fetus in Fig. 2). Specifically, the concentration in the exposure group was the highest just after birth, but decreased at PND1. The concentration then increased from PND1 to PND3, but decreased again with time. In the control pups, the bromine ion concentration was 11.2 ± 7.7 μg/g-brain on PND3.

The bromine ion concentration in pup stomachs with milk from the exposure group on PND1 was 830.6 ± 188.8 μg/g-stomach, which was about twice as much as that in the mother brain at GD20 (Fig. 2).

Discussion

The one-compartment model was applied to analyze the bromine ion concentration in the brains of virgin females, mothers, fetuses, and pups. Equations (3) and (4) have

two parameters, the excretion rate constant k and the 1-BP uptake rate R. The excretion rate constant, k, can be easily calculated from equation (5) by using the biological half-life $T_{1/2}$ (days). In our previous work[11], $T_{1/2}$ for male rats was 4.7–15.0 days in blood and 5.0–7.5 days in urine. Therefore, $T_{1/2}$ = 7.0 days was used for mothers and virgin females in this study. $T_{1/2}$ in pups was 3.1 days, obtained by experimental data. Equation (4) was applied to the data from PND1 for the exposure group and from PND4 and PND8 for the gestation exposure group as shown in Fig. 3. $T_{1/2}$ = 3.1 days was also used for fetuses. The half-lives of between GD20 for fetuses and PND1 for the exposure group were excluded from the calculation because of the time lag due to birth.

As shown in Fig. 2, the bromine ion concentration in the brains of mothers was lower than that in the brains of virgin females. A reason for this might be that the bromine ion concentration was diluted because of increasing body weight. The average body weight of pups, w (g), was expressed using the following equation (Fig. 1):

$$w = 0.00028T^{3.31} \tag{6}$$

The average body weight of mothers, W (g), was calculated as the sum of that of virgin females (ρV = 271.1 g) and of pups, w, (interpolated value for GDs):

$$W = 271.1 + 27w \tag{7}$$

where 27 is the constant, which was determined to give the best fit for the experimental data as shown in Fig. 1.

For virgin females, the uptake rate, R, of 2853 μg/h was obtained to give the best fit

of equations (3) and (4) for the experimental data on GD21 in Fig. 2. Therefore, $R/\rho V = R/W = 2853/271.1 = 10.5$ μg/(h·g) for virgin females, and $R/\rho V = 2853/(271.1 + 27w)$ for mother rats was used in equation (3). For fetuses, R (bromine ion uptake rate from mothers) was assumed to be proportional to body weight, and $R/\rho V = R/w = 22.0$ μg/(h·g) was applied, which was obtained to give the best fit for the experimental data on GD20 in Fig. 2. On PNDs, suckling (exposure to bromine ion from milk) was assumed to occur at 2-h intervals. As shown in Fig. 3, the curve of bromine ion concentration in the brains of the postnatal exposure group is convex. In addition, on PND1, the concentration in pup stomachs with milk was high, and the level was higher than that in the mother brain, as calculated using the one-compartment model (486.2 μg/g-brain). Therefore, we assume that the uptake rate R of pups is high at first and then decreases. In this work, R in the postnatal exposure group can be expressed by the following equation:

$$R = 388e^{-0.126(t-32)} \tag{8}$$

where 32 is the initial suckling (h) and 388 and 0.126 are the constants determined experimentally. The bromine ion concentration in the exposure group was calculated as the sum of the concentrations in the gestation exposure and postnatal exposure groups. Conditions of the one-compartment model and the values of parameters obtained are listed in Table 2. Solid, broken, and dotted lines in Fig. 2 indicate calculated lines for fetuses, mothers, and virgin females, respectively. In Fig. 3, solid, broken, and dotted lines indicate calculated lines of exposure, postnatal exposure, and gestation exposure groups, respectively. The lines calculated using the proposed model could be estimated from the experimental data with acceptable precision as shown in both figures.

The calculated bromine ion uptake rates per weight, $R/\rho V$, for adults and fetuses were 10.5 and 22 μg/(h·g), respectively. This result suggests that the bromine ion easily transfers from mothers to fetuses, and the concentration in fetuses was higher than that in mothers. R in postnatal exposure group was expressed as an exponential function, and $R/\rho V$ of 55 μg/(h·g) was obtained at initial suckling time. This value was large compared to 22 μg/(h·g), the calculated value at GD20, before birth. This suggests that uptake rate of bromine ion via milk was higher than that via the placenta, and the bromine ion concentration in the exposure group could be explained as the sum of that in the gestation and postnatal exposure groups, which is shown in Fig. 3.

In summary, the results of this study suggest (1) the concentration of bromine ion in mother rats was lower than that in virgin female rats, (2) bromine ion easily transferred from mothers to fetuses and accumulated before birth, (3) bromine ion was concentrated more in milk than in the brains of the mothers, and (4) bromine ion uptake rate in pups was high immediately after birth.

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References

1) Ichihara G. Neuro-reproductive toxicities of 1-bromopropane and 2-bromopropane. Int Arch Occup Environ Health 2005; 78; 79-96.

2) The Japan Society for Occupational Health. Recommendation of occupational exposure limits (2013-14). J Occup Health 2013; 55; 422-41.

3) Ohnishi A, Ishidao T, Kasai T, Arashidani K, Hori H. Neurotoxicity of 1-bromopropane in rats. J UOEH 1999; 21; 23-8.

4) Fueta Y, Ishidao T, Kasai T, Hori H, Arashidani K. Decreased paired-pulse inhibition in the dentate gyrus of the brain in rats exposed to 1-bromopropane vapor. J Occup Health 2000; 42; 149-51.

5) Fueta Y, Ishidao T, Arashidani K, Endo Y, Hori H. Hyperexcitability of the hippocampal CA1 and the dentate gyrus in rats subchronically exposed to a substitute for chlorofluorocarbons, 1-bromopropane vapor. J Occup Health 2002; 44; 156-65.

6) Fueta Y, Fukuda T, Ishidao T, Hori H. Electrophysiology and immunohistochemistry in the hippocampal CA1 and the dentate gyrus of rats chronically exposed to 1-bromopropane, a substitute for specific chlorofluorocarbons. Neuroscience 2004; 124; 593-603.

7) Fueta Y, Ishidao T, Ueno S, Yoshida Y, Kunugita N, Hori H. New approach to risk assessment of central neurotoxicity induced by 1-bromopropane using animal models. Neurotoxicology 2007; 28; 270-3.

8) Ueno S, Yoshida Y, Fueta Y, Ishidao T, Liu JQ, Kunugita N, Yanagihara N, Hori H. Changes in the function of the inhibitory neurotransmitter system in the rat brain following subchronic inhalation exposure to 1-bromopropane. Neurotoxicology 2007; 28; 415-20.

9) Yoshida Y, Liu JQ, Nakano Y, Ueno S, Fueta Y, Ishidao T, Kunugita N, Yamashita U, Hori H. 1-BP inhibits NF-kB activity and Bcl-xL expression in astrocytes in vitro and reduces Bcl-xL expression in the brains of rats in vivo. Neurotoxicology 2007; 28; 381-6.

10) Yoshida Y, Nakano Y, Ueno S, Liu JQ, Fueta Y, Ishidao T, Kunugita N, Yanagihara N, Sugiura T, Yamashita U, Hori H. Effects of 1-bromopropane, a substitute for chlorofluorocarbons, on brain-derived neurotrophic factor (BDNF) expression. Int Immunopharmacol 2009; 9; 433-8.

11) Ishidao T, Kunugita N, Fueta Y, Arashidani K, Hori H. Effects of inhaled 1-bromopropane vapor on rat metabolism. Toxicol Lett 2002; 134; 237-43.

12) Ichihara G, Miller JK, Ziolkowska A, Itohara S, Takeuchi Y. Neurological disorders in three workers exposed to 1-bromopropane. J Occup Health 2002; 44; 1-7.

13) Ichihara G, Li W, Ding X, Peng S, Yu X, Shibata E, Yamada T, Wang H, Itohara S, Kanno S, Sakai K, Ito H, Kanefusa K, Takeuchi Y. A survey on exposure level, health status, and biomarkers in workers exposed to 1-bromopropane. Am J Ind Med 2004; 45; 63-75.

14) Li W, Shibata E, Zhou Z, Ichihara S, Wang H, Wang Q, Li J, Zhang L, Wakai K, Takeuchi Y, Ding X, Ichihara G. Dose-dependent neurologic abnormalities in workers exposed to 1-bromopropane. J Occup Environ Med 2010; 52; 769-77.

15) Sekiguchi S, Suda M, Zhai YL, Honma T. Effects of 1-bromopropane, 2-bromopropane, and 1,2-dichloropropane on the estrous cycle and ovulation in F344 rats. Toxicol Lett 2002; 126; 41-9.

16) Yamada T, Ichihara G, Wang H, Yu X, Maeda K, Tsukamura H, Kamijima M, Nakajima T, Takeuchi Y. Exposure to 1-bromopropane causes ovarian dysfunction in rats. Toxicol Sci 2003; 71; 96-103.

17) Furuhashi K, Kitoh J, Tsukamura H, Maeda K, Wang H, Li W, Ichihara S, Nakajima T, Ichihara G. Effects of exposure of rat dams to 1-bromopropane during pregnancy and lactation on growth and sexual maturation of their offspring. Toxicology 2006; 224; 219-28.

18) Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicological Sci 2003; 74; 382-92.

19) Hori H, Hyakudo T, Oyabu T, Ishimatsu S, Yamato H, Tanaka I. Effects of inhaled methyl bromide gas on the metabolic system and kinetics of bromine ion in rats. J UOEH 2002; 24; 151-60.

Table 1. Experimental groups and ages of adult and fetal rats exposed to 700 ppm of 1-BP and of pups on sampling day

Groups (n)		Age (n) on sampling day
Virgin female	Exposure (5)	GD21 (2)
Mother	Exposure (11) Control (5)	GD20 (3)
Fetus	Exposure	GD20 (13)
Pup†	Exposure Postnatal exposure Gestation exposure Control	PND1 (10), PND3 (10), PND5 (5), PND7 (5) PND2 (5), PND4 (5), PND8 (5) PND4 (5), PND8 (5) PND3 (5)

1-BP: 1-bromopropane, GD: gestation day, PND: postnatal day, †: Exposure = 1-BP exposed pups were raised by their birth mother exposed to 1-BP, Postnatal exposure = control pups were raised by 1-BP exposed mother, Gestation exposure = 1-BP exposed pups were raised by control mother, Control = control pups were raised by control mother

Table 2. Parameters of the one-compartment model

Groups		$T_{1/2}$ (days)	ρV (g)	R (μg/h)	Results
GD	Virgin female	7.0	271.1	2853	Fig. 2
	Mother	7.0	271.1+27w	2853	Fig. 2
	Fetus	3.1	w	22w	Fig. 2
PND	Gestation exposure	3.1			Fig. 3
	Postnatal exposure	3.1	w	$388e^{-0.126(t-32)}$	Fig. 3
	Exposure	Gestation exposure + Postnatal exposure			Fig. 3
	Mother	7.0			Text†

†: the concentration in mother brain corresponding to PND1 (486.2 μg/g-brain),
$w=0.00028T^{3.31}$ by equation (6)

Figure Captions

Fig. 1. The average body weight of mothers (W) exposed to 700 ppm of 1-BP up to GD20 and that of pups (w) after exposure. 1-BP: 1-bromopropane; GD: gestation day; PND: postnatal day

Fig. 2. Change in bromine ion concentration in rat brain exposed to 700 ppm of 1-BP on GDs. Symbols represent experimental data: ●, fetus; ▲, mother; △, virgin female. Solid, broken, and dotted lines indicate calculated lines for fetuses, mothers, and virgin females, respectively. 1-BP: 1-bromopropane; GD: gestation day

Fig. 3. Change in bromine ion concentration in pup brain during PNDs. Symbols represent experimental data: ●, exposure group (1-BP exposed pups were raised by their birth mother exposed to 1-BP); ◇, postnatal group (control pups were raised by 1-BP exposed mother); □, gestation exposure (1-BP exposed pups were raised by control mother). Solid, broken, and dotted lines indicate calculated lines for exposure, postnatal exposure, and gestation exposure groups, respectively. 1-BP: 1-bromopropane; PND: postnatal day

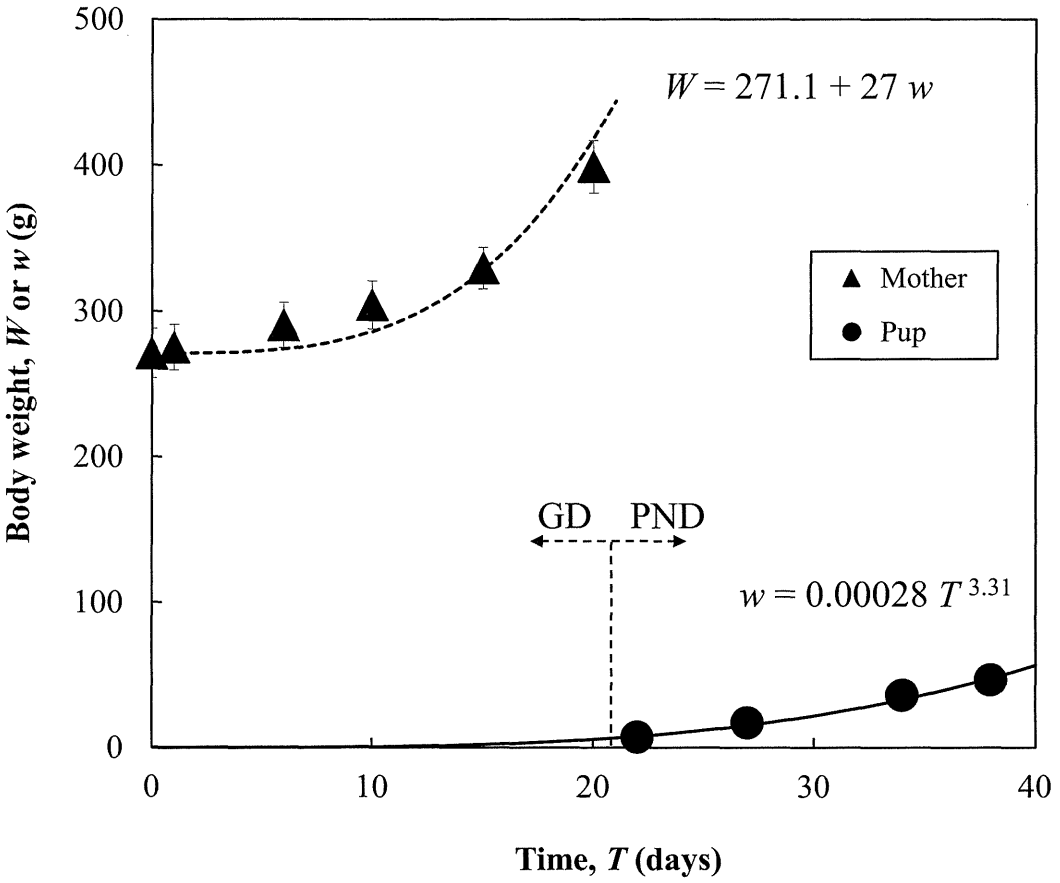
500
400
300
200
100
0
0
10
20
30
40
Body weight, W or w (g)
Time, T (days)
W = 271.1 + 27 w
Mother
Pup
GD
PND
w = 0.00028 T 3.31

Fig. 1.

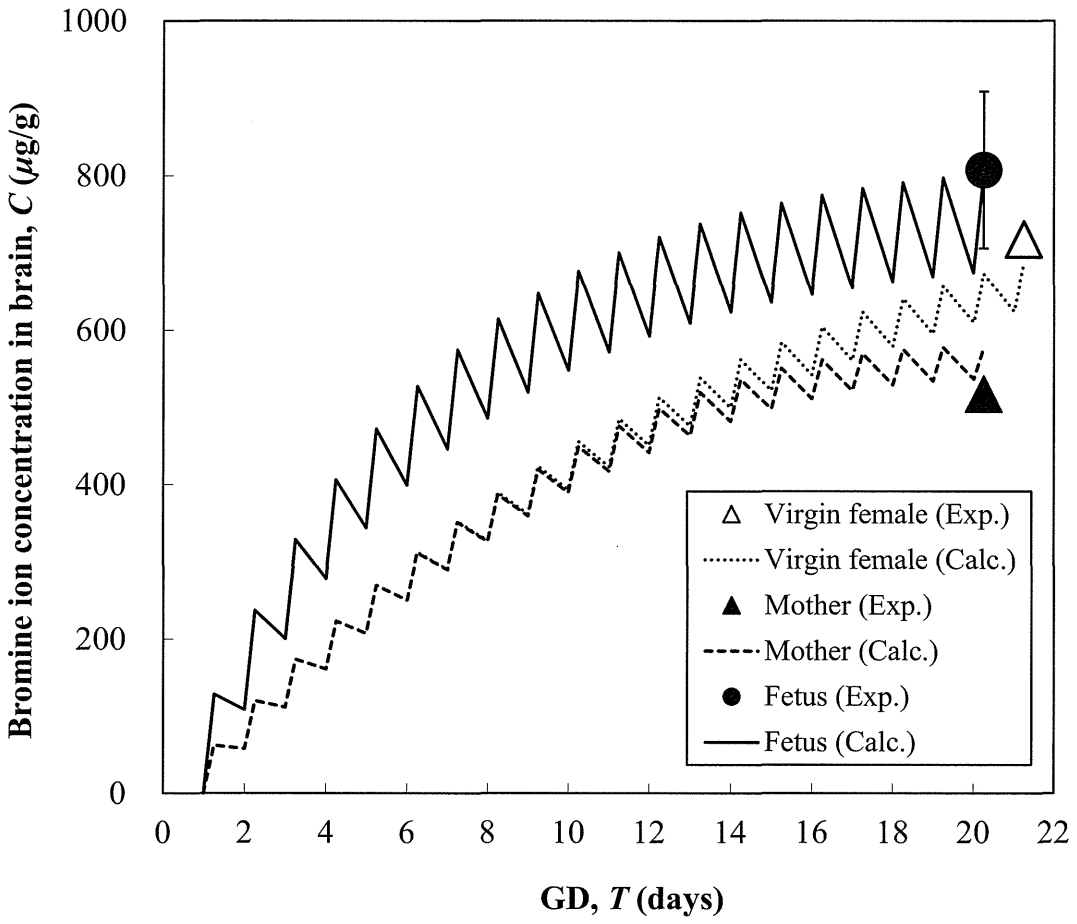
Bromine ion concentration in brain, C (μg/g)
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
22
GD, T (days)
Virgin female (Exp.)
Virgin female (Calc.)
Mother (Exp.)
Mother (Calc.)
Fetus (Exp.)
Fetus (Calc.)

Fig. 2.

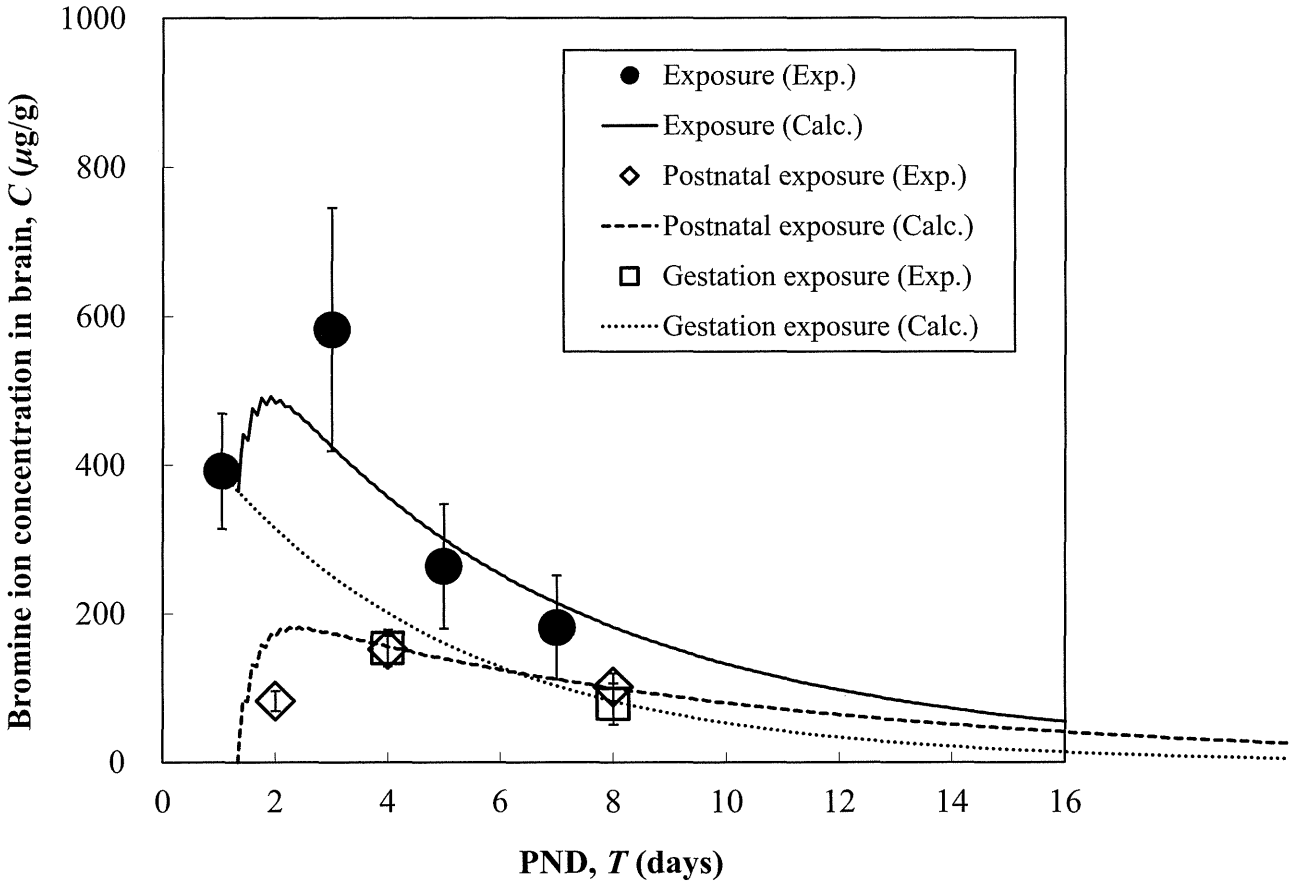
Bromine ion concentration in brain, C (μg/g)
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
PND, T (days)
Exposure (Exp.)
Exposure (Calc.)
Postnatal exposure (Exp.)
Postnatal exposure (Calc.)
Gestation exposure (Exp.)
Gestation exposure (Calc.)

Fig. 3.

[Research Note]

Prenatal Exposure to 1-Bromopropane Suppresses Kainate-Induced Wet Dog Shakes in Immature Rats

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Abstract : 1-Bromopropane (1-BP) is used in degreasing solvents and spray adhesives. The adverse effects of 1-BP have been reported in human cases and adult animal models, and its developmental toxicity has also been reported, but its effects on developmental neurotoxicity have not been investigated in detail. We evaluated the effects in rat pups of prenatal exposure to 1-BP on behaviors such as scratching and wet dog shakes (WDS), which were induced by injection of kainate (KA). Pregnant Wistar rats were exposed to vaporized 1-BP with 700 ppm from gestation day 1 to day 20 (6 h/day). KA at doses of 0.1, 0.5, and 2.0 mg/kg were intraperitoneally injected into a control group and a 1-BP-exposed group of pups on postnatal day 14. There was no significant difference in scratching between the control and the prenatally 1-BP-exposed groups, while suppression of the occurrence ratio of WDS was observed at the low dose of 0.1 mg/kg of KA in the prenatally 1-BP-exposed pups. Our results suggest that prenatal exposure to 1-BP affects neurobehavioral responses in the juvenile period.

Keywords : 1-bromopropane, prenatal exposure, developmental neurotoxicity, wet dog shake, rats.

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Introduction

The volatile organic compound 1-bromopropane (CH_3-CH_2-CH_2Br; 1-BP), a substitute for specific chlorofluorocarbons, is mainly used in degreasing solvents and spray adhesives. It has been reported that occupational exposure to 1-BP causes neurotoxicity, such as numbness, gait disturbance, prolongation of distal latency and memory dysfunction [1].

Animal models exposed to 1-BP have also shown central neurotoxicity, including ataxic gait, prolongation of distal latency, alteration of mRNA levels of neurotransmitter receptors [1], and hippocampal disinhibition [2]. *In vitro* studies have revealed that the direct application of 1-BP enhanced the currents mediated by the activation of A type γ-aminobutyric acid ($GABA_A$) receptors, suppressed the currents mediated by neuronal nicotinic acetylcholine receptors, and potentiated feedback inhibition in the cornu ammonis 1 (CA1) subfield of hippocampal slices [3]. The gene expression of the B-cell lymphoma-extra large molecule (Bcl-xl), and the activity of nuclear factor-kappa B (NF-κB), were suppressed in *in vitro* and *in vivo* studies [4]. The developmental effects of 1-BP have also been investigated [5], but little is known about the developmental neurotoxicity in offspring.

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In our previous study of the developmental neurotoxicity of 1-BP, prenatal exposure to 1-BP altered hippocampal excitability and the gene expression of the Na^+ channel [6] and glutamate receptor subunits on postnatal day (PND) 14 [7]. These results raised the possibility that prenatal exposure to 1-BP affects brain development and its related behaviors. However, conventional behavioral tests for rodents are difficult to apply to pups. Thus, we focused on the particular behaviors of scratching and wet dog shakes (WDS), which can be observed in pups.

Scratching is defined as repetitive and quick flexion-extension movements of the hind limbs toward the neck or the head region. This behavior has been shown to be spontaneously induced in normal as well as pathological conditions and is used as an itch model in rodents [8], although the behavior in pups remains to be analyzed. WDS is characterized as brief and fierce shaking of the head, neck, and trunk, appearing when rodents are wet, as the name suggests [9]. Interestingly, it has been reported that both scratching and WDS can be induced by electrical stimulation of limbic structures [10], and by several pharmacological interventions, such as kainate (KA) [9, 11] and pentylenetetrazole. KA is the agonist of ionotropic glutamate receptors, which mediate excitatory neurotransmission and are predominantly distributed in the hippocampus, inner lamina of the neocortex, and ventral thalamus [12]. Thus, scratching and WDS induced by KA could be useful indices of changes in the excitatory neurotransmission of neuronal networks in pup brains. In this study, we examined the effect of prenatal exposure to 1-BP on behaviors in pups by evaluating the incidences of scratching and WDS induced by KA.

Materials and Methods

Animals and 1-BP inhalation

Thirty-two female and 16 male Wistar rats (designated the parental (P) generation) purchased from Kyudo *Co.* (Tosu, Japan) at 11 weeks of age were housed in plastic cages with paper-made chips (ALPHA-dri, Shepherd Specialty Papers, Milford, USA) on a 12 h light/dark cycle (light period: 07:00-19:00). The room temperature was kept at 23 ± 1°C. The relative humidity was about 70%. The animals had free access to food and water. Proestrus stage was verified with an impedance checker (MK-10B, Muromachi Kikai *Co., Ltd.*, Tokyo, Japan). When the impedance was over three kΩ, the F0 female rats were mated with male rats. In the morning of the following day, the existence of sperm in the vaginal smear or vaginal plug was verified as the gestation day (GD) 0. Fourteen dams from the colony were used in the experiment. The pregnant rats of the P generation were randomly divided into two groups (7 rats in each): one group as the control and the other for exposure to 1-BP.

1-BP was purchased from Kanto Chemical *Co., Ltd.* (Tokyo, Japan). Seven dams were exposed to 1-BP vapor at a concentration of 700 ppm (6 h/day) for 20 days from GDs 1 to 20 in an exposure chamber [13], whereas the other seven dams were provided fresh air in the same type of chamber. Both P generation groups were not allowed access to food and water during the inhalation period. Four weeks of 1-BP inhalation (700 ppm) resulted in apparent effects on the hippocampus in the adult rats [2]. Therefore, we first chose the concentration of 700 ppm to study the possible underlying mechanism of developmental neurotoxicity in prenatally 1-BP-exposed rats. The concentration of 1-BP was monitored with a gas chromatograph (GC353B FSL, GL Sciences *Inc.*, Japan) equipped with a flame ionization detector.

All the pregnant rats gave birth to offspring (termed the first filial (F1) generation) on GD 21. The day of birth was defined as PND 0. We randomly gathered 26 F1 rats from the 7 control litters and 22 F1 rats from the 7 1-BP-exposed litters. All the F1 pups were bred with their mother rats during the lactation period. In this study, 24 female and 3 male F1 rats were obtained from the 7 dams in the control group, and 18 female and 4 male F1 rats were obtained from the 7 dams in the 1-BP-exposed group, respectively. We examined the F1 rats for the general toxicity of 1-BP inhalation exposure, such as litter size, sex ratio, testicular descent, vaginal opening, ear opening, and survival rate. The body weight of the F1 rats was measured on PND 14.

KA administration and behavioral observation

KA was obtained from Wako Pure Chemical Industries, *Ltd.* (Osaka, Japan). KA (0.1, 0.5, and 2.0 mg/kg) was dissolved in phosphate buffered saline (PBS).

PBS or KA was intraperitoneally injected to the F1 rats at PND 14, after which the F1 rats were placed in a clear plastic cage, and the scratching and WDS were observed by video-recording for 180 min in a room for the behavioral observation. The room temperature was kept at about 25°C. The behavioral observation was conducted for 180 min between 09:30 and 15:30. The number of F1 rats that showed the scratching and the WDS behavior was counted and then the occurrence ratio was calculated. The duration and frequency of scratching and WDS were also measured. This experiment was approved by the Ethics Committee for Animal Care and Experimentation in accordance with the University of Occupational and Environmental Health, Japan.

All the chemicals used in this study were a reagent grade and purchased from commercial sources.

Statistical analysis

The difference in bodyweight between the F1 control and F1 1-BP-exposed groups was analyzed by Student *t*-test. The Mantel-Haeinzel procedure was utilized to see the whole effect of the prenatal inhalation of 1-BP on the occurrence ratio of scratching and WDS. When appropriate, Fisher's exact test determined significant differences. A two-way analysis of variance (ANOVA) was performed to clarify the effects of prenatal exposure to 1-BP and/or a dose of KA on the frequency and the duration of scratching and WDS. When appropriate, *post hoc* analysis by Scheffe's test determined significant differences, respectively. The criteria of significant difference was $P < 0.05$ in all the statistical analyses. Data represent mean ± standard error of the mean (SEM).

Results and Discussion

General toxicity of 1-BP inhalation exposure in F0 and F1 generations

There were no outward pathological signs related to 1-BP in the F0 rats. The body weights of the P generation dams treated with 1-BP were not significantly different from those in the control (fresh air) group (data not shown). None of the F1 rats died during the experimental period, indicating that the exposure seemed to cause little stress on the dams in this study. There was no difference in the sex ratio, survival rate, or other clinical signs between the F1 control and F1 1-BP-exposed groups, with the exception of body weight. The body weight in the female F1 1-BP-exposed group (32.5 ± 0.5 g) was significantly lower ($P < 0.01$, Student *t*-test) than that in the female F1 control group (35.0 ± 0.4 g). The 1-BP-exposed male F1 rats also had a lower body weight (33.5 ± 0.3 g) compared to the male F1 control group (37.0 ± 0.8 g) ($P < 0.01$, Student *t*-test). Our results were consistent with previous studies showing that prenatal exposure to 1-BP has no effects on postnatal survival rate, excluding the body weight [5].

Effect of prenatal exposure to 1-BP on behavioral responses

KA administration elicits immobilization, followed by scratching, WDS, forelimb clonus, and status epilepticus (continuous chronic-tonic posturing of all 4 limbs) [11]. It is also known that a low dose less than 3 mg/kg of KA elicits scratching and WDS but hardly ever elicits epileptic convulsions. Our preliminary study also showed that doses of KA higher than 4 mg/kg induced convulsive behaviors as well as scratching and WDS, thus we chose doses of 0.1, 0.5, and 2.0 mg/kg of KA.

Behavioral data obtained from both genders is combined in Tables 1 and 2, because it has been reported that there are no sex differences in KA induced-behaviors in pups [14].

In the F1 control group, all of the tested pups showed scratching during the 180 min after injection of PBS or KA. The frequency and duration of the scratching was significantly higher only at the dose of 2.0 mg/kg (Table 1). WDS were observed in 80% of the PBS-injected control pups and in all of the KA-injected control pups. A significantly higher frequency of WDS was observed at the dose of 2.0 mg/kg (Table 2). The behavioral changes induced by the KA doses of 0.1 and 0.5 mg/kg were similar to those of PBS, thus it can be said that these two doses are subclinical.

Spontaneous scratching and WDS were also observed in the F1 1-BP-exposed group. The occurrence ratio of scratching was 100% at all doses of KA (Table 1), whereas that of WDS was 40 to 60% in 0 to 0.5 mg/kg and 100% in 2.0 mg/kg of KA (Tables 2). The effect of prenatal exposure to 1-BP was observed