

Original

## A cross-fostering analysis of bromine ion concentration in rats that inhaled 1-bromopropane vapor

Toru Ishidao<sup>1</sup>, Yukiko Fueta<sup>1</sup>, Susumu Ueno<sup>2</sup>, Yasuhiro Yoshida<sup>3</sup> and Hajime Hori<sup>1</sup>

<sup>1</sup>Department of Environmental Management, School of Health Sciences, University of Occupational and Environmental Health, Japan, <sup>2</sup>Department of Occupational Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan and <sup>3</sup>Department of Immunology and Parasitology, School of Medicine, University of Occupational and Environmental Health, Japan

**Abstract: Objective:** Inhaled 1-bromopropane decomposes easily and releases bromine ion. However, the kinetics and transfer of bromine ion into the next generation have not been clarified. In this work, the kinetics of bromine ion transfer to the next generation was investigated by using cross-fostering analysis and a one-compartment model. **Methods:** Pregnant Wistar rats were exposed to 700 ppm of 1-bromopropane vapor for 6 h per day during gestation days (GDs) 1-20. After birth, cross-fostering was performed between mother exposure groups and mother control groups, and the pups were subdivided into the following four groups: exposure group, postnatal exposure group, gestation exposure group, and control group. Bromine ion concentrations in the brain were measured temporally. **Results:** Bromine ion concentrations in mother rats were lower than those in virgin rats, and the concentrations in fetuses were higher than those in mothers on GD20. In the postnatal period, the concentrations in the gestation exposure group decreased with time, and the biological half-life was 3.1 days. Conversely, bromine ion concentration in the postnatal exposure group increased until postnatal day 4 and then decreased. This tendency was also observed in the exposure group. A one-compartment model was applied to analyze the behavior of bromine ion concentration in the brain. By taking into account the increase of body weight and change in the bromine ion uptake rate in pups, the bromine ion concentrations in the brains of the rats could be estimated with acceptable precision.

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**Key words:** 1-Bromopropane inhalation, Cross-fostering, Bromine ion concentration, One-compartment model, Animal experiment

1-Bromopropane (1-BP, CAS no. 106-94-5) is widely used as a substitute for chlorofluorocarbons, which destroy the ozone layer. The toxicity of 1-BP has been reviewed<sup>1)</sup>, and the Japan Society for Occupational Health recommends an occupation exposure limit of 0.5 ppm<sup>2)</sup>. Previously, we studied the effects of inhaled 1-BP vapor in male rats on the nervous<sup>3-8)</sup> and immune systems<sup>9,10)</sup>.

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<sup>11)</sup>, 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<sup>12-14)</sup>, 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<sup>15-17)</sup>; 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,

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Correspondence to: Toru Ishidao, Department of Environmental Management, School of Health Sciences, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, 807-8555, Japan (e-mail: ishidao@health.uoeh-u.ac.jp)

**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)	GD20 (3)
	Control (5)	
Fetus	Exposure	GD20 (13)
Pup†	Exposure	PND1 (10), PND3 (10), PND5 (5), PND7 (5)
	Postnatal exposure	PND2 (5), PND4 (5), PND8 (5)
	Gestation exposure	PND4 (5), PND8 (5)
	Control	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

they were housed in pairs in animal rooms under 12-h light-dark cycle conditions at  $22 \pm 1^\circ\text{C}$  and  $55 \pm 5\%$  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<sup>17,18)</sup> 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

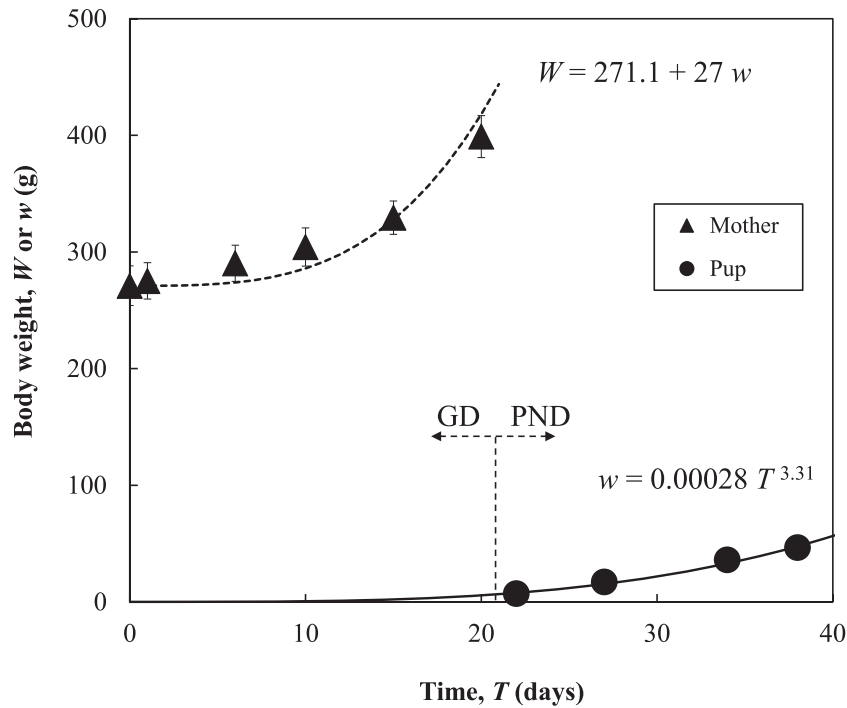
this apparatus and procedure have been given elsewhere<sup>11)</sup>. In order to study change in bromine ion in blood and brain when the condition of dysfunction of feedback inhibition (i.e., disinhibition) was confirmed, exposure concentration was designed to be 700 ppm, which was higher than LOAEL (400 ppm) for disinhibition<sup>7)</sup>. The actual concentration of 1-BP vapor in the chamber was  $701.3 \pm 5.2$  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 GD 1-20 (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 ml) at  $0^\circ\text{C}$ . The sample (1 ml) was dispensed into a vial, and 0.1 ml of dimethyl sulfate was added to convert bromine ion to methyl bromide. Then, 0.1 ml of an aqueous solution of isopropyl alcohol (0.5 volume percent) was added as an internal standard. The vial was heated at  $50^\circ\text{C}$  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)<sup>11)</sup>.

#### 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<sup>19)</sup>. We assumed the bromine ion uptake rate, i.e., the genera-



**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

tion rate of bromine ion, is equal to the 1-BP uptake rate because 1-BP is decomposed quickly<sup>(1)</sup> and releases bromine ion. Under this assumption, mass balance equations of bromine ion during exposure and clearance periods respectively were as follows:

$$\frac{dx}{dt} = R - kx \quad (1)$$

$$\frac{dx}{dt} = -kx \quad (2)$$

where  $x$  is the amount of bromine ion ( $\mu\text{g}$ );  $t$  is time (h);  $R$  is the generation rate of bromine ion ( $\mu\text{g/h}$ ), 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  $C$  ( $\mu\text{g/g}$ ) during exposure and clearance respectively were obtained as follows:

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

$$C = C_0 e^{-kt} \quad (4)$$

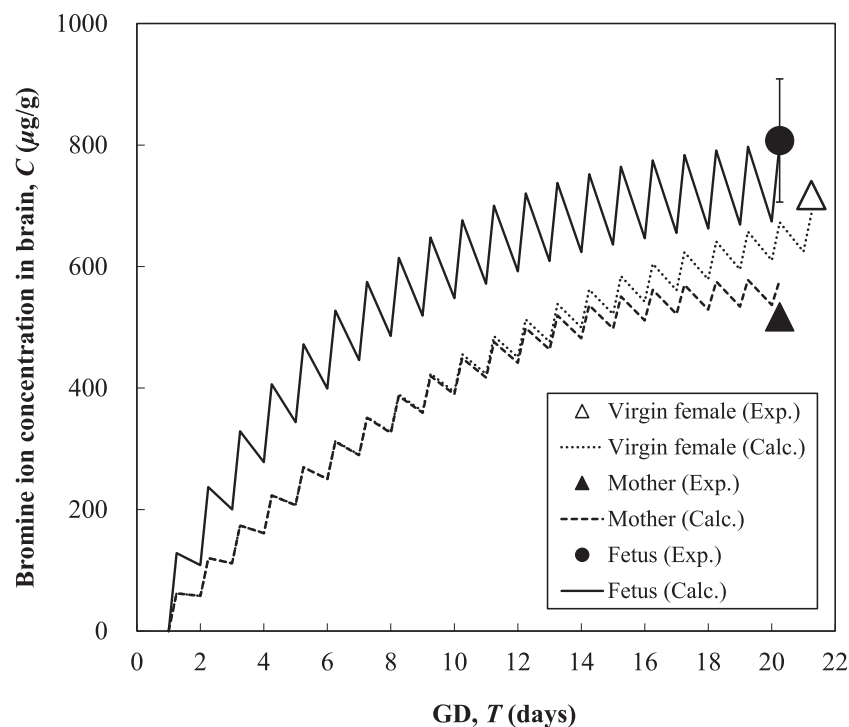
where  $V$  is the volume of the compartment (ml),  $\rho$  is the density of the compartment (g/ml), and  $C_0$  is the initial concentration during clearance ( $\mu\text{g/g}$ ). The excretion rate constant  $k$  is given by the biological half-life,  $t_{1/2}$  (h) or  $T_{1/2}$  (days).

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

## 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  $15.0 \pm 2.8$  and  $14.9 \pm 2.5$  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 ( $271.1 \pm 17.0$  g) during GD1-20.

Bromine ion concentration in the rat brain ( $\mu\text{g/g-brain}$ ) 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 ob-



**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

served 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  $11.2 \pm 7.7 \mu\text{g/g-brain}$  on PND3.

The bromine ion concentration in pup stomachs with milk from the exposure group on PND1 was  $830.6 \pm 188.8 \mu\text{g/g-stomach}$ , 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<sup>(1)</sup>,  $T_{1/2}$  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):

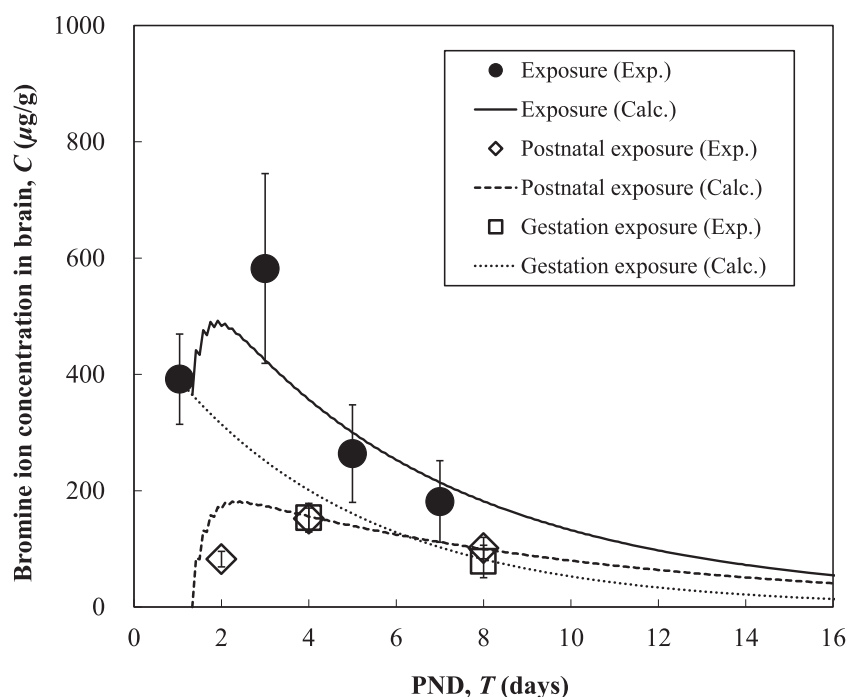
$$w=0.00028T^{3.31} \quad (6)$$

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

$$W=271.1+27w \quad (7)$$

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  $2853 \mu\text{g/h}$  was obtained to give the best fit of equations (3) and (4) for the experimental data on GD21 in Fig. 2. Therefore,  $R/\rho V=R/W=2853/271.1=10.5 \mu\text{g}/(\text{h} \cdot \text{g})$  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,



**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

and  $R/\rho V = R/w = 22.0 \mu\text{g}/(\text{h} \cdot \text{g})$  was applied, which was obtained to give the best fit for the experimental data on GD 20 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 PND 1, 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 ( $486.2 \mu\text{g}/\text{g}\text{-brain}$ ). 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:

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

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  $22 \mu\text{g}/(\text{h} \cdot \text{g})$ , 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  $55 \mu\text{g}/(\text{h} \cdot \text{g})$  was obtained at initial suckling time. This value was large compared to  $22 \mu\text{g}/(\text{h} \cdot \text{g})$ , 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 up-

**Table 2.** Parameters of the one-compartment model

	Groups	$T_{1/2}$ (days)	$\rho V$ (g)	$R$ ( $\mu\text{g}/\text{h}$ )	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  $\mu\text{g}/\text{g}$ -brain),  $w=0.00028T^{3.31}$  by equation (6)

take rate in pups was high immediately after birth.

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**Conflict of Interest:** None declared.

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