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Effects of 2-Bromopropane on the Female Reproductive Function in Sprague-Dawley Rats

Cheol Hong LIM, Seung Hee MAENG, Jun Yeon LEE, Yong Hyun CHUNG, Tae Gyun KIM, Jung Han PARK, Young Hahn MOON and Il Je YU*

Department of Industrial Toxicology, Industrial Health Research Institute, KISCO, 34-6 Kusan-dong, Bupyung-gu, Inchon 403-120, Korea

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Abstract: 2-Bromopropane (2BP) has been implicated to be the reason for the mass intoxication of workers at an electronic company in Korea. A case series study indicated that 2BP was the possible causative chemical for reproductive toxicity, causing severe anemia accompanied by amenorrhea among female workers, and azoospermia or oligospermia among male workers. To clarify the effect of 2BP on the female reproductive function, repeated doses of 2BP were tested on female Sprague-Dawley rats for 21 days. Ten rats were assigned to each treatment group. The rats were maintained in a 12 hr: 12 hr light-dark cycle and vaginal smears were monitored daily for 3 cycles. After the rats had completed 3 estrous cycles, vehicle control olive oil, 300 mg, 600 mg, and 900 mg/kg of 2BP were injected into intraperitoneum for 14 days. The female rats were then mated with male rats on a 1:1 ratio basis. The treatment continued for an additional 7 days. Rats treated with 2BP experienced a significant decrease in body weight gain depending on the dose of 2BP. The estrous cycles of the rats continued at a normal duration of time before the initiation of treatment, showing 4.32 days for the control group, 4.79 days for the low dose, 4.63 days for the middle dose, and 4.75 days of estrous cycle for the high dose group. 2BP treatment, however, induced a significant delay of the estrous cycle in the high dose treated group, showing 11.1 ±3.82 days of the estrous cycle. 2BP decreased the fertility and tended to decrease in the number of pups born, depending on the dose. A 900 mg/kg treatment of 2BP decreased ovarian weight, but 2BP did not affect the length of gestation. Our results indicated that 2BP seemed to be the causative agent for amenorrhea observed in female workers.

Key words: 2-Bromopropane, Isopropyl bromide, Female reproductive toxicity, Estrous cycle, Fertility, Pups

Introduction

A recent case report on the mass intoxication of workers at an electronic company at Yangsan in Korea reported that 17 of 25 female workers appeared to have an ovary dysfunction accompanying amenorrhea[1]. Eight workers with amenorrhea showed simultaneously findings of pancytopenia. Six of 8 male workers showed oligospermia or azoospermia. One worker with azoospermia was also shown to have pancytopenia[1]. The report further indicated that 2-bromopropane (2BP), a major component of Solvent 5200 which had been used as a cleaning solution in a tact switch manufacturing process, might be the possible causative chemical for reproductive and hematopoietic toxicity[1]. Little is known about the reproductive damage and other toxicities caused by 2BP. NIOSH RTECS described 2BP with 4,837 mg/kg of LD_{50} and 36 g/m^3 of LC_{50}[2]. A recent study on the LC_{50} of 2BP in ICR mice turned out to be 31,171 ppm[3].

*To whom correspondence should be addressed.

2BP was tested positive in the Ames test, and proved negative in both the micronucleus test which used experimental animals, and the chromosomal aberration test which used cultured Chinese hamster lung cells[4]. Recently the testicular and hematopoietic toxicities of 2BP have been identified[5–7]. The reports suggested that 2BP mainly targeted the reproductive and hematopoietic organs[5–7].

To clarify the effect of 2BP on the female reproductive function, female SD rats were repeatedly treated with 2BP for 21 days while their estrous cycles were monitored. The reproductive function of female rats were further studied by mating them with male rats. In this report, we describe the results of the female reproductive function experiment.

Method

Chemicals

2BP was obtained from Tokyo Chemical Industry (Japan). The purity of 2BP was 99.0% and no significant contaminants were detected. Vehicle, olive oil was purchased from Yakuri Pure Chemical Co. (Japan).

Animals and treatment

Female Sprague-Dawley (Crj:CD) rats (6 weeks old) were obtained from Daehan Animal Center (Korea) and allowed to acclimate for 2 weeks in a safety clean rack (MJ-721 CS(P), Myung-jin, Korea). Temperature and relative humidity were regulated within limits of 23 ± 2°C and 55 ± 7%, respectively. Male rats, used for mating, were obtained from Daehan Animal Center (Korea) when they were 8 weeks old and allowed to acclimate for 1 week. Rats were group-housed in poly propylene cages. Each cage contained 5 female rats and 3–4 male rats. Nesting material, CareFRESH (U.S.A) was obtained from Daehan Animal Center (Korea). Purina rodent chow (Korea) and water were provided *ad libitum*. The rats were kept in a controlled environment with a 12-hr light/dark cycle (light was between 7:00 and 19:00). Vaginal smears were made and monitored daily for 2 weeks. These 2 weeks were also used to allow the estrous cycle to complete 3 times before the initiation of treatment. Vaginal smears were made and monitored until the end of mating period. Rats were assigned randomly to four treatment groups (10/group) using a randomization system stratified by body weight one day before the initiation of 2BP treatment. The dose levels were 0, 300, 600, and 900 mg/kg body weight. 2BP was injected intraperitoneally once a day for 14 days until mating. Injection volume was adjusted to 2 ml/kg body weight using vehicle, olive oil. The animals were then allowed to mate with male rats on a one to one basis. Additional treatment was continued for 7 days.

Animals were examined daily for evidence of any treatment related effects including any respiratory, dermal, behavioral, nasal, or genitourinary changes suggestive of irritancy. Body weights were evaluated at the time of purchase and grouping, twice a week after the initiation of the experiment, and before necropsy.

Vaginal smear

Vaginal epithelial smears were made from each rat daily between 9:00 a.m. and 11:00 a.m. The vaginal smears were made from 2 weeks before the treatment and 21 days after initiation of the treatment. In order to cycle more regularly for female rats, male rats were housed in the same room with female rats during experiment period. The success of mating was determined by finding the sperm at the vaginal smear. The vaginal cavity of rat was thoroughly lavaged with 0.1 ml of phosphate buffered saline[8]. The cells taken were dropped over glass slides. The smears were then fixed with methanol and stained in Giemsa staining solution (Merck, Germany). The phases of the estrous cycle were determined by microscopic examination. The time intervals from the estrous to the next estrous were measured to calculate the mean length of estrous cycle.

Mating and fertility

To determine the fertility of females, mating was done by placing one adult male and one female into individual cages for 7 days. The presence of spermatozoa in the vaginal smear the following morning was defined as day 0 of pregnancy. Pregnant females were separated from male rats on day 0 of pregnancy and nonpregnant rats were separated from male rats after 7 days of co-habitation. Pregnant females were observed for weight development, pregnancy period and signs of intoxication.

Observation of dams and offsprings

Animals that had pups were anesthetized with diethyl ether (Junsei Chemical Co., Japan) and were sacrificed one day after the pup was born. All remaining animals were sacrificed 28 days after mating. During the autopsy of the animals, the weight of the ovaries, kidneys, spleen, and liver were measured. The fertility index, the number of pups born, the weight of pups born, pairing days until copulation, and the length of gestation were compared between the control and treatment groups. The pups born were counted, measured for their body weight, and examined for any morphological

abnormality.

Statistical analysis

Two-way ANOVA and Duncan's multiple range test were used to compare the body weight of the control with other three groups. Chi-square test was used to compare copulation index, fertility index and other parameters between the control and other experiment groups.

Results

Body and organ weight development

One female rat in 600 mg/kg treatment group had died due to internal bleeding caused by an injection needle perforating blood vessel during treatment. Female rats treated with 600 mg/kg and 900 mg/kg fell into a narcotic stage after 15 min of injection. Female rats treated with 2BP showed a significant decrease in body weight gain depending on the dose of 2BP (Fig. 2 and Table 1). Although female rats in 0, 300, and 600 mg/kg treated groups showed an increase of body weight during the gestation period, there was no increase of body weight at 900 mg/kg treatment group during the gestation period (Fig. 2). 2BP significantly decreased ovarian weight in the 900 mg/kg treated group (Table 1). Other organs including the kidneys, liver, spleen

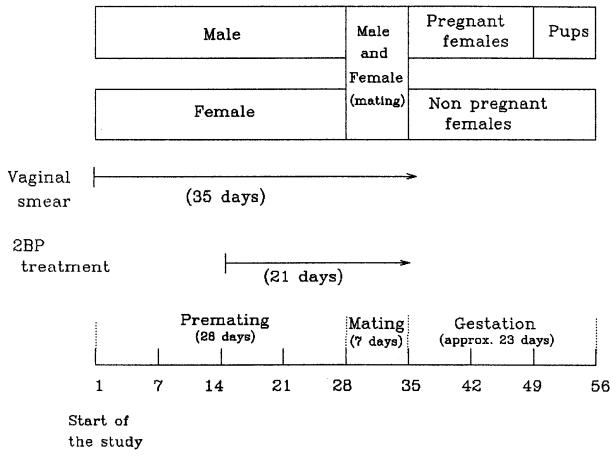

Fig. 1. Scheme of experiment

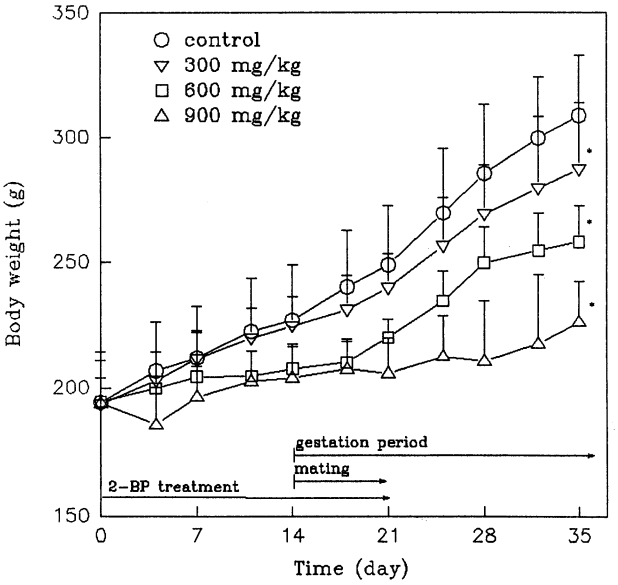

Fig. 2. Body weight change of female rats during 21 days of 2BP treatment and gestation period

Female rats in separate group were treated with 0, 300, 600, and 900 mg/kg of 2BP for 21 days. 10 female rats were assigned to each treatment group. The body weight was measured at the time of grouping and twice per week after the initiation of the experiment. *indicates $p<0.05$ versus control. The vertical bars indicate standard deviation.

Table 1. Effect of 2BP on the relative organ weight of female rats

	Control	300 mg/kg	600 mg/kg	900 mg/kg
No. of animal	10	9	9	7
Initial body weight (g)	194.3 ± 20.1	193.6 ± 10.5	194.8 ± 16.1	193.4 ± 10.2
Terminal body weight (g)	280.2 ± 15.9	270.8 ± 20.9	261.9 ± 10.5**	227.6 ± 37.6**
% body weight				
Ovary R	0.045 ± 0.008	0.049 ± 0.012	0.037 ± 0.009	0.029 ± 0.008**
Ovary L	0.046 ± 0.008	0.044 ± 0.012	0.037 ± 0.009	0.032 ± 0.010**
Kidney R	0.332 ± 0.028	0.350 ± 0.019	0.340 ± 0.035	0.349 ± 0.047
Kidney L	0.345 ± 0.026	0.353 ± 0.014	0.324 ± 0.023	0.359 ± 0.043
Spleen	0.296 ± 0.072	0.276 ± 0.030	0.276 ± 0.044	0.323 ± 0.066
Liver	4.377 ± 0.902	4.330 ± 0.567	4.292 ± 0.266	4.650 ± 0.908

Values are mean ± S.D. ** indicates $p<0.01$ versus control.

Table 2. Effect of 2BP on the relative ovary weight of female rats

	Control	300 mg/kg	600 mg/kg	900 mg/kg
Pregnant female				
No. of animals	9	6	3	1
Terminal body weight (g)	279.3 ± 16.6	270.7 ± 11.4	264.0 ± 16.8	217
Ovary R.	0.046 ± 0.008	0.052 ± 0.004	0.045 ± 0.009	0.039
Ovary L.	0.046 ± 0.009	0.046 ± 0.004	0.050 ± 0.005	0.048
Nonpregnant female				
No. of animals	1	3	6	6
Terminal body weight (g)	288	271.0 ± 37.7	260.8 ± 7.8	229.3 ± 40.9
Ovary R.	0.037	0.043 ± 0.020	0.034 ± 0.006	0.028 ± 0.008
Ovary L.	0.042	0.035 ± 0.016	0.037 ± 0.006	0.029 ± 0.008

did not show statistically significant organ weight changes (Table 1). When we compared the ovarian weight dividing the pregnant and the non-pregnant females, there was no statistical significant decrease of ovarian weight in the pregnant females (Table 2). Since only one female in the 900 mg/kg treatment group was pregnant and only one female in the control group was nonpregnant, it was difficult to compare the statistical significance among treated groups (Table 2).

Estrous cycle

The result of the estrous cycle monitoring is shown in Figure 3 and summarized in Table 3. The control and 300 mg/kg treatment did not affect the duration of the estrous cycle, but 900 mg/kg treatment delayed the estrous cycle significantly. The reason for the delay in the estrous cycle was mainly due to the prolongation of the diestrus stage. In the 900 mg/kg treatment group, the estrous cycles in many of the animals were sustained in the diestrus stage.

Effect of 2BP on dams and offsprings

After 14 days of 2BP treatment, female rats were then caged in pairs with male rats. Although all animals were successfully mated except 2 pairs; one was in the 300 mg/kg treatment group and another was in the 900 mg/kg treatment group, 2BP treatment resulted in a dose dependent decrease in pregnancy (Table 4). Among the 900 mg/kg treatment group only 1 female produced a pup. 2BP treatment showed a tendency in the number of pups to decrease depending on the dose. Four females died during post-treatment periods with unknown causes. One was among the 300 mg/kg treatment group and it included 5 dead fetuses. The others belonged to the 900 mg/kg treated group and they had no fetuses. Although necropsy was performed, no

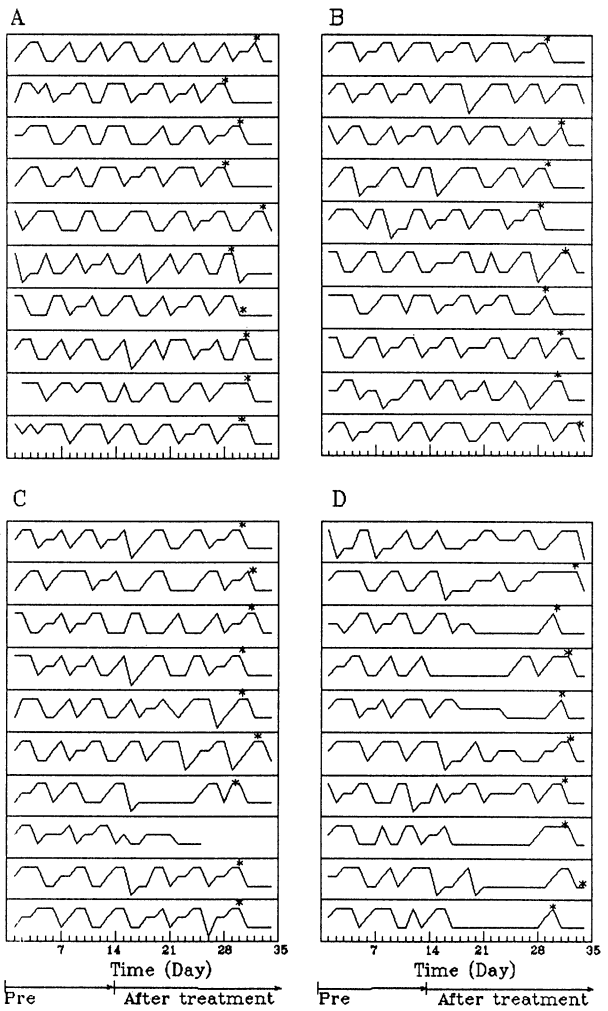

Fig. 3. The effect of 2BP on the estrous cycle
A, control; B, 300 mg/kg; C, 600 mg/kg; D, 900 mg/kg treated group. The Graph begins from the metestrus (Bottom) and goes to the diestrus, proestrus, and estrous (Top) stage. *indicates day 0 of pregnancy.

Table 3. Effect of 2BP on the estrous cycle

	Control	300 mg/kg	600 mg/kg	900 mg/kg
	(days)	(days)	(days)	(days)
No. of animals	10	10	9	10
Before treatment	4.32 ± 0.47	4.79 ± 0.25	4.63 ± 0.54	4.75 ± 0.49
After treatment	4.58 ± 0.40	4.94 ± 0.51	5.89 ± 2.37	11.05 ± 3.82*

( ) indicate the length of estrous cycle. Values are mean ± S.D. *indicates $p<0.05$ versus control.

Table 4. Effect of 2BP on the reproductive effects of female rats

	Control	300 mg/kg	600 mg/kg	900 mg/kg
No. of mated pairs	10	10	9	10
No. of copulated pairs	10	9	9	9
Copulation index(%)[(b)]	100	90	100	90
No. of pregnant females	9	7	3	1
Fertility index(%)[(a)]	90	78	33	11
Pairing days until copulation	2.2 ± 1.4	2.8 ± 1.4	2.8 ± 1.1	3.2 ± 1.2
Gestation length in days	23 ± 1.2	23 ± 0.8	23 ± 0.8	24
No. of pregnant females with pups alive	9	6	3	1
No. of pups born	9.8 ± 5.5	7.3 ± 5.0	6.7 ± 2.1	1
No. of pups born alive	9.8 ± 5.5	7.2 ± 4.8	6.0 ± 1.0	1
Weight of pups born	7.0 ± 1.2	7.0 ± 4.4	7.5 ± 0.5	4.7
Weight of pups born alive	7.0 ± 1.2	6.6 ± 1.3	7.4 ± 1.1	4.7

Values are mean ± S.D. [(a)], No. of pregnant animals/no. of animals with successful copulation. [(b)], No. of copulated pairs/no. of mated pairs.

other distinct gross pathological findings were observed. One dead pup was found in the 300 mg/kg treated group and two were found in the 600 mg/kg treated group, but there were no abnormal pups. It is unclear whether the weight of the pups was affected by 2BP treatment.

Discussion

Our study indicated that the reproductive toxicity was induced by 21 days of 2BP treatment in female rats. The normal estrous cycle of female rats 4–5 days were disrupted and were extended to 11 ± 2.8 days in the high dose of 2BP treatment. Our findings are very similar to those human female workers with secondary amenorrhea. Secondary amenorrhea states the absence of menstruation for 3 months or more in women with past menses[9]. Secondary amenorrhea showing high FSH levels greater than normal prolactin levels and symptoms suggestive of menopause was not due to strenous exercise or environmental stress but to ovarian failure[10, 11]. Although our results showed some accordance in disruption of normal estrus cycle and decrease of ovarian weight with the ovarian failure found in the female electronic workers, it was difficult to interpret that ovarian failure was caused by the administration of 2BP to female rats, since gonadotropin levels which are important diagnostic criteria were not measured in our experiment.

An additional important consequence of reproductive toxicity caused by 2BP is the decrease in the rate of pregnancy and the reduction in number of pups. It has been suggested that few live pups in treated females indicated a female reproductive toxicant[12], thus altered estrous cyclicity would substantiate this result. Althought our results did not have the necessary basic data including number of corpora lutea and number of implantation sites to attribute the reproductive toxicity of 2BP to the rate of pregnancy and the reduction in number of pups, the disruption of the estrous cycle and the decrease of ovary weight due to 2BP may induce the decrease in the fertility index and in the number of pups born.

Fertility and the number of pups born were also found to

decrease when other chemicals such as TCDD, 2.4-D, DDT and PCB were used, which caused a disruption of the estrous cycle[13–15]. Environmental pollutants such as PCB, 2.4-D and TCDD have been known to cause reproductive toxicity in female animals. TCDD has been known to disturb the estrous cycle in female rats by prolonging periods of diestrus and thus reducing the ovulatory rate[13]. 2.4-D has also been known to delay the diestrus period, and causes an increase in embryonal death[14]. DDT and PCB disturbed the estrous cycle and elevated the formation of follicular cysts, and altogether abolished litter production[15]. Although reproductive toxicity caused by environmental chemicals has been studied rather extensively, there are few reports regarding on female reproductive toxicity caused by DBCP or other halogenated propanes[16–19]. Ethylene bromide has been known to prolong the estrous cycle of rats[18]. 1,2-Dibromopropane disturbed the estrous cycle, reduced fertility, and increased postnatal mortality among the offsprings[19]. Interestingly no external abnormal pups were born from 2BP treated rats. It is speculated that 2BP may not be mutagenic in mammals since both chromosomal and micronucleus tests showed negative results[4].

2BP is now known to cause male reproductive toxicities including atrophy of the testes accompanying necrosis of germ cells in seminiferous tubules[5–7]. Hematopoietic toxicity of 2BP also has been confirmed[5–7]. Therefore, the main targets of 2BP are the reproductive organs and hematopoietic organs. To confirm the ovarian failure rather extensively, further studies on hormonal levels of luteinizing hormone and follicle-stimulating hormone in 2BP treated rats should be done. Teratogenicity of 2BP also should be studied extensively using more animals per dose group.

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