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Sprague-Dawley 랫드에서 2-Bromopropane의 배자치사 및 최기형성 효과

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Embryo lethality and teratogenicity of 2-Bromopropane in the Sprague-Dawley rat

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Abstract : The present study was undertaken to evaluate the potential adverse effects of 2-BP on pregnant dams and embryo-fetal development after maternal exposure during the gestational days (GD) 6 through 19 in Sprague-Dawley rats. The test chemical was administered subcutaneously to pregnant rats at dose levels of 0, 375, 750 and 1250 mg/kg/day. During the test period, clinical signs, mortality, body weights and food consumption were examined. All dams were subjected to caesarean section on GD 20 and their fetuses were examined for external, visceral and skeletal abnormalities. At above 750 mg/kg, toxic effects including signs of toxicity, suppressed body weight, decreased gravid uterine weight and reduced food intake were observed in pregnant dams. An increase in the fetal deaths, a decrease in the litter size, a reduction in the fetal body weight and an increase in the incidence of fetal morphological alterations were also found. There were no adverse effects on either pregnant dams or embryo-fetal development at a dose level of 375 mg/kg. These results suggest that a 14-day subcutaneous dose of 2-BP is embryolethal and teratogenic at above 750 mg/kg/day in pregnant rats. In the present experimental condition, the no-observed-adverse-effect level of 2-BP is considered to be 375 mg/kg/day for dams and embryo-fetuses, respectively.

Key words : 2-bromopropane, maternal toxicity, developmental toxicity, teratogenicity, rats

Introduction

2-Bromopropane (2-BP, CAS No. 75-26-3), a halogenated propane analogue, is a substitute for chlorofluorocarbones (CFCs) which have a great potential to destroy the ozone layer and to warm the earth's environment. Because this chemical is nonflammable and volatile and is easily broken down in the environment and is

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less destructive to the ozone layer than CFCs, it has been used as one of the alternative solvents. In 1995 a cluster of patients with amenorrhea, oligozoospermia, and anemia was discovered in Korean workers exposed to solvent containing 2-BP. Epidemiological studies suggested that 2-BP might be the causative agent of these health disorders [14, 24]. Since the outbreak of reproductive disorders after exposure to 2-BP in Korea, several extensive animal studies have been conducted to determine the potential adverse effects of 2-BP on reproductive, hematopoietic, central nervous, and immune systems [6, 23, 27, 30-32]. Maeng and Yu [16] reported that 2-BP exhibited no mutagenic effects on mouse bone marrow cells as determined by *in vivo* chromosome aberration and *in vivo* micronucleus tests, but Ishikawa *et al.* [7] showed an induction of micronuclei formation in preimplantation mouse embryos after maternal treatment with 2-BP accompanied by a decrease in embryo cell number. Recent *in vitro* studies also showed that 2-BP is an apparent DNA damaging agent [30, 32]. These positive results strongly suggest that the DNA damage by 2-BP might be involved in the various toxicities induced by 2-BP. Reproductive organ toxicity studies showed that the testicular or ovarian dysfunction induced by 2-BP exposure resulted from damaging the early types of spermatogenic cells in male rats or primordial follicles and their oocytes in female rats [23, 31]. According to a recent pre- and postnatal developmental toxicity study [8], repeated subcutaneous injection of 2-BP to pregnant/lactating female rats showed decreased delivery rate, increased peri- and postnatal deaths, suppressed body weight development, and increased incidence of reproductive organ dysfunction of F1 offspring at dose levels of 405 mg/kg or greater. However, the potential adverse effects of 2-BP on embryo-fetal development have never been studied yet.

This study was conducted to determine the potential effects of 2-BP on pregnant dams and embryo-fetal development in Sprague-Dawley rats when administered from days 6 through 19 of gestation.

Materials and Methods

Animal husbandry and maintenance

Nulliparous male and female Sprague-Dawley rats aged 10 weeks were obtained from a specific pathogen free colony at Bio Genomics Inc. (Seoul, Korea) and used after one week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23±3°C and a relative humidity of 50±10% with artificial lighting from 08:00 to 20:00 and with 13~18 air changes per hour. Only healthy animals were assigned to the study. For mating, two females were placed into stainless-steel wire-mesh cages of one male overnight. Successful mating was ascertained by the presence of sperm in a vaginal smear, and the following first 24h was designated as day 0 of gestation. Mated females were housed singly in clear polycarbonate cages with stainless steel wire lids and were allowed sterilized tap water and commercial rodent chow (PMI Nutrition International, IN, USA) *ad libitum*. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International, and animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals [22].

Test chemical and treatment

2-BP was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). The chemical purity was >99% by gas chromatography. The test chemical was dissolved in a corn oil (Sigma Chemical Co., MO, USA) solution as a vehicle and was freshly prepared before the treatment. The daily application volume was calculated in advance based on the most recently recorded body weight of the individual animal. 2-BP was administered subcutaneously to pregnant rats from GD 6 through 19 with a dose volume of 5 ml/kg body weight. The vehicle control rats received an equivalent volume of corn oil alone.

Experimental groups

Healthy female rats were assigned randomly to four experimental groups: three treatment groups of 2-BP 375, 750 and 1250 mg/kg and a vehicle control group (n=12 inseminated females per group).

Selection of doses

The dose levels were determined based on the results of a previous study [7] in which 2-BP at above 900 mg/kg caused an induction of micronuclei formation in preimplantation mouse embryos after maternal treatment with 2-BP accompanied by a decrease in embryo cell number. A dose of 1250 mg/kg was selected as the highest dose and doses of 750 and 375 mg/kg were selected

as middle and low doses, respectively. This range of dose levels encompassed the highest dose levels used by Ichihara *et al.* [6], Omura *et al.* [23], and Kang *et al.* [8] to determine the reproductive toxic potential of 2-BP.

Observation of dams

All pregnant females were observed daily throughout gestation for mortality, moribundity, general appearance and behavior. Maternal body weights were measured on GD 0, 6, 9, 12, 15 and 20 and individual food consumption were determined on GD 1, 7, 10, 13, 16 and 20. At scheduled termination (GD 20), all pregnant females were euthanized by carbon dioxide overdose and subjected to external and internal macroscopic examination.

Caesarean section

The ovaries and uterus of each female were removed and examined for the number of corpora lutea and the status of all implantation sites, i.e., live and dead fetuses, early and late resorptions and total implantations. Resorption was classified as "early" when only placental tissue was visible and "late" when placental and embryonic tissue were visible at caesarean section. Live fetuses and their placentas were weighed individually. All live fetuses were sexed and evaluated for external morphological abnormalities including cleft palate. Alternate fetuses were selected for either skeletal or visceral examination. The skeletal evaluation of 5% formalin-fixed fetuses was performed after staining the skeleton with Alizarin Red S and clearing with potassium hydroxide solution by the modified Dawson's method [4]. For the visceral examination of Bouin's fluid-fixed fetuses, we adapted a freehand razor sectioning technique [28] for the head and abdomen, and Nishimura's method [21] for the thorax. External, visceral and skeletal findings were classified as developmental malformations, variations, or retardations. We have used the terminology suggested in an internationally developed glossary of terms for structural developmental abnormalities in common laboratory mammals [29].

Statistical analysis

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SAS software [25]. The unit of comparison was the pregnant dam or the litter. Continuous data variables such as maternal body weight, food consumption, fetal body weight and placental weight were subjected to one-way analysis of variance (ANOVA), and Scheffe's multiple comparison test was conducted when analytic results were significant [26]. The numbers of corpora lutea, total implantations, live and dead fetuses were statistically evaluated using the Kruskal-Wallis nonparametric ANOVA [15], followed by the Mann-Whitney U test when appropriate. Incidence data such as external, visceral and skeletal abnormalities were compared using the Fisher's exact probability test [5]. Male-to-female sex ratio and the proportions of litters with malformations and developmental variations were compared using the chi-square test and Fisher's exact probability test. The difference was considered statistically significant when $P \leq 0.05$.

Results

Maternal toxicity

The maternal findings for the pregnant rats treated with 2-BP subcutaneously on days 6 through 19 of pregnancy are presented in Table 1. All females survived in both control and treated groups throughout the study. Pregnant rats of the 750 and 1250 mg/kg groups showed treatment-related clinical signs such as dull fur and reddish tear from the eyes, which were dose-dependent in incidence and severity (data not shown). No treatment-related clinical findings were observed in the 375 mg/kg group. Maternal body weights on GD 15 and 20 in the 750 and 1250 mg/kg groups were significantly suppressed when compared with the vehicle control group. Maternal body weight gain for the intervals GD 6-20 (treatment period) and corrected body weight in the groups were also significantly lower than those in the vehicle control group. Food consumption was significantly decreased on GD 20 in the high dose group, in comparison with the vehicle control group. At autopsy of dams, no treatment-related pathological alterations were observed in any treated group (data not shown).

Developmental toxicity

The reproductive findings for the pregnant rats treated with 2-BP subcutaneously on days 6 through 19 of pregnancy are summarized in Table 2. No significant differences were observed in the number of corpora lutea, implantation, and sex ratio in the treatment groups, compared with those of the vehicle control group. However, the number of fetal deaths

Table 1. Maternal findings of pregnant rats treated with 2-bromopropane during gestational days 6 through 19[a]

Parameters	2-Bromopropane (mg/kg/day)			
	0	375	750	1250
Number of pregnant animals	10	11	12	11
Body weight (g)				
Day 0	228.6±12.8	227.2±14.4	228.2±15.3	226.7±13.8
Day 6	264.5±13.0	260.7±14.0	261.1±11.0	256.8±13.9
Day 9	280.2±12.7	278.2±13.6	273.9±16.3	269.4±15.7
Day 12	298.8±14.8	293.6±15.8	290.0±11.8	281.4±20.9
Day 15	334.1±13.6	325.4±16.0	303.9±15.4**	290.6±19.1**
Day 20	400.6±20.5	386.8±22.3	342.1±30.0**	316.3±35.6**
Body weight gain (g)				
Days 0- 6 (pre-treatment period)	35.9±6.0	33.5±6.5	32.9±8.4	30.1±4.4
Days 6-20 (treatment period)	126.1±16.8	116.1±17.8	81.0±25.3**	59.4±33.6**
Gravid uterine weight (g)	95.3±10.9	89.7±12.1	44.5±29.3**	23.8±26.5**
Corrected body weight (g)[b]	305.3±30.4	297.1±34.4	297.6±29.2	292.5±18.4
Food consumption (g)				
Day 1	20.3±2.94	21.8±3.10	21.1±2.66	22.4±2.52
Day 7	23.8±3.66	23.7±3.78	22.1±3.06	23.6±2.85
Day 10	26.4±4.25	25.5±3.05	23.1±1.70	25.6±3.23
Day 13	26.2±3.17	26.8±4.11	25.1±2.91	27.7±5.53
Day 16	25.8±3.64	26.0±3.88	24.0±4.15	21.3±6.01
Day 20	28.1±3.27	27.5±5.41	25.6±3.90	18.9±5.64**

[a]Values are presented as means±SD for all pregnant animals.
[b]Corrected body weight = body weight on gestational day 20 - gravid uterine weight
**Significant difference at $p<0.01$ level compared with the vehicle control group.

Table 2. Reproductive findings of pregnant rats treated with 2-bromopropane during gestational days 6 through 19

Parameters	2-Bromopropane (mg/kg/day)			
	0	375	750	1200
No. of females mated	12	12	12	12
No. of pregnant animals	10	11	12	11
No. of corpora lutea[a]	16.5±2.03	17.2±2.65	16.8±2.44	16.3±1.68
No. of implantations[a]	15.2±2.17	15.0±3.32	14.0±2.95	15.9±1.76
No. of fetal deaths[b]	0.5±1.06	0.6±0.95	6.9±4.89**	10.6±6.09**
Resorptions: Early	0.5±1.06	0.6±0.95	0.8±1.59	3.4±6.97
Late	0	0	6.1±5.04**	7.1±6.00**
Dead fetuses	0	0	0	0.2±0.60
Postimplantation loss (%)[a,c]	3.2±7.43	4.0±9.17	53.5±39.96**	68.5±38.66**
No. of litters totally resorbed	0	0	4	5
Litter size[a]	14.5±2.28	12.5±3.50	6.8±6.56**	5.1±6.39**
Male/female	75/74	71/67	41/44	29/27
Sex ratio (male/female)	1.01	1.06	0.93	1.07
Fetal body weight (g): Male[a]	3.93±0.17	3.76±0.20	3.20±0.53**	2.45±0.55**
Female[a]	3.78±0.14	3.57±0.15	3.15±0.48**	2.50±0.43**

[a]Values are presented as means±SD.
[b]Fetal deaths=resorptions+death fetuses
[c]Post-implantation loss (%) = [(no. of implantation sites - no. of live fetuses)/no. of implantation sites]×100
**Significant difference at $p<0.01$ level compared with the vehicle control group.

Table 3. External alterations in fetuses from pregnant rats treated with 2-bromopropane during gestational days 6 through 19

Parameters	2-Bromopropane (mg/kg/day)			
	0	375	750	1250
Fetuses examined	149	138	85	56
Litters examined	10	11	8	6
Fetuses with malformations (%)[a]	0	0	13**(15.3)	4** (7.1)
Litters affected (%)[b]	0	0	4 (50.0)	3 (50.0)
Kinked tail	0	0	7	2
Acaudate	0	0	1	2
Protruding tongue	0	0	5	0

[a]A single fetus may be represented more than once in listing individual defects.
[b]Includes litters with one or more affected fetuses.
**Significant difference at $p<0.01$ level compared with the vehicle control group.

Table 4. Visceral alterations in fetuses from pregnant rats treated with 2-bromopropane during gestational days 6 through 19

Parameters	2-Bromopropane (mg/kg/day)			
	0	375	750	1250
Fetuses examined	73	67	41	27
Litters examined	10	11	8	6
Fetuses with malformations (%)[a]	0	0	3 (7.3)	0
Litters affected (%)[b]	0	0	3 (37.5)	0
Microphthalmia	0	0	1	0
Anophthalmia	0	0	2	0
Fetuses with variations (%)[a]	5 (6.8)	6 (9.0)	10* (24.4)	5 (18.5)
Litters affected (%)[b]	4 (40.0)	5 (45.5)	6 (75.0)	3 (50.0)
Dilated renal pelvis	0	2	0	1
Dilated ureter	2	4	6	2
Misshapen thymus	4	5	4	3

[a]A single fetus may be represented more than once in listing individual defects.
[b]Includes litters with one or more affected fetuses.
*Significant difference at $p<0.05$ level compared with the vehicle control group.

exhibited a dose-related increasing trend and was significantly increased in the 750 and 1250 mg/kg groups. Because of the high percentage of resorptions, the numbers of live fetuses were significantly decreased in the middle and high dose groups, in comparison with the vehicle control group. The body weights of male and female fetuses were statistically significantly decreased in the 750 and 1250 mg/kg groups when compared with those in the vehicle control group.

The types and incidences of fetal external malformations are shown in Table 3. The incidences of malformed fetuses in the 750 and 1250 mg/kg groups were significantly increased when compared with those in the vehicle control group. Although the number of litters with affected fetuses in the groups were also higher than controls, the difference was not statistically significant among the groups. External malformations appeared in 13 of the 85 fetuses, in 4 of the 8 litters, at a dose level of 750 mg/kg and 4 fetuses of the 56 fetuses, in 3 of the 6 litters examined, at 1250 mg/kg. Major external anomalies observed were kinked tail, acaudate and protruding tongue.

As shown by the data in Table 4, there were no statistically significant increases in the number of malformed fetuses or of litters with viscerally malformed fetuses among the groups. Visceral malformations occurred 3 of the 41 fetuses, in 3 of the 8 litters, at 750 mg/kg. The incidences of visceral variations were 5 (6.8%), 6 (9.0%), 10 (24.4%), and 5 (18.5%) in the vehicle control, 375, 750, and 1250 mg/kg groups, respectively. The

Table 5. Skeletal alterations in fetuses from pregnant rats treated with 2-bromopropane during gestational days 6 through 19

Parameters	2-Bromopropane (mg/kg/day)			
	0	375	750	1250
Fetuses examined	76	71	44	29
Litters examined	10	11	8	6
Fetuses with malformations (%)[a]	0	1 (1.4)	2 (4.5)	3 (10.3)
Litters affected (%)[b]	0	1 (9.1)	2 (25.0)	3 (50.0)
Absent cervical arch	0	0	1	0
Fused cervical arch	0	0	0	1
Absent sacral/caudal vertebra	0	0	1	2
Hemicentric thoacic centrum	0	1	0	0
Fetuses with variations (%)[a]	12 (15.8)	8 (11.3)	7 (15.9)	13* (44.8)
Litters affected (%)[b]	6 (60.0)	5 (45.5)	3 (37.5)	6 (100.0)
Enlarged fontanel	0	1	0	0
Bipartite ossification of thoracic centrum	3	2	3	5
Bipartite ossification of sternebra	1	0	1	1
Cervical rib	0	2	3	3
Misshapen sternebra	0	1	0	1
Misaligned sternebra	0	1	0	0
Short supernumerary rib	9	4	0	3
Short 13th rib	1	1	0	0
Unossified pubis	1	0	0	0
Fetuses with retardations (%)[a]	13 (17.1)	14 (19.7)	11 (25.0)	11 (37.9)
Litters affected (%)[b]	7 (70.0)	7 (63.6)	6 (75.0)	5 (83.3)
Dumbbell ossification of lumbar centrum	1	2	0	0
Dumbbell ossification of thoracic centrum	10	9	6	9
Incomplete ossification of interparietal	1	1	0	2
Incomplete ossification of pubis	0	2	7	3
Incomplete ossification of supraoccipital	0	2	2	1
No. of ossification centers[c]				
Sternebra	4.9±0.56	4.8±0.64	4.1±0.57*	3.5±0.68**
Metacarpals in both forelimbs	7.3±0.71	7.3±0.79	6.3±0.78	7.2±0.94
First phalanges in both forelimbs	1.7±1.96	1.6±1.54	1.5±2.77	0.2±0.41
Metatarsals in both hindlimbs	8.0±0.09	8.0±0.55	7.8±0.71	7.5±0.86
First phalanges in both hindlimbs	0.3±0.55	0.2±0.84	0	0
Sacral and caudal vertebra	7.9±0.45	8.0±0.27	7.6±0.60	7.1±0.69*

[a]A single fetus may be represented more than once in listing individual defects.
[b]Includes litters with one or more affected fetuses.
[c]Values are presented as means±SD.
*Significant difference at $p<0.05$ level compared with the vehicle control group.
** Significant difference at $p<0.01$ level compared with the vehicle control group.

number of fetuses with visceral variations at 750 mg/kg was significantly increased when compared with the vehicle control group.

The types and incidences of fetal skeletal malformations, variations, and retardations are shown in Table 5. Although the incidences of fetal skeletal malformations did not differ significantly among the groups, these findings including absent cervical arch, fused cervical arch and absent sacral/caudal vertebra observed in the 750 and 1250 mg/kg groups are uncommon in normal Sprague-Dawley rats [1, 11, 17, 19]. Skeletal malformations occurred in 1 of the 71 fetuses, in 1 of

the 11 litters, at 375 mg/kg, 2 of the 44 fetuses, in 2 of the 8 litters, at 750 mg/kg, and 3 of the 29 fetuses, in 3 of the 6 litters examined, at 1250 mg/kg. The incidences of skeletal variations were 12 (15.8%), 8 (11.3%), 7 (15.9%) and 13 (44.8%); retardations 13 (17.1%), 14 (19.7%), 11 (25.0%), and 11 (37.9%); in the vehicle control, 375, 750 and 1250 mg/kg groups, respectively. The incidence of fetuses with skeletal variations in the 1250 mg/kg group was significantly higher than that in the vehicle control group. There was some evidence of treatment-related reductions in the ossification of fetal skeleton. The number of ossification center of sternebra in the 750 and 1250 mg/kg groups and sacrocaudal vertebra in the 1250 mg/kg group was significantly lower than that in the vehicle control group.

Discussion

The present study was conducted to evaluate the potential developmental toxicity of 2-BP injected sucbcutaneously to Sprague-Dawley rats at dose levels of 375, 750, and 1250 mg/kg/day on days 6 through 19 of pregnancy. The results of the study showed that the administration of 2-BP during pregnancy resulted in significant developmental toxicity in rats.

The maternal toxicity observed in the 750 and 1250 mg/kg groups included abnormal clinical signs, suppressed body weight and decreased food intake. Treatment related clinical signs on pregnant rats, as evidenced by increased incidence of dull fur and reddish tear, were observed at dose levels of 750 or greater. These clinical signs observed in this study were indications of stress induced by the treatment of 2-BP. The dose-dependent suppression of body weight and body weight gain with increasing dose indicates that this finding is caused by the administration of 2-BP. The decrease of food intake observed in the high dose group was also considered to be a treatment-related effect because the change was consistent with the suppressed body weight and showed a dose-response relationship. The decreased gravid uterine weight observed in the middle and high dose groups may be largely due to the increased post-implantation loss and the decreased fetal body weight. This is well supported by the fact that the corrected body weight in the groups was not affected by 2-BP treatment.

The developmental toxicity of 2-BP included an increase in the fetal deaths, a decrease in the litter size, a reduction in the fetal body weight and an increase in the incidence of fetal morphological alterations at dose levels of above 750 mg/kg. The dose-dependent suppression of male and female fetal body weights with increasing dose indicates that this finding is caused by the administration of 2-BP. It is well known that a reduction in the fetal body weight is an indicator of intrauterine retardation effects. The reduction in fetal body weight was consistent with the fetal ossification delay, i.e., decreased ossification centers of sternebra and sacrocaudal vertebra. The dose-related increase in the incidence of malformed fetuses suggests that the teratogenic effect is closely related to the administration of 2-BP. The predominant signs of fetal abnormal development observed in this study were detected in vertebra and rib.

According to the results of previous toxicity studies [6, 23, 27, 30-32], administration of 2-BP to experimental animals caused various adverse effects on reproductive organs, bone marrow, central nervous system, and immune system. Reproductive organ toxicity studies showed that the testicular or ovarian dysfunction induced by 2-BP treatment resulted from damaging the early types of spermatogenic cells in males or primordial follicles and their oocytes in females, indicating that highly proliferating cells/organs are primary targets of 2-BP [23, 31]. Recently, it was reported that 2-BP induces DNA damage, impairs functional antioxidant cellular defenses, and enhances the lipid peroxidation in cultured Leydig cells [30]. More recently, Zhao *et al.* [32] reported the formation of N^7-isopropyl guanine as an adduct product with a reaction of 2`-deoxyguanosin and 2-BP at a physiological condition. The above results strongly suggest that the DNA damage by 2-BP might be involved in various toxicities induced by 2-BP exposure. It has been well described that most of the DNA damaging agents are highly embryotoxic and/or teratogenic in experimental animals [3, 12]. As for the adverse effects of 2-BP on embryo-fetal and F1 offspring development, a recent study by Ishikawa *et al.* [7] showed an induction of micronuclei formation in preimplantation mouse embryos after maternal treatment with 2-BP accompanied by a decrease in embryo cell number. According to a pre- and postnatal developmental toxicity study by Kang *et al.* [8], repeated subcutaneous injection of 2-BP to pregnant/lactating female rats resulted in a decrease

in delivery rate, an increase in peri- and postnatal deaths, and an increase in reproductive organ dysfunction of F1 offspring at a dose level of 1215 mg/kg. The results of above investigators and ours clearly showed that the environmental pollutant 2-BP is an apparent developmental toxicant in rat embryo-fetuses having a very high cell proliferation rate.

In the standardized developmental toxicity study [12, 13], it is difficult to distinguish between a direct effect of a test chemical on embryo-fetus and a secondary effect to maternal toxicity when developmental effects are observed in the presence of maternal toxicity. According to the reports of Khera [9], maternal toxicity caused by diverse chemical and physical agents invariably causes increased incidence of malformed fetuses and increased number of embryonal resorptions and fetal deaths. In contrast, Chernoff *et al.* [2] reported that overt maternal toxicity as defined by maternal lethality and decreased maternal body weight gain is not always associated with the same defined syndrome of adverse developmental effects. Thus, the relationship between maternal toxicity and adverse developmental toxicity during pregnancy still remains a critical issue in developmental toxicity studies [1]. Meanwhile, transfer and compartmentalization of the chemical from pregnant dam to fetus through the placenta is an important factor for determining embryo-fetal developmental toxicity [10, 20]. Placental transfer is significantly moderated by free drug characteristics (such as lipid solubility, degree of ionization and molecular weights) and placental properties (maternal and fetal blood flow, drug metabolism and placental age). It is evident that chemicals with a molecular weight less than 600 may readily transmigrate through the placenta [18]. In the case of the present study, although adverse effects of 2-BP on embryo-fetal development appeared to be accompanied by maternal toxicity at above 750 mg/kg, the maternal toxic effect of 2-BP was much lesser than the developmental toxic effect of 2-BP. Moreover, the molecular weight of 2-BP is 123 (less than 600), indicating that this chemical can readily pass the blood-placental barrier. Therefore, it is considered that the developmental toxicity induced by 2-BP is a direct effect on rat embryo-fetuses and that rat embryo-fetuses are more susceptible than their dams to adverse effects of 2-BP treatment, indicating that this chemical has a high potential for causing developmental toxicity when administered to pregnant dams.

Based on the results, it was concluded that 2-BP was embryolethal and teratogenic at a minimally maternally toxic dose in Sprague-Dawley rats. In the present experimental condition, the no-observed-adverse-effect level of 2-BP is considered to be 375 mg/kg/day for dams and embryo-fetuses, respectively.

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