J. Toxicol. Pub. Health
Vol. 19, No. 3, pp. 227-234 (2003)

Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Assessment of Embryotoxicity of 2-Bromopropane in ICR Mice

Jong-Choon Kim[1], Dong-Ho Shin[1], Sung-Ho Kim[1], Ki-Seok Oh[1], Hyeon-Yeong Kim[2], Jeong-Doo Her[3], Cheng-Zhe Jiang[3] and Moon-Koo Chung[3]

[1]College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea
[2]Industrial Chemicals Research Center, Industrial Safety and Health Research Institute, Korea Industrial Safety Corporation, Daejeon 305-380, Korea
[3]Korea Institute of Toxicology, KRICT, Daejeon 305-600, Korea

Received August 4, 2003; Accepted August 21, 2003

ABSTRACT. 2-Bromopropane (2-BP), 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. 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 17 in ICR mice. The test chemical was administered subcutaneously to pregnant mice at dose levels of 0, 313, 625 or 1,250 mg/kg/day. All dams were subjected to caesarean section on GD 18 and their fetuses were examined for external, visceral and skeletal abnormalities. In the 1,250 mg/kg group, maternal toxicity included an increase in the incidence of abnormal clinical signs and a decrease in the maternal body weight, body weight gain, and corrected body weight. Developmental toxicity included a decrease in the fetal body weight, a reduction in the placental weight, an increase in the fetal skeletal variation and ossification delay. There were no adverse effects on either pregnant dams or embryo-fetal development in the 313 and 625 mg/kg groups. These results suggest that a 12-day subcutaneous dose of 2-BP is embryotoxic at a maternally toxic dose (i.e., 1,250 mg/kg/day) in ICR mice. In the present experimental condition, the no-observed-adverse-effect level of 2-BP is considered to be 625 mg/kg/day for dams and embryo-fetuses, respectively.

Keywords: 2-Bromopropane, Maternal toxicity, Developmental toxicity, Teratogenicity, Pregnant mice.

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 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 were 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 (Kim *et al.*, 1996; Park *et al.*, 1997). 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 (Ichihara *et al.*, 1997; Omura *et al.*, 1999; Son *et al.*, 1999; Wu *et al.*, 2002; Yu *et al.*, 1999; Zhao *et al.*, 2002). Maeng and Yu (1997) 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.* (2001) 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 (Wu *et al.*, 2002; Zhao *et al.*, 2002). 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

Correspondence to: Jong-Choon Kim, Department of Toxicology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea
E-mail: toxkim@chonnam.ac.kr

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 (Omura *et al.*, 1999; Yu *et al.*, 1999). According to a recent pre- and postnatal developmental toxicity study (Kang *et al.*, 2002), 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.

The aim of the present study was to determine the potential effects of 2-BP on pregnant dams and embryo-fetal development in ICR mice when administered on days 6 through 17 of gestation.

MATERIALS AND METHODS

Animal Husbandry and Maintenance

Nulliparous male and female ICR mice 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 to 18 air changes per hour. Only healthy animals were assigned to the study. For mating, two females were placed into the cage of each male overnight. Successful mating was ascertained by the presence of a vaginal plug, and the following first 24 h 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* (NRC, 1996).

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) aqueous vehicle and was freshly prepared daily 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 mice from GD 6 through 17 with a dose volume of 5 ml/kg body weight. The vehicle control mice received an equivalent volume of corn oil alone. Although the major exposure route of 2-BP is dermal, the subcutaneous route was selected by reasons that a greater amount of the chemical can be dosed by the subcutaneous route rather than the dermal route, that the subcutaneous dose provides accuracy in estimating the amount of test article taken into the organism, and that the absorption route of these routes may be similar.

Experimental Groups

Healthy female mice were assigned randomly to four experimental groups: three treatment groups of 2-BP receiving 313, 625 or 1,250 mg/kg/day and a vehicle control group (n=11 inseminated females per group).

Selection of Doses

The dose levels were determined based on the results of a previous study (Ishikawa *et al.*, 2001) 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 1,250 mg/kg/day was selected as the highest dose and doses of 625 and 313 mg/kg/day were selected as middle and low doses, respectively, using a common ratio of 2. This range of dose levels encompassed the highest dose levels used by Ichihara *et al.* (1997), Omura *et al.* (1999), and Kang *et al.* (2002) to determine the reproductive toxic potential of 2-BP.

Observation of Dams

All pregnant females were observed daily throughout gestation for mortality, morbundity, general appearance and behavior. Maternal body weights were measured on GD 0, 6, 9, 12, 15 and 18 and individual food consumption was determined on GD 1, 7, 10, 13, 16 and 18. At scheduled termination (GD 18), 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 implanta-

tions. 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 (1926). For the visceral examination of Bouin's fluid-fixed fetuses, we adapted a freehand razor sectioning technique (Wilson, 1965) for the head and abdomen, and Nishimura's method (1974) 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 (Wise *et al.*, 1997).

Statistical analysis

Statistical analyses were performed by comparing the treatment groups with the vehicle control group using SAS software (SAS Institute, Inc., 1997). 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 (Scheffe, 1953). The numbers of corpora lutea, total implantations, live and dead fetuses were statistically evaluated using the Kruskal-Wallis nonparametric ANOVA (Kruskal and Wallis, 1952), 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 (Fisher, 1970). 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 mice treated with 2-BP subcutaneously on days 6 through 17 of pregnancy are presented in Table 1. All females survived in both control and treated groups throughout the study. Pregnant mice of the 1,250 mg/kg group showed treatment-related clinical signs such as dull fur, reddish

Table 1. Maternal findings of pregnant mice treated with 2-bromopropane during gestational days 6 through 17[a]

Parameters	2-Bromopropane (mg/kg/day)			
	0	313	625	1250
Number of pregnant animals	11	11	11	11
Body weight (g)				
Day 0	31.5 ± 1.96	31.4 ± 2.17	31.7 ± 2.67	30.7 ± 2.19
Day 6	33.5 ± 2.25	33.5 ± 2.39	33.8 ± 2.78	32.4 ± 2.10
Day 9	36.7 ± 2.34	37.2 ± 2.91	37.3 ± 2.64	34.6 ± 2.71
Day 12	43.0 ± 3.31	43.6 ± 3.62	43.9 ± 3.01	39.2 ± 4.54
Day 15	52.2 ± 4.76	53.0 ± 4.54	53.6 ± 3.70	44.8 ± 8.10*
Day 18	63.1 ± 7.10	64.4 ± 6.77	63.0 ± 6.11	49.8 ± 10.9**
Body weight gain (g)				
Days 0~6 (pre-treatment period)	2.1 ± 1.16	2.1 ± 1.27	2.1 ± 1.56	1.7 ± 1.15
Days 6~18 (treatment period)	29.6 ± 5.58	30.9 ± 5.20	29.2 ± 1.39	19.6 ± 9.13**
Corrected body weight[b]	40.0 ± 2.45	39.2 ± 2.03	39.4 ± 4.84	34.3 ± 5.33**
Food consumption (g)				
Day 1	6.1 ± 1.30	5.8 ± 1.54	5.4 ± 1.04	5.9 ± 0.92
Day 7	6.6 ± 1.01	6.8 ± 1.42	7.2 ± 1.49	7.0 ± 1.11
Day 10	6.6 ± 1.36	6.9 ± 1.77	7.4 ± 1.45	7.1 ± 1.07
Day 13	8.1 ± 1.44	8.7 ± 1.69	9.0 ± 1.37	8.4 ± 1.86
Day 16	10.4 ± 2.18	9.8 ± 2.74	11.3 ± 3.49	9.2 ± 2.84
Day 18	8.3 ± 2.89	8.8 ± 2.68	9.3 ± 3.55	8.1 ± 2.01

[a]Values are presented as means ± SD for all pregnant animals.
[b]Corrected body weight=body weight on gestational day 18 - gravid uterine weight.
*Significant difference at $P<0.05$ level compared with the control group.
**Significant difference at $P<0.01$ level compared with the control group.

tear, and swelling, induration, crust formation and ulceration at the injection sites, which were dose-dependent in incidence and severity (data not shown). No treatment-related clinical findings were observed at 625 and 313 mg/kg. Maternal body weight on GD 15 and 18 in the 1,250 mg/kg group was significantly suppressed when compared with the vehicle control group. Maternal body weight gain for the intervals GD 6-18 (treatment period) and corrected body weight in the group were also significantly lower than those in the vehicle control group. On the contrary, there were no statistically significant differences in food consumption between the vehicle control and treatment groups. At autopsy of dams, no treatment-related pathological alterations were observed in any treated group (data not shown).

Table 3. External alterations in fetuses from pregnant mice treated with 2-bromopropane during gestational days 6 through 17

Parameters	2-Bromopropane (mg/kg/day)			
	0	313	625	1250
Fetuses examined	143	136	152	122
Litters examined	11	11	11	11
Fetuses with malformations (%)[a]	0	0	0	3 (2.5)
Litters affected (%)[b]	0	0	0	3 (27.3)
Hematoma	0	0	0	1
Umbilical hernia	0	0	0	2
Malrotated hindlimb	0	0	0	1

[a]A single fetus may be represented more than once in listing individual defects.
[b]Includes litters with one or more affected fetuses.

Developmental Toxicity

The reproductive findings for the pregnant mice treated with 2-BP subcutaneously on days 6 through 17 of pregnancy are summarized in Table 2. No significant differences were observed in the number of corpora lutea, implantation, resorptions, dead fetuses, and sex ratio in the treatment groups, compared with those of the vehicle control group. The number of live fetuses per litter in the high dose group was slightly decreased when compared with the controls (11.1 *versus* 13.0 for controls), but it was not considered to be related to treatment of 2-BP since it was not significantly different in comparison with controls and did not exhibit a dose-response relationship. However, the body weights of male and female fetuses and the placental weight were statistically significantly decreased in the 1,500 mg/kg group, compared with those of the vehicle control group.

The types and incidences of fetal external malformations are shown in Table 3. Although the numbers of malformed fetuses or of litters with affected fetuses in the 1,250 mg/kg group were increased slightly, the differences were not statistically significant between the groups. External malformations occurred in 3 of the 122 fetuses, in 3 of the 11 litters, at 1,250 mg/kg. Although the incidence for fetal external malformations was low, these findings including hematoma, umbilical hernia, and malrotated limb are uncommon in normal ICR mice (MARTA, 1997; Nakatsuka *et al.*, 1997; Chahoud *et al.*, 1999).

As shown by the data in Table 4, visceral malformation and variation were observed only a single fetus,

Table 2. Reproductive findings of pregnant mice treated with 2-bromopropane during gestational days 6 through 17

Parameters	2-Bromopropane (mg/kg/day)			
	0	313	625	1250
No. of females mated	11	11	11	11
No. of pregnant animals	11	11	11	11
No. of corpora lutea[a]	15.5 ± 1.81	15.1 ± 1.74	16.4 ± 2.16	14.1 ± 1.68
No. of implantations[a]	15.0 ± 2.05	14.4 ± 1.93	15.9 ± 2.21	13.1 ± 2.04
No. of fetal deaths	22	22	23	22
Resorptions: Early	15	11	11	15
Late	2	7	3	3
Dead fetuses	5	4	9	4
No. of live fetuses per litter[a]	13.0 ± 3.41	12.4 ± 2.38	13.8 ± 2.64	11.1 ± 2.73
Male/female	77/66	70/66	76/76	61/61
Sex ratio (male/female)	1.16	1.06	1.00	1.00
Fetal body weight (g): Male[a]	1.42 ± 0.08	1.41 ± 0.07	1.39 ± 0.09	1.32 ± 0.09*
Female[a]	1.37 ± 0.12	1.35 ± 0.08	1.33 ± 0.10	1.26 ± 0.08*
Placental weight (g)[a]	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01**

[a]Values are presented as means ± SD.
*Significant difference at $P<0.05$ level compared with the control group.
**Significant difference at $P<0.01$ level compared with the control group.

Table 4. Visceral alterations in fetuses from pregnant mice treated with 2-bromopropane during gestational days 6 through 17

Parameters	2-Bromopropane (mg/kg/day)			
	0	313	625	1250
Fetuses examined	66	65	76	61
Litters examined	11	11	11	11
Fetuses with malformations (%)[a]	0	0	0	1 (1.6)
Litters affected (%)[b]	0	0	0	1 (9.1)
Hypoplasia of lung	0	0	0	1
Fetuses with variations (%)[a]	0	0	0	1 (1.6)
Litters affected (%)[b]	0	0	0	1 (9.1)
Dilated renal pelvis	0	0	0	1
Dilated ureter	0	0	0	1

[a]A single fetus may be represented more than once in listing individual defects.
[b]Includes litters with one or more affected fetuses.

respectively; malformation observed was hypoplasia of the lung and variation dilated renal pelvis and dilated ureter.

The types and incidences of fetal skeletal malformations, variations, and retardations are shown in Table 5. No skeletal malformations were observed in any treatment group. The incidences of skeletal variations were 25 (32.5%), 24 (33.8%), 33 (43.4%) and 39 (63.9%); retardations 0, 0, 1 (1.3) and 4 (6.6%); in the vehicle control, 313, 625 and 1,250 mg/kg groups, respectively. The incidences of fetuses with skeletal variations and retardations in the 1,250 mg/kg group were significantly increased when compared with those in the vehicle control group. The predominant signs of skeletal variations and retardations were found in rib, vertebra and sternebra. Although the incidences of litters with skeletal variations and retardations in the group were also higher than those of the vehicle control group, the differences were not statistically significant. There was some evidence of treatment-related reductions in the ossification of fetal skeleton. The number of ossification centers

Table 5. Skeletal alterations in fetuses from pregnant mice treated with 2-bromopropane during gestational days 6 through 17

Parameters	2-Bromopropane (mg/kg/day)			
	0	313	625	1250
Fetuses examined	77	71	76	61
Litters examined	11	11	11	11
Fetuses with malformations (%)[a]	0	0	0	0
Litters affected (%)[b]	0	0	0	0
Fetuses with variations (%)[a]	25 (32.5)	24 (33.8)	33 (43.4)	39** (63.9)
Litters affected (%)[b]	10 (90.9)	9 (81.8)	10 (90.9)	11 (100)
Short 13th rib	1	0	0	0
Cervical rib	0	1	0	2
Full/short supernumerary rib	22	19	22	26
Msshapen sternebra	0	3	3	10
Enlarged fontanel	0	0	0	1
Misaligned sternebra	1	2	2	0
Bipartite ossification of thoracic centrum	0	0	0	1
Bipartite ossification of sternebra	1	1	4	2
Extra sternebral ossification site	0	1	3	2
Fetuses with retardations (%)[a]	0	0	1 (1.3)	4* (6.6)
Litters affected (%)[b]	0	0	1 (9.1)	3 (27.3)
Incomplete ossification of supraoccipital	0	0	1	3
Dumbbell ossification of lumbar centrum	0	0	0	1
No. of ossification centers[c]				
Sternebra	6.0	6.0 ± 0.04	6.0 ± 0.12	6.0 ± 0.05
Metacarpals in both forelimbs	8.0	8.0	8.0	8.0
First and 2nd phalanges in both forelimbs	13.7 ± 0.86	13.5 ± 0.63	13.3 ± 1.03	12.7 ± 1.19*
Third phalanges in both forelimbs	10.0	10.0	10.0	9.9 ± 0.15
Metatarsals in both hindlimbs	10.0	10.0	10.0	10.0
First and 2nd phalanges in both hindlimbs	12.5 ± 1.90	11.8 ± 2.11	11.0 ± 2.02	10.7 ± 0.72*
Third phalanges in both hindlimbs	10.0	10.0	9.9 ± 0.33	9.7 ± 0.83
Sacral and caudal vertebra	12.0 ± 0.92	12.3 ± 1.67	12.2 ± 1.36	12.4 ± 0.94

[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 control group.
**Significant difference at $P<0.01$ level compared with the control group.

of phalanges in fore- and hindlimbs in the 1,250 mg/kg group was significantly decreased in a dose-dependent manner, but no significant decrease in fetal ossification was observed in the 313 and 625 mg/kg groups.

DISCISSION

The present study was conducted to evaluate the potential developmental toxicity of 2-BP injected sucbcutaneously to ICR mice at dose levels of 313, 625, and 1,250 mg/kg/day on days 6 through 17 of pregnancy. The results of the study showed that a 12-day subcutaneous dose of 2-BP to mice during pregnancy (days 6 through 17) produced significant embryotoxicity at a minimally maternotoxic dose in ICR mice.

Treatment related clinical signs, as evidenced by increased incidence of dull fur, reddish tear, swelling, induration, crust formation and ulceration at the injection sites, were observed in the 1,250 mg/kg group. These findings were limited only at the injection sites and were maybe attributed to the irritating effects of 2-BP. Of the adverse clinical signs observed in this study, increased incidence of swelling at the injection site was maybe attributed to a direct irritating effect of 2-BP. Some kinds of clinical signs such as dull fur and reddish tears were indications of stress induced by the treatment of 2-BP. The significant maternal toxicity of 2-BP, as evidenced by suppression or decrease in the body weight, body weight gain and corrected body weight, was also found in the highest dose group. The significant suppression of maternal body weight, body weight gain and corrected body weight observed in the highest dose group indicates that this finding is closely related to the administration of test chemical, because this change was remarkable and showed a dose-response relationship.

The developmental toxicity of 2-BP included a reduction in fetal body weight and placental weight, an increase in the incidence of fetal skeletal variations and retardation, and a fetal ossification delay in the 1,250 mg/kg group. 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 phalanges in fore- and hindlimbs. The dose-related increase in the fetal morphological alternations with increasing dose suggests that the embryotoxic effect is closely related to the administration of 2-BP. The predominant signs of fetal abnormal development observed at 1,250 mg/kg were detected in rib, vertebra, and sternebra.

According to the results of previous toxicity studies, administration of 2-BP to experimental animals caused various adverse effects on reproductive organs, bone marrow, central nervous system, and immune system (Ichihara *et al.*, 1997; Omura *et al.*, 1999; Son *et al.*, 1999; Wu *et al.*, 2002; Yu *et al.*, 1999; Zhao *et al.*, 2002). 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 (Omura *et al.*, 1999; Yu *et al.*, 1999). Recently, it was reported that 2-BP induces DNA damage, impairs functional antioxidant cellular defenses, and enhances the lipid peroxidation in cultured Leydig cells (Wu *et al.*, 2002). More recently, Zhao *et al.* (2002) 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 in experimental animals (Chung *et al.*, 1998; Kim *et al.*, 2003). As for the adverse effects of 2-BP on embryo-fetal and F1 offspring development, a recent study by Ishikawa *et al.* (2001) 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.* (2002), 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 1,215 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 (Kim *et al.*, 2001, 2003), 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 (1987), 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.* (1989)

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 (Chahoud *et al.*, 1999). In the case of the present study, because the adverse effects of 2-BP on embryo-fetal development appeared to be accompanied by maternal toxicity at a dose level of 1,250 mg/kg, the exact cause and effect relationship between maternal and developmental toxicities could not be elucidated. Further *in vivo* and *in vitro* teratogenicity studies are presently underway to better understand the potential teratogenic effects of 2-BP on embryo-fetal development and to determine whether the effects on the conceptus were maternally mediated.

Based on the results, it was concluded that 2-BP was embryotoxic at a minimally maternally toxic dose (1,250 mg/kg/day) in ICR mice. In the present experimental condition, the no-observed-adverse-effect level of 2-BP is considered to be 625 mg/kg/day for dams and embryo-fetuses, respectively.

ACKNOWLEDGEMENTS

This study was financially supported by Chonnam National University in the program, 2002.

REFERENCES

Chahoud, I., Buschmann, J., Clark, R., Druga, A., Falke, H., Faqi, A., Hansen, E., Heinrich-Hirsch, B., Hellwig, J., Lingk, W., Parkinson, M., Paumgartten, F.J., Pfeil, R., Platzek, T., Scialli, A.R., Seed, J., Stahlmann, R., Ulbrich, B., Wu, X., Yasuda, M., Younes, M. and Solecki, R. (1999): Classification terms in developmental toxicology: need for harmonisation. Report of the Second Workshop on the Terminology in Developmental Toxicology, *Reprod. Toxicol.*, **13**, 77-82.

Chernoff, N., Rogers, J.M. and Kavlock, R.J. (1989): An overview of maternal toxicity and prenatal development: considerations for developmental toxicity hazard assessments, *Toxicology*, **59**, 111-125.

Chung, M.K., Kim, J.C. and Roh, J.K. (1998): Embryotoxic effects of SKI 2053R, a new potential anticancer agent, in rats, *Reprod. Toxicol.*, **12**, 375-381.

Dawson, A.B. (1926): A note on the staining of the skeleton of cleared specimens with Alizarin Red S, *Stain Technol.*, **1**, 123-124.

Fisher, R.A. (1970): Statistical methods for research workers (14th edition), Oliver and Boyd, Edinburgh, UK.

Ichihara, G., Asaeda, N., Kumazawa, T., Tagawa, T., Kamijima, M., Yu, X., Kondo, H., Nakajima, T., Kitoh J., Yu, I.J., Moon, Y.H., Hisanaga, N. and Takeuchi, Y. (1997): Testicular and haematopoietic toxicity of 2-bromopropane, a substitute for ozone layer-depleting chlorofluorocarbons, *J. Occup. Health*, **39**, 57-63.

Ishikawa, H., Tian, Y. and Yamauchi, T. (2001): Induction of micronuclei formation in preimplantation mouse embryos after maternal treatment with 2-bromopropane, *Reprod. Toxicol.*, **15**, 81-85.

Kang, K.S., Li, G.X., Che, J.H. and Lee, Y.S. (2002): Impairment of male rat reproductive function in F1 offspring from dams exposed to 2-bromopropane during gestation and lactation, *Reprod. Toxicol.*, **16**, 151-159.

Khera, K.S. (1987): Maternal toxicity in human and animals: effects on fetal development and criteria for detection, *Teratogen. Carcinogen. Mutagen.*, **7**, 287-295.

Kim, J.C., Shin, D.H., Kim, S.H., Ahn, T.H., Kang, S.S., Jang, B.S., Kim, C.Y. and Chung, M.K. (2003): Developmental toxicity evaluation of the new fluoroquinolone antibacterial DW-116 in rats, *Teratogen. Carcinogen. Mutagen.*, **23(Suppl. 1)**, 123-136.

Kim, J.C., Shin, H.C., Cha, S.W., Koh, W.S., Chung, M.K. and Han, S.S. (2001): Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy, *Life Sci.*, **69**, 2611-2625.

Kim, Y., Jung, K., Hwang, T., Jung, G., Kim, H., Park, J., Kim, J., Park, J., Park, D., Park, S., Choi, K. and Moon, Y. (1996): Hematopoietic and reproductive hazards of Korean electronic workers exposed to solvents containing 2-bromopropane, *Scan. J. Work Environ. Health*, **22**, 387-391.

Kruskal, W.H. and Wallis, W.A. (1952): Use of ranks in one criterion variance analysis, *J. Am. Statist. Assoc.*, **47**, 614-617.

Maeng, S.H. and Yu, I.J. (1997): Mutagenicity of 2-bromopropane, *Ind. Health*, **35**, 87-95.

MARTA (Middle Atlantic Reproduction Teratology Association), (1997): Appendix B: Historical Control Data in *Handbook of Developmental Toxicology* (Hood, R.D. ed.), CRC Press, New York, pp. 716-724.

Nakatsuka, T., Horimoto, M., Ito, M., Matsubara, Y., Akaike, M. and Ariyuki, F. (1997): Japan pharmaceutical manufacturers association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice, *Cong. Anon.*, **37**, 47-138.

Nishimura, K.A. (1974): Microdissection method for detecting thoracic visceral malformations in mouse and rat fetuses, *Cong. Anom.*, **14**, 23-40.

NRC (National Research Council), (1996): Guide for the Care and Use of Laboratory Animals. National Research Council. National Academy, Washington, USA.

Omura, M., Romero, Y., Zhao, M. and Inoue, N. (1999): Histopathological evidence that spermatogonia are the target cells of 2-bromopropane, *Toxicol. Lett.*, **104**, 19-26.

Park, J.S., Kim, Y.H., Park, D.W., Choi, K.S., Park, S.H. and Moon, Y.H. (1997): An outbreak of hematopoietic and reproductive disorders due to solvents containing 2-bromopropane in an electronic factory, South Korea: Epidemiological survey, *J. Occup. Health*, **39**, 138-143.

SAS Institute, Inc. (1997): *SAS/STAT Software: Changes and Enhancements Through Release 6.12.* SAS Institute,

Cary, NC.

Scheffe, H. (1953): A method of judging all contrasts in the analysis of variance, *Biometika*, **40**, 87-104.

Son, H.Y., Kim, Y.B., Kang, B.H., Cho, S.W., Ha, C.S. and Roh, J.K. (1999): Effects of 2-bromopropane on spermatogenesis in the Sprague-Dawley rat, *Reprod. Toxicol.*, **13**, 179-187.

Wilson, J.G. (1965): Methods for administering agents and detecting malformations in experimental animals in *Teratology. Principles and Techniques* (Wilson, J.G. and Warkany, J. eds.). University of Chicago Press, Chicago and London, pp. 262-277.

Wise, L.D., Beck, S.L., Beltrame, D., Beyer, B.K., Chahoud, I., Clark, R.L., Clark, R., Druga, A.M., Feuston, M.H., Guittin, P., Henwood, S.M., Kimmel, C.A., Lindstrom, P., Palmer, A.K., Petrere, J.A., Solomon, H.M., Yasuda, M. and York, R.G. (1997): Terminology of developmental abnormalities in common laboratory mammals (Version 1), *Teratology*, **55**, 249-292.

Wu, X., Faqi, A.S., Yang, J., Pang, B.P., Ding, X., Jiang, X. and Chahoud, I. (2002): 2-Bromopropane induces DNA damage, impairs functional antioxidant cellular defenses, and enhances the lipid peroxidation process in primary cultures of rat Leydig cells, *Reprod. Toxicol.*, **16**, 379-384.

Yu, X., Kamijima, M., Ichihara, G., Li, W., Kitoh, J., Xie, Z., Shibata, E., Hisanaga, N. and Takeuchi, Y. (1999): 2-Bromopropane causes ovarian dysfunction by damaging primordial follicles and their oocytes in female rats, *Toxicol. Appl. Pharmacol.*, **159**, 185-193.

Zhao, L.X., Kim, E.K., Lim, H.T., Moon, Y.S., Kim, N.H., Kim, T.H., Choi, H., Chae, W., Jeong, T.C. and Lee, E.S. (2002): Synthesis, characterization and in vitro identification of N^7-guanine adduct of 2-bromopropane, *Arch. Pharm. Res.*, **25**, 39-44.