

# Research on the Reproductive and Developmental Toxicity of Benzyl Butyl Phthalate

JI Yu-bin<sup>1,2,3</sup>, SHEN Lin-jing<sup>1</sup>, YU Lei<sup>1,2,3</sup>, LI Wen-lan<sup>1,2,3</sup>

<sup>1</sup>Center for Life Science and Environment Science, Harbin University of Commerce, Harbin, China, 150076

<sup>2</sup>Workstation for Post-doctoral Scientific Research, Drug Research Center of Harbin University of Commerce, Harbin, China, 150076

<sup>3</sup>Natural Anti-tumor Drug Engineering Research Center of Ministry of Education, Heilongjiang Harbin, China, 150076

Email: yulei912@163.com

**Abstract:** Benzyl butyl phthalate (BBP) becomes a global organic pollutants, which is widely used and has a mass production. As an environmental estrogen, BBP has picked up great attention of scholars all over the world. Reproductive and developmental toxicities are introduced in this paper. It can cause decline of the testosterone concentrations, the count and vitality of the sperm, and enlargement of the liver in the male rats and mice. It could also lead to the decrease of the progesterone level and uterine weight in the female. All these can be seen in the offspring, such as the decrease of the birth weight, anogenital distance (AGD), testicular weight, and the increased of teratogenic. In addition, BBP can also affect the sexual differentiation in the male offspring.

**Key words:** toxicology; butyl benzyl phthalate; developmental toxicity

## 1 Introduction

As a member of phthalate esters (PAEs), butyl benzyl phthalate (BBP for short) is a kind of environmental estrogens<sup>[1]</sup>, which is also considered as a kind of priority pollutant by the United States Environmental Protection Agency (EPA). It is usually used in flooring, wall sheet plastic, paint, paste and plasticizer because of its processing performance, oil resistance and pollution resistance. At the same time, it is widely used in medical equipment, synthetic rubber, industrial dyes, food containers, automobiles, clothing, furniture, cosmetics, defoaming agents, lubricants and pesticides etc. as a kind of resin, dyes or additives. It is commonly found in municipal sewage sludge and the atmosphere<sup>[2,3]</sup>. Because its photolysis, hydrolysis and evaporation rates are slow, it has a long-term impact on the human body. Current studies on BBP are not much reported. So it is essential to conduct an in-depth study of BBP in order to reveal the mechanism, help with the prevention and resolution of BBP's poisoning effect on the human body.

## 2 Physical and Chemical Properties

It is a kind of colourless transparent oily liquid with micro-fragrance. And the molecular formula is C<sub>19</sub>H<sub>20</sub>O<sub>4</sub> with the molecular weight 312.40. Its structural formula is as shown in Figure 1. The melting point is -35°C, and the boiling point is 370°C. The other qualities are as follows: relative density, 1.111-1.119 (25/4°C); refractive index, 1.5336-1.5376 (25°C); flash point, 200°C (open cup); solubility at 30°C in water,

0.0003% (weight); soluble in organic solvents.

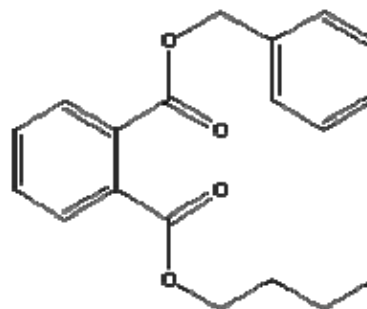


Figure 1. Structure Formula of Butyl Benzyl Phthalate (BBP)

## 3 Reproductive Toxicity

A large number of studies on animals have shown that BBP has reproductive toxicity not only to the male but also to the female. And most of them were about the male, especially focusing on the rodents.

### 3.1 Reproductive Toxicity on the Male

BBP multigeneration effects on reproduction in mammals are available. A two-generation reproductive study<sup>[4]</sup> was conducted in male and female Sprague-Dawley rats using oral doses of 0, 20, 100, and 500 mg/kg/day BBP. In the parent animals (F(0)), a decrease in body weight gain was observed in males in

the 500 mg/kg/day group, although no significant decrease in food consumption was found. No dose-related changes were observed in estrous cyclicity, fertility, or lactation. A dose-dependent increase in kidney weight in rats of both sexes, an increase in liver weight in males, and a decrease in the weight of the ovaries in females were observed. No macroscopic or microscopic changes were found in the reproductive system of males or females. Oral administration of BBP caused a decrease in the serum concentration of testosterone, and an increase in FSH. In the next generation (F(1)), the body weight of male and female offspring at birth in the 100 and 500 mg/kg groups was significantly decreased, and the body weight in the 500 mg/kg group was lower throughout the study, while viability was not affected. Anogenital distance (AGD) at birth was decreased in male pups and was increased in female pups of the 500 mg/kg/day group. Preputial separation for male offspring in the 500 mg/kg/day group was delayed, while vaginal opening for female offspring in this group was not affected. BBP did not affect reproductive ability, including delivery and lactation, at any dose whereas macroscopic and microscopic changes of the testis, and decreased serum concentrations of testosterone were observed in male offspring of the 500 mg/kg/day group after puberty. From these data, it would appear that 20 mg/kg BBP is a no observed adverse effect level (NOAEL) for reproductive effects on parent animals and the next generation.

Another study<sup>[5]</sup> showed that, in the parental animals, the no observed effect level (NOEL) and the no observed adverse effect level (NOAEL) were less than 100 mg/kg/day, and no serious effects on the reproductive capacity were induced at doses less than 200 mg/kg/day. The NOEL and NOAEL for the growth and development of offspring were concluded to be less than 100 mg/kg/day.

Effects of subchronic exposure of laboratory mice to benzylbutyl phthalate (BBP) on the quantity and quality of male germ cells were studied<sup>[6]</sup>. Sperm counts were diminished 4 and 8 weeks after the start of exposure to BBP. In the same time decrease in sperm motility and dose-dependent increase in the frequency of abnormal sperm heads and slight increase in DNA damage were noted. 4 weeks after the end of exposure, slight decrease in sperm counts in the group of 1/4 LD50 was observed, only. Correlation between sperm count and testes and epididymes weight were noted. The most sensitive to BBP exposure occurred spermatozoa and spermatids.

Rats when exposed to BBP during pregnancy, concentration of testosterone decreased in the male embryos<sup>[7]</sup>. The effect of BBP on the development of male reproductive system was cumulative. With the increasing of BBP dose, the anti-male role it played strengthened.

The study<sup>[8]</sup> of Wu Dan et al showed that a higher level of BBP could cause significant decline of LDH, SDH, Ca-Mg-ATPase activity in mice testicular tissues, indicating BBP interfered the anaerobic energy supply and the aerobic metabolism of testicular tissue, and at the same time suppressed the energy use of the male reproductive cells, resulting in the damage on male reproductive system.

### 3.2 Reproductive Toxicity on the Female

Ema M et al has studied the reproductive effects of BBP in pregnant and pseudopregnant rats<sup>[9]</sup>. Rats were given BBP by gastric intubation at 0, 250, 500, 750, or 1000 mg/kg on Days 0 to 8 of pregnancy and the pregnancy outcome was determined on Day 20 of pregnancy. The same doses of BBP were given to pseudopregnant rats, with an induced decidual cell response on Days 0 to 8 of pseudopregnancy, and the uterine weight on Day 9 served as an index of the uterine decidualization. BBP caused significant increases in the incidences of preimplantation loss in females successfully mated at 1000 mg/kg and of postimplantation loss in females having implantations at 750 mg/kg and above. Uterine decidual growth in pseudopregnant rats was significantly decreased at 750 mg/kg and above. Findings suggest that early embryonic loss due to BBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function.

### 4 Estrogenic Activity

BBP could increase the expression of progesterone receptor messenger ribonucleic acid in the preoptic area of adult ovariectomized rats<sup>[10]</sup>, and inhibit pulsatile luteinizing hormone secretion under an insulin-induced hypoglycaemic state in ovariectomized rats<sup>[11]</sup>. Jobling et al<sup>[12,13]</sup> carried out the proliferation experiments with MCF-7 which was sensitive with estrogen, and the results were all positive. The experiment<sup>[14]</sup> of LI Wen-lan on mice observing the weights of uterus has also given a support. Besides, some studies<sup>[15,16]</sup> showed that BBP had estrogenic activity at a high concentration level, while was anti-estrogenic when the concentration was low.

### 5 Developmental Toxicity

Ema M et al has compared the developmental toxicity of BBP and DBP and concluded that they may have the same mechanism<sup>[17]</sup>.

Normal sexual development of rats exposed to BBP from conception to weaning has been studied<sup>[18]</sup>. BBP has been administered in drinking water (1000 micrograms/liter) to pregnant AP rats during gestation and lactation. The sexual development of the pups was then monitored until their termination at postnatal day 90

(pnd 90). The body weights of the BBP pups were marginally increased at birth, but this difference resolved by pnd 90. A 1.1-day advance in the average day of vaginal opening and a small increase in male AG distance on pnd 2 came out. These last two effects are related to the increased weight of the BBP pups. The incidence of FSH-containing cells in the pituitary gland of animals from each group was unaffected at pnd 90. However, the absence of an effect of BBP administration on pup testis weight and testicular sperm count at pnd 90 is in contrast to reductions in these measurements reported earlier by testicular sperm count et al.

The manifestation of deviant development induced by BBP varies with the developmental stage at the time of administration and that BBP induces two discrete responses from embryos to teratogenicity during early and late organogenesis<sup>[19]</sup>.

Developmental toxicity of BBP in the rat using a multiple dose study design was studied and it showed that fetotoxicity included increased resorptions, reduced fetal weights, increased incidence of skeletal anomalies, and reduced fetal testis weights in the presence of an increased incidence of retarded testicular descent<sup>[20]</sup>.

Perinatal exposure to BBP could alter sexual differentiation of the male rat<sup>[21]</sup>. And BBP given in the late pregnancy could produced adverse effects on the development of the reproductive system in male offspring<sup>[22]</sup>.

The no-observable-effect-levels (NOEL) of BBP in rats were 0.5 and 1.0% BBP in the diet for maternal and embryofetal toxicity, respectively<sup>[23]</sup>. Teratogenic evaluation of BBP in rats was studied via gastric intubation<sup>[24]</sup>. In the 0.5 g/kg group, food consumption during the administration period was significantly decreased, but no adverse effect on the embryo-fetus was detected. High maternal lethality and complete resorption of implanted embryos in all the surviving dams were observed in the 1.0 g/kg group. Increased embryo-fetal death and decreased fetal weight were found at a dose of 0.75 g/kg which also caused reductions in maternal body weight gain and food consumption. A significantly and markedly increased incidence of fetal malformations was also detected in the 0.75 g/kg group. Cleft palate, fusion of the sternebrae and dilatation of the renal pelvis were mostly observed.

The administration of BBP during the first and second half of pregnancy produced embryoletality and teratogenicity, respectively<sup>[25]</sup>. And the susceptibility to the teratogenicity of BBP varies with the developmental stage at the time of administration<sup>[26,27]</sup>. Embryoletality of BBP during early pregnancy in rats shows that the postimplantation embryonic loss due to BBP during early pregnancy is mediated via the reduction in plasma progesterone levels, an impairment of luteal function<sup>[28]</sup>.

## 6 Conclusions

Nowadays, with the development of science, technology, and industry, BBP has been widely used. However, most of the time, it is hardly got a tense attention. It becomes very hot to get the answers to whether it can affect our health, and how. Therefore, it is necessary to study its distribution in the environment, accumulation in human body, the toxicological effect to human and the mechanism with the help of experiments.

## References

- [1] Li Wenlan, Wang Shuaishuai. Toxicological Studies of Environmental Hormone Butyl Benzyl Phthalate(BBP) [J]. JOURNAL OF HARBIN UNIVERSITY OF COMMERCE(NATURAL SCIENCES EDITION). 2005 4,21(2):130(Ch).
- [2] Environmental Factors and Reproductive Health [M]. Beijing: Chemical Industry Press.2002-5-1,202(Ch).
- [3] Mo Cehui, Cai Quanying, Wu Qitang, et al. Studies of the Phthalate Esters in the Urban Sewage Sludge[J]. China Environmental Science.2001,21 (4) :362-366(Ch).
- [4] Nagao T.Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study[J]. Reprod Toxicol.2000 Nov-Dec,14(6):513-32.
- [5] Aso S, Ehara H, Miyata K et al.A two-generation reproductive toxicity study of butyl benzyl phthalate in rats[J]. J Toxicol Sci.2005 Dec:39-58.
- [6] Tyrkiel EJ, Dobrzyńska MM, Derezińska E et al. Effects of subchronic exposure of laboratory mice to benzyl butyl phthalate (BBP) on the quantity and quality of male germ cells[J]. Roczn Panstw Zakl Hig.2007,58(4):677-86.
- [7] Hotchkiss AK, Parks SLG, Ostby JS, et al. A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion[J].Biol Reprod.2004,71:1852-1861.
- [8] Wu Dan, Li Yan, Tang Bei, et al. Effect of Butyl Benzyl Phthalate on Enzymes Related to Mice Testicular Energy Metabolism[J]. Asian Journal of Ecotoxicolog.2007 12,2(4):440-444(Ch).
- [9] Ema M, Miyawaki E, Kawashima K.Reproductive effects of butyl benzyl phthalate in pregnant and pseudopregnant rats[J]. Reprod Toxicol.1998 Mar-Apr,12(2):127-32.
- [10] Funabashi T, Kawaguchi M, Kimura F.The endocrine disrupters butyl benzyl phthalate and bisphenol A increase the expression of progesterone receptor messenger ribonucleic acid in the preoptic area of adult ovariectomized rats[J]. Neuroendocrinology.2001 Aug,74(2):77-81.
- [11] Kawaguchi M, Funabashi T, Aiba S et al.Butyl benzyl phthalate, an endocrine disrupter, inhibits pulsatile luteinizing hormone secretion under an insulin-induced hypoglycaemic state in ovariectomized rats[J]. J Neuroendocrinol.2002 Jun,14(6):486-91.
- [12] Picard K, Lhuguenot JC, Lavier-Canivenc MC et al.Estrogenic activity and metabolism of n-butyl benzyl phthalate in vitro: identification of the active molecule(s)[J]. Toxicol Appl Pharmacol.2001 Apr 15,172(2):108-18.
- [13] Jobling S, ReynoldsT, White R, et al. A variety of environmentally persistent chemicals, including some phthalate plasticizers, Are Weakly Estrogenic[J]. Environmental Health Perspect. 1995, 103(6): 582-587.
- [14] Li Wenlan, Ji Yubin, Yang Bo,et al. Estrogen Biological Activities of Butyl Benzyl Phthalate in the Environment[J].Urban Environment and Urban Ecology.2003 2,16(1):22-24(Ch).
- [15] Okubo T, Suzuki T, Yokoyama Y, et al. Estimation of estrogenic and anti-estrogenic activities of some phthalate di-esters and monoesters by MCF-7 cell proliferation assay in vitro[J].Biol Pharm Bull.2003,26(8):1219.

- [16] P Sohon, JP Sumpster. Several environmental oestrogens are also anti-androgens[J]. *Journal of Endocrinology*.1998 ,158: 327-339.
- [17] Ema M, Kurosaka R, Amano H et al.Comparative developmental toxicity of n-butyl benzyl phthalate and di-n-butyl phthalate in rats[J]. *Arch Environ Contam Toxicol*. 1995 Feb,28(2):223-8.
- [18] Ashby J, Tinwell H, Lefevre PA et al.Normal sexual development of rats exposed to butyl benzyl phthalate from conception to weaning[J]. *Regul Toxicol Pharmacol*.1997 Aug,26(1 Pt 1):102-18.
- [19] Ema M, Miyawaki E, Kawashima K.Developmental effects of plasticizer butyl benzyl phthalate after a single administration in rats[J]. *J Appl Toxicol*.1999 Sep-Oct,19(5):357-65.
- [20] Piersma AH, Verhoef A, te Biesebeek JD et al.Developmental toxicity of butyl benzyl phthalate in the rat using a multiple dose study design[J]. *Reprod Toxicol*.2000 Sep-Oct,14(5):417-25.
- [21] Gray LE Jr, Ostby J, Furr J et al.Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat[J]. *Toxicol Sci*. 2000 Dec,58(2):350-65.
- [22] Ema M, Miyawaki E.Effects on development of the reproductive system in male offspring of rats given butyl benzyl phthalate during late pregnancy[J]. *Reprod Toxicol*.2002 Jan-Feb,16(1):71-6.
- [23] Ema M, Murai T, Itami T et al.Evaluation of the teratogenic potential of the plasticizer butyl benzyl phthalate in rats[J]. *J Appl Toxicol*.1990 Oct,10(5):339-43.
- [24] Ema M, Itami T, Kawasaki H.Teratogenic evaluation of butyl benzyl phthalate in rats by gastric intubation[J]. *Toxicol Lett*.1992 Jun,61(1):1-7.
- [25] Ema M, Itami T, Kawasaki H.Embryo lethality and teratogenicity of butyl benzyl phthalate in rats[J]. *J Appl Toxicol*.1992 Jun,12(3):179-83.
- [26] Ema M, Itami T, Kawasaki H.Effect of period of exposure on the developmental toxicity of butyl benzyl phthalate in rats[J]. *J Appl Toxicol*.1992 Feb,12(1):57-61.
- [27] Ema M, Itami T, Kawasaki H.Teratogenic phase specificity of butyl benzyl phthalate in rats[J]. *Toxicology*.1993 Mar 30,79(1):11-9.
- [28] Ema M, Kurosaka R, Amano H et al.Embryo lethality of butyl benzyl phthalate during early pregnancy in rats[J]. *Reprod Toxicol*.1994 May-Jun,8(3):231-6.