Hazard assessment of benzyl butyl phthalate

[Benzyl butyl phthalate, CAS No. 85-68-7]

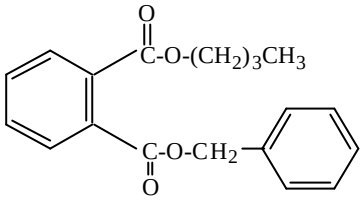
Chemical name: Benzyl butyl phthalate

Synonyms: Butyl benzyl phthalate; Phthalic acid butyl benzyl ester; 1,2-Benzenedicarboxylic acid, Butyl benzyl ester; BBP

Molecular formula: $C_{19}H_{20}O_4$

Molecular weight: 312.4

Structural formula:



Appearance: Clear oily liquid[1)]

Melting point: -35°C [2)]

Boiling point: 370°C [1) 2)]

Specific gravity: d_4^{25} =1.113 - 1.121 [1)]

Vapor pressure: 1.15×10^{-3} Pa (20°C)[1)]

Partition coefficient: Log Pow = 4.91 (measured value)[1)]

Degradability: Hydrolyzability: No report.

Biodegradability: Easily biodegradable (BOD=81%, 14 days)[2)]

Solubility: Water: 0.71 mg/*l* [1)]

Organic solvents: No report.

Amount of Production/import: 1998: 291 t (Production 0 t, Import 291 t)[3)]

Usage: Plasticizer for vinyl chloride and nitrocellulose resin.

Because BBP is highly resistant to oil and abrasion, BBP is used for coating electric wire[1)].

Applied laws and regulations: Law Concerning Reporting, etc. of Release of Specific Chemical Substances to the Environment and Promotion of the Improvement of Their Management; Marine Pollution Prevention Law

[1)] HSDB, 2001 [2)] "Tsusansho Koho" (daily), 1975; [3)] Ministry of International Trade and Industry, 1999

1. Toxicity Data

1) Information on adverse effects on human health

In a skin patch test in 15-30 volunteers, butyl benzyl phthalate (BBP) is reported to be a moderate skin irritant (Mallette & von Haam, 1952).

In another skin patch test, 200 volunteers were topically sensitized with BBP by applying the patch three times weekly for 24 hours over 5 weeks and then challenged by applying the BBP patch 2 weeks later, showing no skin irritating or sensitizing potential (Hammond et al., 1987).

The cases of hazardous effects of phthalate esters including BBP reported so far are presented below, although these may not be the effects of BBP alone.

In an epidemiological study involving young children who developed respiratory disorders during the first 2 years of life, children living in the room carpeted with polyvinyl (PVC) containing phthalate ester plasticizers such as BBP are reported to have increased risk of respiratory disorders than those living in the absence of PVC flooring (odds ratio calculated as the index for disease risk = 1.89, 95% confidence interval 1.14-3.14) (Jaakkola et al., 1999).

In a large-scale epidemiological study, the workers engaged in PVC production using phthalate ester plasticizers such as BBP (type of phthalate esters other than BBP is not specified) for 5 years or more are reported to have a significantly increased risk of multiple myeloma as compared with the control workers (CERHR, 2000).

In a large-scale cohort study in Cape Cod, Massachusetts, U.S.A., the percentage of the BBP-exposed female workers among full-time female workers with breast cancer (n = 261) was not different from the percentage of BBP-exposed female workers in the control females (n = 753). Therefore, occupational exposure to BBP is reported to be unrelated to the increased incidence of breast cancer (Aschengrau et al., 1998).

The occupational exposure to phthalate ester mixtures containing BBP (types of phthalate esters other than BBP are not specified) in PVC production is reported to be associated with increased incidences of menstrual disorders and spontaneous abortions among female workers as compared with those not exposed to BBP (CERHR, 2000).

2) Information on endocrine system and reproductive system

(1) *in vitro* test results related to receptor binding (Attachment-1)

In a receptor binding assay using fused proteins consisting of estrogen receptor ligand

binding domains linked to glutathione-S-transferase (GST), BBP weakly bound to human, mouse and chicken estrogen receptors (Matthews et al., 2000). In the binding assays using human estrogen receptor and uterine homogenates from immature SD rats, the binding affinities of BBP were about 1/31,000 and 1/28,000 – 1/80,000 of those of 17 β-estradiol, respectively (Zacharewski et al., 1998; Blair et al., 2000; Hashimoto et al., 2000; CERI, 2001b).

In yeast two-hybrid assays, BBP activated gene transcription (the activation activity was 1/1,700,000 of E2 by Nishihara et al)(Nishihara et al., 2000; Hashimoto et al., 2000). In the estrogen-dependent yeast growth assay with *Saccharomyces. cerevisiae* strain PL3 transfected with human estrogen receptor gene, and proliferating response to ligand binding to human estrogen receptors, BBP had a weak growth-promoting activity at 10 μM (Zacharewski et al., 1998).

In reporter gene assays using MCF-7 cells and HeLa cells, the potential of BBP at 10 μM to activate transcription of the reporter gene were 46% and 34% those of 10nM 17 β-estradiol, respectively (Zacharewski et al., 1998). In a similar reporter gene assay using HeLa cells, weak ERE-dependent activation of gene transcription (1/410,000 of E2) was observed (CERI, 2001b).

In cell proliferation assays, the relative potency of BBP was reported to be 1/25,000-1/1,000,000 of E2 in the recombinant yeast cells (Coldham et al., 1997; Harris et al., 1997) and 1/250,000 - 1/100,000 that of E2 in human breast cancer cells (MCF-7, ZR-75-1) (Soto et al., 1995, 1997; Korner et al., 1998).

In a reporter gene assay using yeast cells transfected with human androgen receptor gene, BBP was reported to antagonize androgen-like activity of dihydrotestosterone (anti-androgen-like activity) (Sohoni & Sumpter, 1998).

In a reporter gene assay using yeast cells transfected with human progesterone receptor gene, BBP did not activate gene transcription (Tran et al., 1996).

(2) in vivo test results in mammals (Attachments-2 (1), (2), (3), -3, -4 and -5)

In a uterotrophic assay, (in accordance with the OECD guidelines), BBP given subcutaneously at 0, 0.05, 0.5 and 5 mg/mouse for 4 days had no effect on uterus weight of female CFLP mice (aged 18 days) in any groups (Coldham et al., 1997).

In a similar uterotrophic assay, BBP given subcutaneously at 0, 500, 1,000 and 2,000

mg/rat for 3 days had no effect on uterus weight in female SD rats (aged 20 days) in any groups. For the detection of antiestrogenic activities subcutaneous administration of BBP at 0, 500, 1,000 and 2,000 mg/rats in combination with ethinylestradiol at 0.6 μg/kg/day to juvenile female SD rats (aged 20 days) caused no change in uterus weight in any groups, either (CERI, 2001a). For the detection of estrogenic activity in an assay using ovariectomized female SD rats (aged 31 days), oral administration of BBP at 0, 1, 20, 200 and 2,000 mg/kg/day for 4 days had no effect on uterus weight in any groups (Zacharewski et al., 1998).

In a Hershberger assay, an androgenicity and anti-androgenicity screening assay, BBP given orally to castrated SD rats (aged 7 weeks) at 0, 40, 200 and 1,000 mg/kg/day for 10 days caused no weight changes in male accessory reproductive organs. Further, for the detection of antiandrogenic activity BBP was administered orally to castrated SD rats (aged 7 weeks) at 0, 40, 200 and 1,000 mg/rats for 10 days in combination with subcutaneous testosterone propionate at 0.4 mg/kg/day. The animals in 200 mg/kg or higher groups showed decrease in absolute and relative weights of anterior lobe of prostate, relative weight of seminal vesicle, absolute and relative weights of bulbourethral gland and bulbospongiosus muscle + levator ani muscle weight, indicating the possibility of anti-androgenic activity of BBP, although the results were not clearly reproducible (CERI, 2001a).

The reproductive toxicities of BBP probably due to its anti-androgenic activity have been reported in the F_1 males. That is, female SD rats were orally gavaged with 750 mg/kg/day of BBP from gestation day 14 to postnatal day 3, resulting in decreased testis weight, reduced ano-genital distance (AGD) and increased incidence of nipple retention (postnatal day 13) in the F_1 males (Parks et al., 1999).

In another perinatal exposure study in which female SD rats were gavaged with 750 mg/kg/day of BBP from gestation day 15 to postnatal day 3, birth weight decreased in both male and female F_1 offsprings, and reduced AGD and increased incidences of retained areolae and nipples were observed in F_1 males (Gray et al., 2000).

The effects of BBP on endocrine and reproductive systems in repeated-dose toxicity and reproductive/developmental toxicity studies are presented as in the following.

In a 14-day dosed feed study, male F344 rats (aged 12-15 weeks) were exposed to BBP in diet at 0, 0.625, 1.25, 2.5 and 5.0% (equivalent to 0, 447, 890, 1,338 and 1,542 mg/kg/day). BBP caused decrease in testis, epididymis, prostate and seminal

vesicle weight, atrophy of testis, prostate and seminal vesicle, immature spermatid production, epithelial degeneration of seminiferous tubules and increase in luteinizing hormone and follicle stimulating hormone levels in 2.5% or higher groups and epididymal atrophy and decreased plasma testosterone level in 5% group (Kluwe et al., 1984; Agarwal et al., 1985).

In a peripubertal male rat assay performed according to procedures proposed by EDSTAC, male SD rats were given 500 mg/kg/day of BBP by gavage for 14 or 20 days immediately after weaning, but no abnormal changes were observed in testis and accessory reproductive organs (Ashby & Lefevre, 2000).

In a 26-week feeding study in male F344/N rats (aged 6 weeks), dietary exposure to BBP at 0, 300, 900, 2,800 and 25,000 ppm (equivalent to 0, 30, 60, 180, 550 and 1,650 mg/kg/day) was associated with decrease in testis, seminal vesicle and epididymis weights, degeneration of testis and epididymis, atrophy of seminiferous tubules and decreased sperm count in the 25,000 ppm group (NTP, 1997).

In a 106-week feeding study in which male F344/N rats (aged 6 weeks) were exposed to BBP in diet at 0, 3,000, 6,000 and 12,000 ppm (equivalent to 0, 120, 240 and 500 mg/kg/day), epididymal weight increased in 6,000 ppm or higher groups (NTP, 1997).

In a mating study, male F344/N rats (aged 6 weeks) were exposed to BBP in diet at 0, 300, 2,800 and 25,000 ppm (equivalent to 0, 20, 200 and 2,200 mg/kg/day) for 10 weeks and then mated with two untreated females. Males in 25,000 ppm group showed decreased sperm count, decreased relative prostate and testis weights, decreased epididymis and seminal vesicle weights and degeneration of testis and epididymis. In females mated with males in 25,000 ppm group, infertility rate increased (10/30 females), and this increase was thought to be due to toxic effect of BBP on male reproductive system (NTP, 1997).

In another mating study, male and female Wistar rats (age, unspecified) were exposed to BBP in diet at 0, 0.2, 0.4 and 0.8% (equivalent to 0, 106, 217 and 446 mg/kg/day). The females were mated after 2 weeks of dietary administration and were continued to be exposed to BBP in diet (equivalent to 0, 116, 235 and 458 mg/kg/day during the gestation period and to 0, 252, 580 and 1,078 mg/kg/day during the lactation period) through gestation and lactation periods. In this study, the dams in 0.8% group exhibited increased relative liver weight and suppressed body weight gain during gestation and lactation periods, but BBP had no effect on the fetuses (TNO, 1993).

In a reproduction study in WU rats (aged 10-11 weeks), male and females were gavaged orally with BBP at 0, 250, 500 and 1,000 mg/kg/day for 2 weeks and then mated with the same dose animals. The suppressed body weight gain, decreased testis and epididymis weights, Leydig cell hyperplasia and testicular degeneration were observed in F_0 males in 1,000 mg/kg/day group. In the F_0 females in the 1,000 mg/kg/day group, decreased fertility rate, suppressed body weight gain during gestation and decreased number of live pups at birth were observed. The pup body weight at birth decreased in F_1 males and F_1 females in 500 mg/kg/day or higher groups, and the pup body weight on postnatal day 6 decreased in F_1 males and females in 1,000 mg/kg/day group (Piersma, 1995).

In a reproduction study in SD rats (male: aged 6 weeks, female: aged 13 weeks), males and females were orally gavaged for 12 and 2 weeks, respectively with BBP at 0, 20, 100 and 500 mg/kg/day, and then paired and mated within the same dose group. The ovary weight increased in F_0 females in 100 mg/kg/day or higher groups. Among F_1 animals, abnormal changes included decreased body weight in both sexes in 100 mg/kg/day or higher groups, reduced AGD at birth in both sexes in 500 mg/kg/day group and delayed prepuce separation and decrease in serum testosterone in males in 500 mg/kg/day group (Nagao et al., 2000).

In a developmental toxicity study, female CD-1 mice were exposed to BBP in diet at 0, 0.1, 0.5 and 1.25% (equivalent to 0, 182, 910 and 2,330 mg/kg/day) from gestation days 6 through 15. The maternal body weight gain was suppressed in dams in 0.5% or higher groups. As for the effects on fetuses, increased number of dead embryos/fetuses and increased incidences of skeletal malformations involving ribs, sternum and vertebral column (control: 31%; 0.5% group: 61%; 1.25% group: 100%) were observed in 0.5% or higher groups, and the fetal body weight gain was suppressed in the 1.25% group (Price et al., 1990).

In a developmental toxicity study in which female CD rats were exposed to BBP in diet at 0, 0.5, 1.25 and 2.0% (equivalent to 0, 420, 1,100 and 1,640 mg/kg/day) on gestation days 6 through 15, maternal toxicities included suppressed body weight gain, increased food consumption and water intake and increased relative liver weight in 1.25% or higher groups and motor ataxia, gait disturbance and increased relative kidney weight in 2.0% group. As for embyro-fetotoxicities, decreased body weight, increased resorptions and increased incidence of malformations in urinary tract, eye and vertebral column (control: 2%; 2.0% group: 53%) were reported in 2.0% group (Field et al., 1989).

In a teratogenicity study, female Wistar rats were fed diets containing BBP at 0, 0.25, 0.5, 1.0 and 2.0% (equivalent to 0, 185, 375, 654 and 974 mg/kg/day) on gestation days 0 to 20. Among dams, suppressed body weight gain and decreased food consumption were observed in 1.0% or higher groups. The fetal toxicities included decreased number of live fetuses in 0.5% or higher groups, decreased body weight in 1.0% group and increased post-implantation losses (%) in 2.0% group (Ema et al., 1990).

In another teratogenicity study in which female Wistar rats were gavaged with BBP at 0, 500, 750 and 1,000 mg/kg/day on gestation days 7 to 15, maternal body weight gain was suppressed in dams in 750 mg/kg/day group. The embryo-fetotoxicities included decreased fetal body weight, increased number of resorbed embryoes (3/10 dams), increased number of dead fetuses, increased post-implantation losses and increased number of fetuses with external, skeletal and visceral malformations (control group: 1, 750 mg/kg/day group: 20) in 750 mg/kg/day group and resorptions in all dams (6) in 1,000 mg/kg/day group (Ema et al., 1992a).

The studies were also performed to assess effects of BBP in terms to dose and exposure period on post-implantation loss and manifestation of malformations (see Attachment-2 (3), Ema et al., 1991; 1992b; 1992c; 1994).

BBP is metabolized into two monoester metabolites, i.e., monobutyl and monobenzyl phthalates. The developmental toxicities of these metabolites are also reported.

In a developmental toxicity study in which female Wistar rats were gavaged with monobutyl phthalate at 0, 250, 500 and 625 mg/kg/day on gestation days 7 to 15, suppressed maternal body weight gain and decreased food consumption were observed in 500 mg/kg/day or higher groups. Among fetuses, weight loss, increased post-implantation losses, decreased number of live fetuses and increased incidences of skeletal malformations and dilated renal pelvis were observed (Ema et al., 1995a) (Attachment-2 (3)). In another study in which female Wistar rats were gavaged orally with monobenzyl phthalate at 0, 250, 313, 375, 438 and 500 mg/kg/day on gestation days 7 to 15, maternal body weight gain was suppressed in the 313 mg/kg/day or higher groups, and food consumption decreased in 250 mg/kg/day or higher groups. The fetal toxicities included increases in post-implantation losses and incidence of external malformations in 438 mg/kg/day or higher groups, increased incidence of skeletal malformations in 313 mg/kg/day or higher groups and increased incidence of visceral malformations in 375 mg/kg/day or higher groups (Ema et al., 1996c) (Attachment-4).

In a study in female Wistar King rats, prenatal exposure to monobutyl phthalate given by oral gavage at about 1,000 mg/kg/day on gestation days 15 to 18 resulted in (on postnatal days 30-40) in undescented testis in 87% of postnatal male fetuses (Imajima et al., 1997).

Based on these results, these two monoester metabolites of BBP appeared to be almost equally toxic to dams and fetuses as their parent compound.

The effects of low doses of BBP given to pregnant rats on maturation of male reproductive organs and perinatal mortality of male offspring were reported.

In a study by gestational and lactational exposure, female Wistar rats were exposed to BBP in drinking water at 1 mg/*l* (postnatal days 1-2, 10-12 and 20-21; equivalent to 0.126, 0.274 and 0.336 mg/kg/day) for 2 weeks and then mated, and the exposure was continued during gestation and lactation periods. No toxic effect was observed in F_0 females. However, offspring body weight increased (postnatal day 22) in F_1 males and F_1 females, and absolute and relative testis weights decreased in F_1 males. In a replicate study in which the above dams were again mated with F_0 males after weaning of F_{1a} offspring, the F_1 males also showed decreased absolute and relative testis weights and decreased daily sperm production (Sharpe et al., 1995).

The above study was replicated using increased number of animals/group and with appropriate control compound after performing purity test of the test substance. That is, female Wistar AP rats were exposed to BBP in drinking water at 0 and 1 mg/*l* (equivalent to 0 and 0.183 mg/kg/day) from gestation day 1 until postnatal day 20. The F_1 males showed increased body weight on postnatal day 2, reduced AGD and increased relative liver weight, and F_1 females early vaginal patency. However, sperm count or testis weight were not affected in male offspring (Ashby et al., 1997).

Female Wistar outbred rats were exposed to BBP in drinking water at 0.1, 1 and 3 mg/*l* (equivalent to 0.012, 0.14 and 0.385 mg/kg/day) for 2 weeks and then mated, and the exposure was continued during gestation and lactation periods. Among F_1 animals, no differences were observed in sperm morphology, sperm count, sperm motility, estrus cycle and sexual maturation in any groups as compared with the control group (CERHR, 2000).

In another study, female Wistar rats were exposed to BBP in drinking water or in diet at 1 and 3 ppm for 2 weeks and then mated with untreated males, and exposure was continued throughout gestation and lactation periods. BBP had no effect on dams or fetuses (CERHR, 2000).

The NTP (National Toxicology Program)-CERHR (Center for Evaluation of Risk to Human Reproduction) Expert Panel concluded that the data of Sharpe et al. on the BBP toxicities in male reproductive organs of F_1 offspring are not to be evaluated for the reproductive toxicities because (1) the dose-response data are absent, (2) the analytical data of BBP concentration in drinking water are absent, (3) the results are not reproduced within the same laboratory, (4) the results are not reproduced in other laboratories, etc. (CERHR, 2000).

In these replicate studies, pup mortality within 4 postnatal days was reported to be significantly increased. Female Wistar outbred rats were exposed to BBP in drinking water at 0, 0.1, 1 and 3 mg/*l* (equivalent to 0, 0.012, 0.14 and 0.385 mg/kg/day) and then mated, and exposure was continued throughout gestation and lactation periods, resulting in a significant increase in number of dead pups within 4 days after birth in 1 mg/*l* or higher groups. In the 3 mg/*l* group, increased number of hypothermic pups (postnatal day 1), increased number of large pups (postnatal day. 4) and increased hair loss were observed (CERHR, 2000). The NTP (CERHR) Expert Panel considers that the reliability of these data is low since the results are not reproducible in other laboratories, but designates the NOAEL of 0.385 mg/kg/day and 0.14 mg/kg/day for dams and fetuses, respectively, which are 3 orders of magnitude lower than the previously reported NOAELs (see Attachment-2 (3), 182-500 mg/kg/day for dams, 182-420 mg/kg/day for fetuses).

3) Information on general toxicity

(1) Acute toxicity (Table 1)

The acute toxicity of BBP is relatively low. In rats dosed orally, weight loss, loss of vitality and leukocytosis are reported. Histopathologically, congestive encephalopathy, myelin degeneration and splenitis due to neuroglial cell proliferation and degeneration of central nervous system structures are also observed (IPCS, 1999).

Table 1 Results of acute toxicity studies

	Mouse	Rat	Rabbit
Oral LD_{50}	—	2,000-20,000 mg/kg*	—
Inhalation LD_{50}	—	—	—
Percutaneous LD_{50}	6,700 mg/kg	6,700 mg/kg	—
Intraperitoneal LD_{50}	—	—	—

*: Variable depending on the studies.

(2) Repeated-dose toxicity (Attachment-3)

In a 106-week feeding study male and female B6C3F$_1$ mice (aged 4-5 weeks) given BBP at 0, 6,000 and 12,000 ppm (equivalent to 0, 1,029 and 2,058 mg/kg/day in males and to 0, 1,037 and 2,074 mg/kg/day in females), body weight decreased dose-dependently in both sexes (NTP, 1982).

In a 14-day feeding study male F344 rats (aged 12-15 weeks) given diets containing BBP at 0, 0.625, 1.25, 2.5 and 5.0% (equivalent to 0, 447, 890, 1,338 and 1,542 mg/kg/day), liver and kidney weights increased in 0.625% or higher groups, and body weight decreased in 2.5% or higher groups. In 5% group, multifocal and chronic hepatitis and cortical lymphocytosis in thymus were observed (Kluve et al., 1984; Agarwal et al., 1985).

In a 21-day feeding study in male and female F344 rats (age, unspecified), BBP at 0, 1.2 and 2.5% developed peroxisome proliferation (Barber et al., 1987).

In a 1-month feeding study in female F344 rats (age, unspecified) BBP at 0, 6,000, 12,000 and 24,000 ppm (equivalent to 0, 300, 600 and 1,200 mg/kg/day) induced peroxisome proliferation (NTP, 1997).

In a 3-month feeding study in male and female Wistar rats (aged 4-6 weeks), BBP at 0 and 2,500-12,000 ppm (equivalent to 0, 151, 381 and 960 mg/kg/day in males and to 0, 171, 422 and 1,069 mg/kg/day in females) caused relative liver weight increase in males in 151 mg/kg/day group and females in 171 mg/kg/day, or higher groups, and increased relative kidney weight, histopathological lesions in pancreas (islet cell enlargement, vacuolation, congestion, inflammation, fibrosis) and decreased urinary pH (males) were observed in males in the 381 mg/kg/day group and females in the 422 mg/kg/day or higher groups. Hepatic necrosis and anemia were observed in males in 960 mg/kg/day group and females in 1,069 mg/kg/day or higher groups (Hammond et al., 1987).

In another 3-month feeding study, male and female SD rats (aged 4-6 weeks) were given BBP at 0 and 2,500-20,000 ppm (equivalent to 0, 188, 375, 750, 1,125 and 1,500 mg/kg/day), resulting in increased relative weights of kidney and liver in males in 750 and 1,125 mg/kg/day or higher groups, respectively, and increased liver weight in females in 750 mg/kg/day or higher groups. However, the histopathological changes of pancreas seen in the above Wistar rats were not observed (Hammond et al., 1987).

In a 26-week feeding study, male F344/N rats (aged 6 weeks) were exposed to BBP at 0, 300, 900, 2,800, 8,300 and 25,000 ppm (equivalent to 0, 30, 60, 180, 550 and 1,650 mg/kg/day). The liver weight increased in 8,300 ppm or higher groups, and decreased body weight, increased relative kidney weight and increased incidence of macrocytic anemia were observed in 25,000 ppm group (NTP, 1997).

In a 52-week feeding study, the dietary BBP exposure of female F344 rats (aged 6 weeks) at 0, 6,000, 12,000 and 24,000 ppm (equivalent to 0, 300, 600 and 1,200 mg/kg/day) was associated with peroxisome proliferation (NTP, 1997).

In a 106-week feeding study, male and female F344 rats (aged 6 weeks) were given BBP at 0, 3,000, 6,000 and 12,000 ppm in males and at 0, 6,000, 12,000 and 24,000 ppm in females (equivalent to 0, 120, 240 and 500 mg/kg/day in males and to 0, 300, 600 and 1,200 mg/kg/day in females). The kidney weight increased in males in 3,000 ppm or higher groups, and nephropathy was observed in females in 6,000 ppm or higher groups. The liver weight increased in males in 12,000 ppm group, and decreased body weight, renal tubule pigmentation, hepatic granuloma and splenic hyperplasia were observed in males in 12,000 ppm group and females in 24,000 ppm group (NTP, 1997).

In a 3-month feeding study, male and female dogs (adult) were exposed to BBP at 0 and 10,000-50,000 ppm (equivalent to 0, 400, 1,000 and 1,852 mg/kg/day in males and to 0, 700, 1,270 and 1,973 mg/kg/day in females based on the figures in one of the Table). The body weight decreased in males in 400 and 1,852 mg/kg/day groups and females in 1,270 mg/kg/day or higher groups (Hammond et al., 1987).

In a 13-week inhalation study, male and female SD rats (aged 6-8 weeks) were exposed to BBP mists at aereal concentrations of 0, 50, 218 and 789 mg/m^3 (equivalent to 0, 9.2, 39.4 and 143 mg/kg/day in males and to 0, 9.8, 42 and 152 mg/kg/day in females) for 6 h/day. The liver and kidney weights decreased in both sexes in 789 mg/m^3 group, with decrease in blood glucose in males alone (Hammond et al., 1987).

4) Information on mutagenicity/genotoxicity and carcinogenicity

(1) Mutagenicity/genotoxicity (Table 2)

In *in vitro* studies, BBP was negative in the reverse mutation test using *Salmonella typhimurium* strains (Litton Bionetics Inc., 1976; Rubin et al., 1979; Zeiger et al., 1982; 1985; Kozumbo et al., 1982).

In gene mutation assay with mouse lymphoma cells, positive results without metabolic

activation were reported (Myhr et al., 1986; Myhr & Caspary, 1991). However, since BBP was tested at such high concentrations that produced precipitation, NTP considered that the assay is invalid (NTP, 1997).

In the BALB/3T3 cell transformation assay, BBP was reported to be negative (Litton Bionetics Inc., 1977; Barber et al., 2000). BBP was also negative in chromosomal aberration test and sister chromatid exchange test using Chinese hamster ovary cells (CHO cells) in culture (Galloway et al., 1987).

In *in vivo* studies, BBP was weakly positive in chromosomal aberration test and sister chromatid exchange test using bone marrow cells from mice treated once intraperitoneally at 1,250-5,000 mg/kg (NTP, 1997). In sex-linked recessive lethal test in *Drosophilia melanogaster*, BBP was negative (Valencia et al., 1985; IPCS, 1999).

Table 2 Results of mutagenicity/genotoxicity assays

Test method		Cells/animal species used	Results*	References
In vitro	Reverse mutation test	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1539, S9(+/-) and 10-10,000 μg/ml	-	Litton Bionetics Inc., 1976; Rubin et al., 1979; Zeiger et al., 1982; 1985 ; Kozumbo et al., 1982
	Gene mutation test	L5178Y mouse lymphoma cells, S9 (+/-)	-	Litton Bionetics Inc., 1977; Hazleton Biotechnologies Company, 1986; Barber et al., 2000
		L5178Y mouse lymphoma cells, S9(+/-). (Negative under the condition of +S9)	+ -	Myhr et al., 1986; Myhr & Caspary, 1991
	Cell transformation assay	BALB/3T3 cells	-	Litton Bionetics Inc., 1977; Barber et al., 2000
	Chromosomal aberration test	CHO cells S9 (+/-)	-	Galloway et al., 1987
	Sister chromatid exchange test	CHO cells S9 (+/-)	-	Galloway et al., 1987
In vivo	Chromosomal aberration test	Mouse bone marrow cells, 1,250-5,000 mg/kg×1, i.p.	+w	NTP, 1997
	Sister chromatid exchange test	Mouse bone marrow cells, 1,250-5,000 mg/kg×1, i.p.	+w	NTP, 1997
	Sex-linked recessive lethal test	*Drosophilia melanogaster*	-	Valencica et al., 1985
	Micronucleus test	Mouse	-	IPCS, 1999

*-: Negative +: Positive +w: Weakly positive

(2) Carcinogenicity (Table 3, Attachment-4)

With respect to carcinogenicity of BBP in rodents, the results of NTP studies on carcinogenicity are available (Attachment-4).

In a 103-week feeding study, male and female B6C3F_1 mice (aged 5-6 weeks) were given BBP at 0, 6,000 and 12,000 ppm (equivalent to 0, 1,029 and 2,058 mg/kg/day in males and to 0, 1,037 and 2,074 mg/kg/day in females). No histopathological changes were observed in any of the BBP groups (NTP, 1982).

In another 103-week feeding study, male and female F344 rats (aged 5 weeks) were given BBP at 0, 6,000 and 12,000 ppm (equivalent to 0, 474 and 948 mg/kg/day in males and to 0, 550 and 1,100 mg/kg/day in females) (all males were sacrificed at dosing weeks 29-30 due to significant death). The incidence of mononuclear cell leukemia (MNCL) increased in females in 12,000 ppm group (NTP, 1982).

However, in a second 106-week feeding study in which male and female F344 rats (aged 6 weeks) were exposed to BBP in diet at 0, 3,000, 6,000 and 12,000 ppm in males

and at 0, 6,000, 12,000 and 24,000 ppm in females (equivalent to 0, 120, 240 and 500 mg/kg/day in males and to 0, 300, 600 and 1,200 mg/kg/day in females), the incidence of mononuclear cell leukemia (MNCL) in females was not different among the groups. In males, meanwhile, combined incidences of pancreatic acinar cell tumors and carcinomas in the control and 3,000, 6,000 and 12,000 ppm groups were 3/50, 2/49, 3/50 and 10/50, respectively, indicating obvious increase in the highest group. The incidences of hyperplastic lesions of pancreatic acinar cells in the control and 3,000, 6,000 and 12,000 ppm groups were 4/50, 0/49, 9/50 and 12/50, respectively, indicating obvious increase in the highest group. Based on these results, NTP concluded that BBP is carcinogenic in male rats (NTP, 1997).

Despite the negative results in most of mutagenicity and genotoxicity studies, BBP is carcinogenic in rats. It is therefore likely that BBP acts as a promoter in the multistep processes of carcinogenicity. However, no data are available concerning the promoting effect of BBP.

No data are found concerning the potential carcinogenicity of BBP in humans.

Table 3 Evaluation of carcinogenicity by national and international organizations

Organization	Category	Significance	References
EPA	Group C	Possible human carcinogen.	IRIS, 2002
EU	—	No evaluation.	ECB, 2000
NTP	—	No evaluation.	NTP, 2000
IARC	Group 3	Unclassifiable as to carcinogenicity to humans	IARC, 2001
ACGIH	—	No evaluation.	ACGIH, 2001
Japan Society for Occupational Health	—	No evaluation.	Japan Society for Occupational Health, 2001

5) Information on immune system

At present, no data are found concerning the potential immunotoxicity of BBP.

6) Fate and Metabolism

In a dermal toxicity study, 49 mg/kg of BBP labeled with ^{14}C in the benzene ring was applied to dorsal skin of male F344 rats, and the application site was covered. By 7 days after application, 27% of the dose was absorbed. The majority of the remaining radiocarbons was recovered from the application site (Elsisi et al., 1989). In oral gavage

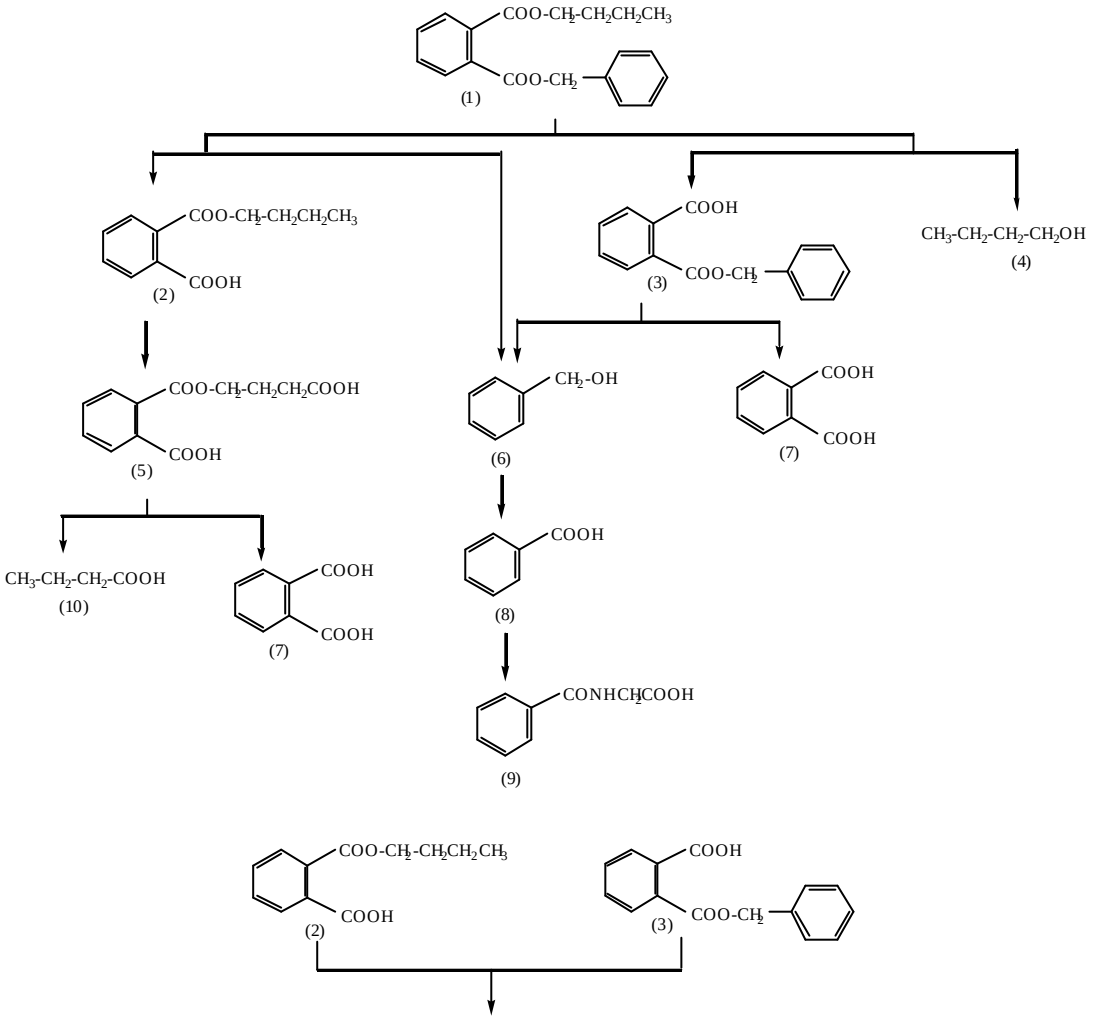
study in which dogs were given with 5,000 mg/kg of BBP, only 10% of the dose was absorbed (Erickson, 1965; CERHR, 2000).

Male F344 rats were dosed once orally with 2, 20, 200 or 2,000 mg/kg of BBP. The fraction of the dose absorbed was 61-74% after 2-200 mg/kg, but only 16% after 2,000 mg/kg/day (Eigenberg et al., 1986).

Male F344 rats were treated intravenously with 20 mg/kg of BBP labeled with ^{14}C in benzene ring. The radioactivity was detected in blood, liver, kidney, muscle, skin, small intestine, adipose tissue, brain, lung, testis and spleen immediately after dosing, but the concentrations in blood, liver, kidney, muscle, skin and small intestine declined to 1/2 or less than the peak level within 30 minutes after dosing (Eigenberg et al., 1986).

In male Wistar rats, BBP orally given was promptly hydrolyzed by esterases secreted from the small intestine to its monoester phthalates (monobutyl and monobenzyl phthalates), which were absorbed and excreted into urine as free monoesters or as glucuronide conjugates (Fig. 1) (Mikuriya et al., 1988). In another metabolism study in female Wistar rats given BBP by oral gavage at 150, 475, 780 and 1,500 mg/kg/day for 3 days, urine contained monobutyl phthalate, monobenzyl phthalate, hippuric acid, phthalic acid, benzoic acid and carboxypropyl phthalate, and ω-oxidation product of monobutyl phthalate, but no glucuronide metabolites. The absence of glucuronidation of the monoesters is due to sex difference (Fig. 1) (Nativelle et al., 1999).

In a study with male F344 rats exposed to 2-200 mg/kg of BBP, about 90% of the dose was eliminated within 24 hours after dosing, with 80% and 20% of this fraction being eliminated via urine and feces, respectively. In another study in male F344 rats treated intravenously with 20 mg/kg of BBP ^{14}C-labeled in its benzene ring, 55% and 34% of the administered radioactivity were recovered in bile and urine, respectively (Eigenberg et al., 1986).



(1) Butyl benzyl phthalate
(2) Monobutyl phthalate
(3) Monobenzyl phthalate
(4) Butyl alcohol
(5) Carboxypropyl phthalate
(6) Benzyl alcohol
(7) Phthalic acid
(8) Benzoic acid
(9) Hippuric acid
(10) Butylic acid

Fig. 1 Metabolic pathways for butyl benzyl phthalate

2. Hazard assessment at present

Concerning the effects on endocrine and reproductive systems, there is no report which clarifies causal relationship of the effects with BBP exposure.

Although BBP had a weak binding affinity to estrogen receptors (1/28,000－1/80,000 of E_2) in *in vitro* binding assay which was performed to assess the effect on endocrine system, it showed no estrogenic activity when tested *in vivo* by uterotrophic assay. In the Hershberger assay in castrated male rats administered BBP orally, weights of male accessory reproductive organs decreased, and oral administration to pregnant rats resulted in manifestation of reproductive toxicities in F_1 males. These findings suggest the potential anti-androgenic activity of BBP.

As for major effect of BBP on reproductive system, repeated-dose studies indicated weight decrease and degeneration in male rat testis and accessory reproductive organs at higher doses of 1,000 mg/kg/day or more. In reproductive & developmental toxicity tests, it was reported that body weight of pups decreased at doses of 100 mg/kg/day or more, and number of surviving pups decreased at doses equivalent to 375 mg/kg/day or more. Most of the findings on decrease in fertility index, increase in resorbed embryo, decrease in surviving pups and survival rate, as well as external, skeletal and visceral malformations in fetuses were reported for rather high doses(equivalent to 654－1,640 mg/kg/day or more). Additionally, increase in infertility considered to be related to the effects on male reproductive organs of the parental animals was observed at higher dose equivalent to 2,200 mg/kg/day.

As the information related to hazardous effects of BBP in humans, there are discrepant reports indicating that it is irritating to skin and that it is neither skin irritating nor sensitizing. In the animal studies, mainly liver and kidney were affected after repeated oral and dermal administration. In mutagenicity studies, BBP was generally negative in a series of both *in vitro* and *in vivo* assays, but also reported to be weakly positive in some assays. In carcinogenicity studies, no histopathological changes were observed in mice. In rats, however, increased incidence of mononuclear cell leukemia (MNCL) and development of pancreatic adenoma/carcinoma were observed in males, suggesting the possibility that BBP may acts as a promotor.

3. Risk assessment and other necessary future measures

On BBP two-generation reproductive toxicity study is going on. Since it is suggested that there is a possibility that BBP has anti-androgenic action, endocrine disruption and associated toxicities of BBP will be comprehensively assessed by incorporating the result

of the above two-generation reproductive toxicity test.

On the other hand, since findings accumulated so far suggest that BBP has reproductive & developmental toxicity regardless of the interaction with endocrine systems, risk assessment based on the hazard and exposure assessment is to be conducted and appropriate risk management be examined.

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Attachment-1 Results of *in vitro* assays on receptor binding

Item	Test methods and conditions	Results	Conclusion	References
ER binding assays	Methods: Competitive binding assay with [^{3}H]-E2 as a ligand. Receptor: Uterine homogenate from immature SD rats Temperature: 30°C pH: 7.6 Exposure concentration: 10^{-6} - 10^{-3} M	IC50 value: 3.6×10^{-5} M (E2: 1.3×10^{-9} M)	BBP has a binding affinity for ER. (The binding affinity is 1/28,000 that of E2.)	Zacharewski et al., 1998
	Methods: Competitive binding assay with [^{3}H]-E2 as a ligand. Receptor: Uterine homogenate from immature SD rats pH: 7.4 Temperature: 4°C	IC50 value: 7.2×10^{-5} M (E2: 8.99×10^{-10} M)	BBP has a binding affinity for ER. (The binding affinity is 1/80,000 that of E2.)	Blair et al., 2000
	Methods: Competitive binding assay with [^{3}H]-E2 as a ligand. Receptor: GST-ERdef fused protein (human, mouse and chicken) Temperature: 4°C	BBP binds very weakly within concentration range of 10^{-9}-10^{-4} M.	BBP has a binding affinity for ER.	Matthews et al., 2000
	Methods: Competitive binding assay with a fluorescent ligand (ES1) as a ligand. Receptor: Human ERα Temperature: 25°C	The inhibition of ligand binding increased at 5×10^{-5} M or above.	BBP has a binding affinity for ER.	Hashimoto et al., 2000
	Methods: Human ER binding assay (recombinant ERα ligand domain)	IC50: 4.0×10^{-5} M (E2: 1.3×10^{-9} M) RBA: 0.0032%	BBP has binding affinity for ER. (The binding affinity is 1/31,000 that of E2.)	CERI, 2001b
Yeast two-hybrid assay	Cells: Yeast cells transfected with Gal4 DNA binding domain/human ER ligand binding domain genes, Gal4 activation domain/coactivator TIF2 genes and β-galactosidase reporter gene.	REC10: 5×10^{-4} M (E2: 3×10^{-10} M)	BBP activates ER-mediated transcription.	Nishihara et al., 2000
	Cells: Yeast cells transfected with Gal4 DNA binding domain/human ER ligand binding domain genes, Gal4 activation domain/coactivator TIF2 genes and β-galactosidase reporter gene. Concentration: 5×10^{-7} - 5×10^{-3} M (BBP)	Weak β-galactosidase activity was detected at 5×10^{-5} M and 5×10^{-4} M. (When the response to 10^{-7} M E2 is assumed as 100%, the relative value of the maximal response to 5×10^{-4} M BBP was 10%.)	BBP activates ER-mediated transcription.	Hashimoto et al., 2000
Human ER responsive yeast cell growth assay	Cells: *S. cerevisiae* strain PL3 transfected with human ER. Exposure concentration: 10^{-5} M (BBP), 10^{-9} M (E2), incubation: 5 days	Weak growth of the yeast cells was detected from day 3 of incubation. (Distinct growth of the yeast cells for E2 was detected from day 3 of incubation)	BBP has weak cell growth-promoting activity.	Zacharewski et al., 1998

Item	Test methods and conditions	Results	Conclusion	References
Reporter gene assay with recombinant yeast cells	Cells: Yeast cells transfected with human progesterone receptor gene and β-galactosidase reporter gene. Concentration: 10^{-6} M (BBP), 10^{-8}M (progesterone), 10^{-6} M (BBP)+10^{-8} M (progesterone) Incubation : 12 hr	When the cells were exposed to progesterone at 10^{-8} M at which no significant activity is detected upon exposure to 10^{-6} M BBP in combination with 10^{-6} M BBP, the progesterone activity was not affected.	BBP does not activate progesterone receptor-mediated transcription.	Tran et al., 1996
	Cells: Yeast cells transfected with human ER gene and β-galactosidase reporter gene. Concentration: 10^{-11} - 10^{-5} M (BBP), 10^{-13} - 10^{-7} M (E2) Incubation: 18 hr	The β-galactosidase activity increased dose-dependently within a range of 10^{-8}-10^{-5} M. (When the response to E2 is assumed as 100%, the relative value of the maximal response to 10^{-5} M BBP is 5.3%.)	BBP activates the ER-mediated transcription of the reporter gene. (The potency of transcription activation was 1/250,000 that of E2.)	Coldham et al., 1997
	Cells: Yeast cells stably transfected with human ER gene and β-galactosidase reporter gene. Concentration: 5×10^{-7} - 10^{-3} M (BBP), 4.8×10^{-12} - 10^{-8} M (E2) Incubation: 4 - 6 days	β-galactosidase activity increased dose-dependently within a range of 10^{-6}-10^{-3} M. (When the response to E2 is assumed as 100%, the relative value of the maximal response to 10^{-3} M BBP was 50%.)	BBP activates the ER-mediated transcription. (The potency of transcription activation was 1/1,000,000 that of E2.)	Harris et al., 1997
Reporter gene assay with recombinant cell cultures	Cells: MCF-7 cells transiently transfected with Gal4-human ER gene and Gal4-regulated promoter containing luciferase reporter gene and HeLa cells stably transfected with these genes. Concentration:10^{-7}, 10^{-6}, 10^{-5} M (BBP) 10^{-12} - 10^{-8} M (E2) Incubation: 24 hr	MCF-7 cell assay: The luciferase activity was detected at 10^{-5} M (the activity was 46% of that induced by 10^{-8} M E2.) HeLa cell assay: The luciferase activity was detected at 10^{-5}M (the activity was 34% of that induced by 10^{-8} M E2). (Upon exposure to E2, the % transcription activation increased dose-dependently within a range of 10^{-12} - 10^{-8} M. At E2=10^{-8} M, the transcription activation of the reporter gene increased 23-fold in the MCF-7 cells and 11-fold in the HeLa cells.)	BBP activates the ER-mediated transcription.	Zacharewski et al., 1998
	Cells: Yeast cells stably transfected with human androgen receptor (AR) gene and β-galactosidase reporter gene. Concentration: 2×10^{-8} - 5×10^{-5}M (BBP), 1.25×10^{-9} M (DHT) Incubation: 4 - 6 days	Agonist activity: Negative within a range of 2×10^{-8}-5×10^{-8} M. Antagonist activity: BBP antagonized the agonist effect of 1.25×10^{-9} M DHT within a range of 2×10^{-8}-2×10^{-5} M.	BBP does not activate the AR-mediated transcription. (BBP antagonizes the effect of DHT. BBP has no agonist activity.)	Sohoni & Sumpter, 1998

Item	Test methods and conditions	Results	Conclusion	References
	Cells: HeLa cells incorporated with ER expression plasmid and ER response element. Concentration: 10^{-11} - 10^{-5} M	PC50: 4.1×10^{-6} M (E2: $<10^{-11}$ M)	BBP activates ER-mediated transcription. (The potency of activating the gene transcription is 1/410,000 that of E2.)	CERI, 2001b
Human breast cancer cell proliferation assay	Cells: Human breast cancer cells (MCF-7 cells and E-screen assay) Concentration: 10^{-5} M (BBP) 10^{-10} M (E2) Incubation: 5 days	The cell proliferation was detected at 10^{-5}M (When the response to E2 is assumed as 100%, the relative value of the maximal response to 10^{-5} M BBP was 90%.)	BBP induces cell proliferation. (The potency of activating gene transcription is 1/100,000 that of E2.)	Soto et al., 1995, 1997
	Cells: Human breast cancer cells (MCF-7 and ZR-75 cells) MCF-7: Concentration: 10^{-5} M (BBP), 10^{-8} M (E2) Incubation: 11 days ZR-75-1: Incubation: 10^{-5} M, 10^{-6} M, 10^{-7} M (BBP), 10^{-8} M, 10^{-10} M, 10^{-12} M (E2) Incubation: 10 days	MCF-7 cell assay: Cell proliferation was detected at 10^{-5} M. ZR-75 cell assay: Cell proliferation was detected at 10^{-5} M. (Upon exposure to E2, dose-dependent cell proliferation was detected within a range of 10^{-8}-10^{-12} M.)	BBP induces cell proliferation.	Harris et al., 1997
	Cells: Human breast cancer cells (MCF-7) Concentration: 10^{-10} -10^{-5} M (BBP), 10^{-14} -10^{-8} M (E2) Incubation: 6 days	Dose-dependent cell proliferation was detected within a range of 10^{-10}-10^{-6} M. (When the maximal response to E2 was assumed as 100%, the relative value for 10^{-3}M BBP was 80%.) (Upon exposure to E2, dose-dependent cell proliferation was detected within a range of 10^{-14}-10^{-11} M.)	BBP induces cell proliferation.	Jones et al., 1998
	Cells: Human breast cancer cells (MCF-7) Concentration: $\leq10^{-4}$ M (BBP), 10^{-12} - 10^{-8} M (E2) Incubation: 5 days	Cell proliferation was detected at 3×10^{-5} M. (When the maximal response to E2 was assumed as 100%, the relative value for 3×10^{-5}M BBP was 80%.)	BBP induces cell proliferation. (The potency of BBP was 1/250,000 that of E2.)	Korner et al., 1998
Reporter gene assay with recombinant cell cultures	Cells: Human breast cancer cell line, MCF-7 cells stably transfected with human ER gene and luciferase reporter gene (MVLN cells) Concentration: 10^{-10} -10^{-5} M (BBP), 10^{-9} M (E2) Incubation: 24 hr	Significant luciferase activity was detected upon exposure to BBP. (When the maximal response to E2 was assumed as 100%, the relative value for 10^{-3} M BBP was 65%.)	BBP activates the ER-mediated transcription. (The activity of transcription activation is 1/2,100 that of E2.)	Itoh et al., 2000

ER: Estrogen receptor E2: 17β-estradiol REC10: Concentration equivalent to that produces 10% of the activity of 10^{-7} M E2 PC50: Concentration equivalent to that produces 50% of the maximal response to E2 IC50: 50% inhibition concentration

Attachment-2 Results of the fertility and reproductive toxicity studies in mammals

(1) Results of fertility studies by screening technique

Animal species	Administration method	Administration period	Dose	Results	References
Mouse (CFLP, female) 7 mice/group	s.c. (Uterotrophic assay)	Mice were exposed for 3 days from pnd. 18, and uterus was removed on the 4th day and weighed.	0, 0.05, 0.5 and 5 mg/mouse	No effect on uterus weight.	Coldham et al., 1997
Rat (SD, female) 6 rats/group	s.c. (Uterotrophic assay)	Rats were exposed for 3 days from pnd. 20, and uterus was removed 24 hr after the final dose and weighed.	0, 500, 1,000 and 2,000 mg/kg	No effect on uterus weight.	CERI, 2001a
			0, 500, 1000 and 2000 mg/kg + Ethinylestradiol, 0.6 μg/kg/day, s.c.	No effect on uterus weight.	
Rat (SD, female) 10 rats/group	Gavage (Uterotrophic assay) (Ovariectomized on pnd. 19.)	Rats were exposed for 4 days from pnd. 31, and uterus was removed on the 5th day and weighed.	0, 1, 20, 200 and 2,000 mg/kg	No effect on uterus weight.	Zacharewski et al., 1998
Rat (SD, male) Castrated at the age of 6 weeks	Gavage (Hershberger assay)	Rats were exposed for 10 days from 8 days after castration and necropsied about 24 hr after the final dose.	0, 40, 200 and 1,000 mg/kg/day	No effects on weights of male accessory reproductive organs.	CERI, 2001a
			BBP 0, 40, 200 and 1,000 mg/kg/day + testosterone propionate 0.4 mg/kg/day, s.c.	Decreases in the absolute and relative weights of abdominal lobe of prostate, relative weight of seminal vesicle, absolute and relative weights of bulbourethral gland and weight of the bulbospongiosus muscle + levator ani muscle in the 200 mg/kg or higher groups (the dose-dependency is obscure).	
Rat (SD, female)	Gavage (in corn oil)	gd. 14 – pnd. 3. AGD and testis weight were determined on pnd. 2.	0, 750 mg/kg/day	F_1: Decreased testis weight, reduced ano-rectal distance (AGD) and increased incidence of nipple retention (pnd. 13)	Parks et al., 1999
Rat (SD, female)	Gavage (in corn oil)	gd. 15 – pnd. 3	0, 750 mg/kg/day	F_1: Decreased birth weight in both sexes, reduced AGD in males, decreased testis weight and increased incidences of areolar and nipple retention.	Gray et al., 2000

(2) Results of repeated-dose and fertility toxicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Rat (F344, male) (aged 12-15 weeks) 10 rats/group	By feeding	14 days	0, 0.625, 1.25, 2.5, 5.0% (Corresponding to 0, 447, 890, 1,338 and 1,542 mg/kg/day)	Decreased weights of testis, epididymis, prostate and seminal vesicle, atrophy of testis, prostate and seminal vesicle, immature epididymal spermatocytes, epithelial necrosis of seminiferous tubules and increases in luteinizing hormone and follicle-stimulating hormone at 2.5% or above. Atrophy of epididymis and decrease in plasma testosterone at 5%.	Kluwe et al., 1984; Agarwal et al., 1985
Rat (Alpk:Apf SD, male)	Gavage	Pnd. 22-23, 14 days	0, 500 mg/kg/day	No effect on testis or accessory reproductive organs.	Ashby & Lefevre, 2000
		Pnd. 35-36, 14 days	0, 500 mg/kg/day	No effect on testis or accessory reproductive organs.	
		Pnd. 35-36, 20 days	0, 500 mg/kg/day	No effect on testis or accessory reproductive organs.	
Rat (F344/N, male) (aged 6 weeks) 11-15 rats/group	By feeding	26 weeks	0, 300, 900, 2,800, 8,300, 25,000 ppm (Corresponding to 0, 30, 60, 180, 550 and1,650 mg/kg/day)	Decreases in testis, seminal vesicle and epididymis weights, degeneration of testis and epididymis, atrophy of seminiferous tubules and decreased sperm count at 25,000 ppm.	NTP, 1997
Rat (F344/N, male) (aged 6 weeks)	By feeding	106 weeks	Male: 0, 3,000, 6,000, 12,000 ppm (Male: Corresponding to 0, 120, 240 and 500 mg/kg/day)	Increased epididymis weight at 6,000 ppm or above.	NTP, 1997
Rat (F344/N, male) (aged 6 weeks) 15 rats/group	By feeding	For 10 weeks before mating, mated with 2 untreated females for 7 days. Females were necropsied on gd. 13 (numbers of corpora lutea and implanations). Males were necropsied after mating.	0, 300, 2,800, 25,000 ppm (Corresponding to 0, 20, 200 and 2,200 mg/kg/day)	F_0 males: Decreased sperm concentration, decreases in relative prostate weight and relative testis weight, decreases in epididymis and seminal vesicle weights, degeneration of testis and epididymis and increased infertility rate in females (10/30 females) mated with males in the 25,000 ppm group NOAEL: 200 mg/kg/day LOAEL: 2,200 mg/kg/day	NTP, 1997
Rat (Wistar, male and female) (age, unspecified) Female: 24 females/group, Male: 12 males/group	By feeding	Male: 10 weeks before mating. Female: 2 weeks before mating and through the gestation and lactation period. Females were mated again with the F_0 males after weaning of the F_{1a} pups.	0, 0.2, 0.4, 0.8% [Male (in terms of mg/kg/day): Before mating - 0, 108, 206, 418. Female (in terms of mg/kg/day): Before mating - 0, 106, 217, 446. Gestation period - 0, 116, 235, 458. Lactation period - 0, 252, 580, 1,078]	F_0 females: Suppressed body weight gain during the gestation and lactation periods at 0.8%. F_{1a}: No effect.	TNO, 1993

Animal species	Administration method	Administration period	Dose	Results	References
				F_0 females: No effect. F_{1b}: Decreased body weight (pnd. 21). NOAEL: (Male) 418 mg/kg/day (Female) 446 mg/kg/day	
Rat (WU, male and female) (aged 10-11 weeks) 15 rats/sex/group	Gavage (in corn oil)	2 Weeks before mating. Mating was continued for a maximal of 2 weeks. F_0 males were necropsied 29 days after the start of dosing. In F_0 females, gavage administration was continued until day 6 postpartum and necropsied. F_1 offsprings were necropsied on pnd. 6.	0, 250, 500, 1,000 mg/kg/day	F_0 males: Decreased testis and epididymis weights, Leydig cell hyperplasia and testicular degeneration at 1,000 mg/kg/day. F_0 females: Decreased fertility at 1,000 mg/kg/day. F_1 males and females: Decreased birth weight at 500 mg/kg/day. Decreased number of live pups/litter (at birth and on pnd. 6) and decreased body weight at birth and on pnd. 6 at 1,000 mg/kg/day. NOAEL: 500 mg/kg/day LOAEL: 1,000 mg/kg/day	Piersma, 1995
Rat (SD, male and female) (male: aged 6 weeks, female: aged 13 weeks) 25 rats/sex/group	p.o. (in corn oil)	F_0 males and F_0 females were exposed for 12 and 2 weeks, respectively, before mating, and the pairs were allowed to mate for a maximum of 2 weeks. F_0 females were treated throughout the gestation, delivery and lactation periods and necropsied after weaning of the F_1. F_1 after weanlings were mated within the same dose group and necropsied as described for the F_0 animals.	0, 20, 100, 500 mg/kg/day	F_0 females: Increased ovary weight at 100 mg/kg/day or above. F_1 males and females: Decreased body weight at 100 mg/kg/day or above and reduced AGD at birth at 500 mg/kg/day. F_1 males: Delayed prepuce separation and decreased serum testosterone at 500 mg/kg/day. NOAEL: 20 mg/kg/day	Nagao et al., 2000

(3) Results of developmental toxicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Mouse (CD-1, female) 30 mice/group	By feeding	gd. 6-15 (cesarean section on gd. 17) Examination of F_0 dams (body weight, liver, kidney and uterus weights, number of corpora lutea, number of implantations). Examination of fetuses (body weight, gross examination for external, visceral and skeletal malformations).	0, 0.1, 0.5, 1.25% (Corresponding to 0, 182, 910 and 2,330 mg/kg/day)	F_0 females: Suppressed body weight gain at 0.5% or above. F_1: Increased number of dead fetuses and increased incidence of malformations (rib, sternum, vertebral column) (control: 31%, 0.5% group: 61%, 1.25% group: 100%). Suppressed body weight gain at 1.25%. NOAEL: 182 mg/kg/day (dams) 182 mg/kg/day (fetuses) LOAEL: 910 mg/kg/day (dams) 910 mg/kg/day (fetuses)	Price et al., 1990
Rat (CD, female) 30 rats/group	By feeding	gd. 6-15 (cesarean section on gd. 20) Examination of F_0 dams (body weight, liver, kidney and uterus weights, number of corpora lutea, number of implantations). Examination of all fetuses (body weight, gross examination of external, visceral and skeletal malformations) .	0, 0.5, 1.25, 2.0% (Corresponding to 0, 420, 1,100 and 1,640 mg/kg/day)	F_0 females: Suppressed body weight gain, increased food consumption and water intake and increased relative liver weight at 1.25% or above. Motor ataxia, gait disturbance and increased relative kidney weight at 2.0%. F_1: Decreased body weight, increased number of resorptions and increased incidence of malformations (urinary tract, eye, vertebral column) (control: 2%, 2.0% group: 53%). NOAEL: 420 mg/kg/day (dams) 420 mg/kg/day (fetuses) LOAEL: 1,100 mg/kg/day (dams) 1,100 mg/kg/day (fetuses)	Field et al., 1989
Rat (Wistar, female) 15-18 rats/group	By feeding	gd. 0-20 (cesarean section on gd. 20) F_0 dams: Body weight, food consumption and necropsy (number of implantations). Fetuses: Sex, body weight, skeletal malformations and visceral malformations.	0, 0.25, 0.5, 1.0, 2.0% (Corresponding to 0, 185, 375, 654 and 974 mg/kg/day)	F_0 females: Suppressed body weight gain and decreased food consumption at 1.0%. Decreased body weight at 2.0%. F_1: Decreased number of live fetuses at 0.5% or above. Decreased fetal body weight at 1.0%. Increased post-implantation losses (%) at 2.0%. NOAEL: 375 mg/kg/day (dams) 185 mg/kg/day (fetuses) LOAEL: 654 mg/kg/day (dams) 375 mg/kg/day (fetuses)	Ema et al., 1990

Animal species	Administration method	Administration period	Dose	Results	References
Rat (Wistar, female) 10 rats/group	Gavage	gd. 7-15 (cesarean section on gd. 20) F_0: Body weight, food consumption and necropsy (number of implantations). Fetuses: Sex, body weight, skeletal malformations and visceral malformations.	0, 500, 750, 1,000 mg/kg/day	F_0 females: Suppressed body weight gain at 750 mg/kg/day. Deaths in 4/10 dams and litter resorptions in 6 dams at 1,000 mg/kg/day. Increased number of resorptions (3/10 dams), increased number of dead fetuses, increased post-implantation losses and increased number of malformed fetuses (external, skeletal, visceral) (control group: 1 fetus, 750 mg/kg/day group: 20 fetuses) at 750 mg/kg/day. F_1 male and female fetuses: Decreased body weight at 750 mg/kg/day. NOAEL: 500 mg/kg/day (dams) 500 mg/kg/day (fetuses) LOAEL: 750 mg/kg/day (dams) 750 mg/kg/day(fetuses)	Ema et al., 1992a
Rat (Wistar, female)	p.o.	gd. 0-20	0, 2.0% (Corresponding to 0 and 974 mg/kg/day)	Resorptions in all dams.	Ema et al., 1991;1992b
		gd. 0-11		Resorptions in all dams.	
		gd. 11-20		Increases of malformed fetuses (cleft palate, sternal fusion).	
Rat (Wistar, female)	p.o.	gd. 0-7	0, 2.0% (Corresponding to 0 and 974 mg/kg/day)	Increased resorptions.	Ema et al., 1992c
		gd. 7-16		Increased resorptions, increase in malformed fetuses (cleft palate, sternal fusion).	
		gd. 16-20		No effect.	
Rat (Wistar, female)	p.o.	gd. 0-7	0, 2.0% (Corresponding to 0 and 954 mg/kg/day)	Decreased uterus and ovary weights, decreased plasma progesterone level.	Ema et al., 1994
		gd. 0-9		Decreased uterus and ovary weights, decreased plasma progesterone level.	
		gd. 0-11		Decreased uterus and ovary weight, decreased plasma progesterone level. Increased post-implantation losses .	
Rabbit (New Zealand)	p.o. (capsule)	gd. 6-18	0, 3, 10 mg/kg/day	F_0: No effect. F_1: No effect.	Monsanto, 1978

<Results of developmental toxicity of mono-n-butyl phthalate>

Animal species	Administration method	Administration period	Dose	Results	References
Rat (Wistar, female)	p.o.	gd. 7-15	0, 250, 500, 625 mg/kg/day	F_0: Suppressed body weight gain and decreased food consumption at 500 mg/kg/day or above. F_1: Increased post-implantation losses (%), decreased number of live fetuses, decreased body weight and increases in skeletal malformations (cleft palate) and dilated renal pelvis at 500 mg/kg/day or above. NOAEL: 250 mg/kg/day (dams) 250 mg/kg/day (fetuses) LOAEL 500 mg/kg/day (dams) 500 mg/kg/day (fetuses)	Ema et al., 1995a
Rat (Wistar, female)	p.o.	gd. 7-15	0, 250, 313, 375, 438, 500 mg/kg/day	F_0: Decreased food consumption at 250 mg/kg/day or above. Suppressed body weight gain at 313 mg/kg/day or above. F_1: Increase in skeletal malformations at 313 mg/kg/day or above. Increase in visceral malformations at 375 mg/kg/day or above. Increase in post-implantation losses and increase in external malformations at 438 mg/kg/day or above.	Ema et al., 1995b
Rat (Wistar, female)	p.o.	gd. 7-9	0, 250, 375, 500, 625 mg/kg/day	F_0: Suppressed body weight gain at 375 mg/kg/day or above. F_1: Skeletal malformations and dilated renal pelvis at 375 mg/kg/day or above.	Ema et al, 1996a
		gd. 10-12	0, 250, 375, 500, 625 mg/kg/day	F_0: Suppressed body weight gain at 500 mg/kg/day or above. F_1 : No malformation.	
		gd. 13-15	0, 250, 375, 500, 625 mg/kg/day	F_0: Suppressed body weight gain at 250 mg/kg/day or above. F_1: Cleft palate and sternal fusion at 375 mg/kg/day or above.	
Rat (Wistar, female)	p.o.	gd. 7-9	0, 500, 625, 750 mg/kg/day	F_0: Suppressed body weight gain at 625 mg/kg/day or above. F_1: Skeletal malformations at 500 mg/kg/day or above. Increased resorptions and external malformations at 625 mg/kg/day or above.	Ema et al, 1996b
		gd. 10-12	0, 500, 625, 750 mg/kg/day	F_0: Suppressed body weight gain at 625 mg/kg/day or above. F_1: Increased resorptions at 625 mg/kg/day or above. No malformations.	
		gd. 13-15	0, 500, 625, 750 mg/kg/day	F_0: Suppressed body weight gain at 500 mg/kg/day or above. F_1: Increased resorptions at 500 mg/kg/day or above. Cleft palate	

Animal species	Administration method	Administration period	Dose	Results	References
				and sternal fusion at 625 mg/kg/day or above.	
Rat (Wistar-King A, female)	p.o. (in sesame oil)	gd. 15-18 F_1 males were examined for the position of the testis on gd. 20 and pnd. 30-40.	About 1,000 mg/kg/day	F_1 males: Undescented testis (87% on pnd. 30-40).	Imajima et al., 1997

<Results of developmental toxicity of monobenzyl phthalate>

Animal species	Administration method	Administration period	Dose	Results	References
Rat (Wistar, female)	p.o.	gd. 7-15	0, 250, 313, 375, 438, 500 mg/kg/day	F_0: Decreased food consumption at 250 mg/kg/day or above. F_1: Increase in skeletal malformations at 313 mg/kg/day or above. Increase in visceral malformations at 375 mg/kg/day or above. Increase in post-implantation losses (%) and increase in external malformations at 438 mg/kg/day or above. NOAEL: 250 mg/kg/day (fetuses) LOAEL: 250 mg/kg/day (dams) 313 mg/kg/day (fetuses)	Ema et al., 1996c

<Results of reproductive/developmental toxicity studies of low-dose BBP>

Animal species	Administration method	Administration period	Dose	Results	References
Rat (Wistar, female) 5-6 rats/group	p.o. (in drinking water)	2 weeks before mating and during gestation and lactation periods (untreated during the mating period). F_1 males were necropsied on pnd. 90-95. F_0 females were mated again after weaning of F_{1a} offspring.	0, 1 mg/*l* (pnd. 1-2: eq. to 0.126 mg/kg/day, pnd. 10-12: eq. to 0.274 mg/kg/day, pnd. 20-21: eq. to 0.336 mg/kg/day)	F_0: No effect F_{1a}: Increased body weight (pnd. 22), increased absolute and relative testis weights.	Sharpe et al., 1995
			Positive control: DES 0.0011 mg/kg/day	F_{1a}: Decreased body weight (pnd. 22), decreased absolute and relative testis weights.	
			0, 1 mg/*l* (pnd. 1-2: eq. to 0.126 mg/kg/day, pnd. 10-12: eq. to 0.274 mg/kg/day, pnd. 20-21: eq. to 0.336 mg/kg/day)	F_0: No effect F_{1b}: Increased body weight (pnd. 22), decreased absolute and relative testis weight, decreased daily sperm production.	
			Positive control: DES 0.0011 mg/kg/day	F_{1b}: Increased body weight (pnd. 22), decreased relative testis weight, decreased daily sperm production.	
Rat (Wistar AP, female) 18-19 rats/group	p.o. (in drinking water)	gd. 1 – pnd. 20. F_0 females were necropsied after weaning of their pups (liver enzyme activities, hematology, micronucleated erythrocytes). F_1 animals were sexed, weighed and necropsied for their sexual maturity.	0, 1 mg/*l* (Corresponding to 0 and 0.183 mg/kg/day)	F_0: No effect. F_1 males: Increased body weight (pnd. 2), reduced AGD, increased relative liver weight. F_1 females: Early vaginal patency.	Ashby et al., 1997
			Positive control: DES 0.0086 mg/kg/day	F_0: Decreased body weight F_1 male: Decreased body weight, reduced AGD, delayed prepuce separation, decreases in testis, epididymis, seminal vesicle and prostate weights, decreased sperm count. F_1 female: Increases in uterus weight and uterotrophic response, increased absolute ovary weight, early vaginal patency.	
Rat (Wistar outbred, female) 22-25 rats/group	p.o. (in drinking water)	2 weeks before mating and during gestation and lactation periods (rats were exposed during mating but not exposed for 1 week during the co-housing period.) F_0 females were necropsied after weaning of their pups. F_1 offsprings were necropsied on pnd. 89-101 (body weight, abnormalities, sexual maturation, functions). F_0 females were mated again after weaning of F_{1a} pups.	0, 0.1, 1, 3 mg/*l* (Corresponding to 0, 0.012, 0.14 and 0.385 mg/kg/day)	F_0: No effect. F_{1a}: No difference in sperm morphology, sperm count, sperm motility, estrus cycle and sexual maturity in any groups as compared with the control. Increased postnatal deaths within 4 days after birth and increased number of large pups (pnd. 4) at 1 mg/*L*. Increased postnatal deaths within 4 days after birth, increased number of hypothermic pups, increased number of large pups (pnd. 4) and increased incidence of hair loss at 3 mg/L.	CERHR, 2000

Animal species	Administration method	Administration period	Dose	Results	References
			Positive control: DES 0.0011-0.0055 mg/kg/day	(Positive control) F_0: Suppressed body weight gain, prolonged gestation period. F_1: Increased postnatal deaths within 4 days after birth, decreased number of live pups, suppressed body weight gain, delayed prepuce separation, decreased sperm count, decreased testis weight.	
			0, 1, 3 mg/*l* (Corresponding to 0, 0.14 and 0.385 mg/kg/day)	F_0: No effect. F_{1b}: Increased postnatal deaths within 4 days after birth at 1 mg/*l*. Increased postnatal deaths within 4 days after birth and increased stillborn pups at 3 mg/ *l*. NOAEL: 0.385 mg/kg/day (dams) 0.14 mg/kg/day (fetuses) LOAEL: 0.385 mg/kg/day (fetuses)	
Rat (Wistar, female) 21-25 rats/group	p.o. (in drinking water or in diet)	Females were exposed for 2 weeks before mating and during mating, gestation and lactation periods (mating period: maximum of 3 weeks). F_0 dams were necropsied after weaning of their pups. F_1 pups were examined for the number of live pups, body weight and malformations, and the number of live pups and their body weight gain were followed until pnd. 21.	0, 1, 3 ppm 1 ppm In diet (in terms of mg/kg/day) 0.08-0.09 (before mating) 0.06-0.07 (during gestation) 0.11-0.06 (during lactation) In drinking water (in terms of mg/kg/day) 0.10-0.12 (before mating) 0.11-0.11 (during gestation) 0.17-0.24 (during lactation) 3 ppm In diet (in terms of mg/kg/day) 0.27-0.28 (before mating) 0.19-0.25 (during gestation) 0.34-0.39 (during lactation) In drinking water (in terms of mg/kg/day) 0.34-0.35 (before mating) 0.35-0.35 (during gestation) 0.54-0.80 (during lactation)	F_0: No difference in body weight gain, fertility and food consumption in any groups as compared with those in the control. F_1: No great difference in the % resorptions, viability on pnd. 4 and body weight in any groups as compared with the control. NOAEL: 0.34 - 0.49 mg/kg/day (dams, in diet) 0.54 - 0.80 mg/kg/day (dams, in drinking water) 0.34 - 0.49 mg/kg/day (fetuses, in diet) 0.54 - 0.80 mg/kg/day (fetuses, in drinking water)	CERHR, 2000

Attachment-3 Results of repeated-dose toxicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Mouse ($B6C3F_1$, male and female) aged 4-5 weeks 50 mice/group	By feeding	106 weeks	0, 6,000, 12,000 ppm (Male: 0, 1,029, 2,058 mg/kg/day; Female: Corresponding to 0, 1,037 and 2,074 mg/kg/day)	Decreased body weight at 6,000 ppm or above. LOAEL (male) = 1,029 mg/kg/day (female) = 1,037 mg/kg/day	NTP, 1982
Rat (F344, male) aged 12-15 weeks 10 rats/group	By feeding	14 days	0, 0.625, 1.25, 2.5, 5.0% (Corresponding to 0, 447, 890, 1,338 and 1,542 mg/kg/day)	Increased liver and kidney weights at 0.625% or above. Decreased body weight at 2.5% or above. Multifocal and chronic hepatitis and cortical lymphocytosis in the thymus at 5%. LOAEL = 447 mg/kg/day	Kluwe et al., 1984; Agarwal et al., 1985
Rat (F344, male and female) age unspecified 5 rats/group	By feeding	21 days	0, 1.2, 2.5%	Peroxisome proliferation (increases in palmitoyl CoA oxidase, lauric acid 11-hydroxylase and lauric acid 12-hydroxylase)	Barber, 1987
Rat (F344, female) age unspecified 5 rats/group	By feeding	1 month	0, 6,000, 12,000, 24,000 ppm (Corresponding to 0, 300, 600 and 1,200 mg/kg/day)	Peroxisome proliferation (increases in palmitoyl CoA oxidase and carnitine acetyl transferase)	NTP, 1997
Rat (Wistar, male and female) aged 4-6 weeks 27-45 rats/group	By feeding	3 months	0, 2,500-12,000 ppm (Male: 0, 151, 381, 960 mg/kg/day; Female: Corresponding to 0, 171, 422 and 1,069 mg/kg/day)	Increased relative liver weight in males at 151 mg/kg/day and females at 171 mg/kg/day or above. Increased relative kidney weight, histopathological changes in the pancreas (islet cell enlargement, vacuolation, congestion, inflammation, fibrosis) and decreased urinary pH (in males alone) in males at 381 mg/kg/day and females at 422 mg/kg/day or above. Hepatic necrosis and anemia in males at 960 mg/kg/day and females at 1,069 mg/kg/day or above. LOAEL (male) = 151 mg/kg/day (female) = 171 mg/kg/day	Hammond et al., 1987
Rat (SD, male and female) aged 4-6 weeks 10 rats/group	By feeding	3 months	0, 2,500-20,000 ppm (Corresponding to 0, 188, 375, 750, 1,125 and 1,500 mg/kg/day)	Increased relative kidney weight in males at 750 mg/kg/day or above. Increased relative liver weight in males at 1,125 mg/kg/day or above. Increased liver weight in females at 750 mg/kg/day or above. LOAEL (male) = 750 mg/kg/day (female) = 750 mg/kg/day	Hammond et al., 1987

Animal species	Administration method	Administration period	Dose	Results	References
Rat (F344/N, male) aged 6 weeks 11-15 rats/group	By feeding	26 weeks	0, 300, 900, 2,800, 8,300, 25,000 ppm (Corresponding to 0, 30, 60, 180, 550 and 1,650 mg/kg/day)	Increased liver weight at 8,300 ppm. Decreased body weight, increased relative kidney weight and increased incidence in macrocytic anemia at 25,000 ppm. LOAEL = 550 mg/kg/day	NTP, 1997
Rat (F344, female) aged 6 weeks 5 rats/group	By feeding	52 weeks	0, 6,000, 12,000, 24,000 ppm (Corresponding to 0, 300, 600, 1,200 mg/kg/day)	Peroxisome proliferation (increases in palmitoyl CoA oxidase and carnitine acetyl transferase)	NTP, 1997
Rat (F344/N, male and female) aged 6 weeks 60 rats/group	By feeding	106 weeks	Male: 0, 3,000, 6,000, 12,000 ppm Female: 0, 6,000, 12,000, 24,000 ppm (Corresponding to male: 0, 120, 240 and 500 mg/kg/day; female: 0, 300, 600 and 1,200 mg/kg/day	Increased kidney weight in males at 3,000 ppm or above. Nephropathy in females at 6,000 ppm or above. Increased liver weight in males at 12,000 ppm. Decreased body weight, renal tubule pigmentation, hepatic granuloma and splenic hyperplasia in males at 12,000 ppm and females at 24,000 ppm. LOAEL (male) = 120 mg/kg/day (female) = 300 mg/kg/day	NTP, 1997
Dog (beagle, male and female) adult 3 dogs/group	By feeding	3 months	0, 10,000-50,000 ppm (Corresponding to male: 0, 400, 1,000 and 1,852 mg/kg/day; female: 0, 700, 1,270 and 1,973 mg/kg/day)	Decreased body weight in males at 400 and 1,852 mg/kg/day and females at 1,270 mg/kg/day or above.	Hammond et al., 1987
Rat (SD, male and female) aged 6-8 weeks 25 rats/group	Inhalation	6 hr/day 13 weeks (5 days/week)	0, 50, 218, 789 mg/m^3 (Corresponding to male: 0, 9.2, 39.4 and 143 mg/kg/day; female: 0, 9.8, 42 and 152 mg/kg/day)	Decreased liver and kidney weights and decreased blood glucose (males alone) in both sexes at 789 mg/m^3.	Hammond et al., 1987

Attachment-4 Results of carcinogenicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Mouse ($B6C3F_1$, male and female) aged 5-6 weeks 50 mice/group	By feeding	103 weeks	0, 6,000, 12,000 ppm (Corresponding to male: 0, 1,029 and 2,058 mg/kg/day; female: 0, 1,037 and 2,074 mg/kg/day)	No histopathological changes.	NTP, 1982; CERHR, 2000
Rat (F344/N, male and female) aged 5 weeks 50 rats/group	By feeding	103 weeks Males were killed at weeks 29-30.	0, 6,000, 12,000 ppm (Corresponding to male: 0, 474 and 948 mg/kg/day; female: 0, 550 and 1,100 mg/kg/day)	Increased incidence of mononuclear cell leukemia (MNCL) in females at 12,000 ppm.	NTP, 1982; CERHR, 2000
Rat (F344/N, male and female) aged 6 weeks 60 rats/group	By feeding	106 weeks	Male: 0, 3,000, 6,000, 12,000 ppm Female: 0, 6,000, 12,000, 24,000 ppm (Corresponding to male: 0, 120, 240 and 500 mg/kg/day; female: 0, 300, 600 and 1,200 mg/kg/day)	Increase in the combined incidence of acinar cell tumor/carcinoma of pancreas (3/50, 2/49, 3/50 and 10/50 in the control and 3,000, 6,000 and 12,000 ppm groups, respectively) and increase in incidence of hyperplastic lesion of pancreatic acinar cells (4/50, 0/49, 9/50 and 12/50 in the control and 3,000, 6,000 and 12,000 ppm groups, respectively) in males in the highest dose group.	NTP, 1997