

## Hazard assessment of benzophenone

[Benzophenone, CAS No. 119-61-9]

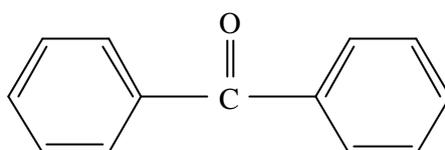
Generic name: Benzophenone

Synonyms: Diphenylmethanone, Diphenyl ketone, Benzoylbenzene, BZP

Molecular formula:  $C_{13}H_{10}O$

Molecular weight: 182.22

Structural formula:



Appearance: White crystals<sup>1)</sup>

Melting point: 48.5°C<sup>1)</sup>

Boiling point: 305.4°C (1.013×10<sup>5</sup> Pa)<sup>1)</sup>

Specific gravity:  $d_4^{18} = 1.1108$ <sup>1)</sup>

Vapor pressure: 1.33×10<sup>2</sup> Pa (108.2°C)<sup>1)</sup>

Partition coefficient: Log Pow = 3.18 (observed value)<sup>2)</sup>

Degradability: Hydrolyzability: No Report

Biodegradability: Poorly biodegradable (BOD=0%, 14 days)<sup>3)</sup>

Solubility: Water: 137 mg/l (25°C) (observed value)<sup>2)</sup>

Organic solvents: Soluble in acetone and benzene<sup>1)</sup>.

Amount of production/import:(1998): 322 t (Production 0 t, Import 322 t)<sup>4)</sup>

Usage: Raw material for synthesis of drugs and insecticides. Fragrance enhancer, UV screen<sup>1)</sup>

Applied laws and regulations: None.

<sup>1)</sup> HSDB, 2001; <sup>2)</sup> PHYSPROP, 2000; <sup>3)</sup> Tsusansho Koho (Daily), 1982; <sup>4)</sup> Ministry of International Trade and Industry, 1999; <sup>5)</sup> NTP, 2000

## 1. Toxicity Data

### 1) Information on adverse effects on human health

In a sensitization study (maximization test) of a 6% solution of benzophenone (BZP) involving 25 volunteers, no positive reaction was observed (Kligman, 1966, 1970).

### 2) Information on endocrine system and reproductive system

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

In binding assay using ligand binding domain of the human estrogen receptor, BZP did not bind to estrogen receptor up to 0.1 mM (CERI, 2001a). In yeast two-hybrid assay, BZP did not activate gene transcription (Nishihara et al., 2000).

The *in vitro* assays of BZP derivatives are now under way. In binding assay using human estrogen receptor ligand binding domain, 10 hydroxy derivatives of BZP and one dibromo-BZP were shown to bind to the estrogen receptor (Relative potency of binding affinity is 1/1,100 – 1/44,000 of that of E2)(CERI, 2001a). Benzhydrol arising from reduction of BZP did not bind to estrogen receptor (Nakagawa & Tayama, 2001). In reporter gene assay with yeast cells transfected with human estrogen receptor, BZP *per se* did not activate estrogen responsive element (ERE)-dependent transcription of reporter gene, but its derivatives (4-hydroxy-, 3-hydroxy-, 2-hydroxy-, 4-amino-, 4,4'-dihydroxy-, 4,4'-diamino-, 4-chloro-4'-hydroxy-, 2,3,4-trihydroxy-, 2,4,4'-trihydroxy- and 2,2',4,4'-tetrahydroxy-BZPs) activated ERE-mediated gene transcription (Schultz et al., 2000).

There exist the data suggesting that BZP *per se* did not activate gene transcription in reporter gene assay with HeLa cells (human cervix carcinoma cell line) incorporated with human or rat estrogen receptor expression plasmids and estrogen receptor responsive element, but 3-hydroxy-, 4-hydroxy-, 4,4'-dibromo-, 4,4'-dihydroxy-, 4-chloro-4'-hydroxy-, 2,4,4'-trihydroxy-, 4-fluoro-4'-hydroxy-, 2,4-dihydroxy- and 2,2',4,4'-tetrahydroxy-BZPs exhibited binding affinity for human estrogen receptor in binding assays and activate transcription of reporter gene in reporter gene assay with HeLa cells transfected with human estrogen receptor expression plasmids and estrogen receptor responsive element. 2,3,4-trihydroxy-BZP and 2,3,4,4'-tetrahydroxy-BZP bound to human estrogen receptor but did not activate gene transcription in the reporter gene assay (CERI, 2001a).

In MCF-7 cells, estrogen-dependent human breast cancer cell line, BZP had no cell proliferative activity, whereas 4-hydroxybenzophenone induced cell proliferation at high

concentrations (10-100  $\mu$ M) (Nakagawa et al., 2000).

In reporter gene assay in yeast cells transfected with human progesterone receptor, BZP did not activate progesterone responsive element (PRE)-dependent gene transcription, nor did it antagonize gene transcription activation mediated by progesterone (Tran et al., 1996).

## (2) *in vivo* test results in mammals (Attachment-2)

In uterotrophic assay (in accordance with the OECD draft guidelines), estrogenicity and anti-estrogenicity screening test, ovariectomized female SD rats were exposed subcutaneously to BZP at 0, 5, 50 and 500 mg/kg/day for 7 days. In 500 mg/kg/day group, uterine weight increased slightly. When BZP was administered subcutaneously at 0, 5, 50 and 500 mg/kg/day in combination with ethinylestradiol at 0.3  $\mu$ g/kg/day, s.c., for 7 days (to assess the anti-estrogenic effect on BZP), uterine weight decreased slightly in 50 mg/kg/day and higher groups (CERI, 2001b).

In another uterotrophic assay, juvenile female SD rats were exposed subcutaneously to BZP at 0, 2, 20 and 200 mg/kg/day for 3 days, but uterine weight remained unchanged (CERI, 2001a). On the other hand, subcutaneous administration of 4-hydroxybenzophenone, metabolite of BZP, at 0, 100, 200 and 400 mg/kg/day for 3 days resulted in a dose-dependent increase in uterine weight, but benzhydrol did not cause change in uterine weight at 400 mg/kg/day (Nakagawa & Tayama, 2001).

In Hershberger assay (in accordance with the OECD draft guideline), androgenicity and anti-androgenicity screening test, the androgenic effect of BZP was assessed in castrated male SD rats by gavage with BZP at 0, 1, 10 and 100 mg/kg/day for 10 days, but weights of the male accessory reproductive organs remained unchanged. When BZP was administered by oral gavage at 0, 1, 10 and 100 mg/kg/day in combination with testosterone propionate at 0.4 mg/kg/day, s.c., for 10 days (in order to assess the anti-androgenic effect), weights of male accessory reproductive organs remained unchanged (CERI, 2001b).

## 3) Information on general toxicity

### (1) Acute toxicity (Table 1)

In acute toxicity study, male Swiss mice (19-25 g) given BZP (suspended in 5% gum

arabic) orally or intraperitoneally showed sedation, decreased motor activity, unstable gait, shivering and reduced respiration rate (Cprino et al., 1976). The LD<sub>50</sub> values after various dosing routes are reported for mice, rats and rabbits (NTP, 2000).

**Table 1 Results of acute toxicity studies**

	Mouse	Rat	Rabbit
Oral LD <sub>50</sub>	2,895 mg/kg	1,900 mg/kg	-
Inhalation LD <sub>50</sub>	-	-	-
Percutaneous LD <sub>50</sub>	-	-	3,535 mg/kg
Subcutaneous LD <sub>50</sub>	727 mg/kg	-	-

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

In a 28-day feeding study in which both sex of SD rats were given BZP in diet at 0, 100 and 500 mg/kg/day, decrease in red blood cell count and hematocrit, increase in urea nitrogen, bilirubin, total protein and albumin, increases in liver and kidney weights and hepatocellular hypertrophy were observed in 100 mg/kg/day or higher groups, and decreases in hemoglobin and alkaline phosphatase and increase in glucose in 500 mg/kg/day group. In a 90-day dosed feeding study in SD rats exposed to BZP in diet at 0 and 20 mg/kg/day, no abnormal changes were observed (Burdock et al., 1991).

In a 14-week feeding study, both sex of F344 rats were given BZP at 0, 1250, 2500, 5000, 10000 and 20000 ppm (equivalent to 0, 75, 150, 300, 700 and 850 mg/kg in males and 0, 80, 160, 300, 700 and 1,000 mg/kg in females, respectively). Body weight decreased in 20,000 ppm group, and body weight gain was suppressed in 2,500 ppm and higher groups. In 1,250 ppm and higher groups, liver showed increased weight and hepatocellular hypertrophy and vacuolation, and kidney showed increased weight, protein casts in tubular lumen, dose-related dilatation of renal tubules and renal papillary necrosis (NTP, 2000).

In a 14-week feeding study, both male and female B6C3F<sub>1</sub> mice were given BZP in diet at 0, 1250, 2500, 5000, 10000 and 20000 ppm (equivalent to 0, 200, 400, 800, 1600 and 3300 mg/kg in males and 0, 270, 540, 1000, 1900 and 4200 mg/kg in females, respectively). The decreased body weight and death occurred in 20,000 ppm group, and body weight gain was suppressed in 5,000 ppm and higher groups. The increased kidney weight was observed in 2,500 ppm and higher groups, and increased liver weight and hepatocellular hypertrophy in 1,250 ppm and higher groups (NTP, 2000).

In a study in male guinea pigs given BZP intraperitoneally at 0 and 0.5 mg/kg/day for

15 days, liver was grossly enlarged, and histopathologically, liver showed hepatocellular degeneration and necrosis, connective tissue proliferation and proliferation of bile duct epithelial cells (Dutta et al., 1993).

#### 4) Information on mutagenicity/genotoxicity and carcinogenicity

##### (1) Mutagenicity/genotoxicity (Table 2)

BZP is not mutagenic either in *in vitro* or *in vivo* assays (Martinez et al., 2000; NTP, 2000).

**Table 2 Results of mutagenicity/genotoxicity studies**

Test method		Test conditions	Results*	References
<i>in vitro</i>	Reverse mutation test	<i>Escherichia coli</i> WP2uvrA/pKM101, IC203. S9(-), 200 µg/plate	-	Martinez et al., 2000
		<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535 and TA1537. S9(-/+) 1-1000 µg/plate	-	NTP, 2000
<i>in vivo</i>	Micronucleus test	B6C3F <sub>1</sub> mouse. Administered intraperitoneally at 200-500 mg/kg 3 times at 24-hr intervals. Bone marrow cells	-	NTP, 2000

\*-: Negative

##### (2) Carcinogenicity (Table 3)

Female Swiss mice (aged 7 weeks) were dermally given 0, 5, 25 and 50% BZP (solvent: acetone, 50 mice/group) twice weekly over their whole life, resulting in no BZP-related increase in tumor incidence (Stenback & Shubik, 1974).

**Table 3 Carcinogenicity assessment by national and international organizations**

Organization	Category	Significance	References
EPA	-	No evaluation	IRIS, 2002
EU	-	No evaluation	ECB, 2000
NTP	-	No evaluation	NTP, 2000
IARC	-	No evaluation	IARC, 2001
ACGIH	-	No evaluation	ACGIH, 2000
Japan Society for Occupational Health	-	No evaluation	Japan Society for Occupational Health, 2001

### 5) Information on immune system

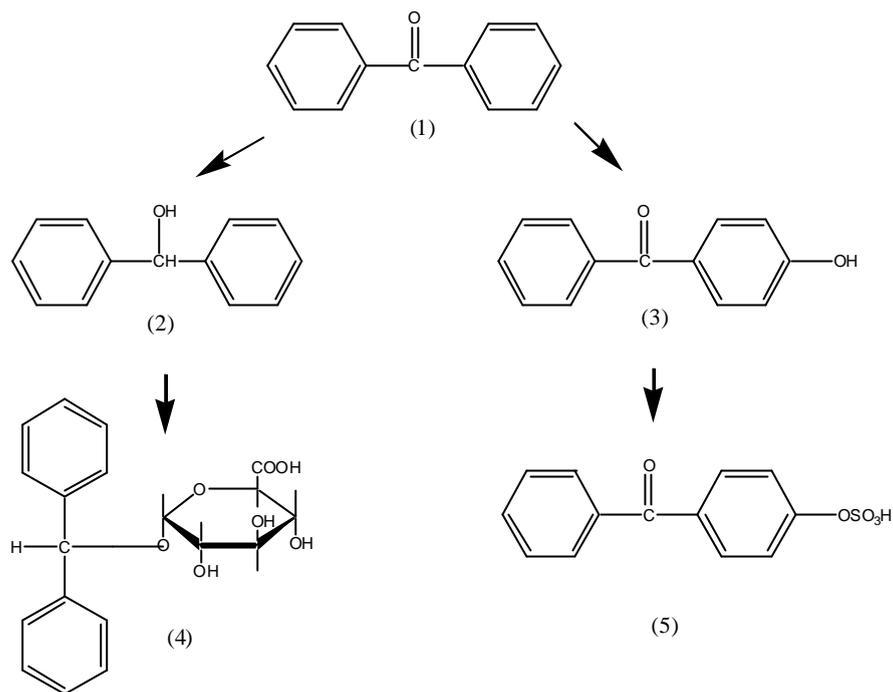
At present, no data are available on the effect on immune system.

### 6) Fate and Metabolism

In a dermal toxicity study in Bengal monkeys, the dermal absorption of BZP is reported to be 44% and 69% of the dose under the conditions of open-patch and closed-patch application, respectively (NTP, 2000).

In feeding study in rabbits, the carbonyl group of BZP was reduced to yield benzhydrol, which was then conjugated with glucuronic acid and excreted into urine. The urinary excretion was 41-61% of the dose (NTP, 2000). In oral administration study in male SD rats, 1% of the dose was excreted into urine as 4-hydroxybenzophenone (NTP, 2000). In *in vitro* study with the rat primary hepatocyte cultures, benzhydrol, 4-hydroxybenzophenone and their sulfate conjugates were detected in cell suspensions added with 0.25 mM BZP (Nakagawa et al., 2000).

The binding of BZP with the human placental aromatase and consequent inhibition of the enzyme activity were investigated *in vitro*. When added to aromatase solution, BZP altered absorption spectrum of aromatase even in the presence of its substrate, androst-4-ene-3,17-dione, and antagonized aromatase activity. These findings indicate that BZP binds to the active site of aromatase molecule and thereby inhibits the enzyme activity (Vaz et al., 1992).



- (1) Benzophenone (BZP)  
 (2) Benzhydrol  
 (3) 4-hydroxybenzophenone

- (4) Glucuronide conjugate  
 (5) sulfate conjugate

**Fig. 1 Metabolic pathways of benzophenone**

## 2. Hazard assessment at present

No data are available on the effects of BZP on human endocrine and reproductive systems.

In *in vitro* studies to assess endocrine effect, BZP does not bind to human estrogen receptor and thus does not activate gene transcription mediated by human estrogen receptor. BZP does not induce proliferation of MCF-7 cells either. 4-Hydroxybenzophenone and other BZP derivatives arising from BZP metabolism bind to human estrogen receptor and thus cause gene transcription activation mediated by human estrogen receptor and proliferation of MCF-7 cells. In addition, BZP *per se* antagonizes *in vitro* activity of aromatase involved in estrogen biosynthesis from testosterone.

In *in vivo* studies, administration of BZP alone induced a slight increase in uterine weight in uterotrophic assay using ovariectomized rats, but concomitant administration of BZP plus 17 $\beta$ -ethinylestradiol caused decrease in uterine weight. In Hershberger assay

in castrated rats, BZP alone or in combination with testosterone propionate had no effect on male accessory reproductive organs. In the uterotrophic assay of 4-hydroxybenzophenone, a metabolite of BZP, in immature rats, uterine weight increased dose-dependently.

These findings suggest that BZP has weak estrogenic and anti-estrogenic effects, but does not have androgenic or anti-androgenic effects. Since BZP *per se* does not bind to estrogen receptor, this estrogenic activity is not attributable to BZP *per se* but to its breakdown products. The inhibitory effect of BZP on aromatase suggests that BZP may depress estrogen biosynthesis in the body, but relationship of this effect with the anti-estrogenic effect seen in uterotrophic assay remains unknown at present.

So far, no data are available concerning reproductive/developmental toxicities. At present, therefore, there are not sufficient data for evaluation of the endocrine disrupting effects of BZP.

As the information relevant to hazardous effects of BZP, mainly hepatotoxicities and nephrotoxicities in rodents are reported. In mutagenicity studies, BZP is reported to be negative both in *in vitro* and *in vivo* assays. As for the carcinogenicity of BZP, no human data are available, and the tumor incidence does not increase by dermal application in experimental animals.

### **3. Risk assessment and other necessary future measures**

2-Generation reproduction toxicity study for BZP is currently on-going. Since the existing results of animal studies suggest possibility that metabolites of BZP have weak estrogenic activity, and anti-estrogenic effect of BZP is suggested, endocrine-disrupting effect of BZP and its toxicities consequent to endocrine disruption will be comprehensively evaluated by taking into consideration of the result of the above 2-generation reproductive toxicity study.

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

Item	Test methods and conditions	Results	Conclusion	References
ER binding assay	Human ER binding assay (recombinant ER $\alpha$ ligand domain)	BZP: IC50 value: $>10^{-4}$ M (E2: $1.7 \times 10^{-9}$ M)	BZP has no binding affinity for human ER.	CERI, 2001a
		BZP derivatives (IC50 value) 4-Hydroxy-BZP: $1.3 \times 10^{-5}$ M (E2: $1.4 \times 10^{-9}$ M; RBA: 0.011% ) 3-Hydroxy-BZP: $1.0 \times 10^{-5}$ M (E2: $1.3 \times 10^{-9}$ M; RBA: 0.013% ) 4,4'-Dihydroxy-BZP: $7.3 \times 10^{-6}$ M (E2: $1.2 \times 10^{-9}$ M; RBA: 0.017% ) 2,4-Dihydroxy-BZP: $8.9 \times 10^{-6}$ M (E2: $1.2 \times 10^{-9}$ M; RBA: 0.014% ) 2,4,4'-Trihydroxy-BZP: $1.7 \times 10^{-6}$ M (E2: $1.2 \times 10^{-9}$ M; RBA: 0.074% ) 2,3,4,4'-Tetrahydroxy-BZP: $4.3 \times 10^{-6}$ M (E2: $1.1 \times 10^{-9}$ M; RBA: 0.025% ) 2,2',4,4'-Tetrahydroxy-BZP: $1.4 \times 10^{-6}$ M (E2: $1.3 \times 10^{-9}$ M; RBA: 0.093% ) 4-Chloro-4'-hydroxy-BZP: $1.9 \times 10^{-5}$ M (E2: $1.6 \times 10^{-9}$ M; RBA: 0.0081% ) 4-Fluoro-4'-hydroxy-BZP: $4.8 \times 10^{-5}$ M (E2: $1.1 \times 10^{-9}$ M); $2.7 \times 10^{-5}$ M (E2: $1.1 \times 10^{-9}$ M); RBA: 0.0031% 2,3,4-Trihydroxy-BZP: $1.8 \times 10^{-5}$ M (E2: $1.6 \times 10^{-9}$ M; RBA: 0.0088% ) 4,4'-Dibromo-BZP: $1.7 \times 10^{-5}$ M (E2: $1.4 \times 10^{-9}$ M; RBA: 0.0082% )	BZP derivatives (4-hydroxy/3-hydroxy/4,4'-dihydroxy/2,4-dihydroxy/2,4,4'-trihydroxy/2,3,4,4'-tetrahydroxy/2,2',4,4'-tetrahydroxy/4-chloro-4'-hydroxy/4-fluoro-4'-hydroxy/2,3,4-trihydroxy/4,4'-dibromo derivatives of BZP) have binding affinity for ER (the binding affinities were 1/1,100 - 1/44,000 of that of E2).	
		BZP IC50: $>5 \times 10^{-4}$ M	No effect	Nakagawa & Tayama, 2001
		4-Hydroxy-BZP: $5 \times 10^{-5}$ M	Binding affinity +	
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 $\beta$ -galactosidase reporter gene	REC10: $>3 \times 10^{-3}$ M (E2: $3 \times 10^{-10}$ M)	BZP does not activate ER-mediated gene transcription.	Nishihara et al., 2000

## Benzophenone

Item	Test methods and conditions	Results	Conclusion	References
Reporter gene assay in yeast cells	Bioassay using yeast cells transfected with human estrogen receptor expression plasmid and estrogen receptor responsive element	BZP (1 $\mu$ M) is negative for agonist activity. Some of the BZP derivatives have estrogen activity. The EC <sub>50</sub> values for each of these derivatives are as shown below. 4-Hydroxy-BZP: $1.12 \times 10^{-6}$ M 3-Hydroxy-BZP: $2.57 \times 10^{-6}$ M 4-Amino-BZP: $6.34 \times 10^{-5}$ M 4,4'-Dihydroxy-BZP: $2.53 \times 10^{-6}$ M 4,4'-Diamino-BZP: $5.89 \times 10^{-5}$ M 4-Chloro-4'-hydroxy-BZP: $2.88 \times 10^{-7}$ M 2,4-Dihydroxy-BZP: $2.4 \times 10^{-6}$ M 2,3,4'-Trihydroxy-BZP: $5.08 \times 10^{-6}$ M 2,4,4'-Trihydroxy-BZP: $5.64 \times 10^{-7}$ M 2,2',4,4'-tetrahydroxy-BZP: $7.92 \times 10^{-6}$ M	BZP does not activate ER-mediated gene transcription.	Schultz et al., 2000
	Bioassay using the yeast cells transfected with human progesterone receptor expression plasmid and progesterone receptor responsive element	BZP (1 $\mu$ M) is negative for either agonist or antagonist activity.	BZP does not activate progesterone receptor-mediated gene transcription.	Tran et al., 1996
Reporter gene assay in recombinant cell cultures	Cells: HeLa cells transfected with human estrogen receptor expression plasmid and estrogen receptor responsive element. Exposure concentration: $10^{-11}$ - $10^{-5}$ M	BZP is negative for agonist activity within a range of $10^{-11}$ - $10^{-5}$ M. Some of the BZP derivatives have the ability of gene transcription activation. The PC <sub>50</sub> <sup>(4)</sup> values for each of these derivatives are as shown below. 4-Hydroxy-BZP: $2.6 \times 10^{-6}$ M 3-Hydroxy-BZP: $2.6 \times 10^{-6}$ M 4,4'-Dihydroxy-BZP: $1.6 \times 10^{-6}$ M 2,4-Dihydroxy-BZP: $2.4 \times 10^{-6}$ M 2,4,4'-Trihydroxy-BZP: $3.7 \times 10^{-7}$ M 2,2',4,4'-Tetrahydroxy-BZP: $3.3 \times 10^{-7}$ M 4-Chloro-4'-hydroxy-BZP: $1.8 \times 10^{-6}$ M 4-Fluoro-4'-hydroxy-BZP: $2.0 \times 10^{-6}$ M 4,4'-Dibromo-BZP: $2.7 \times 10^{-6}$ M	BZP does not activate ER-mediated gene transcription.	CERI, 2001a
	Cells: HeLa cells transfected with rat ER expression gene and ER responsive element. Exposure concentration: $10^{-11}$ - $10^{-5}$ M	BZP is negative for agonist activity in the range of $10^{-11}$ - $10^{-5}$ M. (E <sub>2</sub> : PC <sub>50</sub> : $<10^{-9}$ M)	BZP does not activate ER-mediated gene transcription.	Yamasaki et al., 2001
Human breast cancer cell proliferation assay	Bioassay using the proliferation of human breast cancer cells (MCF-7 cells) as the index.	4-Hydroxybenzophenone, a BZP derivative, has the proliferative activity (10-100 $\mu$ M)(equivalent to 80% of 1 nM E <sub>2</sub> at 100 $\mu$ M of 4-Hydroxybenzophenone).	BZP has no proliferative activity.	Nakagawa et al., 2000

ER: Estrogen receptor; E<sub>2</sub>: 17 $\beta$ -Estradiol; REC<sub>10</sub>: Concentration that produces activity equivalent to 10% of the activity of  $10^{-7}$  M E<sub>2</sub>; PC<sub>50</sub>: Concentration that produces activity equivalent to 50% of the activity of  $10^{-7}$  M E<sub>2</sub>.

**Attachment-2 Results of studies on mammalian endocrine and reproductive systems**

Animal species	Administration method	Administration period	Dose	Results	References
Rat (SD, female) 6 rats/group	s.c. (Uterotrophic assay) (Ovariectomized rats, Ovariectomized at the age of 6 weeks)	BZP was administered for 7 days from the age of 8 weeks, and uterus was removed on the 8th day and weighed.	BZP 0, 5, 50, 500 mg/kg/day	Slight increase in uterine weight at 500 mg/kg/day. (estrogenic effect)	CERI, 2001b
			BZP 0, 5, 50, 500 mg/kg/day + 17 -Ethinylestradiol 0.3 µg/kg/day (s.c.)	Slight decrease in uterine weight at 50 mg/kg/day or above. (anti-estrogenic effect)	
Rat (SD, female) 6 rats/group	s.c. (Uterotrophic assay)	BZP was administered for 3 days from postnatal day 20, and the uterus was removed on the 4th day and weighed.	BZP 0, 2, 20, 200 mg/kg/day	No effect on uterine weight.	CERI, 2001a
Rat (SD, female)	s.c. (Uterotrophic assay)	BZP derivatives were administered for 3 days from postnatal day 21, and the uterus was removed 6 hr after the final dose and weighed.	4-Hydroxy-BZP at 0, 100, 200 and 400 mg/kg/day	The uterine weight increased dose-dependently. (estrogenic effect)	Nakagawa & Tayama, 2001
			Benzhydrol 400 mg/kg/day	No effect on uterine weight.	
Rat (SD, male) 6 rats/group	Oral gavage (Hershberger assay) (Castrated rats, castrated at the age of 6 weeks)	BZP was administered for 10 days from the age of 7 weeks, and male accessory reproductive organs were weighed on the 11th day.	BZP 0, 1, 10, 100 mg/kg/day	No effect on weights of male accessory reproductive organs.	CERI, 2001b
			BZP 0, 1, 10, 100 mg/kg/day + Testosterone propionate 0.4 mg/kg/day (s.c.)	No effect on weights of male accessory reproductive organs.	

## Attachment-3 Results of repeated-dose toxicity studies

Animal species	Administration method	Administration period	Dose	Results	References
Rat (SD, male and female)	By feeding	Administration was started at the age of 6 weeks. 28 days	0, 100, 500 mg/kg/day	Decreases in red blood cell count and hematocrit, increases in urea nitrogen, bilirubin, total protein and albumin, increases in liver and kidney weights and hypertrophy of liver cells in the 100 and 500 mg/kg/day groups. Decreases in hemoglobin and alkaline phosphatase and increase in glucose in 500 mg/kg/day group.	Burdock et al., 1991
Rat (SD, male and female)	By feeding	Administration was started at the age of 6 weeks. 90 days	0, 20 mg/kg/day	No abnormality.	Burdock et al., 1991
Rat (F344, male and female)	By feeding	Administration was started at the age of 8-9 weeks. 14 weeks	0, 1,250, 2,500, 5,000, 10,000, 20,000 ppm (Male: Corresponding to 0, 75, 150, 300, 700 and 850 mg/kg/day; Female: Corresponding to 0, 80, 160, 300, 700 and 1000 mg/kg/day)	Hepatic and renal toxicities in 1,250 ppm and higher groups. Suppressed body weight gain in 2,500 ppm and higher groups. Decreased body weight in 20,000 ppm group.	NTP, 2000
Mouse (B6C3F <sub>1</sub> , male and female)	By feeding	Administration was started at the age of 8-9 weeks. 14 weeks	0, 1,250, 2,500, 5,000, 10,000, 20,000 ppm (Male: Corresponding to 0, 200, 400, 800, 1600 and 3300 mg/kg/day; Female: Corresponding to 0, 270, 540, 1000, 1900 and 4200 mg/kg/day)	Hepatic toxicities in 1,250 ppm and higher groups. Renal toxicities in 2,500 ppm and higher groups. Suppressed body weight gain in 5,000 ppm and higher groups. Decreased body weight gain and death in 20,000 ppm group.	NTP, 2000
Guinea pig (male, 550-600 g)	i.p.	15 days	0, 5 mg/kg/day	Hepatocellular degeneration and necrosis, connective tissue proliferation, proliferation of bile duct epithelial cells.	Dutta et al. , 1993