Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

Butylbenzyl Phthalate

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
GPO Box 58, Sydney NSW 2001, Australia www.nicnas.gov.au

Preface

This report was compiled under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with the Department of the Environment, Water, Heritage and the Arts, which carry out the environmental assessment for NICNAS. NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia.

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Overview

This review of butylbenzyl phthalate (BBP) is a health hazard assessment only. For this assessment, two key reviews on BBP prepared by the European Chemicals Bureau and the US Centre for the Evaluation of Risks to Human Reproduction were consulted. Information from these reviews was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.

According to the American Chemistry Council, the largest use of BBP is in vinyl tiles. BBP is used as a plasticiser in PVC for food conveyor belts, carpet tiles, artificial leather, tarps, automotive trim, weather stripping, traffic cones and vinyl gloves. BBP is also used in some adhesives, food wrap or food packaging and has been reported at low concentrations in baby equipment and children toys, probably as byproducts/impurities. Current EU legislation restricts the use of BBP in toys and childcare articles, and prohibits its use in cosmetics.

In Australia, BBP is mainly imported as finished products or mixtures. BBP is used industrially in automotive coating pigments, adhesives, sealants, paints, parquetry adhesives and coatings, construction sealants, marine coatings and road-marking paints. BBP is also used in military specified topcoats for metal substrates. It serves as a speciality plasticiser for nitrocellulose lacquers and acrylic coatings, screen printing chemicals (mostly for T-shirts) and polyurethane forklift wheels. PVC consumer products containing BBP as a plasticiser include gumboots, toys, play and exercise balls.

Structurally, phthalate esters are characterized by a diester structure consisting of a benzenedicarboxylic acid head group linked to two ester side chains. BBP possesses one linear and one ringed structure ester side chains.

BBP is absorbed and excreted rapidly following ingestion. In rats, low doses are excreted predominantly via urine and high doses predominantly via faeces. Dermally, BBP is slowly absorbed with small amounts distributed to several organs. BBP is metabolised initially to monobutyl phthalate (MbuP) or monobenzyl phthalate (MBzP) by hepatic and intestinal mucosal cells. There is no evidence of tissue accumulation. Available data suggest a half-life of BBP following oral exposure of less than 24 hours.

In experimental animals, BBP exhibits low acute oral, dermal and intraperitoneal toxicity. Data on toxicity following acute inhalation exposure are not available.

BBP produced minimal skin and eye irritation in animals. No data were available on respiratory irritation potential. No skin sensitisation was reported with BBP in two human patch tests. A single old study in rabbits noted slight sensitising effects. More recent ear swelling tests showed negative results. Based on weight-of-evidence, BBP is not a skin sensitiser.

Data from a well performed 3-month oral repeated dose dietary study in rats revealed a NOAEL of 151 mg/kg bw/d with a LOAEL of 381 mg/kg bw/d based on significant increases in relative kidney weight and histopathological changes in the pancreas and liver in males. A 90-day rat inhalation study established a NOAEC of 218 mg/m3 and a LOAEC of 789 mg/m3 based on increased kidney and liver weight in both male and females. Several repeated dose studies showed peroxisome proliferation following BBP administration.

BBP was investigated in a variety of in vitro and in vivo genotoxicity studies. Overall, based on all data available and on a weight-of-evidence basis, BBP is considered to be non-genotoxic.

With regards to carcinogenicity, data from in vitro and in vivo studies provides limited evidence of carcinogenicity in animals.

Extensive studies have been performed to study reproduction, fertility and developmental toxicity of BBP in laboratory animals. Human data are insufficient to draw any conclusions.

In animals, effects of BBP on fertility or reproductive organs in rats following oral or inhalation exposure include reduced mating and fertility indices, decreases in testes weight, histopathological changes in testes and hormonal changes. These effects occurred, in the majority of studies (conducted in rats), at BBP doses equal to or greater than doses that induced systemic toxicity. A NOAEL for fertility effects derived from a well-conducted two-generation reproduction study was 200 mg/kg bw/d with a LOAEL of 400 mg/kg bw/d based on increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells in the F1 generation.

In vivo developmental toxicity studies in rats and mice indicate an anti-androgen type activity for BBP. Effects include reduced testicular weight, reduced anogenital distance (AGD), and retarded transabdominal descent of testes in male offspring exposed to BBP during the organogenic period and/or the late prenatal early postnatal period. The incidence of these effects was dependent on dose and developmental age.

In a two-generation study in rats, a NOAEL of 50 mg/kg bw/d for developmental effects was determined based on a statistically significant dose-related reductions in AGD in both F1 and F2 offspring from 250 mg/kg bw/d in the absence of maternal toxicity. A LOAEL of 100 mg/kg bw/d was determined from another two-generation study for developmental effects based on decreased body weight and AGD.

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Acronyms and Abbreviations

AGD	anogenital distance
BBP	butylbenzyl phthalate
bw	body weight
C	Celsius
CA	chromosomal aberrations
CAS	Chemical Abstracts Service
CERHR	Centre for the Evaluation of Risks to Human Reproduction
CHO	Chinese hampster ovary
d	day
DAP	diallyl phthalate
DBP	dibutyl phthalate
DEHP	diethylhexyl phthalate
DIDP	di-isodecyl phthalate
DIOP	diiso-octyl phthalate
DMBA	dimethylbenz(a)anthracene
DNA	deoxyribonucleic acid
DOP	dioctyl phthalate
ECB	European Chemicals Bureau
EU	European Union
f	female
F0	parental generation
F1	filial 1 (first generation)
F2	filial 2 (second generation)
FSH	follicle-stimulating hormone
g	gram
GD	gestation day
GLP	good laboratory practice
h	hour
IgE	immunoglobulin E
ip	intraperitoneal
kg	kilogram
kPa	kilopascals
L	litre

LC50	median lethal concentration
LD50	median lethal dose
LH	luteinizing hormone
LOAEC	lowest-observed-adverse-effectconcentration
LOAEL	lowest-observed-adverse-effect level
m	male
MBuP	monobutyl phthalate
MBzP	monobenzyl phthalate
MCL	mononuclear cell leukaemia
mg	milligram
mL	millilitre
mRNA	messenger ribonucleic acid
Mt	metallothionein
nL	nanolitre
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
PND	post-natal day
ppm	parts per million
PVC	polyvinyl chloride
sc	sub-cutaneous
SCE	sister chromatid exchange
SIDS	Screening Information Data Set
w/v	weight per volume
w/w	weight per weight
Zn	zinc
µl	microlitre
µg	microgram

1. Introduction

This review of butylbenzyl phthalate (BBP) is a health hazard assessment only. For this assessment, two key reviews on BBP prepared by the European Chemicals Bureau (ECB, 2004) (reviewed by SCHER, 2005) and the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) were consulted. Information from these reviews was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.

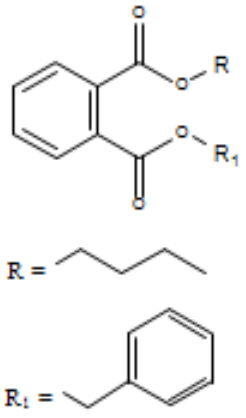
Information on Australian uses was compiled from data supplied by industry in 2004 and 2006.

References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information from this assessment is published also in the form of a phthalate hazard compendium providing a comparative analysis of key toxicity endpoints for 24 *ortho*-phthalate esters (NICNAS, 2008).

2. Identity

2.1 Identification of the substance

CAS Number:	85-68-7
Chemical Name:	1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester
Common Name:	Butyl benzyl phthalate (BBP)
Molecular Formula:	$C_{19}H_{20}O_4$
Structural Formula:	
Molecular Weight:	312.35
Synonyms:	Benzyl n-butyl phthalate, n-butyl benzyl phthalate, butyl phenylmethyl 1,2-benzenedicarboxylate, phthalic acid-benzyl butyl ester
Purity/Impurities/Additives:	Purity: >98.5% w/w Impurities: <1.0% dibenzyl phthalate (CAS No. 523-31-9), <0.5% benzyl benzoate (CAS No. 120-51-4), <0.5% dibutyl phthalate (CAS No. 84-74-2), <2 ppm α-clorotoluen (CAS No. 100-44-7), <2 ppm α-α- diclorotoluen (CAS No. 98-87-3) Additives: <0.5 ppm pentaerythritol tetrakis (3-(3,5-di-tert-butyl-4-hydoxyphenyl) propionate) (CAS No. 6683-19-8)

2.2 Physico-chemical properties

Table 1: Summary of physico-chemical properties

Property	Value
Physical state	Clear oily liquid
Melting point	<-35°C
Boiling point	370°C at 1.01 kPa
Density	1114-1122 kg/m^3 (25°C)
Vapour pressure	8.0 x10^{-8} kPa (25°C)
Water solubility	0.0028 g/L (20°C)
Partition coefficient n-octanol/water (log Kow)	4.84 (temperature not available)
Henry's law constant	No data
Flash point	198°C

Source: CERHR (2003); ECB (2004)

3. Uses

CERHR (2003) notes that according to the American Chemistry Council, the largest use of BBP is in vinyl tiles. BBP is also used as a plasticiser in PVC for food conveyor belts, carpet tiles, artificial leather, tarps, automotive trim, weather stripping, traffic cones and vinyl gloves. BBP is also used in some adhesives, food wrap or food packaging and has been reported at low concentrations in baby equipment and children toys, probably as byproducts/impurities (ECB, 2004). Current EU legislation restricts the use of BBP in toys and childcare articles, and prohibits its use in cosmetics.

In Australia, BBP is mainly imported as finished products or mixtures. BBP is used industrially in automotive coating pigments, adhesives, sealants, paints, parquetry adhesives and coatings, construction sealants, marine coatings and road-marking paints. BBP is also used in military specified topcoats for metal substrates. It serves as a speciality plasticiser for nitrocellulose lacquers and acrylic coatings, screen printing chemicals (mostly for T-shirts) and polyurethane forklift wheels. PVC consumer products containing BBP as a plasticiser include gumboots, toys, play and exercise balls.

4. Human Health Hazard

4.1 Toxicokinetics

Previous evaluations

Oral

In a study by Anderson et al. (2000*), 7 volunteers/group were given a single dietary dose of stable isotope-labelled BBP at doses of 0, 168-225 and 336-510 µg. Background levels of unlabelled monobutyl phthalate (MBuP) and monobenzyl phthalate (MBzP) were detected in most of the urine samples 24 hours before dosing. The majority of labelled phthalate monoesters was excreted in the first 24 hours. The formation and excretion of MBzP was high after 24 hours, 67% and 78% on a molar basis at the low and high dose, respectively. In contrast, only 6% was excreted as MBuP in the high dose group 24 hours after dosing. On day 2 and 6 after dosing, no labelled phthalate monoester excretion was measured.

In an excretion and metabolism study, male Fischer-344 rats were dosed orally with ring-labelled ^{14}C-BBP at 2, 20, 200 or 2000 mg/kg bw or 20 mg/kg bw intravenously. In 24 h, 61%-74% of the dose was excreted in the urine and 13%-19% in faeces at 2-200 mg/kg. At 2000 mg/kg, 16% of the ^{14}C was excreted in the urine and 57% was excreted in faeces. Increases in faecal elimination at 2000 mg/kg bw may be due to incomplete absorption of administered BBP or BBP metabolites during the enterohepatic circulation. Urinary ^{14}C was composed of monophthalate derivatives (10%-42% of the dose) and glucuronides of these derivatives (2%-12% of the dose). At 4 hours after intravenous administration of 20 mg/kg BBP, 53%-58% of the dose was excreted in the bile. No parent compound was found in the bile, but MBuP-glucuronide and MBzP-glucuronide (26% and 13% of the dose) and trace amounts of free monoesters (2% of the dose) and unidentified metabolites (14% of the dose) were present. Larger quantities of MBuP (44%) than of MBzP (16%) were formed. The half-life of the parent BBP and the monoesters were approximately 6 hours in all tissues (Eigenberg et al., 1986*).

To determine excretion and tissue distribution of BBP, rats were treated orally with 16, 160 or 1600 mg/kg bw ^{14}C-BBP and the urine and faeces collected for 5 days. The majority of radioactivity remaining in the animals at sacrifice were located in liver, kidney, small intestine and total gut contents. However, the radioactive BBP residues present in any of the tissues were very small and no evidence indicated tissue accumulation. Excretion in urine was rapid and appeared to be largely independent of the dose of BBP administered. More than 80% of the administered dose was excreted in the urine within 5 days, the bulk of the remainder being excreted by faeces (Lake et al., 1978*; BIBRA, 1978*).

In another metabolism study in male rats, BBP was reported to be partially hydrolysed by intestinal esterases, primarily to MBuP and benzyl alcohol, with MBzP and n-butanol as minor products of hydrolysis. There was a preference for hydrolysis of benzyl ester, resulting in a preponderance (~3:1) of MBuP in the urine compared to MBzP (Agarwal et al., 1985).

In a study by Nativelle et al. (1999*), female Wistar rats were dosed with BBP (150, 475, 780, and 1500 mg/kg bw/d) by gavage for 3 consecutive days. Six metabolites,

MBuP (29%-34%), MBzP (7%-12%), hippuric acid (51%-56%), phthalic acid (2%-3%), an ω-oxidised metabolite of MBuP (1%-2%) and benzoic acid (very small amount) were identified in urine after 24 h. Parent BBP was not present. The recovered metabolites in the urine represented between 58% and 30% of the doses from 150 to 1500 mg/kg bw/d. After 24 hours the metabolites recovered in the urine were similar in the rats exposed to two lowest doses (except for MBuP). At 780 mg/kg bw/d hippuric acid was detected in a lower amount. At the highest dose all levels of metabolites decreased significantly compared to the levels at the two lowest doses. After 48 hours the level of hippuric acid started to decrease significantly at 475 mg/kg bw/d, however, elimination of MBuP and MBzP was similar at the two lowest doses. Elimination of MBuP and MBzP and hippuric acid was similar for the two highest doses but in a lower amount compared to the first dose. In contrast, after 72 hours the levels of metabolites excreted were constant with the dose of BBP. As regard the time dependency, treatment with 475 mg/kg bw/d resulted in a steady-state level of urinary excretion of metabolites within 72 hours. Multiple dosing with 1500 mg/kg bw/d showed identical levels of excretion of all metabolites at 48 h, whereas after 72 h, the levels of MBuP, MBzP and hippuric acid were increased 1.9, 2.4 and 1.4 fold, respectively.

Groups of 5 immature female Alpk:APfSD rats were given a single oral dose of BBP (1, 10 and 100 mg/kg bw). Twenty-four hours after dosing, plasma levels of BBP and MBzP were below the limit of detection (0.04 mg/L) in all dose groups. Levels of MBuP averaged 0.14 mg/L after exposure to 100 mg/kg bw BBP. The urinary excretion of MBuP after exposure to 1, 10 or 100 mg/kg bw BBP was 10.4, 2.5 and 1.8% of the dose, respectively, and for MBzP, 5.0, 0.5 or 0.5%, respectively. Following administration of 1, 10 or 100 mg/kg bw BBP the percentage of the dose recovered in the urine was 15.4, 3.0 and 2.3%, respectively. The study suggested that the urinary excretion of MBuP was higher than MBzP after exposure to BBP in immature rats (Monsanto, 1997*).

Pharmacokinetic studies in four beagle dogs given a divided oral dose equivalent to 5000 mg BBP/kg bw over a period of 4 hours demonstrated recovery of 88% (males) and 91% (females) of unchanged BBP from the faeces; approximately 4% was excreted as urinary phthalic acid (Ericson, 1965*).

Dermal

In a dermal absorption study, male Fischer-344 rats were given 157 µmol/kg bw ^{14}C-BBP for 7 days giving an applied dermal dose of 5-8 mg/cm^2. After 7 days, approximately 30% of the applied dose was excreted in the urine or faeces, 4.6% was found in the muscle, 0.5% was found in the brain, spinal cord and testes, and 45% was found at the skin area of application. This study indicates that BBP is slowly absorbed by the dermal route (Elsisi et al., 1989*).

In vitro

In an in vitro study, ^{14}C-BBP was hydrolysed by both rat hepatic and intestinal mucosal cell preparations. The rate of hydrolysis was proportional to both the tissue concentration and the incubation time. Free phthalic acid appeared to be absent from both hepatic and intestinal mucosal cell tissue incubations, indicating that BBP was being metabolised to either MBuP and/or MBzP (Lake et al., 1978*; BIBRA, 1978*).

Data not reported in previous evaluations

No data.

Conclusion

The kinetics of BBP after oral administration is dose-dependent. The majority of BBP (up to 74%) is excreted in the urine in the dose-range of 2 to 200 mg/kg bw after 24 hours. However, after administration of 2000 mg/kg bw, only 16% is excreted in urine with the majority in faeces indicating incomplete absorption of BBP or metabolites during enterohepatic circulation. Dermal absorption is much slower than oral absorption. After dermal application, only 30%-40% of the applied amount reaches the systemic circulation and is distributed to multiple tissues after 7 days. Data on absorption of inhaled BBP are not available.

BBP is metabolised initially to MBuP or MBzP by hepatic and intestinal mucosal cells. In adult and immature rats, the ratio of MBuP to MBzP in the urine is 3:1. There is no evidence of tissue accumulation. In contrast to rats, BBP is mainly metabolised to MBzP in humans. Available animal and human data suggest a half-life of BBP following oral exposure of less than 24 hours.

4.2 Acute toxicity

Previous evaluations

Table 2: Summary of acute toxicity studies

Study	Species	Results (LD50/LC50)	References
Oral	Rat (Sprague Dawley)	20400 mg/kg bw	Hammond et al., 1987*; Monsanto, 1976a*
	Rat (Fisher 344)	2330 mg/kg bw	NTP, 1982a*
	Guinea pig	>13750 mg/kg bw	RTECS, 2005
	Mouse	6160 mg/kg bw (m), 4170 mg/kg bw (f)	NTP, 1982a*
Intraperitoneal	Rat	>1800 mg/kg bw	Mallette & Von Haam, 1952*
	Mouse	>4000 & <5000 mg/kg bw	Monsanto, 1983*
	Mouse	3160 mg/kg bw	Calley et al., 1966*
Dermal	Rat	6700 mg/kg bw	Statsek, 1974*
	Rabbit	>10000 mg/kg bw	Hammond et al., 1987*, Monsanto, 1976a*

Source: ECB (2004)

Data not reported in previous evaluations

No data.

Conclusion

BBP exhibits low acute oral, dermal and intraperitoneal toxicity. Rat oral LD50 values range from 2330-20400 mg/kg bw. The dermal LD50 value for rats is 6700 mg/kg bw. Data on toxicity following acute inhalation exposure are not available.

4.3 Irritation

4.3.1 Skin irritation

Previous evaluations

A repeated insult human patch test was performed with undiluted BBP in 200 human volunteers. Twenty-four hour patch applications were repeated 3 times a week over 5 weeks with re-challenge after a 2 week rest period. No primary irritation reactions were observed during induction or challenge periods (Monsanto, 1980*; Hammond et al., 1987*).

In another human skin irritability and sensitisation test, a 10% concentration of BBP induced slight irritation reactions in 12% of the subjects (15-30 humans) tested. No other details were provided (Mallette & Von Haam, 1952*).

Two old studies using rabbits (one poorly described patch test and one intradermal injection test) reported that BBP had a moderate skin irritating effects (Mallette & Von Haam, 1952*; Calley et al., 1966*). In contrast, two Draize tests in rabbits on intact and abraded skin (Monsanto, 1976a*; Hammond et al., 1987*) and one mouse ear epicutaneous test showed no irritation or swelling (Monsanto, 1983*).

Data not reported in previous evaluations

No data.

Conclusion

Weight-of-evidence indicates that BBP induces minimal skin irritation in animals and humans.

4.3.2 Eye irritation

Previous evaluations

Acute eye irritation was evaluated in Draize tests in 2 groups of 6 New Zealand white rabbits. Undiluted BBP (0.1 mL) produced a slight degree of irritation at 1 and 24 h, which subsided within 48 h (Monsanto, 1976a*; Hammond et al., 1987*). No other details were provided.

Data not reported in previous evaluations

No data.

Conclusion

Limited information indicates that BBP induces minimal eye irritation in animals.

4.4 Sensitisation Previous evaluations

Assessments of sensitisation were conducted two weeks after a primary irritation test on 15-30 humans with 10% BBP. BBP had no sensitising effect in humans (Mallette & Von Haam, 1952*).

In a repeated human insult patch test, undiluted BBP were applied to the skin of 200 human volunteers for 24 hours, 3-times a week for 5 weeks. Following a 2-week rest period, human test subjects were re-challenged BBP for 24 hours to virgin sites on the skin. Neither primary irritation nor sensitisation reactions were observed (Monsanto, 1980*; Hammond et al., 1987*).

In an old study (Mallette & Von Haam, 1952*), BBP had a slight skin sensitising effect in rabbits when applied 2 weeks after a primary irritation test. No other details were provided.

BBP was negative in the various studies performed to assess skin sensitisation potential of BBP in mice and guinea pigs (Monsanto, 1983*). No conclusions could be established when BBP was tested for antibody formation in mice using a passive cutaneous anaphylaxis test (Monsanto, 1983*).

Data not reported in previous evaluations

Adjuvant properties of BBP were investigated in mice sensitised to ovalbumin. In contrast to DEHP, BBP did not stimulate levels of serum ovalbumin-specific IgE, IgG1 or IgG2 antibodies (Larsen et al., 2003).

In a recent sensitization assay, 50 µl undiluted BBP were applied to the skin of both ears of female B6C2F1 mice 5 times/week for 2 weeks (Butala et al., 2004). Seven days later, the animals were challenged and after a further 7 days the animals were sacrificed. Treatment with BBP did not result in significant elevations in total serum IgE, or auricular lymph node IL-4 or IL-3 or corresponding mRNA.

Conclusion

No tests conducted to standard protocols are available. In an old poorly reported study, BBP was reported to have a slight skin sensitising effect in rabbits. However, in more recent studies, BBP tested negative in the various ear swelling tests in mice and guinea pigs. BBP did not induce IgE antibody or Th2 cytokine responses in mice nor show adjuvant activity for IgE, IgG1 or IgG2 antibodies. No skin sensitisation was reported with BBP in two human patch tests. Weight-of-evidence indicates that BBP is not a skin sensitiser.

4.5 Repeated dose toxicity

4.5.1 Oral

Previous evaluations

Short-term, subchronic and two chronic studies were available for assessment. Only those key studies being well reported and conducted according to Good Laboratory Practice (GLP) are described below. These and other studies are briefly summarised in Table 3.

Short-term: Adult male F344 rats (10/group) were fed BPP in the diet at 0, 0.625, 1.25, 2.5, or 5.0% (0, 312, 625, 1250 or 2500 mg/kg/bw/d)for 14 days (Agarwal et al., 1985). Decreased food intake and decreased body weights seen at the two highest doses may be due to unpalatability of the food, the severity of which precluded associating any effects of BBP treatment at the highest dose.

All treated rats showed dose-related increase in relative liver and kidney weights. No histopathology or haematology changes were observed at ≤ 1.25%. However, at ≥ 2.5%, relative decreases in testes, seminal vesicle and thymus weight were noted; relative epididymal weight was reduced at the high dose. Dose-related histopathological changes in seminal vesicles, testes, and prostate were observed, as was a decrease in bone marrow cellularity at the two highest doses. Mild multifocal hepatitis and cortical lymphocytolysis in the thymus were also observed at the high dose. Increases in luteinizing hormone (LH) were observed at the lowest dose and the two highest doses. An increase in follicle stimulating hormone (FSH) was observed at the two highest doses and a decrease in testosterone was observed at the high dose. The LOAEL was 0.625% (312 mg/kg bw/d) based on increases in liver and kidney weights and increased LH levels.

In a 28-day gavage study, Cpb-WU male rats (28 days of age, 3/group) were given BBP (0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 2100 mg/kg bw/d) (Piersma et al., 1999). No changes in food consumption were reported. Relative liver weight was statistically significantly increased at 750 mg/kg bw/d and above. Liver palmitoyl CoA (PCoA), an index of peroxisome proliferation showed a similar response. A dose-dependent increase in relative kidney weight and a decrease in thymus and thyroid weight were reported from 750 mg/kg bw/d. None of these changes were statistically significant.

There was a dose-related decrease in relative testes weight at 750 mg/kg bw/d and above. This reached statistical significance at 1250 mg/kg bw/d. Histopathological analysis of the testes revealed severe atrophy from 970 mg/kg bw/d. FSH was significantly increased from 1250 mg/kg bw/d and a statistically significant decrease in testosterone levels were reported from 450 mg/kg bw/d. The NOAEL was 350 mg/kg bw/d based on decreased testosterone levels from 450 mg/kg bw/d.

Subchronic: In a 3-month dietary study, male and female Wistar rats were given BBP in the diet at approximately 0, 151, 381, or 960 mg/kg bw/d (Hammond et al., 1987*). Reductions in body weight gain were reported in all BBP treated groups. At the highest dose, no reduction in food consumption was apparent, suggesting that the reduced body weight gain at this dose may have been a toxic effect. Observations included slight anemia in males at the highest dose and decreased urinary pH in males at the mid and high doses. A similar significant increase in relative kidney weight occurred in a dose-related manner in both sexes at 151 mg/kg bw/d (8% increase) and above. Relative liver and cecum weights were significantly increased at all dose levels in females (4% for liver and 12% for cecum at 151 mg/kg bw/d) and relative liver weights were significantly increased at the highest dose in males. Gross pathological lesions were limited to increased incidence of red spots on the liver of mid- and high-dose males. The liver of high-dose males additionally had small areas of cellular necrosis. Histopathological lesions of the endocrine pancreas were observed in males at the mid and high doses and included islet enlargement with cell vacuolization and peri-islet congestion. No histopathological lesions were described for females. The NOAEL was 151 mg/kg bw/d for males based on increases in relative kidney weight and histopathological alterations in the pancreas and

histopathological and gross pathological alterations in the liver at 381 mg/kg bw/d (LOAEL). For females, the LOAEL was 151 mg/kg bw/d based on marginal increases in relative liver and cecum weights unaccompanied by histopathological or gross pathological changes.

In the same study, Sprague-Dawley rats (10/sex/group) were fed diets containing 0, 188, 375, 750, 1125, or 1500 mg/kg bw/d. There were no changes in pancreatic, hepatic, or testicular lesions, caecal enlargement, urinary pH or haematological parameters. There was a significant increase in relative liver weight in females at 750 mg/kg bw/d and higher and males at 1125 mg/kg bw/d. There was a significant increase in relative kidney weight in females only at 750 mg/ kg bw/d and higher. Sprague-Dawley rats were therefore less sensitive to BBP than Wistar rats. The NOAEL was set at 375 mg/kg bw/d in females and 750 mg/ kg bw/d in males, based on changes in relative liver weight at higher doses (Hammond et al., 1987*).

B6C3F1 mice were exposed to 240, 464, 946, 1875 and 3750 mg/kg bw/d of BBP in diet for 90 days. A decreased body weight gain was observed at all doses in males and at 1875 mg/kg bw/d and higher in females. Otherwise, no adverse toxicological effects were observed at any dose. The LOAEL for males was 240 mg/kg bw/d, and the NOAEL for females was 946 mg/kg bw/d based on decreased body weight gain (NTP, 1982b*; Hammond et al., 1987*).

In a NTP (1997*) study, groups of 15 F344/N male rats were fed BBP in diet at 0, 300, 900, 2800, 8300, or 25000 ppm (0, 30, 60, 180, 550 or 1650 mg/kg bw/d) for 26 weeks. At the high-dose, decreases in total body weight (due to decreased food intake) were observed as well as increases in relative liver and kidney weight. At 550 mg/kg bw/d, increases in absolute and relative liver weights were observed. An increased incidence of macrocytic anaemia was observed on days 30-180. The testes was a primary target organ based on decreased weight and sperm concentrations and histopathological findings at the high dose. Decreases in relative testes, absolute epididymis, and absolute seminal vesicle weight were observed, as were atrophy of seminiferous tubules and degenerative changes in testes and epididymis. No histological changes in other body tissues were seen at this dose. A NOAEL for systemic toxicity was established at 180 mg/kg bw/d and a LOAEL at 550 mg/kg bw/d based on increases in mean cell haemoglobin and relative liver weights.

Chronic studies: F344 rats (50/sex/group) were fed 6000 or 12000 ppm BBP (360 and 720 mg/kg bw) 7 days/week, for 28 or 103 weeks. Mean body weights of dosed females were lower than those of controls during most of the study. After week 14, an increasing number of dosed males died as a result of unexplained internal bleeding. Consequently, all living male rats were sacrificed at 29 to 30 weeks. No NOAEL was established from this study. The LOAEL value was 360 mg/kg bw based on decreased body weight gain (NTP, 1982b*). For further study description see Section 4.7.

In the same study, B6C3F1 mice (50/sex/group) were similarly dosed through feed at concentrations of 6000 or 12000 ppm BBP (approximately 1000 and 2000 mg/kg bw) for 2 years. Dose related decreases in body weight were seen in both male and female mice. No treatment related changes in survival or neoplastic developments were seen. No lesions were observed in reproductive organs.

Table 3: Summary of oral repeated dose toxicity studies in animals (adapted from ECB, 2004)

Study duration/ Species/Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoints	References
Short term				
14-day study (gastric intubation); Sprague Dawley rats; 6 M/dose	0, 160, 480, 1600	160	480; ↓ body weight, liver enlargement and ultrastructural changes with ↑ peroxisome numbers in the liver. Testicular atrophy and ↓ testes weight.	Lake et al., 1978*
14-day study (diet); F344 rats; 10 M/dose	0, 312, 625, 1250 or 2500	not established	447; ↑ liver and kidney weight, ↑ LH	Agarwal et al., 1985
6-week study (diet); Charles River CD rats; 10/sex/dose	0, 500, 1500, 3000	1500	3000; no evidence of neurologic impairment.	Robinson, 1991*
4-week study (diet); Sprague Dawley rats; 5-10/sex/dose	0, 500, 1000, 1500, 2000, 3000, 4000	1000	1500; ↓ body weight, testicular atrophy. Stiffness while walking and bleeding around nares	Hammond et al., 1987*
2-week study (gastric intubation); Wistar rats; 10/sex/dose	0, 480, 1600	480	1600; ↓ body weight, testes atrophy.	Hammond et al., 1987*
4-week study (gavage); Cpb-wU rats 3 M/dose	0, 270, 350, 450, 580, 750, 970, 1250, 1600, 2100	350	450; ↓ testosterone.	Piersma et al., 1999
10-week study (diet); Wistar rats; 15 M/dose	0, 20, 200, 2200	200	2200; changes in absolute and relative organ weights, changes in haematological parameters, and some evidence of minimal anaemia	NTP, 1997*
Subchronic				
3-month study (diet); Sprague Dawley rats; 10/sex/dose	0, 188, 375, 750, 1125, 1500	375 (F) 750 (M)	750; ↑ kidney and liver weight (F); 1125; ↑ liver weight (M)	Hammond et al., 1987*

Study duration/ Species/Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoints	References
3-month study (diet); Wistar rats; 10/sex/dose;	0, 151, 381, 960	151 (M) not established (F)	381 (M); ↑ kidney weight, ↓ urinary pH, histopathological changes in pancreas, and histopathological and gross pathological changes in liver 151 (F); ↑ relative liver and cecum weight (no histopathological changes)	(cont. over page) Hammond et al., 1987*
90-day study (diet); B6C3F1 mice;	0, 240, 464, 946, 1850, 3750	946 (F) not established (M)	1850 (F), 240 (M); ↓ body weight gain	NTP, 1982b*; Hammond et al., 1987*
3-month study (diet); beagle dog; 3/sex/dose	M: 0, 400, 1000, 1852 F: 0, 700, 1270, 1973	1852 (M) 1973 (F)	Not established; ↓ body weight	Hammond et al., 1987*
26-week study (diet); F344/N rats; 15 M/dose	0, 30, 60, 180, 550, 1660	180	550; ↑ cell haemoglobin concentrations and relative liver weight.	NTP, 1997*
Chronic				
2-year study (diet); F344 rats; 50/sex/dose	0, 360, 720	not established	360 (F); ↓ body weight gain in females. Male rats died or sacrificed due to internal bleeding.	NTP, 1982b*
2 year study (diet); B6C3F1 mice; 50/sex/dose	0, 1000, 2000	not established	1000 (approx); ↓ weight gain	NTP, 1982b*
2-year study (diet), F344/N rats; 60/sex/dose	M: 0, 120, 240, 500 F: 0, 300, 600, 1200	240 (M)	500 (M); ↑ relative kidney weight 300 (F); ↑ incidence of nephropathy.	NTP, 1997*

M - males; F – females

In another NTP study, male F344/N rats (60/group) were fed 3000, 6000, 12000 ppm BBP (120, 240, 500 mg/kg bw) and females 6000, 12000, 24000 ppm (300, 600, 1200 mg/kg bw) for 7 days/week for 24 months (NTP, 1997*). The survival rate of rats in all exposed groups was similar to the control group. There were no BBP-related clinical findings in any dosed group. Decreases in body weight were noted in males at 500 mg/kg bw/d and in females at 1200 mg/kg bw/d. Mean body weights at the lower doses were similar to those of the controls throughout the study. In general, haematological changes were sporadic and minor. The absolute right kidney weight of 600 mg/kg bw/d females and the relative right kidney weights of all exposed groups of males and of 1200 mg/kg bw/d females were significantly greater than those of the controls at 15 months. There was a dose-related increase in relative epididymal weight at 240 mg/kg bw/d and relative liver weight at 500 mg/kg bw/d in males. Renal tubule pigmentation in 500 mg/kg bw/d males and in 1200 mg/kg bw/d females was more severe at 15 months and 2 years compared to controls. At 2 years, the incidence of kidney mineralisation in 300 mg/kg bw/d and 1200 mg/kg bw/d females was significantly less than in controls. The incidences of nephropathy in exposed groups of females was significantly higher at 300 mg/kg bw and above after 2 years. An increased incidence of transitional epithelial hyperplasia in the kidney after two years was significant at 600 mg/kg bw. No testicular changes were observed. The NOAEL for male rats was 240 mg/kg bw based on a significant increase (more than 10%) in relative kidney weight at 500 mg/kg bw (LOAEL). The LOAEL for female rats was 300 mg/kg bw based on nephropathy. A NOAEL for female rats could not be established. For further study description see Section 4.7.

Studies of Peroxisome Proliferation: Liver changes were studied in a 21-day feeding study where rats (5/sex/group) were fed BBP at 0, 0.6, 1.2 or 2.5% (BIBRA, 1985; Barber et al., 1987). Another group (5/sex) fed similarly with diethylhexyl phthalate (DEHP) served as positive controls. DEHP caused dose dependent increases in relative liver weights, peroxisome proliferation (scored semiquantitatively via electron microscopy) and induction of cyanide-insensitive palmitoyl-CoA (PCoA) oxidation. BBP also induced increases in relative liver weight, peroxisome proliferation and induction of cyanide-insensitive palmitoyl-CoA (PCoA) oxidation but to a lesser extent than DEHP.

In a 28-day study BBP (0, 0.01, 0.05, 0.1, 0.5 and 1.0%) was fed to groups of 5 male Fisher 344 rats. A further group of 5 males was fed 1.0 % DEHP as a positive control. There was statistically significant reductions in body weight after 28 days of treatment at 0.5% BBP only. Both absolute and relative liver weights were statistically significantly increased with 1.0% BBP and 1.0% DEHP. Testes weight was not affected by treatment of BBP or DEHP. Hepatic PCoA oxidation activity was statistically significantly increased only at 1.0% BBP and 1.0% DEHP. An increase in hepatocyte eosinophila was observed at 1.0% DEHP. No histopathologic changes were reported after exposure to BBP. The NOAEL for induction of increased PCoA oxidation was 0.5% BBP (540 mg /kg bw/d) (BIBRA, 1992*).

Female Fisher 344 rats were fed a diet containing 0, 0.6, 1.2 and 2.4% BBP for 12 months. Evidence of peroxisome proliferation in the liver, as measured as activities of carnitine acetyltransferase (CAT) and PCoA oxidation, was present at $\geq$ 0.6% when measured as CAT and $\geq$ 1.2% when measured as PCoA, after both 1 and 12 months. There was no evidence that BBP treatment produced cell proliferation in the liver at any intervening times examined. No significant immune suppression or

enhancement was observed after BBP treatment for 1 and 12 months. The LOAEL for evidence of peroxisome proliferation was 0.6% BBP (Monsanto, 1994*). The mg equivalent was not stated in ECB (2004).

Data not reported in previous evaluations

No data.

Conclusion

Repeated oral exposure of rats to BBP resulted in decreased body weight gain, alterations in haematological parameters, peroxisomal proliferation and changes to the testes, epididymis, prostate, liver, kidney, spleen and pancreas. Effects on kidney, liver and testes were reported consistently. Mice and dogs appear to be less sensitive to BBP, with the only effects reported being decreased weight gain.

For repeated dose toxicity, a NOAEL of 151 mg/kg bw/d from a 3-month oral dietary study in Wistar rats was derived (Hammond et al., 1987*). The LOAEL was 381 mg/kg bw/d based on significant increases in relative kidney weight and histopathological changes in the pancreas and liver and at this dose and above in males.

4.5.2 Inhalation

Previous evaluations (ECB, 2004)

Studies in animals: In a 4-week study, BBP (0, 360, 1000 and 2100 mg/m^3) was given as an aerosol-vapour to Sprague-Dawley rats (20/sex/group) for 6 h/d, 5 d/week. Seventy percent of particles were 1.1-3.3 µm in size. Toxicological effects such as a statistically significant decrease in body weight gain, death, and in males atrophy of the spleen and reproductive organs, were seen only in the high exposure group. No organ weights were determined. The NOAEC was 1000 mg/m^3 and the LOAEC was 2100 mg/m^3 based on decreased body weight and atrophy of spleen and testes (Monsanto, 1981*).

In another 4-week study, BBP (0, 49, 144 and 526 mg/m^3) was given as an aerosol-vapour to Sprague-Dawley rats (5/sex/group) for 6 h/d, 5 d/week. Body weight gain was reduced relative to controls at 526 mg/m^3 for both sexes. Clinical parameters were not affected by BBP treatment and no changes in organ weights or microscopic abnormalities were observed. The NOAEC was 144 mg/m^3 and the LOAEC was 526 mg/m^3 based on reduced body weight gain (Hammond et al, 1987*).

In a 13-week study, BBP (0, 51, 218 and 789 mg/m^3) was administered as an aerosol-vapour to Sprague-Dawley rats (25/sex/group) in an inhalation chamber for 6 h/d, 5 d/week. Eighty percent of particles were 1.1- 4.7 µm in size. No changes in body weights were observed. Observations of urine-stained fur, piloerection and alopecia were reported at $\geq$ 218 mg/m^3 in both sexes. However, these findings were sporadic and inconsistent and were not considered as reliable indicators of toxicity of the test compound. Significant increases in absolute and/or relative liver and kidney weights were observed at 789 mg/m^3 in both sexes, whereas a significant increase in kidney weight at 218 mg/m^3 was only reported in males at interim sacrifice. Since no change in body weight was reported, the increased liver and kidney weights were considered to be treatment related. A marked decrease in serum glucose was observed in males only at 789 mg/m^3. No compound-related macroscopic or microscopic lesions were detected in any tissues. For both sexes, the NOAEC was 218 mg/m^3 and the LOAEC

was 789 mg/m^3 based on the increased weight of liver and kidney. The study was conducted to GLP (Monsanto, 1982*).

Animal inhalation toxicity studies are summarised in Table 4.

Table 4: Summary of inhalation repeated dose toxicity studies in animals

Study/Species	Doses (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3) & Endpoints	References
4-week Sprague-Dawley rats 20/sex/dose	Dose:0, 360, 1000, 2100 Particle size: 3.3 to 1.1 µm (70%)	1000	2100, ↓ body weight, death and atrophy in spleen and testes (males)	Monsanto, 1981*
4-week Sprague-Dawley rats 5/sex/dose	Dose: 0, 49, 144, 526	144	526; ↓ body weight gain	Hammond et al., 1987
13-week Sprague-Dawley rats 25/sex/dose	Dose: 0, 51, 218, 789 Particle size: 4.7-1.1 µm (80%)	218	789; ↑ kidney & liver weight, ↓ serum glucose (males)	Monsanto, 1982*

Studies in humans: A group of 54 workers (average age 38 years) were exposed to phthalic acid esters (PAE) by inhalation, mainly DEHP, DIDP or BBP in the polyvinyl chloride processing industry (Nielsen et al., 1985*). The subjects had been employed for an average of eight years (range 1-21). Exposures determined by personal sampling ranged from 0.02 to 2 mg/m^3 PAE in different job categories. The exposed workers (18-25 µmol/L) excreted slightly but significantly higher levels of PAE metabolites in urine than controls (17 µmol/L). There were no indications of peripheral nerve or respiratory system effects, although some biochemical tests were reported to be abnormal. However, since the workers were exposed to a mixture of phthalates these data are of limited value.

The health status of 147 female and male workers mostly under 40 years of age was studied (Milkov et al., 1973*). The workers were exposed to phthalate plasticizers, predominantly dibutyl phthalate (DBP) and higher molecular weight alkyl phthalates or periodically to dioctyl phthalate (DOP), diiso-octyl phthalate (DIOP) and BBP. Many workers (65) were exposed for >10 years. The ambient levels of vapours or aerosols of the plasticizers at the working zone ranged from 1.7-66 mg/m^3. In the workers a moderately pronounced toxic polyneuritis was found, the frequency and degree increased with increasing working time. This study is of limited value as the exposure to BBP only was periodic and the main exposure was to DBP or higher alkyl phthalates.

Data not reported in previous evaluations

No data.

Conclusion

The most relevant inhalation study is a 90-day sub-chronic rat study conducted to GLP (Monsanto, 1982*). The NOAEC from the study was 218 mg/m^3 of BBP, and the LOAEC 789 mg/m^3 based on increased kidney and liver weight in both male and female rats.

4.5.3 Dermal

Previous evaluations (ECB, 2004)

A poorly reported dermal toxicity study was carried out with repeated skin applications of BBP at doses of 1, 5, 10 and 100 mg/kg bw for 5 months. No information was provided on the species used. BBP was reported to have a local-irritating action. No mortality was observed during the study (Statsek, 1974*).

Data not reported in previous evaluations

No data.

4.6 Genetic toxicity

Previous evaluations

Eight in vitro studies and four in vivo studies were reported. Experimental details and effect levels for all studies are summarised in Table 5.

In vitro prokaryote and eukaryote studies: Negative results were reported in 3 Ames test for reverse mutation in *Salmonella typhimurium* both with and without metabolic activation (Monsanto, 1976b*; Monsanto, 1976c*; NTP, 1997*). BBP was also negative in mutation tests with *E. coli* (Omori, 1976*) and *Saccharomyces cerevisiae* (Monsanto, 1976b*) and repair tests with *B. subtilis* (Omori, 1976*).

In vitro mammalian cell studies: Negative results have been reported for mouse lymphoma assays with and without metabolic activation (NTP, 1997). In an assay for chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in Chinese hamster ovary cells (Galloway et al., 1987*), there was slight evidence for a trend in one sister chromatid exchange test without activation, but the repeat test was negative as was the single test conducted with S9. The results were negative for CA with or without activation.

In vivo studies: No induction of sex-linked recessive lethal mutations was observed in germ cells from male *Drosophila melanogaster* treated with BBP (500 ppm by injection or up to 50000 ppm in diet) (Valencia et al., 1985*).

In a NTP (1997*) bone marrow SCE study, BBP (0, 1250, 2500, and 5000 mg/kg bw) was given as a single intraperitoneal injection to groups of 5 male B6C3F1 mice. A weak response was reported at 23 and 42 hours but there was no dose response and the study was not repeated.

In a NTP (1997*) bone marrow CA study, BBP (0, 1250, 2500, and 5000 mg/kg bw) was given as a single ip injection to groups of 10 male B6C3F1 mice. There was a positive trend at 17 h post injection with a statistically significant increase in frequency of cells with CA at the highest dose. However, the ratio of CA/cells or the number of CA was not statistically different from the controls. At 36 h the frequency of cells with CA, the number of CA and CA/cells were not significantly different between exposed groups and the controls.

No induction of micronucleus was reported (Ashby et al., 1997*) in a micronucleus test in which 19 female Alpk:APf SD rats (10-12 weeks old) were exposed to BBP (182.6 µg/kg bw/d) via drinking water during gestation and lactation.

In a study for induction of dominant lethal mutations, 24 CD-1 and 36 B6C3F1 male mice were given subcutaneous injections of BBP (400-600, 1280-1840, and 3200-4560 mg/kg bw/d) on days 1, 5, and 10 of the study. Each male was mated for 4 day intervals to three untreated virgin females on days 2, 6, 11, 15, 22, 29, 42, and 49. No increase in foetal deaths and no decrease in various fertility parameters were found (Bishop et al., 1987*).

Data not reported in previous evaluations

No data.

Conclusion

BBP showed no evidence of mutagenicity in *Salmonella typhimurium* or mouse lymphoma cells. Results were considered equivocal in SCE or CA assays in CHO hamster cells.

BBP did not induce sex-linked recessive lethal mutations *in Drosophila melanogaster* or dominant lethal mutations in mice and no inductions of micronucleii were reported in female rats after exposure to BBP. Positive results were obtained in a mouse bone marrow test for SCE, however the responses were weak, and the SCE test has not been repeated. The results for the induction of CA were conflicting when different observations times were used.

Based on all data available and on a weight-of-evidence basis, BBP is considered to be non-genotoxic.

4.7 Carcinogenicity

Previous evaluations

A cell transformation test using Syrian hamster embryo cells treated for 24 h or 7 days was negative at 24 h but showed a positive result at 7 days with concentrations of 2.5 and 10 µg/mL. This was interpreted as transformation occurring via a non-mutagenic mechanism (eg changes in rate of cell proliferation) (Le Boeuf et al., 1996*). No information was available regarding use of an exogenous metabolism system. A negative result was reported in a cell transformation assay using BALB/3T3 cells and 0.49 to 8000 nL/mL BBP (Monsanto, 1985*).

In a carcinogenicity study, F344 rats (50/sex/group) were fed 6000 or 12 000 ppm BBP (360 and 720 mg/kg bw) 7 d/week, for 28 or 103 weeks (NTP 1982b*). Mean body weights of dosed females were lower than the controls during most of the study. After week 14, an increasing number of dosed males died as a result of unexplained internal bleeding. Consequently, all living males were killed at 29 to 30 weeks. Thus, males were not adequately tested for carcinogenicity. Mononuclear cell leukaemia (MCL) occurred at a statistically significant increased incidence in high-dose females (36% vs 14% for low dose and 14% for controls). Both the incidence and the overall trend remained statistically significant when compared with the historical incidence of F344/N female rats with leukaemia in the investigating laboratory. Tumour rates for fibroadenomas of mammary glands were decreased in females.

Table 5: Summary of the genetic toxicity of BBP

Test	Test system (species/strain)	Doses	Metabolic activation	Results	References
In vitro, prokaryotes and lower eukaryotes					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.1 - 10.0 µl/plate	S9 +/-	-ve	Monsanto, 1976b*
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.001 - 10.0 µl/plate	S9 +/-	-ve	Monsanto, 1976c*
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	100 - 10 000 µg/plate	S9 +/-	-ve	NTP, 1997*
Mutation test	*E. coli*	30 mg/plate	Not reported	-ve	Omori, 1976*, original data Kurata, 1975
Repair test	*B. subtilis*	30 mg/plate	Not reported	-ve	Omori, 1976*, original data Kurata, 1975.
Mutation test	*S. cerevisiae* D4	0.1 - 10.0 µl/plate	S9 +/-	-ve	Monsanto, 1976b*
In vitro, mammalian cells					
Mouse lymphoma assay	L5178Y TK	5 - 60 nL/mL.	S9 +/-	-ve	NTP, 1997*
CA and SCE assay	Chinese hamster ovary cell	Up to 1250 µg/mL	S9 +/-	-ve or ?	Galloway, 1987*
In vivo					
Sex-linked recessive lethal mutation	*D. melanogaster*	250, 10000, 50000 ppm BBP in feed.	N/A	-ve	Valencia, 1985*
SCE assay	B6C3F1 mouse bone marrow; 5 male mice	1250, 2500 and 5000 mg/kg bw ip;.	N/A	weak +ve after 23 and 42 hrs	NTP, 1997*
CA assay	B6C3F1 mouse bone marrow; 10 male mice	1250, 2500 and 5000 mg/kg bw; ip injection	N/A	+ve at 17 hrs at highest dose; -ve all doses at 36 hrs.	NTP, 1997*
Micronucleus test	gestation - lactation Alpk:AP$_f$SD (AP) rats 19 female rats	182.6 µg/kg/d; drinking water	N/A	-ve	Ashby et al., 1997*
Dominant lethal mutations	B6C3F1 mice (36), CD-1 mice (24)	400-600, 1280-1840, 3200-4560 mg/kg bw; sc injection	N/A	-ve	Bishop, 1987*

-ve – negative; +ve – positive; ? – equivocal; N/A – not applicable

In an identical study to the above NTP study, B6C3F1 mice (50/sex/group) were fed 6000 or 12 000 ppm BBP (840 and 1680 mg /kg bw) for 24 months (NTP, 1982b*). BBP treatment was not associated with increased incidences of any type of tumour in mice.

In a more recent 2-year carcinogenicity study, groups of 60 male F344/N rats were fed 3000, 6000, 12000 ppm (120, 240, 500 mg/kg bw) and females 6000, 12000, 24000 ppm (300, 600, 1200 mg/kg bw) BBP for 2 years (NTP, 1997*). General toxic effects were described in Section 4.5. At 2 years, the incidences of pancreatic acinar cell adenoma, and adenoma and carcinoma (combined) in 12000 ppm males were significantly greater than in controls (20% vs 6% for controls) and exceeded the historical controls from other 2-year NTP feed studies. One carcinoma was observed in one 12000 ppm male, and two adenomas were observed in the 24000 ppm females. At 2 years, the incidence of focal hyperplasia of the pancreatic acinar cells in 12000 ppm males was significantly greater than in the controls. Transitional epithelial papillomas in the urinary bladder were observed in one control female and in two 24000 ppm females and the incidence of this neoplasm exceeded the range of historical controls from NTP 2-years studies. The incidences of nephropathy in all exposed groups of females and of transitional epithelial hyperplasia in 12000 ppm females were significantly greater than those of the controls. The data suggests that exposure of rats to BBP for 2 years resulted in focal hyperplasia in the pancreas in males and in transitional hyperplasia in the urinary bladder of females.

In a dietary-restriction study, groups of 50-60 F344/N rats were fed BBP, either ad libitum or in amounts that restricted mean body weights to approximately 85% of the mean ad libitum control body weights. The study was conducted to understand the influence of dietary restriction on the sensitivity of the bioassay and the effects of weight-matched controls on the sensitivity of the bioassay. BBP caused an increased incidence of pancreatic acinar cell neoplasms in ad libitum-fed males relative to ad libitum-fed or weight-matched controls. This change did not occur in rats in the restricted feed protocols after 2 years, although acinar cell adenomas were observed in three exposed, feed-restricted males at 30 months. BBP also caused a statistically significant increased incidence (8%) of urinary bladder carcinoma in food restricted females after 32 months, but not after 2 years (NTP, 1995*).

In a 15-week study, groups of 27 female Sprague-Dawley rats (43-day old) were given BBP (0, 250, 500 mg/kg bw/d) intragastrically for 7 days prior to intragastric administration of 31 mg/kg bw/d dimethylbenz(a)anthracene (DMBA). BBP did not affect body weight gain after 15 weeks. The incidence of palpable mammary tumours was significantly inhibited by pre-treatment with BBP. The number of adenocarcinomas per rat was also significantly reduced (Singletary et al., 1997*).

Data not reported in previous evaluations

BBP was tested on Balb/c-3T3 mouse cells in an in vitro mammalian cell transformation assay (Barber et al., 2000). With an exposure period of 72 hours and incubation over 4 weeks BBP did not induce statistically significant increases in transforming activity with concentrations up to 0.16µL/mL.

To induce prostatic neoplasms, male F344 rats were given subcutaneous injections of 25 mg/kg/d DMBA every other week for 10 times in total. One week after the last injection, groups of 12 male F344 rats were given BBP (0, 10 or 100 ppm) in the diet for 40 weeks. BBP did not affect food consumption and body weight gain after 40

weeks. Dietary administration of BBP did not affect the incidence of prostatic adenocarcinoma. The proliferating cell nuclear antigen indexes determined for adenocarcinoma, prostate intra-epithelial neoplasm, and non-lesion glands in BBP-treated rats were slightly lower than that of the DMBA control, but the differences were not statistically significant (Kohno et al., 2004).

Conclusion

BBP was tested for cell transforming potential in 3 mammalian assays in vitro. Two assays were negative whereas one showed transformation activity.

BBP was tested for carcinogenicity in vivo by oral administration in one study in mice and in 3 studies in rats, including a dietary restriction study. No increase in the incidence of tumours was observed in mice.

In rats, a statistically significant increased incidence of MCL was reported in females at 12000 ppm (720 mg/kg bw/d) BBP (NTP, 1982a*). However, in later studies, no significant increase in MCL was seen with the same rat strain, despite a higher concentration of BBP tested. A statistically significant increase in MCL seen in female rats may be equivocal evidence of a substance related effect as this tumour type is spontaneous and highly variable in aged Fischer 344 rats, is rare in other rat strains, has not been found in other mammalian species and has no comparable tumour in humans (Caldwell, 1999).

An increased incidence of pancreatic adenomas occurred in male rats in two studies (LOAEL of 500 mg/kg bw/d) but not after dietary restriction. Neoplasms of the urinary bladder were marginally increased in female rats both in a conventional carcinogenicity study and after 2 year dietary restriction. After 32 months dietary restriction (but not at 2 years), a statistically significant increase in bladder carcinomas in these rats was found. This latter result is difficult to interpret in the absence of historic control data.

In one rat study, BBP significantly inhibited DMBA-induced mammary carcinogenesis. However in a subsequent study, BBP did not significantly alter the incidence of DMBA-induced prostate carcinogenesis. No evidence of carcinogenicity of BBP exists for humans.

Overall, these data provide limited evidence of carcinogenicity in animals.

4.8 Reproductive toxicity

Traditional hazard assessments consider effects on fertility separate from developmental toxicity. Fertility is tested by exposing sexually mature adults to a chemical and examining the effects on reproductive capacity. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. Chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to effects on fertility and the two endpoints cannot be clearly distinguished.

In this hazard assessment, data are presented on the basis of test procedure. Test procedures include repeat dose toxicity studies that dose adult animals for varying durations, two-generation studies, prenatal developmental toxicity studies (only the dam is dosed, study ends before parturition) and postnatal developmental toxicity studies (dam is dosed during gestation and allowed to litter, study ends during

weaning). The effects on fertility (as adults) and development (as foetuses) are then discussed separately.

The effects of BBP on reproductive endpoints have been tested in a variety of species. Reproductive and developmental data are summarised in Table 6.

4.8.1 Human studies

Previous evaluations

Duty et al. (2003a) studied whether the general population levels of phthalate monoesters in urine were associated with altered semen quality. In this study 168 male partners aged 20 to 54 years of sub-fertile couples were recruited. The comparison group was men with all three semen parameters above the reference values. Eight urinary phthalate monoesters were measured in a single spot urine sample collected on the same day as the semen sample [monoethyl phthalate (MEP), monomethyl phthalate (MMP), monoethylhexyl phthalate (MEHP), monobutyl phthalate (MbuP), monobenzyl phthalate (MBzP), monooctyl phthalate (MOP), monoisononyl phthalate (MINP), and monocyclohexyl phthalate (MCHP)]. Semen parameters were dichotomized based on WHO (1999) reference values for sperm concentration and motility and Tygerberg Strict criteria for morphology. Median phthalate levels were dichotomized into high and low categories. The unadjusted median levels of urinary phthalate monoester concentrations in µg/L urine were 156 for MEP, 10.3 for MBzP, 15.9 for MBuP, 5.7 for MEHP, and 7.5 MMP, reflecting exposure to diethyl phthalate, BBP, dibutyl phthalate, diethyl hexyl phthalate and dimethyl phthalate, respectively. The results from this study indicated that median MBuP levels were associated with sperm motility and sperm concentration below the reference values with odds ratio (95% confidence interval) of 2.37 (1.13 – 5.00) and 2.41 (0.80 – 7.23). The median MBzP levels were also associated with sperm motility, morphology, and sperm concentration below the reference values with odds ratio of 1.8, 2.1 and 2.7, respectively. The authors concluded from the study that there were dose-response relations for MBuP and MBzP for one or more of the semen parameters studied and suggestive evidence for MMP for sperm morphology. For the other monoesters, no clear correlations were found.

Data not reported in previous evaluations

A comet assay was used to measure DNA damage in sperm from men attending an andrology clinic (Duty et al., 2003b). At the same time, urine samples were collected and phthalate monoesters levels were determined. Urinary MBzP levels were not associated with increased sperm DNA damage (as measured by comet assay). In a follow-up study with a larger group of men also drawn from an infertility clinic, no consistent relationship was found between MBuP and MBzP and sperm parameters using a computer-aided sperm analysis system (Duty et al., 2004). Non-significant trends between sperm parameters and increased urinary levels of MBuP and MBzP were observed.

Jonsson et al (2005) studied semen parameters and urinary phthalate monoester levels in 234 military recruits. There were no significant associations between highest versus lowest urinary MBzP quartile and any of the semen parameters.

Breast milk samples were analysed for a variety of 6 different phthalate monoesters in a Danish–Finnish cohort study on cryptorchidism, gonadotropins, sex-hormone binding globulin, testosterone and inhibin B (Main et al., 2006). No association was

found between MBzP and cryptorchidism and MBzP and sex-hormone binding globulin, gonadotropins or testosterone.

Association between 11 maternal urinary phthalate monoester concentrations and genital parameters such as anogenital index (AGI) [i.e. anogenital distance (AGD) normalised for body weight] and testicular descent in children was investigated in 85 mother-son pairs (Swan et al., 2005). Urinary MBzP concentration was inversely related to AGI. This study has been criticised by McEwen et al. (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that AGD is more likely to be proportional to height rather than weight and that maternal phthalate urinary concentrations were not normalised for urine volume. The reliability of the measurement of AGD in humans has not been verified. One study of 87 neonates that has assessed the correlation of AGD with body weight found in males a correlation of 0.48 and that body length may be a slightly better predictor for AGD than weight (Salazar-Martinez et al., 2004).

4.8.2 Male reproductive toxicity study

Previous evaluations

In a NTP (1997*) study, groups of 15 male F344/N rats were fed 0, 300, 2800, or 25000 ppm BBP (0, 20, 200, or 2200 mg/kg bw/d) for 10 weeks. After the exposure period the animals recovered for 2 days prior to a 7-day mating period. All rats survived to the end of the study. The final mean body weight of the 25000 ppm group was significantly lower than the controls. No clinical findings related to BBP exposure were noted. A few minor haematological changes (minimal anaemia, and increased platelet count) occurred at 25000 ppm. The absolute and relative prostate gland and testes weight of the 25000 ppm males were significantly less than the controls. Degeneration of the seminiferous tubule epithelium was observed in all males at 25000 ppm. A statistically significant dose-dependent decrease in epididymal spermatozoa concentration was also reported. However, in this study the days of allowed recovery varied within animals and BBP dose. At 2800 ppm a higher number of rats were shown to have a shorter recovery period compared to controls. The epididymal spermatozoa concentration after mating was almost back to normal in the control group after two days, whereas in the 300 and 2800 ppm group it was almost back to normal after 4 or 5 days, however, this information is based on a limited number of observations. When days of recovery were taken into account in a covariate analysis of variance on the epididymal spermatozoa concentration from the control, 300 and 2800 ppm group, the decrease in epididymal spermatozoa concentration was not statistically significant at 2800 ppm, however, a dose-dependent decrease was still evident. Ten females mated to 25000 ppm males were initially found to be sperm-positive; none of these females were pregnant at necropsy. There were no significant differences in litter data between the controls and the 300 and 2800 ppm groups. The NOAEL for fertility was 2800 ppm (200 mg/kg bw/d) as technical errors occurred deriving the epididymal spermatozoa concentration.

4.8.3 Repeat dose toxicity studies

Previous evaluations

In a 28-day repeated dose toxicity study, Cpb-WU male rats (28-day old, 3/group) were given BBP by gavage (0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 2100 mg/kg bw/d) (Piersma et al., 1999). The non-reproductive effects are described in

Section 4.5. A dose-related decrease was reported in relative testes weight from 750 mg/kg bw/d (statistically significant from 1250 mg/kg bw/d). Histopathologic analysis of the testes revealed severe atrophy from 970 mg/kg bw/d. LH and FSH were increased at 1250 mg/kg bw/d and a significant decrease in testosterone level was reported from 450 mg/kg bw/d. Testicular atrophy was reported in the presence of a 20% increase in relative liver weight. The NOAEL for reproductive effects was 350 mg/kg bw/d and the LOAEL was 450 mg/kg bw/d based on decreases in testosterone in males.

In a 14-day dietary fertility study, adult male Fisher 344 rats (10/dose) were given 0, 0.625, 1.25, 2.5 and 5% of BBP in diet (0, 312, 625, 1250 or 2000 mg/kg bw/d; Agarwal et al., 1985). The non-reproductive effects are described in Section 4.5. A significant increase in relative liver and kidney weights in all BBP dose groups were evident with mild multifocal chronic hepatitis at 5%. A significant reduction in total body, and absolute testes, epididymis, prostate and seminal vesicle weights were reported at ≥ 2.5%, whereas a significant decrease in the relative organ weight was only reported in the testes, epididymis and seminal vesicle at ≥ 2.5%. Histology revealed dose-dependent and statistically significant atrophy of the testes, prostate and seminal vesicles at ≥ 2.5%, atrophy of the epididymis at 5%, and the presence of immature sperm cells in the tubular lumen and necrosis of the tubular epithelium in the caput epididymis at ≥ 2.5%. Plasma testosterone concentration was significantly decreased at 5% while follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations were increased at ≥ 2.5%. The NOAEL for reproductive effects was 1.25% (625 mg/kg bw/d) and the LOAEL was 2.5% (1250 mg/kg bw/d) based on changes in male reproductive organ weights and atrophy.

Several studies with BBP were conducted by Lake et al. (1978*), but it is not clear whether these studies were compliant with international recognised guidelines.

4.8.4 One/two-generation reproductive toxicity studies

Previous evaluations

In a two-generation study (Tyl et al., 2004), Sprague-Dawley rats (40-45 days old, 30 animals/sex/dose) were fed BBP at 0, 750, 3750, and 11250 ppm (0, 50, 250 and 750 mg/kg bw/d) for 10 weeks prior to and during a two-week mating period. At 750 mg/kg bw/d, F0 males and females had significantly increased relative liver and kidney weight with minimal hepatic histopathological lesions. Females showed a significantly decreased body weight from day 0 to 70, and during gestation and lactation. Absolute and relative ovary and uterus weights were also statistically significantly increased in F0 females at 750 mg/kg bw/d. No effects on mating, fertility or reproductive organ weights were observed in F0 males. F1 males (but not females) at 250 mg/kg bw/d exhibited significantly increased relative liver and kidney weights. Both males and females at 750 mg/kg bw/d had significantly decreased body weight at necropsy. Histopathological lesions of the liver graded as minimal were only reported in females.

There were significantly reduced mating and fertility indices in F1 parents at 750 mg/kg bw/d. Males had significantly reduced absolute weight in paired testes, paired epididymis, prostate and seminal vesicle with coagulating gland. The number of rats with histopathological changes in testes and epididymis at high dose was significantly increased. The epididymal sperm concentration and the frequency of motile sperm were significantly decreased. A significant increase in the number and

frequency of males with at least one reproductive tract malformations were reported. The number of implantation sites and number of total pups were significantly reduced.

In the F1 progeny, body weight was reduced through much of lactation. At 250 mg/kg bw/d there was a significant increase in uterus weight and a significantly reduced AGD in males (but not females) and one male had a missing testis. In males, AGD was significantly decreased in a dose-related fashion from ≥ 250 mg/kg bw/d. At 750 mg/kg bw/d the number of male pups with ≥1 nipple and the number of nipples per male, the frequency of male pups with ≥1 areolae and the number of areolae per male were significantly increased. Necroscopy at weaning revealed a significant decrease in terminal body weight of both sexes at 750 mg/kg bw/d as well as decreased relative spleen weight. Absolute epididymis and testes weight and absolute ovaries and uterus weight were significantly reduced at weaning at 750 mg/kg bw/d. There was a significant delay in preputial separation in males and vaginal opening in females. In the F2 generation, average pup body weight per litter on post-natal day (PND) 7, 14 and 21 was significantly reduced. A significantly reduced AGD was reported in males at 250 mg/kg bw/d. At weanling necropsy at 750 mg/kg bw/d there was a significantly reduced terminal body weight and relative spleen weight in F2 offspring. Paired testes weight was reduced in the high dose group. At 750 mg/kg bw/d, there was a significant increase in the frequency of male pups with at least one nipple, the number of nipples per male, and the number of areolae per male, reproductive tract anomalies (including missing epididymis or seminal vesicle). In this study, undescended testes was observed in F1 males at 750 mg/kg bw/d on PND 4 and 21 but not as adults and was not observed at any time in any F2 male evaluated in this study at any time or dose.

The NOAEL for maternal toxicity was 250 mg/kg bw/d and the LOAEL was 750 mg/kg bw/d based on organ weight changes and liver histopathological lesions. The NOAEL for fertility was 250 mg/kg bw/d and the LOAEL was 750 mg/kg bw/d based on significantly reduced mating and fertility indices in F1 parents. For developmental toxicity, the NOAEL was 50 mg/kg bw/d and the LOAEL was 250 mg/kg bw/d based on a dose-related reduction in AGD in both F1 and F2 male offspring.

In another two-generation study (Nagao et al., 2000), Sprague-Dawley rats (8-week old, 25/sex/group) were gavaged with 0, 20, 100 or 500 mg/kg bw/d BBP. F0 males were treated for 12 weeks prior to 2-week cohabitation, and until necropsy. F0 females were treated for 2 weeks prior to cohabitation until necropsy. F1 animals were treated by gavage after weaning until necropsy. Mating was permitted at 13 weeks of age. In F0, high dose males but not females, showed a significant decrease in body weight gain. At 500 mg/kg bw/d, there was a statistically significant increase in relative liver weight in males, and a decrease in absolute ovary weight in females. No macroscopic or microscopic changes were observed in the reproductive system of both sexes. A decrease in serum testosterone, T3 and T4 levels at 500 mg/kg bw/d, and an increase in FSH from 100 mg/kg bw/d were reported in males. In females a significant increase in serum concentrations of prolactin, and a significant decrease in T4 were seen at 500 mg/kg bw/d.

The viability of F1 offspring during PND 0-4 was decreased at 500 mg/kg bw/d. Body weight of male and female offspring at birth was decreased at ≥ 100 mg/kg bw/d, and the body weight at 500 mg/kg bw/d was lower throughout the study,

however, the viability was not affected. At 500 mg/kg bw/d a significant decrease in AGD at birth in males, and an increase in AGD in females were reported.

At necroscopy (18 weeks), an increase in relative weight of the thyroid gland, adrenal glands, and liver weights as well as a reduction in the absolute weights of the testes, epididymis and prostate were reported in males at 500 mg/kg bw/d. There was a significant decrease in ovary weight and increase in uterine weight in females at 500 mg/kg bw/d. Furthermore, at 500 mg/kg bw/d, 9/10 males showed bilateral decreases in spermatogonia within the seminiferous tubules. BBP did not, however, affect the reproductive ability. Furthermore, a significant decrease in male in FSH concentration at 500 mg/kg bw/d, in thyroid stimulating hormone (TSH) concentrations at ≥ 100 mg/kg bw/d and a significant decrease in females in T3 level at ≥ 100 mg/kg bw/d, were reported. Histopathologic examination revealed a significant decrease in the numbers of spermatocytes in the seminiferous tubules at
500 mg/kg bw/d compared to controls. Cryptorchidism or hypospadias was not observed in any dose groups. In females no BBP-related histopathologic abnormalities were observed. At PND 40, preputial separation for male offspring in the 500 mg/kg bw/d group was delayed compared to controls, while vaginal opening for female offspring was not affected. No significant adverse effects related to BBP exposure including pup weight, viability and development were reported in the F2 offspring. The NOAEL for reproductive toxicity in males was 100 mg/kg bw/d and the LOAEL was 500 mg/kg bw/d based on atrophy of the testes, epididymis, and seminal vesicle, and reduced reproductive organ weights in the F1 generation. The NOAEL for developmental toxicity was 20 mg/kg bw/d and the LOAEL was 100 mg/kg bw/d based on reduced body weight in offspring of both sexes at birth. There were no dose-related effects on fertility in this study.

In an OECD Guideline validation test, RIVM-bred WU rats (10/sex/group) were exposed by gastric intubation to 250, 500, or 1000 mg/kg bw/d BBP (Piersma et al., 1995). After dosing for 14 days, animals were paired and allowed to mate whilst dosing continued (males for a further 14 days, females until postpartum day 6). At 1000 mg/kg bw/d effects were found on the body weight gain and food consumption in both males and pregnant females, the pregnancy rate was reduced, testes and epididymis weights were significantly reduced, testicular degeneration accompanied by interstitial (Leydig) cell hyperplasia and appearance of cellular debris were increased as were time to conception and post-implantation loss. Corpora lutea, implants per dam, and pre-implantation loss were not different between controls and exposed groups. The number of live pups at day 1 and 6 after birth were significantly lower in the 1000 mg/kg bw/d group compared to controls. Pup weights were reduced in the 500 and 1000 mg/kg groups at PND 1 and reduced in the 1000 mg/kg dose group at PND 6. The NOAEL for effects on reproductive organs and fertility was 500 mg/kg bw/d. The NOAEL for developmental effect was 250 mg/kg bw/d.

In a continuous breeding study, Wistar rats (Crl:WI (WU) BR) (12 males & 24 females/group) were administered BBP in diet (0.2, 0.4 and 0.8%) over one generation producing 2 litters (Monsanto, 1993*). No mortality or clinical signs were considered to be caused by the treatment although one male and one female parent rat in the controls were killed during the study. The live birth index and viability index on PND 4-21 were unchanged. A reduction in mean body weight was observed in the females at 0.8% compared to controls during the gestational and lactational periods. Reduced food consumption was reported at 0.8% during both gestational and lactational periods, and was considered related to BBP exposure. An increase in

relative liver weight was statistically significant in the 0.8% females. Microscopic examination of the organs of the reproductive tract did not reveal any treatment related effects. The NOAEL for parental toxicity was 0.4% based on liver effects, and the NOAEL for reproductive performance and development of the offspring was 0.8% (males: 418 mg/kg bw/d; females: 446 mg/kg bw/d), the highest dose tested.

Data not reported in previous evaluations

In a two-generation reproduction study, Crj:CD (SD)IGS rats (24/sex/group) were gavaged with 0, 100, 200 or 400 mg/kg bw/d for 10 weeks prior to mating, through mating and to weaning (females) or litter delivery (males) (Aso et al., 2005). Gavaging of F1 began at 3 weeks and continued as for F0. In the F0 adults, there was no effect on body weight or body weight gain. Relative liver weight was increased in high dose males and mid- and high dose females. Relative kidney weight was increased in high dose males and females. There was no effect on testes weight but right (but not left) relative epididymal weight was decreased as were spermatozoa in the epididymis. Hyperplasia of Leydig cells was also reported. There was no effect on reproductive endpoints such as mating, number of implantations, number of sperm in testes and epididymis, sperm motility, serum FSH, LH, testosterone or oestradiol.

Foetal body weight was significantly reduced in F1 males and F2 males and females at 100 mg/kg bw/d. Anogenital distance was increased in F1 females exposed to 100 mg/kg bw/d (not dose-related) and decreased in F2 males. In the F1 adults, there was no effect on body weight. Relative liver weight was increased in high dose females, and mid- and high dose males. Relative kidney weight was increased in high dose males and females. Absolute epididymis and seminal vesicle (but not testes) weight was decreased in high dose males. Right (but not left) relative epididymal weight was also reduced in mid-dose F1 males. There was also an increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells. The NOAEL for reproductive effects was 200 mg/kg bw/d and the LOAEL was 400 mg/kg bw/d based on increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells in F1 males. A LOAEL for developmental effects was established at 100 mg/kg bw/d (lowest dose tested) based on decreased foetal body weight in F1 males, and F2 males and females and decreased AGD in F2 males. A NOAEL for development toxicity was not established in this study.

4.8.5 Prenatal developmental toxicity studies

Previous evaluations

In a NTP (1990*) study, timed-pregnant Swiss DC-1 mice (27-30/dose) were fed 0, 0.1, 0.5, 1.25 and 2.0% BBP (0, 182, 910, 2330 and 4121 mg/kg bw/d) from gestation day (GD) 6 to 15. Dams were sacrificed on GD 17. No maternal or embryo/foetal effects were reported at 0.1%. At 0.5% prenatal mortality per litter and malformed foetus per litter were significantly increased. At 0.5% and 1.25% dam weight gain was reduced by 15% and 66% (GD 6-15) respectively. Absolute liver weight decreased and relative liver and kidney weights increased in the absence of treatment-related microscopic lesions; foetal weight decreased and prenatal mortality and malformed foetuses on per litter basis were significantly increased. The 2.0% group was eliminated after evaluation of 14 dams since all conceptuses were resorbed. The NOAEL for developmental toxicity was 182 mg/kg bw/d and the

LOAEL was 910 mg/kg bw/d based increased foetal death and malformed foetuses per litter.

In another NTP (1989*) study, timed-pregnant Sprague-Dawley rats were fed BBP at 0, 0.1, 0.5, 1.25 and 2.0% (0, 419, 1102 and 1641 mg/kg bw/d) from GD 6-15. Dams were sacrificed on GD 20. At 1.25%, dam weight gain (GD 6-15) was reduced, relative liver weight was increased and the frequency of foetuses with variations or malformations per litter was increased. At 2%, dam weight gains decreased and foetal weight, resorptions and malformations on a per litter basis were significantly increased. Maternal food/water intake was unchanged or increased, except for reduced food intake at 2% (GD 6-9). The NOAEL for maternal and developmental toxicity was 0.5% (419 mg/kg bw/d) and the LOAEL was 1.25% (1102 mg/kg bw/d) based on increased frequency of malformations and variations.

In another developmental toxicity study (Piersma et al, 1999), pregnant Cpb-WU rats (8-week old) were gavaged BBP (0, 270, 350, 450, 580, 750, 970, 1250, 1600 or 2100 mg/kg bw/d) from GD 5-15 (short exposure) or GD 5-20 (long exposure).

Maternal effects: Decreased food consumption was observed from 1250 mg/kg bw/d during the first 5 days of dosing. At ≥ 1600 mg/kg bw/d several animals died during the first five days of exposure. Pregnant rats showed a significant dose-related reduction in body weight gain from 750 mg/kg bw/d. A significantly dose-related increase in relative liver weight was reported from 750 and 580 mg/kg bw/d after short- and long-time exposure, respectively. Similar observations were reported in the non-pregnant rats. Peroxisome proliferation was reported only at the three highest doses. A significant reduction in progesterone levels in pregnant rats from 270 mg/kg bw/d after long-time exposure, and from 1250 mg/kg bw/d after short-time exposure were seen. The maternal NOAEL for long exposure was 450 mg/kg bw/d and 580 mg/kg bw/d for short exposure based on significant increased liver weight.

Effects in offspring: No effects on corpora lutea were reported. Early resorptions were increased from 1600 mg/kg bw/d, whereas late resorptions were increased from 750 mg/kg bw/d regardless of the exposure length. Foetal weights were significantly decreased from 450 mg/kg bw/d after short exposure, and from 350 mg/kg bw/d after long exposure. A dose-related decrease in relative foetal testes weight was reported after long exposure from 270 mg/kg bw/d (statistically significant). From 580 mg/kg bw/d the reduction in foetal testes weight was statistically significant. The incidence of retarded foetal testicular descent showed a dose-related increase from 580 mg/kg bw/d, the incidence being higher after long, rather than short, exposure. Skeletal anomalies were increased from 750 mg/kg bw/d. The LOAEL based on a dose-related reduction in relative foetal testes weight was 270 mg/kg bw/d (lowest dose tested). No NOAEL for developmental effects could be established.

In a developmental toxicity study, pregnant rats were given BBP in diet [0, 0.25, 0.5, 1.0 or 2.0% (0, 185, 375, 654 or 974 mg/kg bw/d)] from GD 0-20, and sacrified on GD 20 (Ema et al., 1990*). Reduced maternal weight gain during pregnancy was observed from 375 mg/kg bw/d. However, a reduced adjusted weight gain was only observed at ≥ 654 mg/kg bw/d. Embryo-toxic effects were observed at 375 mg/kg bw/d with a significantly reduced number of live foetuses per litter. At 654 mg/kg bw/d significantly reduced body weights of foetuses, and at 974 mg/kg bw/d complete resorption of all the implanted embryos were reported. Morphological examination of the foetuses revealed no evidence of teratogenesis. A second pairfed group of rats receiving 974 mg/kg bw/d BBP from GD 0-20 showed the same

reduction in weight gain however complete resorption was not found in any of the pairfed rats suggesting that the embryolethality observed in the non-pairfed rats exposed to 974 mg/kg bw/d was due to BBP and not reduced food consumption. The maternal NOAEL was 375 mg/kg bw/d. The NOAEL for offspring was 185 mg/kg bw/d and the LOAEL was 375 mg/kg bw/d based on reduced number of live foetuses per litter.

To study the effect of BBP on development, and on maternal and embryonic zinc metabolism, pregnant Wistar rats were given BBP by gavage (0, 250, 1000, 1500, or 2000 mg/kg bw/d) daily on GD 11-13 (Uriu-Adams et al., 2001). At ≥ 1000 mg/kg bw/d signs of marked maternal toxicity, and reduced activity with 2 deaths at 2000 mg/kg bw/d were reported. The body weight on GD 20 was significantly lower at 2000 mg/kg bw/d. Food intake was lowest at 2000 mg/kg bw/d from GD 12 to 16 compared to the other groups, however, food intake was similar in all groups from GD 17 to 19. No effects on maternal hematocrits, liver or kidney weights were observed. Increased placental weights were seen at ≥ 1 500 mg/kg bw/d. At 2000 mg/kg bw/d some of the placentas had light green patches. BBP affected reproductive outcome in a dose-dependent manner. Reduced foetal weights were observed at ≥ 1500 mg/kg bw/d and at 2000 mg/kg bw/d fewer live foetuses and higher percentages of resorption were seen in the litters compared to the other groups. Gross anomalies were reported in the pups from 1000 mg/kg bw/d. Effects of BBP on skeletal ossification on GD 20 were significant at ≥ 1500 mg/kg bw/d. A higher incidence of skeletal anomalies was reported from 1000 mg/kg bw/d. There was a dose response increase in the incidence of overall rib anomalies (rudimentary and supernumerary ribs; significant from 1000 mgkg bw/d). A dose dependent tendency towards increased concentration of maternal liver metallothionein (Mt) was seen, however, not statistically significant. At 2000 mg/kg bw/d two dams had a six fold higher liver Mt concentration compared to controls, and in these two dams 93%-100% resorptions were reported. Maternal plasma concentrations of Zn were not statistically significant increased among the exposure groups. It was concluded that BBP was not a strong inducer of Mt, and that the teratogenicity of BBP does not appear to be due to alterations in maternal and/or embryonic Zn metabolism. The NOAEL for developmental effects was 250 mg/kg bw/d and the LOAEL was 1000 mg/kg bw/d based on the finding of gross developmental anomalies.

Ema and colleagues performed a number of oral exposure (250-1000 mg/kg bw/d) studies of varying duration encompassing different stages of gestation. Postimplantation loss was significantly increased with as little as 2 days exposure on GD 7-9, 10-12 or 13-15 (Ema et al., 1993*) while exposure in later gestation (GD 11-20 or 16-20) did not significantly increase postimplantation loss (Ema et al., 1992b*; 1992c*). No effect on preimplantation loss per litter was reported on exposure GD 0-20, 0-7 or 0-11 (Ema et al., 1992b*; 1992c*) but a significant increase in the incidence of pre-implantation loss per litter at 1000 mg/kg bw/d following exposure on GD 0-8 was also reported (Ema et al., 1998*). A significant decrease in the uterine and ovarian weight and plasma progesterone levels in all groups exposed to 2% BBP on GD 0-7, 0-9 or 0-11 (Ema et al., 1994*). The post-implantation embryonic loss due to BBP exposure during early pregnancy therefore, may be mediated via the reduction in plasma progesterone levels. Malformations were induced following exposure to 974 mg/kg/d on GD 11-20 or 16-20 (Ema et al., 1992b*; 1992c*) and at 750 or 1000 mg/kg bw/d on GD 7-15, 7-9, 13-15 (Ema et al., 1993*, 1992a*). No malformations were reported after exposure on pregnancy day 10-12 (Ema et al., 1993*).

Data not reported in previous evaluations

In the study by Hotchkiss et al. (2004), pregnant rats were treated with either corn oil, 75 mg/kg bw/d of linuron, 500 mg/kg bw/d of BBP, or a combination from GD 14 to 18. Prenatal exposure to BBP decreased testicular testosterone production and testicular testosterone concentration and induced alterations in androgen-organised tissues. BBP was associated with significantly decreased neonatal AGD and infant areolae retention in males. The frequency of reproductive organ malformations was increased (but not statistically significantly) and reproductive organ weights were reduced. When the androgen receptor antagonist, linuron was added, effects were increased in a dose-additive manner. Maternal body weight gain was unaffected by BBP exposure.

Saillenfait and colleagues (2003) examined the effects of BBP (0, 0.9, 1.8, 3.6 and 5.4 mmol/kg bw) and the metabolites mono-n-butyl phthalate (MBP) and mono-benzyl phthalate (MBzP) in OF1 mice and Sprague-Dawley rats in vivo. Pregnant rats (22-24/group) were given a single dose of 0, 560, 1120 or 1690 mg/kg bw BBP by gavage on GD 10. Pregnant mice were given a single dose of 0, 280, 560, 1120 or 1690 mg/kg bw BBP by gavage on GD 8. While maternal body weight gain was reduced at the two highest doses in mice (including four maternal deaths), no significant difference was noted when body weight was corrected for gravid uterus weight. At the two highest doses, there was a significant decrease in litter size and an increase in post-implantation losses and resorptions. Resorptions were also significantly increased at 560 mg/kg. Foetal weight was significantly decreased at the highest dose only. Half of the foetuses at this dose were malformed. Malformations mainly included exencephaly and imperforate anus associated with absent or vestigial tail. In rats, there were no differences in maternal body weight gain and there were no significant differences in any reproductive or developmental parameters although malformations (mainly exencephaly) occurred in 5% of foetuses at the highest dose. The NOAEL for developmental effects was 280 mg/kg bw/d in mice and 1120 mg/kg bw/d in rats.

Sprague-Dawley outbred CD rats were treated by gavage daily from GD 12 to GD 19 with 500 mg/kg bw/d of BBP. Limited data was presented however, AGD was significantly reduced compared to controls (Liu et al., 2005). Using a microarray analysis it was shown that BBP significantly altered the gene expression of 391 genes (of 30000 queried) relative to controls.

The BBP metabolites, MBuP and MBzP induced similar effects in developmental studies in rats including cryptorchidism (MBuP, Shono et al., 2000*; Imajima et al., 1997*; MBzP, Ema et al., 2003*) and decreased anogenital distance in male pups (MBzP, Ema et al., 2003*).

4.8.6 Postnatal developmental toxicity studies

Previous evaluations

Gray et al. (2000) gavaged pregnant Sprague-Dawley rats (5/group, 2 blocks) with 750 mg BBP /kg bw/d from GD 14 through PND 3 then necroscopied at 3-4 months (block 1) or 4-7 months (block 2). No reduction in maternal weight gain was reported during gestation and up to PND 3. The pup weight per litter was significantly reduced at birth. In male offspring treated with BBP a reduced AGD and reduced paired testes weight per litter at PND 2 were reported. Furthermore, significant decreases in seminal vesicle, ventral prostate, paired epididymis and cauda epididymis were

reported on a per litter basis. As infants, 70% of males displayed female-like areolas/nipples at PND 13 compared to none in the controls on a per litter basis. At necropsy, malformations in the androgen-dependent organs and testes were reported in 84% of male offspring treated with BBP on a per litter basis. Hypospadias were reported in 29% of the male offspring on a per litter basis. The results demonstrated that exposure to 750 mg/kg bw/d BBP from GD 14 through PND 3 severely altered sexual differentiation in the males and that BBP altered male sexual differentiation in an anti-androgenic fashion.

Inhalation

Three repeat dose studies (4-13 week) inhalation studies were available (Monsanto, 1981*; 1982*; Hammond et al., 1987*). Atrophy of the testes and decreased body weight were noted after 4 weeks exposure to 2100 mg/m^3/d (Monsanto, 1981*). The NOAEC was 1000 mg/m^3/d. The latter two studies, using lower concentrations, did not report any reproductive effects.

4.8.7 Mode of action

Previous evaluations

BBP was positive for oestrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000) and showed weak oestrogenic activity in recombinant yeast assay (Harris et al., 1997). The metabolites MBuP and MBzP did not exhibit oestrogenic activity in a recombinant yeast screen assay (Harris et al., 1997; Sohoni and Sumpter, 1998*). BBP was a weak competitive agonist at the oestrogen receptor in an in vitro competitive ligand-binding assay and weakly induced oestrogen receptor-mediated gene expression of luciferase activity in MCF-7 cells (Zacharewski et al., 1998). BBP (but not MBP) increased proliferation of human breast cancer MCF-C7 cells (Okubo et al., 2003; Hong et al., 2005). However, the effects were not replicated in vivo as oral treatment with 600 mg/kg bw/d BBP for 3 days did not increase expression of CaBP-9k mRNA (a gene highly regulated by 17β-oestradiol) in 7 day old female SD rats (Hong et al., 2005). In addition, BBP, for 3-4 days (up to 2000 mg/kg bw/d), did not induce oestrogenic responses in vivo in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998). BBP was shown in vitro to be a potent antiandrogen in yeast cells expressing the androgen receptor (AR) (Sohoni and Sumpter, 1998*).

Data not reported in previous evaluations

BBP (1000 mg/kg) administered orally to rat dams on GD 14-18 significantly reduced both ex vivo testosterone production and insl3 gene expression in foetal rat testes. These effects are likely to result in gubernacular malformations and cryptorchidism in rats (Wilson et al., 2004).

Conclusion

Effects on fertility

In a study of men attending an andrology clinic, an association was found between high levels of MBzP in the urine and altered semen quality including semen concentration, semen mortality and semen morphology (Duty et al., 2003a) but this was not replicated in a later study (Duty et al., 2004) or when a more ‘normal’ population was used (Jonsson et al., 2005). Due to the mixed exposure to various

phthalates it is difficult to conclude that the effect observed on semen quality is related to BBP exposure.

Treatment-related effects on fertility or reproductive organs in rats following oral or inhalation exposure included reduced mating and fertility indices, decreases in testes weight, histopathological changes in testes and hormonal changes. These effects occurred, in the majority of the studies, at BBP doses equal to or greater than doses that induced systemic toxicity (increased liver and kidney weight with histopathological changes).

The NOAEL for fertility effects in a well-conducted two-generation reproduction study was 200 mg/kg bw/d and the LOAEL was 400 mg/kg bw/d based on increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells in F1 (Aso et al., 2005).

Developmental effects

In human studies, urinary MBzP concentrations were inversely related to AGI. However, the use of AGD measurements and the calculation of AGI in humans have been criticised. There was no association between MBzP and cryptorchidism, sex-hormone binding globulin, gonadotropins or testosterone.

In developmental toxicity studies in rats and mice, BBP induced prenatal mortality, reduced foetal weight, decreased AGD in males and increased AGD in females and variations and malformations including undescended testes. The incidence of these effects was dependent on dose and developmental age. The mechanism of action for resorptions has been proposed as reduced circulating progesterone. Maternal toxicity included reduced body weight gain and increased liver weight accompanied by decreased food consumption.

Developmental toxicity was reported in rats and mice exposed in utero to BBP in the absence of marked maternal toxicity suggesting that the teratogenic effect of BBP during the organogenic period is primarily the result of BBP exposure and not a result of reduced body weight gain observed in dams. Both BBP and the metabolites MBP and MBzP have been shown to be embryotoxic in rats and mice (Saillenfait et al., 2003*).

Piersma et al. (1999) in a developmental study of 9 dose levels in rats noted decreased relative foetal testes weights at 270 mg/kg bw/d. A NOAEL could not be derived as this was the lowest dose tested. In other developmental studies, a NOAEL of 185 mg/kg bw/d and a LOAEL of 375 mg/kg bw/d were determined based on decreased litter size in a dietary developmental study in rats (Ema et al., 1990*).

From two-generation studies, a NOAEL of 20 mg/kg bw/d was derived based on decreased foetal body weight in the F0 and F1 offspring at 100 mg/kg bw/d (Nagao et al., 2000). With regards to reproductive organ changes in this study, AGD, testes and epididymis weight and numbers of sperm in the epididymis were decreased in males at 500 mg/kg bw/d. The NOAEL for reproductive organ changes in offspring was 100 mg/kg bw/d. In a two-generation study by Aso et al. (2005), a LOAEL of 100 mg/kg bw/d was also determined based on decreased body weight and AGD in offspring. However, this was the lowest dose tested and so a NOAEL could not be established in this study. Another two-generation rat study (Tyl et al., 2004) derived a NOAEL for developmental toxicity of 50 mg/kg bw/d. The LOAEL was 250 mg/kg

bw/d based on statistically significant, dose-related reductions in AGD in both F1 and F2 males at this dose.

In some in vitro assays, BBP was weakly oestrogenic but this was not replicated in in vivo studies. Therefore no certainty can be associated with the oestrogenic activity observed in vitro. Malformations in reproductive organs and effects on androgen-mediated endpoints in male rats exposed to BBP or its metabolites during prenatal development suggest antiandrogenic activity by BBP. There are in vitro data to support this hypothesis.

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Table 6. Summary of fertility and effects on the reproductive organs with BBP

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
Two-generation studies					
CD Sprague-Dawley rats; 30/sex/group;	diet	0, 750, 3750, 11250 ppm (0, 50, 250, 750)	Mat: 250 Fert: 250 Devp: 50	750; Mat: organ weight changes & minimal liver histopathology. 750; Fert: ↓ mating and fertility indices in F1 parents, reproductive organs malformations in males. 250; Devp: ↓ anogenital distance and reproductive organs weight changes in F1 & F2 males.	Tyl et al., 2004
Sprague-Dawley rats; 25/sex/group	gavage	0, 20, 100, 500	Mat: 100 Fert: 100 Devp: 20	500; Mat: ↓ body wt gain (F_0, F1), ↑ liver wt in F0 males, 500; Fert: ↓ ovary weight in F0 females; ↓ testosterone (F0, F1), ↓ testes, epididymis, and seminal vesicle weight (F1), ↓ number of germ cells in the seminiferous tubules, sperm in the epididymis (F1). No effect on reproductive ability, including delivery and lactation. 100; Devp: ↓ body wt at birth (F1) F2: no significant BBP related effects up to PND 21	Nagao et al., 2000
CD:SD Rats; 20/sex/group	Gavage	0, 100, 200, 400	Mat: 100 Fert: 200 Devp: NE	200; Mat: ↑ rel liver wt (F0 female, F1 male); 400; ↑ rel liver wt (F0 male, F1 female); ↑ rel kidney wt, (F0, F1) 400; Fert: ↓ spermatozoa in epididymis, hyperplasia of Leydig cells (F0), ↓ abs epididymis & seminal vesicle wt (F1), ↑ frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells (F1) 100; Devp: ↓ Foetal body wt (F1 males; F2 males and females), ↑ anogenital distance (F1 females, not dose-related) and ↓ in F2 males (absolute & relative).	Aso et al., 2005

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
Repeat dose studies					
10-week study Fisher 344 rats; 15/male/group	diet	0, 300, 2800, 25000 ppm (0, 20, 200, 2200)	Fert: 200	2200; ↓ epididymal spermatozoa concentration, ↓ body, prostate and testes weight, degeneration in seminiferous tubules, no pregnancy after mating.	NTP, 1997*
26-week study Fisher-344 male rats; 15 male/group	diet	0, 300, 2800, 8300, 25000 ppm (0, 30, 180, 550, 1660)	Fert: 550	1660; ↓ fertility, testes, epididymis weight, epididymal spermatozoa conc. Degenerative changes in testes and epididymis. Other toxic effects see table 3.	NTP, 1997*
28-day study Cpb-WU male rats; 4 weeks of age; 3/group	gavage	0, 270, 350, 450, 580, 750, 970, 1250, 1600, 2100	Fert: 350	450; ↓ testosterone levels from 450 mg/kg bw/d. Severe testicular atrophy from 970 mg/kg bw/d. Dose related ↓ testes weight from 750 mg/kg bw/d (stat sig from 1250 mg/kg bw/d)	Piersma et al., 1999
14–day study Fisher 344 male rats; 10/dose	diet	0, 0.625, 1.25, 2.5 and 5% (0, 312, 625, 1250 and 2500)	Fert: 625	1250; ↓ body, testes, epididymis and prostate weight; histopathologic changes in testes, prostate and seminal vesicle, presence of immature sperm and necrosis in tubular epithelium; ↑ levels of LH and FSH; decreased progesterone levels, general toxicosis at 2500 mg/kg bw/d	Agarwal et al., 1985*
14 days prior to and throughout mating; RIVM-bred WU-rats; 10/sex/group	gavage	0, 250, 500, 1000.	Mat: 500 Fert: 500 Devp: 250	1000; Mat: ↓ body weight, 1000; Fert: pregnancy rate, live pups, epididymis weight, testicular degeneration 500; Devp: ↓ pup weight	Piersma et al., 1995
14-day study; Sprague-Dawley rats; 6 male/group;	gavage	0, 160, 480, 1600	Fert: 160	480; histopathologic changes in testes in one of three rats examined; 1600: ↓ testes weight with testicular atrophy.	Lake et al., 1978*
14-day study,	gavage	0, 480, 1600	Wistar:	480; testicular atrophy in one Sprague-Dawley rat;	Lake et al., 1978*

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
Wistar and Sprague-Dawley rats; 6/male/group;			Fert: 480 Sprague-Dawley: Fert: NE	1600; ↓ testes weight with testicular atrophy in all rats. Sprague-Dawley rats were more severe affected than Wistar rats.	
4-day study; Sprague-Dawley rats; 6/male/group;	gavage	0, 800, 1600	Fert: NE	800; ↓ testes weight and testicular atrophy.	Lake et al., 1978*
4-week study Sprague-Dawley rats; 5-10/sex/group	diet	0, 500, 1000, 1500 2000, 3000, 4000	Fert: 1000	1500; testicular atrophy; ↓ body weight	Hammond et al., 1987*
3 day uterotrophic assay Alkp:ApfSD 20-22 d rats; 6/group	gavage	0, 56, 280, 1120, 2240	Fert: 2240	NE; ↓ body weight at 2240	Monsanto, 1996*
3 day uterotrophic assay Alkp:ApfSD 20-22 d rats; 6/group.	sc	0, 0.5, 5, 50, 500, 5000	Fert: 5000	NE	Zacharewski et al., 1998*
4 days vaginal cell cornification OXV Sprague-Dawley rats; 10/group	gavage	0, 20, 200, 2000	Fert: 2000	NE; No increase in uterine weight	Zacharewski et al., 1998*
Developmental studies					
Single generation, two litters; Wistar rats; 12 M, 24 F	water	0, 0.2, 0.4, 0.8%	Mat: 0.4% Devp: 0.8% [418 (M), 446 (F)]	0.8 % Mat: ↓ body weight gain and food intake in dams, ↑ rel liver wt (female). NE; Devp: No effect	Monsanto, 1993*

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
GD 6-15; Swiss DC-1 mice; 27-30/group	diet	0, 0.1, 0.5, 1.25, 2% (0, 182, 910, 2330, 4121)	Mat: 182 Devp: 182	910; Mat: ↓ dam weight gain at 910; 910; Devp: ↑ prenatal mortality & malformations	NTP, 1990*
GD 6-15 Sprague-Dawley rats; 27-30/group	diet	0, 0.5, 1.25, 2% (0, 419, 1102, 1641)	Mat: 419 Devp: 419	1102; Mat ↓ dam weight gain. 1102; Devp: ↓ foetal weight gain, ↑ resorption & malformations.	NTP, 1989*
GD 5-15 or 5-20 Cpb-WU rats; 10-25/group	gavage	0, 270, 350, 450, 580, 750, 970, 1250, 1600, 2100	Mat: 450 Devp: NE	580; Mat: ↑ liver weight (GD 6-20) & 750 (GD 6-15). 270; Devp: ↓ relative testes weight (GD 6-20), ↓ foetal weight ≥ 350 (GD 6-20) and ≥ 450 (GD 6-15). Effects on testicular migration at 580 (more pronounced after long exp.)	Piersma et al., 1999
GD 0-20 Wistar rats;	diet	0,0.25,0.5,1,2% (0, 185, 375, 654, 974)	Mat: 375 Devp: 185	654; Mat: ↓ adjusted weight gain 375; Devp: ↓ number of foetuses per litter; ↓ adjusted weight gain in dams ≥ 654; ↓ body weight in foetuses 645; complete resorption at 974	Ema et al., 1990*
GD 7-15; Wistar rats;	gavage	0, 500, 750, 1000	Mat: 500 Devp: 500	750; Mat: ↓ food consumption at 500, ↓ food consumption & body wt gain, 750; Devp: complete resorption in some dams, ↓ foetal weight, ↑malformations, ↓ adjusted body weight gain, high maternal mortality, complete resorption in all dams at 1000.	Ema et al., 1992a*
GD 7-9, 10-12, 13-15 Wistar rats;	gavage	0, 600, 750, 1000	Mat: NR Devp: 600	750; Devp: ↓ foetal wt at 750 on GD 7-9 or 1000 on GD 10-12 ↑ number of totally resorbed litters at 1000 on GD 10-12 was; ↑ malformations at on GD 7-9 and 13-	Ema et al., 1993*

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
				15	
GD 0-8; Wistar rats; 10-14/groups	gavage	0, 250, 500, 750, 1000.	Mat: NE Devp: 250	250; Mat: ↓ maternal body weight gain 500; Devp: ↓ foetal wt ≥ 500; ↓ implantations ≥ 750	Ema et al., 1998*
GD 11,12 and 13 Wistar rats; 9-16/group	gavage	0, 250, 1000, 1500, 2000	Mat: 250 Devp: 250	500; Mat: NR 1000; Devp: ↓ foetal weight ≥1500, gross anomalies at 1500 and 2000; skeletal anomalies at 1000	Keen, 1998, Draft*
GD 14 - PND 3 Sprague-Dawley rats; 5/group	gavage	0, 750	Mat: NR Devp: NE	750; Devp: testes malformation (84%), ↓ anogenital distance (absolute & relative), ↓ testes, seminal vesicle, ventral prostate and epididymis weight at PND 2, and males with areolas at PND 13	Gray et al., 2000
GD 0-20; Wistar rats; pair-fed	diet	0, 974	Mat: NE Devp: NE	974; Mat: ↓ body weight, ↓ adjusted body weight gain in dams. Pair fed rats showed the same ↓ in body weight gain. 974; Devp: ↑ post-implantation loss.	Ema et al., 1991*
GD 0-20, 0-7, 7-16, 16-20; Wistar rats; pair-fed	diet	0, 974:	Mat: NE Devp: NE	NE; Mat: No effects in pair-feed pregnant rats. 974; Devp: Post-implantation loss was ↑ on GD 0-20, 0-7, 7-16. Teratogenicity was reported after GD-16-20.	Ema et al., 1992b*
GD 0-20, 0-11, 11-20; Wistar rats;	diet	0, 974	Mat: NE Devp: NE	974; Mat: ↓ body weight gain, ↓ adjusted body weight gain in dams in all groups 974; Devp: Complete resorption after GD 0-20, 0-11. Teratogenic effects after GD 11-20. No effects in pair-fed pregnant rats.	Ema et al., 1992c*
GD 7, 9 or 11;	diet	0, 974	Mat: 974	NE: Mat: no effects in pair-feed pregnant rats.	Ema et al., 1994*

Study design/ Species	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & Endpoint	References
Wistar rats; pair fed			Devp: NE	974; Devp: ↑ post-implantation loss in rats killed on GD 11. ↓ ovarian and uterine wts, plasma progesterone levels all days	
GD 14 to 18 Sprague-Dawley rats 6/group	gavage	0, 75 linuron, 500 BBP, combination.	Mat: 500 Devp: NE	Mat: no effect 500; Devp: ↓ testosterone production, neonatal AGD (M), ↑ infant areolae retention (M), ↑ reproductive organ malformations was increased (but not stat sig), ↓ reproductive organ weights (stat sig for caput and corpus epididymis only)	Hotchkiss et al., 2004
GD 8 OF1 mice; 22-24/group	Gavage	0, 0.9, 1.8, 3.6, 5.4 mmol/kg bw (0, 280, 560, 1120, 1690)	Mat: 560 Devp: 280	1120: maternal death, ↓ maternal body weight gain 560; Devp: ↑ resorptions, ↑ malformations, ↑ post-implantation losses; ↓ litter size at 1120 and 1690	Saillenfait et al., 2003
GD 10 Sprague-Dawley rats; 9-10/group	Gavage	0, 1.8, 3.6, 5.4 mmol/kg (0, 560, 1120, 1690)	Mat: 1120 Devp: 1120	1690: maternal death, 1690; Devp: ↑ malformations	Saillenfait et al., 2003

Mat: maternal; Fert: fertility; Devp: developmental; wt: weight; abs: absolute; rel: relative; NE: not established; PND: postnatal day; GD: gestational day; stat sig: statistically significant; M: male, F: female; NR: not reported

5. Hazard Characterisation

BBP is excreted predominately in urine after low oral doses and predominantly in faeces at high oral doses in rats. BBP is slowly absorbed dermally and is distributed to multiple organs.

BBP is metabolised initially to MBuP or MBzP by hepatic and intestinal mucosal cells. In adult and immature rats, the ratio of MBuP to MBzP in the urine is 3:1. There is no evidence of tissue accumulation. In contrast to rats, BBP is mainly metabolised to MBzP in humans. Available data suggest a half-life of BBP following oral exposure of less than 24 hours.

In experimental animals BBP exhibits low acute oral, dermal and intraperitonal toxicity. Data on toxicity following acute inhalation exposure are not available.

BBP produced minimal skin and eye irritation in animals. No data were available on respiratory irritation potential. No skin sensitisation was reported with BBP in two human patch tests and based on weight-of-evidence BBP is not a skin sensitiser.

Data from a well performed 3-month oral repeated dose dietary study in rats revealed a NOAEL of 151 mg/kg bw/d with a LOAEL of 381 mg/kg bw/d based on significant increases in relative kidney weight and histopathological changes in the pancreas and liver in males (Hammond et al., 1987*). In several studies, higher doses were associated with effects in testes, epididymis, prostate, liver, kidney, spleen and pancreas. A 90-day rat inhalation study established a NOAEC of 218 mg/m^3 and a LOAEC of 789 mg/m^3 based on increased kidney and liver weight in both male and females (Monsanto, 1982*). Several repeated dose studies showed peroxisome proliferation following BBP administration.

BBP was investigated in a variety of in vitro and in vivo genotoxicity studies. Overall, based on all data available and on a weight-of-evidence basis, BBP is considered to be non-genotoxic.

With regards to carcinogenicity, BBP showed cell transformation activity in 1 of 3 in vitro mammalian cell transformation assays. In vivo, BBP was tested for carcinogenicity in one oral administration study in mice and 3 studies in rats, including a dietary restriction study. In rats, a statistically significant increased incidence of MCL was reported in females at 12000 ppm (720 mg/kg bw/d) BBP (NTP, 1982a*). However, in later studies, no significant increase in MCL was seen with the same rat strain, despite a higher concentration of BBP tested. This tumour type is spontaneous and highly variable in aged Fischer 344 rats, is rare in other rat strains, has not been found in other mammalian species and has no comparable tumour in humans (Caldwell, 1999).

An increased incidence of pancreatic adenomas occurred in male rats in two studies (LOAEL of 500 mg/kg bw/d) but not after dietary restriction. Neoplasms of the urinary bladder were marginally increased in female rats both in a conventional study and after 2 year dietary restriction. After 32 months dietary restriction, a statistically significant increase in bladder carcinomas in these rats was found. This latter result is difficult to interpret in the absence of historic control data. In one rat study, BBP significantly inhibited DMBA-induced mammary carcinogenesis. However in a subsequent study, BBP did not significantly alter the incidence of DMBA-induced

prostate carcinogenesis. Overall, these data provide limited evidence of carcinogenicity in animals.

Extensive studies have been performed to study reproduction, fertility and developmental toxicity of BBP in laboratory animals. Human data are insufficient to draw any conclusions.

In animals, effects of BBP on fertility or reproductive organs in rats following oral or inhalation exposure included reduced mating and fertility indices, decreases in testes weight, histopathological changes in testes and hormonal changes. These effects occurred, in the majority of studies (conducted in rats), at BBP doses equal to or greater than doses that induced systemic toxicity (increased liver and kidney weight with histopathological changes). A NOAEL for fertility effects derived from a well-conducted two-generation reproduction study was 200 mg/kg bw/d and the LOAEL was 400 mg/kg bw/d based on increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells in F1 (Aso et al., 2005).

In vivo developmental toxicity studies in rats and mice indicate an anti-androgen type activity for BBP (Piersma et al., 1999; Gray et al., 2000; Parks et al., 1999*; Nagao et al., 2000; Tyl et al., 2004). Effects include reduced testicular weight, reduced anogenital distance, and retarded transabdominal descent of testes in male offspring exposed to BBP during the organogenic period and/or the late prenatal early postnatal period. The incidence of these effects was dose and developmental age dependent. The mechanism of action for resorption has been proposed as reduced circulating progesterone.

Developmental toxicity has been reported in rats and mice exposed in utero to BBP in the absence of marked maternal toxicity. In a two-generation study in rats (Tyl et al., 2004), the NOAEL of 50 mg/kg bw/d for developmental effects was determined based on a statistically significant dose-related reduction in AGD in both F1 and F2 offsprings from 250 mg/kg bw/d in the absence of maternal toxicity. A LOAEL of 100 mg/kg bw/d was determined from Aso et al. (2005) for developmental effects based on decreased body weight and AGD.

5. Human Health Hazard Summary Table

Phthalate	Acute Toxicity	Irritation & Sensitisation	Repeated Dose Toxicity	Genetic Toxicity	Carcinogenicity	Fertility	Developmental Toxicity
Butylbenzyl phthalate (BBP)	Oral Rat: LD50 = 2330-20400 mg/kg bw Dermal Rat: LD50 = 6700 mg/kg bw Inhalation No data	Skin Irritation: minimal effect Eye Irritation: minimal effect Sensitisation: negative	Oral Rat: NOAEL = 151 mg/kg bw/d (m) LOAEL = 381 mg/kg bw/d (m): significantlly ↑ in relative kidney weight, histopathological changes in pancreas and gross pathological changes in liver. Inhalation Rat: NOAEC = 218 mg/m^3 LOAEC = 789 mg/m^3: ↑ liver and kidney weights High doses: testes, epididymis, prostate, liver, kidney, spleen and pancreas effects. PP noted.	*In vitro* Negative in bacterial and mammalian mutation tests Equivocal in sister chromatid and chromosome aberration assays *In vivo* Negative in sex-linked recessive lethal and dominant lethal mutation assays. Negative in a micronuclei test Equivocal in sister chromatid and chromosome aberration assays	*In vitro* Positive in 1 of 3 cell transformation assays. *In vivo* Rat (F344): NOAEL = 360 mg/kg bw/d. LOAEL = 720 mg/kg bw/d: ↑ MCL (f only, not subsequently corroborated); ↑ pancreatic tumours (not with diet restriction). Rat (F344/N): NOAEL = 240 mg/kg bw/d. LOAEL = 500 mg/kg bw/d: ↑ pancreatic cell adenoma and adenoma/carcinoma (combined) (not with diet restriction); Mice (B6C3F1): no effects.	Rat: NOAEL = 200 mg/kg bw/d LOAEL = 400 mg/kg bw/d: atrophy of testes, seminal vesicle and epididymis	two generation study Rat: NOAEL = 50 mg/kg bw/d LOAEL = 100 mg/kg bw/d: ↓ body weight and AGD

PP: peroxisome proliferation; m: male; f: female; ↑: increase; ↓: decrease; AGD: anogenital distance; MCL: mononuclear cell leukaemia.

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Appendix - Robust Study Summaries

Test Substance	**:**	BBP
Type of Test	**:**	Two generation reproductive toxicity
Species	**:**	Rats, Crj:CD(SD)IGS , 24/M/F, 5 weeks, weight unknown. Asutagi breeding centre.
Route of admin.	**:**	Gavage
Study Duration	**:**	10 weeks prior to mating to weaning (females) or delivery (males)
Frequency of treatment	**:**	Daily
Post exposure period	**:**	None
Doses	**:**	0, 100, 200, 400 mg/kg bw/d
Control group	**:**	Olive oil
NOAEL / NOEL	**:**	Repro: 200 mg/kg bw/d Devp: NE mg/kg bw/d.
LOAEL / LOEL	**:**	Repro: 400 mg/kg bw/d Devp: 100 mg/kg bw/d
GLP& QA	**:**	yes
Guidelines	**:**	OECD guideline No. 416
Method	**:**	Rats were fed diet for 10 weeks prior to mating to necroscopy (end of mating period: males; mating, gestation and lactation: females). Administration to F1 started at weaning and continued to necroscopy (as per F0). Sperm motility was tested in F0 and F1 males. Hormone measurement (testosterone, FSH, oestradiol, LH) of 6 male and female F0 and F1 parents were taken at necroscopy. Organ weights were measured at necroscopy & histopathology performed on F0 and F1 parents. Body weight, anogenital distance (on PND 4) and presence of areolae (PND 12-14) were taken.
Result	**:**	In the F0 adults, there was no effect on body weight or body weight gain. Relative liver weight was increased in high dose males and mid- and high dose females. Relative kidney weight was increased in high dose males and females. In the F1 adults, there was no effect on body weight. Relative liver weight was increased in high dose females and mid- and high dose males. Relative kidney weight was increased in high dose males and females. In F0 adults, there was no effect on testes weight but there were decreased spermatozoa in the epididymis and

hyperplasia of Leydig cells. There was no effect on reproductive endpoints such as mating, number of implantations, number of sperm in testes and epididymis, sperm motility, serum FSH, LH, testosterone or oestradiol. In F1 adults, absolute epididymis and seminal vesicle (but not testes) weight was decreased in high dose males. There was also an increased frequency of small testes, diffuse atrophy of seminiferous tubules and hyperplasia of Leydig cells.

Foetal body weight was significantly reduced in F1 males & F2 males and females at 100 mg/kg bw/d. Anogenital distance was increased in F1 females exposed to 100 mg/kg bw/d (not dose-related) and decreased in F2 males.

Conclusion : BBP is a developmental and reproductive toxicant.

Reliability : 1

Reference : Aso S, Ehara H, Miyata K, Hosyuyama S, Shiraishi K, Umano T & Minobe Y (2005) A two-generation reproductive study of butyl benzyl phthalate in rats. J Tox Sc 50: 39-58.