National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR EXPERT PANEL REPORT

on

BUTYL BENZYL PHTHALATE

OCTOBER, 2000 **NTP-CERHR-BBP-00**

PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June, 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

The following seven phthalate esters were selected for the initial evaluation by the Center: butyl benzyl phthalate, di(2-ethylhexyl) phthalate, di-isodecyl phthalate, di-isononyl phthalate, di-n-butyl phthalate, di-n-hexyl phthalate, and di-n-octyl phthalate. Phthalate esters are used as plasticizers in a wide range of polyvinyl chloride-based consumer products. These chemicals were selected for the initial evaluation by the CERHR based on their high production volume, extent of human exposures, use in children's products, published evidence of reproductive or developmental toxicity, and public concern.

This evaluation is the result of three public Expert Panel meetings and 15 months of deliberations by a 16-member panel of experts made up of government and non-government scientists. This report has been reviewed by the CERHR Core Committee made up of representatives of NTP-participating agencies, by CERHR staff scientists, and by members of the Phthalates Expert Panel. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that a given exposure or exposure circumstance may pose a hazard to reproduction and the health and welfare of children; (2) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/development health effects are associated with exposure to specific chemicals or classes of chemicals, including descriptions of any uncertainties that would diminish confidence in assessment of risks; and (3) identify knowledge gaps to help establish research and testing priorities.

The Expert Panel Reports on phthalates will be a central part of the subsequent NTP report that will also include public comments on the Panel Reports and any relevant information that has become available since completion of the Expert Panel Reports. The NTP report will be transmitted to the appropriate Federal and State Agencies, the public, and the scientific community.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.
Reports can be obtained from the website (http://cerhr.niehs.nih.gov**) or from:**

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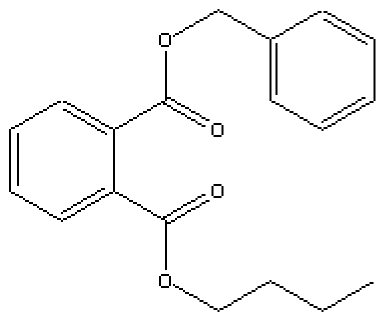
Butyl Benzyl Phthalate

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of Butyl Benzyl Phthalate



Butyl benzyl phthalate (BBP) (CAS 85-68-7) is produced by sequentially reacting butanol and benzyl chloride with phthalic anhydride (*1*).

Table 1: Physicochemical Properties of BBP

Property	**Value**
Chemical Formula	$C_{19}H_{20}O_4$
Molecular Weight	312.35
Vapor Pressure	6×10^{-7} mmHg at 25 ^{o}C
Melting Point	-40.5 ^{o}C
Boiling Point	370 ^{o}C
Specific Gravity	1.12
Solubility in Water	slight – 2.7 mg/L
Log K_{ow}	4.59

(*1*)

1.2 Exposure and Usage

According to the American Chemistry Council (ACC, formerly CMA) (*1*), the largest use of BBP is in vinyl tile. BBP is also a plasticizer in PVC used to manufacture food conveyor belts, carpet tile, artificial leather, tarps, automotive trim, weather stripping, traffic cones, and is used to a limited extent in vinyl gloves. BBP is also used in some adhesives. BBP may be released to the environment during its production and also during incorporation into plastics or adhesives. Because BBP is not bound to the final product, it can be released during the use or disposal of the product. Phthalates that are released to the environment can be deposited on or taken up by crops that are intended for human or livestock consumption, and thus, can enter the food supply.

General Population Exposure

General population exposure to BBP through food has been estimated by at least two authoritative sources: the International Program on Chemical Safety (IPCS) (*2*) and the UK Ministry of Agriculture, Fisheries, and Food (MAFF) (*3-5*).

BBP may enter food by environmental uptake during crop cultivation or by migration from processing equipment or packaging materials. IPCS (*2*) concluded that BBP exposure to the general population is based almost entirely on food intake; these food exposure estimates were based on a survey of 100 food items that were purchased in four Ontario, Canada supermarkets between 1985 and 1988. BBP was only found in yogurt (0.6 µg/g), cheddar cheese (1.6 µg/g), butter (0.64 µg/g), and crackers (0.48 µg/g). Assumptions used to estimate exposure included a 70 kg body weight, and a daily consumption of 13.61 g butter, 3.81 g cheddar cheese, 1.54 g yogurt, 22.73 g pork, and 3.45 g crackers. Adult BBP intake was estimated at 2 µg/kg bw/day and it was stated that exposure to infants and children could be up to three-fold higher.

MAFF (*5*) estimated adult BBP exposure through dietary intake based on a 1993 survey of fatty foods in the United Kingdom. BBP was detected in carcass meat (0.09 µg/g), poultry (0.03 µg/g), eggs (0.09 µg/g), and milk (0.002 µg/g). In calculating dietary food exposures, MAFF assumed that these types of food likely account for 85% of dietary phthalate intake. Food intake levels were obtained from the Dietary and Nutritional Study of British Adults, but the values were not reported by MAFF. Mean and high-level BBP intakes were estimated at 8 µg/person/day and 20 µg/person/day, respectively. Specific details describing the calculations and assumptions used were not provided. Using the IPCS-assumed adult body weight of 70 kg (*2*), the exposure values were converted to 0.11–0.29 µg/kg bw/day.

MAFF also addressed BBP exposure in infants resulting from the consumption of infant formula. A survey published in 1996 reported BBP levels of <0.0044–0.24 µg/g in infant formulas purchased in the UK, while a later survey reported BBP levels of <0.003–0.015 µg/g (*3, 4*). It is speculated that the drop in BBP concentration occurred because infant formula manufacturers were urged to reduce phthalate levels after the MAFF published the results of the 1996 survey (*3*). Based on the results from the 1998 survey and using an assumed body weight of 2.5–3.5 kg at birth and 7.5 kg at 6 months of age, exposure levels were estimated for infants. Formula intake rates were determined from manufacturer instructions. Exposure levels for infants were estimated at 0.2 µg/kg bw/day at birth and 0.1 µg/kg bw/day at 6 months of age. Infants in the United States are likely exposed to lower levels of BBP through formula. In a survey of infant formulas conducted in 1996, BBP levels were below the detection limit of 0.005 µg/g (*6*).

BBP was only detected in one sample (2.8 µg/L) collected in 1991 in a survey of 300 drinking water sites in two Canadian provinces from 1985 to 1994. IPCS (*2*) considered exposure to BBP through drinking water negligible; exposure through soil intake was also considered negligible.

Mouthing of toys and other BBP-containing objects is a potential source of oral phthalate exposure in children. However, BBP is stated not to be used in toys (*7*). In an analysis of 17 plastic toys, BBP was only detected in a PVC doll's head at 0.02% by weight (*8*), a level that suggests contamination rather than planned use.

Off-gassing from building materials has been reported as a potential source of BBP exposure through inhalation; however, exposure has been postulated to be minimal because of BBP's low vapor pressure. The available data, though minimal, support this view. IPCS (*2*) reported that median air levels of 0.034–0.035 ng/m^3 were measured in a survey of 125 California homes. BBP levels in outdoor air were also measured for 65 of these homes and the median BBP level was below the detection limit of 0.051 ng/m^3. The 90^{th} percentile levels of BBP in outdoor air ranged from 5.3 to 6.7 ng/m^3 for daytime to evening. IPCS (*2*) considered BBP exposure through inhalation to be negligible. Pfordt and Bruns-Weller (*9*) measured BBP levels in 3 flooring samples and found BBP in each sample at levels ranging from 10–250 µg/g.

Dermal contact with products containing BBP is possible, but absorption through skin is most likely minimal. Studies in rats have demonstrated that absorption of BBP through skin is fairly slow (approximately 27% in 7 days) (*10*). An *in vitro* study conducted with rat and human skin has demonstrated that permeability of human skin to other phthalates (DBP and DEHP) is much lower than that of rat skin (*11*).

Interpretation of exposure levels for the general population requires caution. The exposure estimates by IPCS and MAFF differed by approximately one order of magnitude. The basis for discrepancies in dietary exposure estimates is difficult to determine for several reasons, including: use of different food types in calculations (e.g., fatty foods vs. a variety of foods); use of different assumptions in calculations; varying BBP levels in foods from different countries; and changing BBP levels in food over time. Dietary intake can vary widely depending on the types of foods eaten and the types of materials in which the foods are packaged. It is noted that the food levels reported by MAFF were collected 12–15 years ago and may not reflect current exposure levels.

<u>Medical Exposure</u>

BBP is not approved by the U.S. Food and Drug Administration for use in medical devices.

<u>Occupational Exposure</u>

Exposure in occupational settings can occur through skin contact and by inhalation of vapors and dusts.

Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tank cars (*1*). Higher exposures to phthalates can occur during the incorporation of the phthalate into the final product if the process is run at a higher temperature than is used in the manufacturing process. The ACC has estimated exposure to BBP in the workplace based upon an assumed level of 1 mg/m^3 during the production of phthalates and 2 mg/m^3 during the manufacture of flexible PVC. An exposure level was estimated by using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 µg/kg bw/workday and 286 µg/kg bw/workday for workers employed in phthalate manufacturing and flexible PVC production operations, respectively. As stated in the General Exposure section, absorption of BBP through skin is expected to be minimal.

The summary for Section 1 is located in Section 5.1.1.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

BBP was not observed to be a primary irritant or sensitizer in skin patch tests with volunteers (*2*). There are no human data on the general toxicity of BBP alone. Occupational exposures to phthalate mixtures containing BBP have been associated in single studies with respiratory/neurological effects and cancer (*2*). In a large, population-based case-control study (*12*), a significant increase in the risk of multiple myeloma has been found among workers employed for 5 or more years in PVC production. In the general population, a significant increase in the risk of bronchial obstruction during the first 2 years of life has been related to presence of PVC flooring (adjusted O.R>=1.89) in a case control study of 251 children and an equal number of matched controls (*13*). The consequences of exposure to children have not been studied.

2.1.2 Experimental Animal Data

Multiple studies in mice and rats are available describing the acute, sub-chronic, and chronic toxicity of BBP. These studies assess oral as well as inhalation routes of exposure. There is a 90-day dietary toxicity study in dogs that includes effects that are possibly related to decreased food consumption.

<u>Acute Studies</u>
Acute toxicity of BBP is low; an oral LD_{50} value for BBP in rats is reported as 2–20 g/kg (*2*). Rabbit dermal and ocular studies revealed no significant concern for BBP-induced sensitization or irritation (*14*).

<u>Sub-chronic Studies</u>
Agarwal et al. (*15*) published a study that explored previous NTP results indicating effects on male fertility and the hematopoietic system (Web Table 1). Adult male F344 rats, 10 per group, were fed diets containing 0, 0.625, 1.25, 2.5, or 5.0% BBP for 14 days. Using actual pre-treatment body weights (200 g) and reported food intake during the 14-day dosing period, equivalent doses of 0, 447, 890, and 1,338 mg/kg bw/day were calculated for the 3 lower dose groups. Since the high-dose group actually lost weight during the study, average weight during the study was used to calculate a dose of 1,542 mg/kg bw/day. All treated rats showed a dose-related increase in relative liver and kidney weights. No histopathology or hematology changes were observed at the 447 or 890 mg/kg bw/day dose levels. However, at doses of 1,338 and 1,542 mg/kg bw/day, relative decreases in testes, seminal vesicle, and thymus weight were noted; relative epididymis 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 two highest doses tested. An increase in follicle stimulating hormone (FSH) was observed in the two highest doses, and a decrease in testosterone was observed at the high dose. The decreased body weight seen at the two highest doses may be due to unpalatability of food; decreased food intake was documented. The severity of the reduced food intake and attendant weight loss precludes associating effects with BBP, or BBP and inanition, at the high dose. The systemic LOAEL determined from these studies is 447 mg/kg bw/day based on increases in organ weight (liver, kidney) and increased LH levels.

Three-month feeding studies were conducted in 4–6 week-old Wistar and Sprague-Dawley (SD) rats fed diets with 2,500–12,000 or 2,500–20,000 ppm BBP, respectively (*14*) (Web Table 2). Male Wistar rats (27–45 rats/sex/group) received doses of 151, 381, or 960 mg/kg bw/day; female doses were 171, 422, or 1,069 mg/kg bw/day. At the low dose, an increase in liver to body weight ratio was seen in both sexes. No histopathology or hematology changes were noted. At the mid-dose, a decrease in body weight was noted in both sexes and increases in liver and kidney to body weight ratios were seen. Pancreatic tissues showed islet cell enlargement, vacuolization, congestion, inflammation, and minor fibrosis. Less frequently, additional pancreatic changes were observed, such as acinar cell atrophy, inflammation, and pyknotic nuclei. A decrease was observed in urinary pH in male rats only. At the highest doses tested, 960 (M) and 1,069 (F) mg/kg bw/day, hepatic necrosis and anemia were observed in addition to the effects seen at lower doses. Cecal enlargement, a finding of uncertain toxicological importance, was reported in this study. The LOAEL for this study was 151–171 mg/kg bw/day based on weight change in the liver.

In this same study, Sprague-Dawley (SD) rats (10/sex/group) were tested at doses of 0, 188, 375, 750, 1,125, or 1,500 mg/kg bw/day. Sprague-Dawley rats were less sensitive to BBP than were Wistar rats, as no pancreatic, hepatic, or testicular lesions, or cecal enlargement were observed. There were no changes in urinary pH or hematological parameters. The NOAEL was set at 375 mg/kg bw/day and the LOAEL at 750 mg/kg bw/day based on increases in organ weight ratios for kidney (male) and liver (female) (*14*).

A 13-week inhalation study was also conducted in groups of 6–8 week-old SD rats (25/sex/group) (*14*) (Web Table 2). The rats were exposed to BBP mists (>90% of aerosol particles <10 µm) at concentrations of 51, 218, or 789 mg/m^3 for 6 hours/day, 5 days/week. Using EPA (*16*) assumptions for rat body weights and daily inhalation rates, estimated exposure doses were 9.2, 39.4, and 143 mg/kg bw/day for males and 9.8, 42.0, and 152 mg/kg bw/day for females. NOAELs of 39.4 (M) and 42.0 (F) mg/kg bw/day were identified in this study. A LOAEL was determined at the highest doses tested, 143 (M) and 152 (F) mg/kg bw/day; this LOAEL was based on increases in liver and kidney organ to body weight changes. Serum glucose levels were also reduced at this dose in male rats only. No body weight changes or histopathological changes were observed.

The NTP (*17*) reported results of a 26-week dietary exposure study in 6-week-old F344/N male rats (Web Table 3). Groups of 15 male rats were fed BBP in the diet at concentrations of 0, 300, 900, 2,800, 8,300, or 25,000 ppm for 26 weeks. The authors calculated doses of 30, 60, 180, and 550 mg/kg bw/day for the 4 lowest exposure levels. A dose was not calculated in the highest exposure group because food intake could not be measured due to an excess scattering of feed. However, a dose of 1,650 mg/kg bw/day was estimated by CERHR based on intake levels observed in the lower dose groups. In the high-dose group, decreases in total body weight (due to decreased food intake) were observed, as were increases in relative liver and kidney weights. An increased incidence of macrocytic anemia was observed on days 30–180. The testis was determined the primary target organ based on weight, sperm concentration, and histopathological findings at the high dose. Decreases in relative testis, absolute epididymis, and absolute seminal vesicle weight were observed, as were atrophy of seminiferous tubules and degenerative changes in testis and epididymis. No histologic changes in other body tissues were seen at this dose. The testis from animals in the lower dose groups were examined histologically and no effects were observed; lowered sperm counts were not seen at the 60, 180, or 550 mg/kg bw/day doses. Absolute and relative liver weight was increased at 550 mg/kg bw/day. A NOAEL was established at 180 mg/kg bw/day[1]. The LOAEL of 550 mg/kg bw/day reflects increases in mean cell hemoglobin after 60–180 days of treatment that may be associated with the macrocytic anemia observed at the next higher dose.

In a 3-month feeding study, 3 adult male and female beagle dogs/group were fed diets with 10,000–50,000 ppm BBP (males: 400, 1,000, or 1,852 mg/kg bw/day; females: 700, 1,270, or 1,973 mg/kg bw/day, as calculated by study authors) (*14*). Food palatability complicated interpretation of reduced body weights in low- and high-dose males and mid- and high-dose females. No other changes were observed for hematological or urinalysis measurements. In high-dose animals there were no histopathological effects in liver, testes, or pancreas.

<u>Chronic Exposure Studies</u>
Two sets of chronic feeding studies have been performed by the NTP (*17, 18*).

Potential BBP carcinogenicity was examined in both B6C3F$_1$ mice and F344/N rats (*18*). Four-to-five week-old B6C3F$_1$ mice (50/sex/group) were dosed through feed at concentrations of 0, 6,000, or

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[1] The NTP (*17*) report stated that epididymal sperm concentration was determined for the lowest and two highest of the treated groups. CMA reports that an audit revealed the original laboratory report, that is the data source for the NTP Report, states that epididymal sperm counts were determined from the three highest dose groups. The data from the original laboratory report are used in this evaluation.

12,000 ppm for 106 weeks. Using EPA assumptions for $B6C3F_1$ mouse body weight and food intake (body weight: 0.03733 kg [M], 0.0353 kg [F]; food intake: 0.0064 kg/day [M], 0.0061 kg/day [F]), dose levels of 0, 1,029, and 2,058 mg/kg bw/day and 0, 1037, and 2,074 mg/kg bw/day were calculated for males and females, respectively. No treatment-related changes in survival or neoplastic developments were seen. Dose-related decreases in body weight were seen in both male and female mice. There were no lesions observed in male or female reproductive organs.

F344/N rats (50/sex/dose) were fed diets containing 0, 6,000, or 12,000 ppm BBP (*18*) for 106 weeks. Using EPA assumptions for F344 rat body weight and food intake, respectively (M:0.380 kg, 0.030 kg/day; F:0.229 kg, 0.021 kg/day), dose levels of 0, 474, and 948 mg/kg bw/day and 0, 550, and 1,100 mg/kg bw/day were estimated for males and females, respectively. Male rats were sacrificed 29–30 weeks into the study because of increases in premature death. Internal hemorrhaging was suspected as the cause of these deaths. Body weight gain and food intake were decreased in both males and females. The female rats were allowed to continue through the 106 weeks of exposure; at necropsy the females exhibited an increased incidence of mononuclear cell leukemia (MNCL). Spleens were examined in the high-dose group and were found to be congested and infiltrated with mononuclear cells. MNCL has been associated with splenomegaly and sometimes hepatomegaly. No evidence of hepatomegaly was reported in these studies.

In another 2-year NTP bioassay (*17*) groups of 60 male Fischer 344/N rats (6 weeks old) were fed BBP in the diet at concentrations of 0, 3,000, 6,000, or 12,000 ppm (0, 120, 240, or 500 mg/kg bw/day) and 60 females (6 weeks old) per group were fed concentrations of 0, 6,000, 12,000, or 24,000 ppm (0, 300, 600, or 1,200 mg/kg bw/day) (Web Table 4) (*17*). After 2 years of exposure to BBP, increases in relative kidney weights were observed in male rats at 120 mg/kg bw/day and represented the lowest observable changes in this study (*17*). Additional dose-related increases included relative epididymis weights at the 240 mg/kg bw/day dose and relative liver weight at the 500 mg/kg bw/day dose in male rats, with total body weight changes in rats occurring only at the highest dose tested, 500 mg/kg bw/day. At the highest dose level, histopathological changes included renal tubule pigmentation, hepatic granulomas, and focal pancreatic hyperplasia with "some evidence" of pancreatic carcinogenicity based on increased incidence of acinar cell adenoma and adenoma or carcinoma (combined). No testicular changes were observed; however, decreases in red blood cells (RBC) and increases in hemoglobin were observed 6 months into the study.

Female F344/N rats exposed to BBP for 2 years showed nephropathy at the 2 lowest doses tested (300 and 600 mg/kg bw/day). At 1,200 mg/kg bw/day, the animals exhibited decreases in body weight and increases in liver and kidney organ to body weight ratios. They also exhibited renal tubule pigmentation (15–24 months), nephropathy, microcytic anemia (15 months), decreases in triiodothyronine, and "equivocal evidence of carcinogenicity" based on pancreatic acinar cell adenoma and urinary bladder transitional cell epithelial papilloma. Pancreatic effects may have been due to chronic stimulation of pancreatic lipase secretion.

In a parallel study at the same laboratory, BBP's ability to induce hepatic peroxisomes was evaluated in female F344/N rats (*17*). Two enzyme markers for peroxisome proliferation, palmitoyl CoA oxidase and carnitine acetyl transferase, were significantly elevated after 1 month and 1 year of exposure in animals exposed to 6,000 ppm BBP and higher (~300 mg/kg bw/day), although the level of induction was lower than that observed after a 3-week exposure to DEHP. The discussion in the NTP report highlights the fact that BBP is a mild peroxisome proliferator compared to DEHP or to hypolipidemic drugs such as clofibrate.

From these 2-year studies, LOAELs for non-cancer, general toxicity effects were determined at 120 (M) and 300 (F) mg/kg bw/day based on kidney organ weight changes in the male and nephropathy in the females. At 500 (M) and 1,200 mg/kg bw/day (F), the highest doses tested, respectively, "some to equivocal evidence" of pancreatic (male and female) and urinary bladder carcinogenicity (female) was observed in rats. No testicular changes were observed at any of the doses tested; however, increases in epididymal weight were seen at the 2 highest doses (240 and 500 mg/kg bw/day). This change in epididymal weight was observed in the absence of total body weight change at the 240 mg/kg bw/day exposure dose.

2.2 Toxicokinetics

Phthalate Moiety

Absorption

Dermal
In a study of dermal absorption of a series of phthalate diesters (*10*), ^{14}C-BBP (157 μmol/kg) was applied to the skin (clipped back) of male F344 rats and the area covered with a perforated cap. Absorption was estimated by the radioactivity eliminated in urine and feces over 7 days, which equaled 27% for BBP. Most of the remainder of the radioactivity was found at the site of application.

Oral
Oral administration of 5 g of BBP/kg to dogs resulted in 10% absorption (*19*). Administration of single oral doses of 2, 20, 200, or 2,000 mg/kg to male Fischer 344 rats showed a dose-dependent increase in the fraction of dose eliminated via the feces (20% at doses from 2–200 mg/kg; 72% at 2,000 mg/kg) and a dose-dependent decrease in the fraction eliminated via the urine (75% at a dose of 2–200 mg/kg and 22% at 2,000 mg/kg), suggesting that absorption through the gut was limited at the high dose (*20*).

Inhalation
There are no reports of the absorption of BBP administered by inhalation. By analogy with other phthalates, di(2-ethylhexyl)phthalate and diisodecylphthalate, BBP would be expected to be absorbed from the lung as the parent compound (*21, 22*).

Biotransformation
Oral studies in rats indicate that BBP is rapidly metabolized by gut enterases to its monoester metabolites (monobutyl and monobenzyl phthalates), which are absorbed and are either excreted in urine as the ester or conjugated with glucuronic acid and then excreted via the urine as the glucuronate (*19, 20, 23*). Urinary metabolites in rats following oral administration of 3.6 mmol BBP/kg/day (1,125 mg/kg bw/day) for 3 days indicated that 70% of the metabolites were monoesters while the remainder were monoester conjugates. The monobutyl ester is generally present in the highest amount; in one study, the ratio of monobutyl to monobenzyl phthalate was 5:3 (*23*). The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats).

BBP and dibutyl phthalate (DBP) share a common metabolite, monobutyl phthalate (MBuP); information from DBP germane to the monoester, and therefore also to BBP, will be presented throughout this evaluation. In addition to the monoesters, the esterase cleavage products, phenol (from the benzyl moiety) and butanol (from the butyl moiety), will be included.

Distribution

Tissue distribution was non-specific for the small amount of dermally absorbed BBP (*10*).

Excretion

Excretion of absorbed BBP and its metabolites is rapid, with approximately 90% eliminated in 24 hours, approximately 80% in urine and 20% in feces, at low doses (2–200 mg/kg). The half-life of BBP in blood is 10 minutes. The blood half-life of the monoester metabolites of BBP is approximately 6 hours (*20*). Following intravenous (IV) administration of 20 mg/kg of ^{14}C-BBP, 55% of the dose was excreted into bile while 34% was excreted in the urine (*20*).

Side Chain-associated Toxicokinetics

Phenol and butanol are products of hydrolysis of the monoesters. Phenol metabolism to polyphenols is well known: butanol is a primary alcohol that is easily oxidized to butyric acid (n-butanoic acid) by alcohol dehydrogenase and aldehyde dehydrogenase. Further metabolism (by α-oxidation pathways) converts butyric acid into acetyl-CoA conjugates in intermediary metabolism pathways with no toxicological importance (*24*).

2.3 Genetic Toxicity

The NTP (*17*) reviewed the genetic toxicity of BBP. An increase in mutations was not observed following treatment of *Salmonella* and L5178Y mouse lymphoma cells with BBP in the presence or absence of S9 activation. BBP treatment with and without S9 activation did not result in sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. However, induction of sister chromatid exchanges and increased chromosomal aberrations in bone marrow cells were observed following a single intraperitoneal (IP) injection of mice with 1,250–5,000 mg/kg bw BBP. There were no increases in sex-linked recessive lethal mutations in the germ cells of *Drosophila* fed or injected with BBP.

Subsequent to the NTP review, BBP tested negative in the L5178Y mouse lymphoma mutation assay with and without activation, and in the Balb/3t3 cell transformation assay (*25*). Ashby et al. (*26*) reported negative results in a micronucleus assay in rats. The IPCS (*2*) review included the publication of Ashby et al. and concluded: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

The summary for Section 2, including general toxicity, toxicokinetics, and genetic toxicity, is located in Section 5.1.2.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

There were no human data located for Expert Panel review.

3.2 Experimental Animal Toxicity

Eleven complete studies and two abstracts were evaluated. Two studies performed through the NTP, were standard prenatal assessment (segment II) studies of BBP administered in the diet of rats and mice. A third was an oral gavage Segment II study in rabbits. There were five studies by Ema et al. in Wistar rats where BBP was administered in the diet or by gavage. Three studies of BBP evaluated drinking water exposure to Wistar rats during gestation and lactation with assessment of adult F_1 males. One abstract evaluated BBP exposure by subcutaneous injection to two strains of male mice ($B6C3F_1$ and CD-1) with subsequent mating to unexposed females (dominant lethal assessment).

3.2.1 Prenatal Development

A dietary study in CD (Sprague-Dawley) rats (*27*) involved exposure of 30 pregnant rats per group to 0, 0.5, 1.25, and 2.0% BBP (0, 420, 1,100, and 1,640 mg/kg bw/day) on gestation day (gd) 6–15. The dams were killed on gd 20, necropsied, and pups examined and evaluated (Web Table 5). Maternal toxicity was expressed in reduced body weights and decreased weight gain, decreased absolute feed consumption (but increased relative feed consumption in g/kg/day), increased relative liver weight (with no histopathological changes), and increased relative water intake at the 1,100 and 1,640 mg/kg bw/day doses. Relative kidney weights were increased at the 1,640 mg/kg bw/day dose. However, the kidneys were not examined histologically. Clinical signs of maternal toxicity, including ataxia and abnormal gait, were also observed at this dose.

At 1,640 mg/kg bw/day, there were increased resorptions and concomitant reduced numbers of live fetuses per litter, reduced fetal body weight, and increased fetal malformations. Urogenital malformations, analyzed separately, were increased; they included distended ureters and distended or absent kidneys. Other fetal malformations at the high dose were anophthalmia (missing eyes), fused or malaligned vertebrae, and fused ribs. There were increased incidences of fetal variations per litter at both the 1,100 and 1,640 mg/kg bw/day doses.

Significant developmental toxicity occurred at the 1,100 and 1,640 mg/kg bw/day doses; teratogenicity was observed at 1,640 mg/kg bw/day. Maternal toxicity was observed at doses that caused developmental toxicity. The maternal and developmental NOAELs were identified at 420 mg/kg bw/day.

Ema et al. (*28*) exposed pregnant Wistar rats, 15–18/group, to BBP in the diet at 0, 0.25, 0.5, 1.0, and 2.0% (intakes of 0, 185, 375, 654, and 974 mg/kg bw/day, respectively) on gd 0–20. Dams were killed on gd 20 and evaluated in a Segment II study design (Web Table 6). There were also pair-fed controls matched with the animals in the highest dose group. No dams died in any group. Adjusted maternal body weight gains (not including gravid uterus weight) and feed consumption were reduced at doses of 654 and 974 mg/kg bw/day. All dams at 974 mg/kg bw/day had fully resorbed litters. There was no treatment-related pre-implantation loss or teratogenicity. The authors concluded that the maternal NOEL was 375 mg/kg bw/day and the developmental toxicity NOEL was 654 mg/kg bw/day. The Expert Panel did not agree with the author's identification of developmental effect levels given that live litter size was reduced at 375 mg/kg bw/day (11.3 vs. control value of 13.9) and 654 mg/kg bw/day (12.3 vs. control value of 13.9); fetal body weights (by sex per litter) were significantly reduced at 654 mg/kg bw/day. The data did support a developmental NOAEL of 185 mg/kg bw/day.

In a second Segment II study, Ema et al. (*29*) treated 10 Wistar rats/group with BBP by gavage with 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–15 (Web Table 7). Dams and fetuses were evaluated following sacrifice on gd 20. Maternal body weight gains were reduced at doses of 750 and 1,000 mg/kg bw/day, but the corrected weight gain (maternal body weight excluding the gravid uterus) was decreased only at the high dose. Food intake was reduced at all dose levels. Four dams in the high-dose group died and entire litters were resorbed in the six surviving dams. Complete litter resorptions were observed in 3/10 dams in the 750 mg/kg bw/day group. Other effects at that dose included increased fetal death due to postimplantation loss, reduced fetal weight, and increased external, skeletal, and internal malformations. The malformations consisted primarily of cleft palate, fused sternebrae, and dilated renal pelves. The maternal and fetal NOAEL was identified as 500 mg/kg bw/day.

The Segment II dietary study in CD-1 mice (*30*) involved exposure of 30 pregnant mice per group to 0, 0.1, 0.5, and 1.25% BBP (0, 182, 910, and 2,330 mg/kg bw/day), on gd 6–15 (Web Table 8). Maternal toxicity was expressed as reduced weight gain at the two highest doses (910 and 2,330 mg/kg bw/day), and increased relative liver and kidney weights and increased relative water intake at the high dose. No histopathological changes were observed in the liver or kidneys.

Embryofetal effects included increased incidences of resorptions and late fetal deaths, with concomitant reductions in live fetuses per litter, and increased malformations (external and skeletal) at 910 and 2,330 mg/kg bw/day. Malformations included exencephaly, short tail, cardiovascular defects, fused ribs, and abnormal or fused sternebrae and vertebrae. Fetal body weight per litter was decreased and fetal variations were increased at the 2,330 mg/kg bw/day dose. As with rats, maternal and developmental toxicity was present at the two highest doses. The maternal and developmental NOAEL was 182 mg/kg bw/day.

A Segment II developmental toxicity study (*31*) was also performed in New Zealand white rabbits. The does, 17/group, were administered BBP (Santicizer 160) orally by gelatin capsule on gd 6–18 at 0, 3.0, or 10 mg/kg bw/day. Does were terminated on gd 29. There was no demonstrable maternal toxicity. There was no demonstrable developmental toxicity, such as effects on fetal body weight, 24-hour survival, or treatment-related external or visceral malformations. Skeletal findings *in toto* were considered equivalent across groups.

Mechanistic Studies

Ema et al. has published a series of articles that focus on three issues: 1) direct vs. indirect toxicity of BBP; 2) the dose and time dependency of the prenatal effects of BBP exposure; and 3) study of the toxic properties of the two monoester metabolites of BBP.

<u>Direct vs. indirect toxicity</u>. Ema (*32*, *33*) exposed Wistar rats to BBP at 2.0% in diet (974 mg/kg bw/day) on gd 0–20, gd 0–11 or gd 11–20. Pair-fed controls received the same amount of diet as treated rats. All dams exposed on gd 0–20 had fully resorbed litters. The pair-fed controls exhibited maternal weight gains comparable to the BBP group, but no treatment-related fetal malformations or resorptions were observed. Dams fed BBP on gd 0–11 also had fully resorbed litters. No increase in postimplantation loss was found in rats exposed on gd 11–20, but the fetuses in this group exhibited malformations, predominantly cleft palate and fused sternebrae. Thus, resorption does not appear to be related to decreased food consumption, but is an effect of the chemical, *per se*.

<u>Time- and dose-dependency</u>. In another dietary study using 2.0 % BBP on gd 0–7, gd 7–16, and gd 16–20 (*34*), postimplantation loss was increased after exposure on gd 0–7 or 7–16; teratogenicity was observed (predominantly cleft palate and fused sternebrae) after exposure on gd 7–16 (*34*). Ema et al. (*29*) also dosed Wistar rats by gavage with BBP in olive oil at 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–15. No live fetuses were present at 1,000 mg/kg bw/day and malformations (cleft palate, fused sternebrae, dilated renal pelves) occurred at 750 mg/kg bw/day accompanied by increased *in utero* death, decreased fetal body weight, and maternal toxicity (reduced weight gain and feed consumption). At 500 mg/kg bw/day, maternal feed consumption during the exposure period was reduced, but no embryofetal effects were observed.

To investigate further the observed embryolethality and teratogenicity, Ema et al. (*35*) exposed Wistar rats to BBP in the diet at 2.0% (954 mg/kg bw/day) during gd 0–7g, gd 0–9, or gd 0–11. Pre-implantation loss was equivalent across all groups. Postimplantation loss was highest for groups treated on gd 0–11. Uterine and ovarian weights were reduced, as was plasma progesterone in all groups (except that ovarian weight was unaffected on gd 7). The authors suggest that the post-implantation loss in early pregnancy was mediated by reduced plasma progesterone levels from impairment in luteal function.

It appears that postimplantation death or the development of malformations is dependent upon both the dose and time during gestation when the exposure occurs.

<u>Studies on monoesters</u>. Ema et al. evaluated the developmental toxicity of the two metabolites of BBP: MBuP (*36*-*38*) and mono-n-benzyl phthalate (MBeP) (*39*) when administered by gavage to Wistar rats.

Ema et al. (*38*) gavaged Wistar rats with MBuP at 0, 250, 500, and 625 mg/kg bw/day on gd 7–15. Maternal toxicity was present at the two highest doses, expressed as reduced body weight gains and reduced feed consumption. At these doses there were also significant increases in postimplantation loss/litter, and decreases in live fetuses/litter and fetal body weight per litter. Fetal malformations were also increased at these doses, with cleft palate, deformed vertebral column, and dilated renal pelves the predominant findings.

Ema et al. (*36*) followed-up with evaluation of stage specificity studies. Wistar rats were dosed with MBuP at 0, 500, 625, or 750 mg/kg bw/day on gd 7–9, gd 10–12, or gd 13–15. Embryolethality was increased at all doses for all dosing intervals. No teratogenicity was observed from the gd 10–12 dosing interval. Increased incidences of fetal external malformations were present in the groups treated with 500 and 750 mg/kg bw/day on gd 7–9 and 13–15. Increased skeletal malformations were observed in groups treated with 500, 625, and 750 mg/kg bw/day on gd 7–9, and with 625 and 750 mg/kg bw/day on gd 13–15. Deformed cervical vertebrae were predominant in groups treated on gd 7–9. Cleft palate and fused sternebrae were observed in groups treated on gd 13–15. These results are consistent with the findings for DBP and BBP, and imply that MBuP (and/or subsequent metabolites) may account for the developmental toxicity (embryolethality and malformations) for both DBP and BBP.

Ema et al. (*39*) also administered MBeP by gavage at 0, 250, 313, 375, 438, and 500 mg/kg bw/day to pregnant Wistar rats on gd 7–15. Decreased maternal weight gain during dosing was present at doses from 313 to 500 mg/kg bw/day, and reduced feed consumption was present from 250 to 500 mg/kg bw/day. Increased postimplantation loss was present at 438 and 500 mg/kg bw/day. Increased incidences of fetal external malformations were present at 438 and 500 mg/kg bw/day, skeletal malformations were present at 313–500 mg/kg bw/day, and visceral ("internal") malformations at 375–500 mg/kg bw/day. The most common fetal findings were effects on cervical and thoracic vertebrae, ribs, and kidney (dilated renal pelves at 375 and 438 mg/kg bw/day, and hypoplasia of the kidney at 500 mg/kg bw/day).

These studies establish a maternal and developmental NOAEL for MBuP of 250 mg/kg bw/day. For MBeP, no maternal NOAEL was identified (effects were observed at 250 mg/kg bw/day); the developmental NOAEL was 250 mg/kg bw/day under the conditions of the study. The finding of fetal kidney effects at 375–500 mg/kg bw/day for MBeP is of concern since the CD rat study (*27*) also found fetal kidney malformations at the high dietary dose (1,640 mg/kg bw/day) and the kidney is a known target organ in adult rats. Cervical ribs are also of concern due to their rarity and proposed mechanism of disruption in gene expression.

An additional study by Ema et al. (*37*) compared effects of BBP and DBP administered by gavage to pregnant Wistar rats at 0, 750, 1,000 or 1,250 mg/kg bw/day on gd 7–9, gd 10–12, or gd 13–15. Increased postimplantation loss was observed for both compounds at all doses from all exposure periods. Malformations were observed in groups treated with both phthalate esters at ≥750 mg/kg bw/day on gd 7–9 (vertebral column and ribs) and on gd 13–15 (cleft palate and fused sternebrae). No malformations were observed with either compound at any dose when they were administered on gd 10–12. The authors concluded that "the similarity in dependence of gestational days of treatment on the manifestations of developmental toxicity and on the spectrum of fetal malformations caused by BBP and DBP suggests that they may act by the same mechanism, possibly via a common metabolite of these two parent compounds."

3.2.2 Postnatal Development

Imajima et al. (*40*) gavaged pregnant Wistar-King A (WKA) rats with MBuP in sesame oil at 0 or 300 mg/day on gd 15–18 (equivalent to approximately 1,000 mg/kg bw/day) (Web Table 16). Male offspring were evaluated on gd 20 and on postnatal days (pnd) 30–40 to determine the position of the testes. In control males, all testes were located in the lower abdomen on gd 20 (19 pups, 3 litters) and had descended into the scrotum on pnd 30–40 (15 pups, 3 litters). In stark contrast, in males exposed *in utero* to MBuP, on gd 20 all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent. On pnd 30–40, MBuP exposed males exhibited cryptorchidism (22/26 pups, 5 litters with uni- or bi-lateral undescended testes); 87% of the undescended testes were in the abdominal cavity, the remaining 13% were located at the external inguinal ring. Testis descent is under androgenic control; the authors suggest that phthalate esters may interfere with FSH stimulation of cAMP accumulation in Sertoli cells, resulting in the reduced secretion of Mullerian inhibiting substance, a putative mediator in transabdominal migration of the testis.

The Panel is aware of data indicating that DEHP, BBP, and diisononylphthalate (DINP), but not diethylphthalate (DEP), or dimethylphthalate (DMP), produced reproductive tract malformations in male offspring of rats gavaged with 750 mg/kg bw/day in corn oil on gd 14 to pnd 3 (*41*). DEHP and BBP are approximately equipotent, resulting in 91 and 84% of the male offspring with multiple malformations, respectively; DINP resulted in 7.7% of the offspring males affected ($p<0.04$) versus 0% in controls. DBP is also active as an anti-androgen with comparable potency to DEHP and BBP.

Since BBP and DBP share a common metabolite, MBuP, the study by Mylchreest et al. (*42*), in which pregnant rats were orally dosed on gd 12–21 with DBP at 0, 0.5, 5, 50, 100, or 500 mg/kg bw/day, is germane. The male offspring were evaluated until puberty. The maternal NOAEL was 500 mg/kg bw/day. The developmental NOAEL was 50 mg/kg bw/day, based on the presence of retained nipples and areolae in pre-weanling males at 100 mg/kg bw/day and malformations of the male reproductive tract, testicular lesions (Leydig cell hyperplasia and one Leydig cell adenoma), increased incidence of undescended testes, reduced anogenital distance, and retained nipples and areolae in males at 500 mg/kg bw/day.

3.2.3 Postnatal Function

This section discusses a series of studies in which pregnant rats were exposed to low doses in drinking water. Two primary issues emerged: effects on male reproductive organs and perinatal mortality.

Sharpe et al. (*43*) reported on adult male offspring from Wistar rat dams exposed 2 weeks prior to mating, and during gestation and lactation, to BBP (in ethanol) in drinking water at 1 mg/L (Web Table 9). This study combined data from the same dams bred twice, with exposure continuing, to assess the effects of BBP. At weaning, male offspring were reared to adulthood, with no further BBP exposure and assessed for reproductive effects. Maternal BBP intake was calculated by weighing water bottles for three 48-hour intervals. On pnd 1–2, pnd 10–12, and pnd 20–21, BBP intake was estimated at 0.126, 0.274, and 0.336 mg/kg bw/day (the latter two measurements were confounded by pups drinking the treated water). At 90–95 days of age, male offspring had significantly smaller testes, but exhibited no effects on body, kidney, or ventral prostate weights. Testicular morphology and seminiferous epithelial tubule cross-sections were unaffected, but the authors reported reduced daily sperm production when compared to controls. This laboratory subsequently reported unexplained fluctuation in testicular weight of control rats (*44*).

Ashby et al. (*26*) attempted to replicate the Sharpe et al. (*43*) findings with larger group sizes and better control and characterization of the dosing material. They exposed 18 AP (Wistar) rats during gestation and lactation to 1 mg/L BBP in drinking water and assessed the F_1 male offspring as adults (Web Table 10). They found no effects of BBP exposure on any endpoints assessed, including testis weights, daily sperm production, caudal epididymal sperm count, accessory sex organ weights, or relative incidence of gonadotrophs (FSH-positive cells) in the pituitary for male or female offspring. This study employed only one dose level. Additional details about study results are included in Web Table 10.

Another replication of the Sharpe et al. (*43*) study was attempted by TNO (*45*) (Web Table 11). They exposed Wistar outbred (Crl:(WI)WU BR) rats, 28 females/group, to BBP in the drinking water at 0.1, 1, and 3 mg/L during premating, gestation, and lactation periods. Doses to dams were estimated at 0, 0.012, 0.14, and 0.385 mg/kg bw/day. No effects were observed on mating index, female fecundity or fertility, or on prenatal postimplantation loss in the parental generation. The study failed to reproduce any effects on F_1 male reproductive organ weights or daily sperm production rates when the F_1 offspring reached adulthood. A decreased number of normal epididymal sperm was found in the low-dose group, and was not considered treatment related. Epididymal sperm motility was normal. Preputial separation in males and estrous cyclicity in females were also unaffected by BBP treatment. Following an evaluation of the Sharpe et al, Ashby et al., and TNO studies, the Expert Panel recommended that the reproductive effects in F_1 males reported by Sharpe et al. (*43*) not be used in assessing the reproductive toxicity of BBP. The bases for the recommendation are: 1) lack of dose-response data (e.g., a single-dose study); 2) no analytical information of BBP levels in drinking water; 3) the original laboratory could not replicate their original findings; and 4) two other respected laboratories have been unable to replicate the effects.

Although no reproductive effects were observed in the TNO (*45*) study, an increase in postnatal pup mortality was noted. There was a significant decrease in postnatal pup survival (by total pups/group) in the 1 and 3 mg/L BBP groups and the DES-positive control (Table 2). According to the authors, the values for BBP were not statistically significant on a per litter basis. The same lab immediately repeated the study according to the same protocol, except that only controls and the 1 and 3 mg/L doses were tested. Pup losses in the second study were significantly decreased compared to control at the 1 mg/L dose and again significantly increased compared to control at the 3 mg/L level (Table 2). Again, statistical significance was reported by the authors as not being achieved when analyzed on a per litter basis. Interestingly, significant effects on decreased pup survival (by total pups/group) were reproduced at the 3 mg/L level. The Panel is aware that the concurrent control values for pnd 0–4 pup loss in these two studies exceeded the historical control values for this laboratory, and that other studies performed at this laboratory during this general time period also experienced high pup losses on pnd 0–4, even in the vehicle control groups.

Table 2: Combined Postnatal Mortality in Two TNO Studies with Wistar Rats

Maternal BBP doses in Drinking Water: mg/L (mg/kg bw/day)	0	0.1 (0.012)[a]	1.0 (0.14)	3.0 (0.385)
Study 1 Pnd 0-4 pup loss/total pups at birth[b]	17/252 (25)	2/233 (23)	30[c]/212 (23)	36[c]/248 (24)
Study 2 Pnd 0–4 pup loss/total pups at birth[b]	42/299 (26)	Not determined	19[d]/248 (23)	70[c]/277 (26)
Combined pnd 0-4 Pup loss /total pups at birth[b]	59/551 (51)	2/233 (23)	49/460 (46)	106/525 (50)
% Pnd 0-4 pup loss	10.7%	0.86%	10.65%	20.19%

[a] This dose only tested in one study; all other doses tested in two studies.
[b] Number in parentheses equals total number of litters.
[c] Significant increase when analyzed by group not significant when analyzed by litter.
[d] Significant decrease when analyzed by group not significant when analyzed by litter.

Bayer (*46*) repeated the study conducted by TNO (*45*) using a different animal supplier to determine if the increased perinatal pup death was reproducible (Web Table 12). In the study 21–25 Wistar SPF-bred Hsd/Cpb: WU(Cpb) rats/group were exposed to 0, 1, or 3 ppm BBP through drinking water or diet (0, 1, or 3 mg BBP/L water or kg food) from 2 weeks prior to mating throughout the gestation and lactation period. The BBP intake values in mg/kg bw/day at 1 and 3 ppm (week 1 and 2: prebreed; weeks 3, 4, 5: gestation; weeks 6, 7: lactation) are provided with exposure in diet: week 1–2, 0.09–0.08 (1 ppm) and 0.28–0.27 (3 ppm); week 3–5, 0.07–0.06 (1 ppm) and 0.25–0.19 (3 ppm); and week 6–7, 0.11–0.16 (1 ppm) and 0.34–0.49 (3 ppm); and with exposure from drinking water: week 1–2, 0.12–0.10 (1 ppm) and 0.35–0.34 (3 ppm); week 3–5, 0.11–0.11 (1 ppm) and 0.35–0.35 (3 ppm); and week 6–7, 0.17–0.24 (1 ppm) and 0.54–0.80 (3 ppm). Pups were evaluated for viability and weight gain until sacrifice on pnd 21. In dams, there were no effects on mating, fertility, gestation, or parturition. Unlike the TNO study, there was no effect on perinatal pup survival during gd 1–4 (Table 3). Postnatal viability index (pnd 0–4), a measure of early pup demise, in the same time frame as in the TNO studies was uniformly high across all groups (97.1–100% for the drinking water component, and 98.5–99.6% for the dietary component). The authors did note increased postimplantation loss in dams treated through drinking water (8.92, 10.68, and 13.33% at 0, 1, and 3 ppm, respectively) and feed (6.38, 10.44, and 8.22% at 0, 1, and 3 ppm, respectively). Because the increases in postimplantation loss were not statistically significant, were within historical control values, and had no effect on litter size at birth, they were not considered to be treatment related. It was concluded that BBP treatment had no effect on litter size, pup survival, or pup weight gain.

Table 3: Pre and Postnatal Mortality in Bayer Study

	Drinking Water			Diet		
ppm	0	1.0	3.0	0	1.0	3.0
BBP intake (mg/kg bw/day)	0	0.17	0.54	0	0.11	0.34
Postimplantation loss per group: number of resorptions (number of implantations)	24(269)	30(281)	40(300)	18(282)	33(316)	25(300)
% Postimplantation loss per group (not statistically significant)	8.92	10.68	13.33	6.38	10.44	8.22
Postnatal viability index % (pnd 0–4)	97.1	100.0	99.6	98.5	99.6	99.3

As reported in an abstract, Parks et al. (*47*) dosed Sprague-Dawley rats by gavage with 750 mg/kg bw/day of BBP, DEHP, or corn oil (vehicle) from gd 14 through pnd 3. On pnd 2, anogenital distance (AGD), and testes weight were measured. Testes weights and AGD were significantly decreased, and the incidence of retained areolae on pnd 13 was increased for both DEHP- and BBP-exposed male pups.

Developmental effects were also reported in a reproductive screening study by Piersma (*48*) and are addressed in Section 4.

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

There were no human data available on the reproductive toxicity of BBP alone. Occupational exposure to phthalate mixtures containing BBP in PVC production has been associated with increased incidence of menstrual disorders and spontaneous abortions among female workers (*49*).

4.2 Experimental Animal Toxicity

Six studies were reviewed in the evaluation of the reproductive toxicity of BBP. No study was definitive and no multigeneration-reproduction study has been published for BBP. Three studies measured reproductive performance. One other reported claims of low-level effects of BBP on reproductive development (discussed in Section 3), but these effects have not been reproduced by separate laboratories.

An assessment of the reproductive toxicity of BBP was reported by Piersma (*48*) (Web Table 13). This standard general and reproductive toxicity screen, conducted according to the OECD 421 protocol, provides useful indications as to major toxic effects. Male and female WU rats (10/sex/group), 10–11 weeks old at the start of exposure, were gavaged for 14 days with BBP in corn oil at dose levels of 0, 250, 500, or 1,000 mg/kg bw/day, and then paired (1:1) and allowed to mate for a maximum of 14 days while dosing continued. Once evidence of mating was observed, the animals were separated. Males continued to be dosed daily, and were then killed and necropsied after a total dosage period of 29 days. Reproductive organs were removed and placed in Bouins fixative. Dosing of females continued until pnd 6, after which the females were killed and necropsied and ovaries and uteri examined. Pups were counted, sexed, weighed, and examined for external malformations on pnd 1 and 6 and then killed.

Body weight gain for the F_0 males was reduced at the high dose (by 21%), whereas the body weight gain of the F_0 females was increased in the second week of dosing (12 g/week compared to 4 g/week for the controls). During pregnancy, the body weight gain of the dams was significantly reduced at the high dose (by 40%). The numbers of animals achieving a pregnancy were 9, 8, 7, and 4 (of 10) in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively. Postnatal pup mortality did not differ across dose groups, but average litter sizes at birth were 9.4, 11.4, 8.4, and 1.5 in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively, with statistical significance achieved at the highest dose. Absolute pup weight was significantly reduced at birth in the high- (29%) and mid-dose (7%) groups. Testicular degeneration accompanied by interstitial cell hyperplasia was significantly increased in the high-dose F_0 males. Ovary structure was not affected by treatment.

In Piersma et al. (*48*), the high-dose group had lower fertility (decreased numbers of litters and decreased numbers of pups per litter) in the F_0 generation with marked histopathology in the testes, but not in the ovaries. F_1 pup weight was reduced at birth in the mid- and high-dose groups and a developmental NOAEL of 250 mg/kg bw/day was identified. The reproductive NOAEL was identified as 500 mg/kg bw/day. The Expert Panel's confidence in the quality of the study is moderate to high; however, because of the design limitations, such as a lack of measures in the F_1 generation, there is uncertainty that these doses correctly represent the reproductive NOAEL.

A one-generation reproduction study following OECD guideline 415 was performed in Wistar rats that were mated twice and produced two litters (*50*) (Web Table 14). BBP was administered in the diet at levels of 0, 0.2, 0.4, and 0.8% to 12 male and 24 female rats per group for 10 and 2 weeks prior to the first mating, respectively. Seven to thirteen days after the first litter was weaned (at pnd 21), the study was repeated with the same rats. Average doses to males during the premating period were estimated by authors at 0, 108, 206, or 418 mg/kg bw/day. Average female doses during the premating, gestation, and lactation periods were estimated at 0, 106, 217, or 446 mg/kg bw/day; 0, 116, 235, or 458 mg/kg bw/day; and 0, 252, 580, or 1,078 mg/kg bw/day, respectively. There were no treatment-related clinical signs or mortality. There were periods of reduced body weight or weight change in females in the high-dose group during gestation and lactation in each of the two matings. A decrease in food consumption during the gd 0–14 period in both matings was considered a substance-related effect. A slight decrease in the number of treated females with litters observed in the first mating was not observed in the second mating. Mean pup weight was slightly decreased in the high-dose group during lactation; this decrease reached statistical significance at pnd 21 in the second litter. The authors attributed the pnd 21 finding to direct consumption of BBP in diet by the pups after pnd 14. All standard reproductive indices (fertility, implantation, and fecundity) were within normal ranges. At necropsy, tissues from male and female reproductive organs were collected and fixed in 4% buffered formalin. Microscopic examination of hematoxylin- and eosin-stained slides from these tissues was performed for control and high-dose rats. Relative liver weights were increased in high-dose females, but examination revealed that the liver and reproductive tissues were normal. The authors concluded that the NOAEL for reproductive performance was 418 mg/kg bw/day in males and 446 mg/kg bw/day in females, with the parental NOAEL being 206 mg/kg bw/day in males and 217 mg/kg bw/day in females.

The NTP (*17*) (Web Table 15) described a 10-week modified mating study. Male F344 rats, 6 weeks old at the commencement of the study, were exposed to BBP (15/group) in the diet at levels of 0, 300, 2,800, or 25,000 ppm for 10 weeks (which delivered approximate doses at 0, 20, 200, 2,200 mg/kg bw/day) and then allowed to recover for 2 days. The rats were then housed individually with two untreated females during a 7–day mating period and females were removed on the first day of a vaginal plug or sperm detection. Females were necropsied on gd 13. After the mating period, 10 and 11 days after receiving the last dose in feed, the males were necropsied and a full histological examination made at 0 and 25,000 ppm only. However, the testis and epididymis, seminal vesicle, and prostate were examined in all groups. The fixative used to preserve the testis was not indicated. Epididymal sperm analysis was also performed on the males; sperm samples were collected for evaluation at the end of the study.

Mean body weights of the high-dose males were 71% of control values at the end of the study, representing a significant reduction. Food consumption differences between the control and high-dose groups at the end of the study were only modestly decreased with treatment when proportionality to body weight is considered. Liver and thymus to body weight ratios were increased in the 2,200 mg/kg bw/day group, whereas absolute and relative testis and prostate weights were reduced. There was marked degeneration in the testis and epididymis at this dose. One animal in the low-dose group had marked testicular atrophy and others had fewer sperm in the epididymis. Epididymal sperm concentration was: 87, 70, and 0.1 % of control at the 20, 200, and 2,200 mg/kg bw/day groups, respectively. Other sperm parameters (motility, morphology) were not measured in the high-dose group due to the absence of sperm; sperm motility and morphology were not different from controls in the other treatment groups. Although 10/30 females mated to high-dose males were sperm-positive during the mating trial, none were pregnant at necropsy. The pregnancy measures of the two lower dose groups were similar to control values.

In the NTP study, the high-dose group (2,200 mg/kg bw/day) had a high rate of infertility (decreased numbers of pregnancies) with marked histopathology in the testes and epididymides and a lowered sperm count. Effects in the 200 mg/kg bw/day group were restricted to a significant reduction in sperm count. However, it was subsequently noted that sperm counts might have been affected by a shorter recovery period from the time between mating to necropsy in the 200 mg/kg bw/day group compared to the other dose groups (*51*). Judd et al. provide the most recent example of a significant body of literature indicating that sperm levels in the cauda epididymis are significantly reduced by ejaculation; in some cases counts are reduced to <50% of control values (*52, 53*). Because epididymal sperm counts in rats have been found to require at least 4–7 days to return to normal after mating (*54*), and 13/15 rats in the 200 mg/kg bw/day group were killed less than 4 days after the detection of a vaginal plug in their mates, while only 7 control males were killed in this same period, the reduction in sperm count in the 200 mg/kg bw/day group in this 10-week study must be considered questionable. Additionally, an expert panel reviewing methods of sperm analysis stated that at least a week should transpire between mating and necropsy in order to avoid ejaculation-induced confounding of sperm count data (*55*). The effects at 2,200 mg/kg bw/day are considered both treatment- and dose-related. A NOAEL of 200 mg/kg bw/day was selected by the Expert Panel. This may not correctly represent the NOAEL because of the lack of measures to assess effects in females and the lack of assessment of reproductive systems in the F_1 generation.

Parallel to the 10-week modified mating study (*17*), a 26-week sub-chronic study was performed where male F344 rats received BBP in the diet at doses of 0, 300, 900, 2,800, 8,300, and 25,000 ppm (0, 30, 60, 188, 550, and 1,650 mg/kg bw/day). The results of this study are presented in the section on General Toxicity and in Web Table 3. While a mating sequence was not part of the 26-week-study design, all other protocol parameters associated with male effects (organ weights, tissues for microscopic evaluation, and epididymal spermatozoal parameters) were identical to the NTP 10-week study. A comparison of results shows similarity in effects on body weight gain, organ weights, histopathological findings, and sperm motility. Interestingly, while sperm concentration in the 200 mg/kg bw/day group was reduced by 30% in the 10-week study, the values for the 550 mg/kg bw/day group in the 26-week study were not reduced. All other measures at this dose were similar to controls. Results of the other two doses, compared to their contemporary controls, were similar.

Agarwal et al. (*15*) (Web Table 1) examined the effect of BBP on the male reproductive system of adult rats. Fischer F344 rats (10 males per group) aged 12–13 weeks were administered BBP at levels of 0, 0.625, 1.25, 2.5, and 5% (0, 447, 890, 1,338, and 1,542 mg/kg bw/day) in the diet for 14 days and killed on day 15. Details of the study and effects on systemic endpoints are provided in Chapter 2. Reproductive effects at the two highest doses included significant weight and histological changes to the testis and accessory sex glands accompanied by changes in circulating FSH and LH levels. An oral NOAEL for reproductive toxicity in this 14-day study in adult male F344 rats was 1.25 % (890 mg/kg bw/day). Expert Panel confidence in the quality of the study is moderate; within design limitations, the study is well conducted and reported. Panel confidence is low that these dose levels correctly represent the NOAEL due to the short exposure time and because guidelines for this type of study do not require assessment of younger animals or the F_1 generation.

Studies on postnatal male fertility, with animals exposed indirectly through maternal consumption, as reported by Sharpe et al. (*43*), and subsequent publications by Ashby et al. (*26*) and TNO (*45*), that failed to reproduce the original findings are presented and discussed in Section 3.2.

According to an abstract, a dominant lethal study was performed (*56*) on $B6C3F_1$ and CD-1 male mice administered BBP by subcutaneous injections on days 1, 5, and 10 of the study at doses equivalent to 400–600, 1,280–1,840, and 3,200–4,560 mg/kg bw/day (triethylene melamine was the positive control). The males were then paired with untreated females every 4 days through day 49; female uterine contents were evaluated on gd 17. BBP did not affect prenatal deaths or fertility in either strain at any dose.

<u>Mode of Action</u>

Several studies have examined the ability of selected phthalate esters to compete with labeled estradiol (E2) for binding to the estrogen receptor (ER). Sources of ER protein included rat uterine cytosol (*57*), rainbow trout hepatic cytosol (*58*), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (*59*, *60*), and rainbow trout ERs expressed in yeast (*61*). Tritiated 17ß-estradiol (E2) was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. BBP has been shown to bind to the estrogen receptor (ER) of rat (*57*) or trout (*58*). The relative binding affinity is approximately 10,000–100,000 times less than (E2).

Selected phthalate esters have been examined in a number of *in vitro* gene expression assays systems. The assays have used stably transfected cells (*57*), transiently transfected cells (*57*, *58*), yeast based assays (*57*, *61*-*63*), and vitellogenin induction in rainbow trout hepatocyte cultures (*61*). BBP induces weak activity in *in vitro* estrogen-mediated gene expression assays in mammalian cell transfection experiments at 10 μM, the highest concentration examined (*57*). In a yeast assay of estrogen-mediated gene expression, the potency of BBP was $1x10^6$–$5x10^7$ less than that of E2, but its metabolites MBuP and MBeP demonstrated no estrogenic activity (*63*). However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in 10 Sprague-Dawley rats/group gavaged with 20, 200, and 2,000 mg/kg bw/day for 4 days (*57*). Moore (*64*) reviewed the data on the estrogenic potential of phthalates and concluded that the estrogenic ability of phthalates identified in the *in vitro* studies is “not relevant to humans or to the environment.”

The summary for Section 4 is located in Section 5.1.4.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

BBP is used in PVC construction materials, automotive materials, and food conveyor belts (*1*). There appears to be no significant use of BBP in toys or medical equipment. It is believed that only negligible amounts of BBP are present in air due to its low volatility; a limited number of air monitoring studies support this view. In a survey of California homes, the median air level of BBP was measured at 0.034–0.035 ng/m^3, and median outdoor air levels of BBP were below the detection limit of 0.051 ng/m^3. However, inhalation exposure to BBP in flexible PVC manufacturing facilities has been estimated at 286 μg/kg bw/workday (*1*). Exposure through contact of BBP-containing materials with skin is negligible due to the relatively slow absorption through skin (*2, 10, 11*).

The IPCS (*2*) concluded that consumption of food containing trace levels of BBP is the only significant source of exposure to the general population. Based on a survey of Canadian foods, IPCS estimated that exposure of adults to BBP is 2 μg/kg bw/day and that exposure levels in children could be up to three-fold higher. Exposures in children may be higher due to dietary differences and intake of BBP through mouthing of BBP-containing objects. MAFF estimated the BBP exposure of adults through diet at 0.11–0.29 μg/kg bw/day and exposure of infants through formula at 0.1–0.2 μg/kg bw/day. In all exposure estimates, it was evident that exposure to the general population, including children, is well below 10 μg/kg bw/day. Discrepancies in food exposure estimates may be due to the inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations.

5.1.1.1 Utility of Data to the CERHR Evaluation

BBP exposures resulting from food intake were estimated by two authoritative sources. There are limitations in these estimates. One agency used 12–15 year-old data which may not reflect current exposure, and the food data were collected in Europe and Canada and estimates may not accurately reflect US dietary patterns.

5.1.2 General Biological and Toxicological Data

Toxicity. There are no human data on exposure to BBP alone. Exposures to BBP-containing phthalate mixtures have been associated with elevated respiratory/neurological morbidity and increased risk of cancer in occupationally exposed population groups (*2*); a single controlled epidemiological study has found an increased risk of bronchial obstruction in young children related to indoor exposures from PVC floor covering (*13*); BBP is a common component of PVC.

In animals BBP is not acutely toxic by the oral or dermal route as evidenced by the LD_{50} value exceeding 2 g/kg bw (*2*). Several subchronic and chronic dietary studies in rats reported consistent adverse effects on body weight and in kidney, liver, and testes (*14*, *15*, *17*). The earliest response was an increase in kidney or liver to body weight ratio(s) observed at doses of 120–151 mg/kg bw/day and higher. Histological changes in the liver were observed in some studies at doses of 960 mg/kg bw/day and higher and changes in kidneys were observed in the chronic study at doses of 500 (M) – 1,200 (F) mg/kg bw/day. Anemia was observed at doses of 500 mg/kg bw/day and higher. The pancreas may also be a target organ in rats, as pancreatic lesions were reported in a subchronic study at 381 mg/kg bw/day. Lesions in testes, seminal vesicles, epididymis, and/or prostate were noted after exposure of rats to 1,338 mg/kg bw/day or higher. In an inhalation study in rats, increases in liver and kidney weights were reported at the maximum dose of 789 mg/m^3 (~150 mg/kg bw/day) (*14*). BBP is considered a weak inducer of peroxisome proliferation in rats.

In repeat-dose studies, mice were less sensitive to toxic effects than were rats. Dietary studies of up to 2-years' duration in $B6C3F_1$ mice showed dose-related reductions in body weight at doses of 1,029 mg/kg bw/day and higher (*18*). There was no clinical or histological evidence of toxicity in tissues, including male and female reproductive organs. Male dogs also appear to be less sensitive than rats because oral doses up to 1,852 mg/kg bw/day for 90 days resulted in reduced body weight but produced no histopathological effects in testes or liver (*14*).

A 2-year dietary study found no evidence of carcinogenicity in $B6C3F_1$ mice and only a marginal increase in mononuclear cell leukemia in F344 female rats (*18*). In a second study in F344 rats, there was some evidence of pancreatic carcinogenicity in males exposed to 500 mg/kg bw/day and equivocal evidence of pancreatic and urinary bladder carcinogenicity in females exposed to 1,200 mg/kg bw/day (*17*).

Toxicokinetics. There are no data from studies in humans. There are no inhalation studies in any species. BBP is rapidly absorbed (at least 75% at doses of 2–200 mg/kg) in orally-dosed rats; this dropped to 22% at 2,000 mg/kg, suggesting saturation at high doses (*20*). BBP is absorbed slowly through the skin (27% in 7 days) of rats (*10*). BBP is rapidly metabolized to monobutyl and monobenzyl esters; by analogy to other phthalate esters, this probably occurs by pancreatic lipase and esterases in the small intestine. The monobutyl ester is usually present in higher amounts, 5:3, than is the monobenzyl ester (*2*). These monoesters are typically conjugated with glucuronic acid and then excreted in the urine (*19*, *20*, *23*). The glucuronidation pathway appears to be saturated at high doses, as noted by the decrease in the glucuronide metabolite relative to the monoester metabolites at high doses (2,000 mg/kg in rats) versus low doses (20 mg/kg in rats). There is no evidence of accumulation in tissues. Excretion of the absorbed BBP and its metabolites is rapid, with approximately 90% elimination in 24 hours. The half-life of BBP in blood is 10 minutes; the blood half-life of the monoester metabolites of BBP is approximately 6 hours.

Genetic toxicity. A recent review by the IPCS (*2*) stated: "Although the weight of evidence of genotoxicity is clearly negative, available data are inadequate to unequivocally conclude that BBP is not clastogenic. However, in the available studies, the activity has been weak and is often consistent with secondary effects of the chemical on DNA."

5.1.2.1 Utility of Data the CERHR Evaluation

The oral subchronic studies in rats and mice are adequate for the evaluation of general toxicity induced by BBP. The database is adequate to determine that the liver is a target organ of toxicity. Some studies were conducted according to GLP standards and relevant exposure routes were utilized. The examination of hepatic effects was adequate and included a limited evaluation of peroxisomal proliferation in rats. There is an inhalation study in rats.

There are acceptable toxicokinetic data for BBP, consisting of absorption, distribution, metabolism, and excretion data following oral and dermal exposure in the rat.

Table 4: Summaries of NOAELs and LOAELs and Major Effects in Oral General Toxicity Studies

Protocol and BBP Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major Effects at Higher Doses
14-day repeat dose dietary study in adult male Fischer 344 rats. 10 rats/group. Doses 0, 447, 890, 1,338, or 1,542 mg/kg bw/day. (*15*)	None	447 ↑LH. ↑Liver and kidney weight.	↓Testes, seminal vesicle, epididymis weight. Histopathological effects in testes, seminal vesicles, and prostate. ↑LH and FSH ↓Testosterone. ↑Liver, kidney, and thymus weight. Histological changes in liver and thymus. ↓Bone marrow cellularity.
3-month repeat dose dietary study in Wistar rats. 4–6 weeks old at start of study. 27–45 rats/sex/group. Doses – M: 0, 151, 381, 960 mg/kg bw/day. F: 0, 171, 422, 1,069 mg/kg bw/day. (*14*)	None	M: 151; F: 171 ↑Liver (4%) weight.	↑Liver and kidney weight. Liver lesions. Pancreatic lesions. Anemia. ↓Urine pH (M). **No** testicular lesions.
90-day repeat dose dietary study in adult Beagles. 3/sex/group. Doses – M: 0, 400, 1,000, 1,852 mg/kg bw/day. F: 0, 700, 1,270, 1,973 mg/kg bw/day. (*14*)	M: None F: 700	M: 400 F: 1,270 Decreased body weight.	**No** histological effects in liver or testes.
26-week dietary study in adult male Fischer 344/N rats. 6-weeks-old at start of study. 15 rats/group. Doses – 0, 30, 60, 180, 550, 1,650. (*17*)	180	550 ↑Liver weight. ↑Hemoglobin.	↓Testis, seminal vesicle, and epididymis weight. Lesions in testis and epididymis. ↓ Sperm counts. ↑ Liver and kidney weight. Anemia.
2-year dietary study in Fischer 344/N rats. 6-weeks-old at start of study. 60 rats/sex/group. Doses – M: 0, 120, 240, 500 mg/kg bw/day; F: 0, 300, 600, or 1,200 mg/kg bw/day. (*17*)	None	M: 120; F: 300 ↑Kidney weight (M). Nephropathy (F).	↑ Liver weight. ↑ Kidney weight. Nephropathy (F). Anemia. ↓ Thyroid hormone (F). Some evidence of pancreatic cancer (M). Equivocal evidence of urinary bladder and pancreatic cancer (F). **No** testicular lesions.
2-year dietary study in $B6C3F_1$ mice. 4–5 weeks old at start of study. 50 mice/sex/group. Doses – M: 0, 1,029, 2,058 mg/kg bw/day; F: 0, 1,037, 2,074 mg/kg bw/day. (*18*)	None	M: 1,029; F: 1,037 ↓Weight gain.	↓ Weight gain. **No** changes in survival or neoplasm development. **No** lesions in male or female reproductive organs.

5.1.3 Developmental Toxicity

Studies of prenatal development consistently show BBP to be embryolethal and teratogenic following exposure to high oral doses in rats and mice on gd 6–15 or 7–15. The incidence of these effects is dependent on dose and developmental age. A maternal and developmental NOAEL in CD-1 mice was 182 mg/kg bw/day (*30*). The Expert Panel noted that there was wide spacing between the NOAEL and the LOAEL of 910 mg/kg bw/day in this study. Effects at the LOAEL and higher doses included increased resorptions and late fetal deaths, reduced number of live fetuses per litter, and increased external and skeletal malformations. The developmental NOAELs in Sprague Dawley and Wistar rats ranged from 420 to 500 mg/kg bw/day, respectively (*27*, *29*). Effects at doses of 750 mg/kg bw/day and higher included increased prenatal mortality, reduced fetal growth, and increased fetal variations and skeletal, visceral, and external malformations. Extending the exposure period to gd 0–20 in Wistar rats resulted in a developmental NOAEL of 185 mg/kg bw/day. An oral prenatal study in rabbits revealed no maternal or developmental toxicity at doses up to 10 mg/kg bw/day; however, utility of the results is limited since no maximum tolerated dose was established (*31*).

Using a prenatal study design similar to that used with BBP (*29*), the monoesters MBuP and MBeP were investigated (*38*, *39*). The developmental toxicity observed with the monoesters was qualitatively similar to that produced by BBP. These data suggest that both monoesters can contribute to the developmental toxicity associated with BBP. Differences in the doses selected for study do not permit a close quantitative comparison of the dose-response relationship between the two monoesters or with BBP. A rat study, using an MBuP dose of 1,000 mg/kg bw/day, reported a subsequent interference with testicular migration and descent (*40*).

Studies in rats indicate that prenatal effects are directly related to the chemical and are not due to decreased food consumption (*32*). The mechanism of action for resorption has been proposed as reduced circulating progesterone due to impaired luteal function (*35*).

The effect of low-dose exposure during mating, gestation, and lactation in Wistar rats has been studied. An increase in postnatal pup mortality was reported (*45*) for rats treated with BBP through drinking water at 1 and 3 mg/L (0.14 and 0.385 mg/kg bw/day). The study was immediately repeated at the same laboratory and only the result at the highest dose (3 mg/L) was replicated. In both studies, statistical significance was not achieved with the litter as the unit of analysis. The Panel noted that concurrent control values for pnd 0–4 pup loss in these two studies exceeded the historical control values for this laboratory. Further, other studies performed at this laboratory during this time period also experienced high pup losses on pnd 0–4, even in the vehicle control groups. Increased pup mortality was not observed in similar studies by Sharpe et al. (*43*) and Ashby et al. (*26*) who dosed Wistar rats with 1 mg/L BBP in drinking water. In addition, a subsequent study by Bayer (*46*) did not result in increased postnatal pup loss at BBP doses of 1 or 3 ppm in drinking water or feed. For the Bayer study, maternal BBP intakes during the first week of lactation (the time of pup losses in the TNO studies) were 0.11 μg/kg bw/day and 0.34 μg/kg bw/day through diet and 0.170 μg/kg bw/day and 0.540 μg/kg bw/day through drinking water.

The Panel is therefore presented with a developmental LOAEL from the two TNO (*45*) studies of approximately 0.385 mg/kg bw/day (3 ppm) and a NOAEL of 0.140 mg/kg bw/day (1 ppm) based on F_1 pup losses on pnd 0–4. There is very low confidence in these values due to the discrepancy between the results of the data when analyzed by group (statistically significant) versus by litter (not statistically significant) and due to the lack of effects in the Bayer (*46*) study. The NOAEL/LOAEL values from the TNO study are approximately 3 orders of magnitude lower (~0.3–0.4 mg/kg bw/day) than the NOAEL values for reproductive/developmental NOAELs in other studies (~185 mg/kg bw/day for developmental toxicity and 500 mg/kg bw/day for reproductive toxicity).

Table 5: Summaries of NOAELs and LOAELs and Major Effects in Developmental Toxicity Studies

Protocol and Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)		Developmental Effects Observed at Higher Dose Levels
		Maternal	Developmental	
Prenatal dietary study in Sprague-Dawley rats. 30 per group received 0, 420, 1,100, or 1,640 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (*27*)	Maternal: 420 Developmental: 420	1,100 ↓ Body weight gain. ↑ Liver weight.	1,100 ↑ Variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ Visceral, skeletal, and external malformations. ↑ Variations.
Prenatal dietary study in Wistar rats. 15–18/group received 0, 185, 375, 654, or 974 mg/kg bw/day on gd 0–20. Dams and pups examined late in gestation. (*28*)	Maternal: 375 Developmental: 185	654 ↓ Weight gain.	375 ↑ Prenatal mortality.	↑ Prenatal mortality. ↓ Decreased fetal weight.
Prenatal gavage studies conducted in Wistar rats. 10/group received BBP 0, 500, 750, or 1,000 mg/kg bw/day (0, 1.60, 2.40, 3.20 mmol/kg bw/day) on gd 7–15. Dams and pups examined late in gestation. (*29*)	Maternal: 500 Developmental: 500 (1.60 mmol/kg bw/day)	750 (2.40 mmol/kg bw/day) ↓ Weight gain.	750 (2.40 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations.	Complete prenatal mortality.
The same study was conducted with MBuP at doses of 0, 250, 500, or 625 mg/kg bw/day (0, 1.13, 2.25, 2.81 mmol/kg bw/day). (*38*)	Maternal: 250 Developmental: 250 (1.13 mmol/kg bw/day)	500 (2.25 mmol/kg bw/day) ↓ Weight gain.	500 (2.25 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.	↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.
The same study was conducted with MBeP at doses of 0, 250, 313, 375, 438, or 500 mg/kg bw/day (0, 0.976, 1.22, 1.46 mmol/kg bw/day). (*39*)	Maternal: None Developmental: 250 (0.976 mmol/kg bw/day)	250 (0.976 mmol/kg bw/day) ↓ Food intake.	313 (1.22mmol/kg bw/day) ↑ Skeletal malformations.	↑ Prenatal mortality. ↑ Internal, external, and skeletal malformations.
Prenatal dietary study in CD-1 mice. 30 per group received 0, 182, 910, or 2,330 mg/kg bw/day on gd 6–15. Dams and pups examined late in gestation. (*30*)	Maternal: 182 Developmental: 182	910 ↓ Weight gain.	910 ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations.	↓ Fetal weight. ↑ Prenatal mortality. ↑ Visceral, skeletal, and external malformations. ↑ Variations.

Postnatal drinking water study in Wistar rats. 28/group received 0, 0.012, 0.140, or 0.385 mg/kg bw/day from 2 weeks prior to mating throughout gestation and lactation. Pups were examined postnatally. The experiment was repeated with the mid and high dose to verify increased postnatal mortality. (*45*)	Maternal: 0.385 Developmental: 0.14	No adverse maternal systemic or reproductive effects.	0.385 ↑ Pup death on pnd 1–4 (12% in treated versus 0.8% in control). ↑ Pup death on pnd 1–4 (17% in treated versus 10% in control) in repeat experiment.	No higher doses.
Postnatal drinking water and dietary study. 21–25/group were treated from 2 weeks prior to mating throughout gestation and lactation. Lactational doses were 0, 0.11–0.16, and 0.34–0.49 through diet and 0, 0.17–0.24, and 0.54–0.80 through drinking water. Pups were examined postnatally. (*46*)	Maternal: 0.34–0.49 (diet), 0.54–0.80 (drinking water) Developmental: 0.34–0.49 (diet), 0.54–0.80 (drinking water)	No adverse maternal systemic or reproductive effects.	No significant effects on development including pup mortality.	No higher doses.

5.1.3.1 Utility of Data to the CERHR Evaluation

The data in rats and mice are adequate for a prenatal assessment of fetal growth, lethality, and teratogenicity. One study examined prenatal effects following exposure during late pregnancy. None of the studies included a postnatal evaluation of androgen-regulated effects (e.g., nipple retention, testicular descent, or preputial separation) that were the most sensitive indicators of developmental toxicity with DBP. BBP and DBP share a common monoester metabolite. Prenatal studies with BBP monoesters (MBuP and MBeP) were sufficient to determine that both metabolites contribute to developmental toxicity. Because of differences in doses administered to the mice and rats, it is not possible to compare sensitivity between the two species.

5.1.4 Reproductive Toxicity

There are no conclusive data in humans that assess the reproductive effects from exposure to BBP alone. All experimental animal studies that assess reproduction have been performed in the rat.

<u>Male reproductive toxicity</u>. Male reproductive performance was evaluated in three rat studies by the oral route of exposure (*17, 48, 50*). There were no effects in reproductive performance in 10 WU rats exposed to up to 500 mg/kg bw/day by gavage for 2 weeks prior to mating. Decreased fertility and testicular histopathology were seen at 1,000 mg/kg bw/day (*48*). No adverse effects were noted in Wistar rats exposed through diet with up to 418 mg/kg bw/day from 10 weeks prior to mating until the birth of a second litter (*50*). Reduced sperm counts were noted in F344 rats exposed to 200 mg/kg bw/day through diet for 10 weeks, but reproductive performance was not affected (*17*). The sperm count effects in the 200 mg/kg bw/day group were considered questionable and not used to determine a NOAEL because: 1) that group had a shorter recovery time from mating to necropsy and the required time to restore cauda epididymal sperm

counts following ejaculation was not reached for most animals (*51, 54*); and 2) no effects on sperm count were reported following exposure to 550 mg/kg bw/day in a 26-week study by the same laboratory (*17*).

Histopathology of male reproductive organs has also been examined in subchronic and chronic F344 rat studies by the oral route; the lowest dose that produced testicular lesions was 1,338 mg/kg bw/day in rats exposed through diet (*15*). The reproductive organs of male $B6C3F_1$ mice were unaffected at dietary doses up to 2,058 mg/kg bw/day and testes of beagle dogs were not affected at dietary doses up to 1,852 mg/kg bw/day. The Expert Panel selected a reproductive NOAEL of 500 mg/kg bw/day for adult male rats. There is uncertainty as to the dose that is without effect on the developing male reproductive tract. The Expert Panel noted that a primary BBP metabolite, MBuP, is likely the active toxicant in DBP studies where exposure during *in utero* development or during the neonatal period of life led to reproductive effects (*42*). Given that MBuP is a metabolite of BBP, the DBP data are relevant to BBP. Similar studies with MBeP, the other metabolite of BBP have not been performed. The existing studies with BBP did not critically examine pups during the sensitive postnatal phases of life. It is probable that such studies would likely result in a lower NOAEL.

<u>Female reproductive toxicity</u>. In a reproductive toxicity screening study in WU rats, decreases in the number of females conceiving and in the number of live pups per litter were observed at an oral gavage dose of 1,000 mg/kg. Clear testicular effects in males suggest that the effect may be due in part to toxicity in the male (*48*). Five hundred mg/kg bw/day was a NOAEL for female fertility in this study, but these data were from a screening study. No effects on implantation, reproductive organ morphology, fertility, or fecundity were seen in a one-generation reproductive toxicity study in Wistar rats that received the highest dietary dose (0.8%), comparable to a BBP intake value 446 mg/kg bw/day in diet during the mating phase of the study (*50*).

<u>Mode of Action</u>

BBP has been shown to bind to the estrogen receptor (ER) of rat and trout (*57, 58*). The relative binding affinity is approximately 10,000–100,000 times lower than 17β-estradiol (E2). BBP also induces weak activity in *in vitro* estrogen-mediated gene expression assays at 10 μM, the highest concentration examined (*57*). In a yeast assay of estrogen-mediated gene expression, the potency of BBP was $1x10^6$–$5x10^7$ less than that of E2, but its metabolites MBuP and MBeP demonstrated no estrogenic activity (*63*). However, no effects on uterine wet weight and vaginal epithelial cell cornification were observed in Sprague-Dawley rats gavaged with 20, 200, and 2,000 mg/kg bw/day for 4 days. Moore (*64*) reviewed the data on the estrogenic potential of phthalates and concluded that the estrogenic ability of phthalates identified in the *in vitro* studies is "not relevant to humans or the environment."

Table 6: Summaries of NOAELs, LOAELs, and Major Effects in Reproductive Toxicity Studies

Protocol & Study	NOAEL (mg/kg bw/day)	Reproductive LOAEL (mg/kg bw/day) and effects	Systemic LOAEL (mg/kg bw/day)and Effects	Reproductive Effects at Higher Doses
One-generation reproductive screening assay in WU rats. 10 pairs/group received 0, 250, 500, or 1,000 mg/kg bw/day by gavage from 2 weeks prior to mating for a total of 29 days (males) or until pnd 6 (females).	Reproductive: 500 Systemic: 500	1,000 ↓Fertility. Testicular lesions. ↓Litter size.	1,000 ↓Weight gain.	No higher doses in study.

(*48*)				
One-generation dietary reproductive toxicity assay in Wistar rats with 12 males and 24 females/group. Males were treated 10 weeks prior to mating with 0, 108, 206, or 418 mg/kg bw/day. Females were treated from 2 weeks prior to mating (0, 106, 217, or 446 mg/kg bw/day), through gestation (0, 116, 235, or 458 mg/kg bw/day) and lactation (0, 252, 580, or 1,078 mg/kg bw/day). (*50*)	Reproductive: 418 (M); 446 (F) Systemic: 206 (M); 217 (F)	No structural or functional effects at any dose.	418 (M); 446 (F) ↓Weight gain (F) ↑Liver weight.	No higher doses in study.
One-generation modified mating study in male F344 rats. 15 males/group treated with BBP through diet at 0, 20, 200, or 2,200 mg/kg bw/day for 10 weeks and then mated with untreated females. (*17*)	Reproductive: 200 Systemic: 200	2,200 ↓ Sperm counts. ↓ Fertility. Testicular and epididymal lesions. ↓ Testis and prostate weight.	2,200 ↓ Weight gain. ↑ Liver weight. Anemia	No higher doses in study.

5.1.4.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of reproductive toxicity in adults. Studies are available that evaluate both structure and reproductive function. In studies with DBP, a phthalate that is also metabolized to MBuP, male rats exposed while *in utero* and during lactation were most sensitive to DBP-induced effects on reproductive structure and function (*65*). Therefore, the most sensitive age for reproductive toxicity was not addressed for BBP. The data was sufficient to demonstrate the testes as a target organ.

5.2 Integrated Evaluation

BBP is primarily used in PVC utilized in the manufacture of construction materials, automotive materials, and food conveyor belts. Exposure of the general population through inhalation is negligible due to the low volatility of BBP. Inhalation exposure to BBP in flexible PVC manufacturing facilities has been estimated at 286 µg/kg bw/workday. Exposure through contact with skin is negligible due to the relatively slow absorption. The IPCS has concluded that consumption of food containing trace levels of BBP is a significant source of exposure to the general population. Estimates based on BBP levels in Canadian and UK foods indicate that exposure to the general population, including children, is below 10 µg/kg bw/day.

There are no human toxicokinetic or toxicity studies for BBP. Studies in rats demonstrate that orally-administered BBP is rapidly converted to the monoester metabolites, MBuP and MBeP, and their respective alcohols within the gut. At low doses, 2–200 mg/kg, approximately 80% of the administered dose is metabolized and the metabolites are absorbed into systemic circulation. The remainder of the dose is excreted in feces unchanged. Absorbed metabolites are glucuronidated and rapidly excreted in urine with no evidence of accumulation. The Expert Panel assumes the toxicokinetic studies in rats to be relevant to human exposure of BBP through food. There are no inhalation toxicokinetic studies.

Prenatal exposure studies in rats and mice have indicated that oral exposure on gd 6–15 or 7–15 to high doses of BBP (> 500 mg/kg bw/day) results in reduced fetal growth, prenatal mortality, and visceral, skeletal, and external malformations. NOAELs of 182 mg/kg bw/day and 500 mg/kg bw/day were identified for mice and rats exposed on gd 6– or 7–15, respectively; however, a comparison of sensitivity between species is not possible due to variations in doses administered. Exposure of Wistar rats during the entire gestation period resulted in a developmental NOAEL of 185 mg/kg bw/day. Oral prenatal studies with the BBP metabolites MBuP and MBeP have demonstrated qualitatively similar results to BBP and suggest that the metabolites are associated with the observed developmental toxicity. None of the studies examined the postnatal effects on the male reproductive system. This is of concern because standard prenatal studies do not detect effects such as altered anogenital distance, retained nipples, delays in acquisition of puberty (preputial separation), and malformation of the post-pubertal male reproductive system. Such effects have been observed with DBP, the monoester metabolite of which is the same as one of the metabolites of BBP. Therefore, the Expert Panel is not confident in the NOAELs obtained from the existing BBP developmental studies. In studies using DBP, a NOAEL of 50 mg/kg bw/day was identified with male reproductive tract anomalies observed at higher doses.

The data indicate that BBP is a reproductive toxicant in adult male rats as evidenced by testicular lesions, reduced sperm counts, and increased infertility following exposure to oral doses exceeding the NOAEL of 500 mg/kg bw/day. Effects on the reproductive system of adult female rats are less certain. There were no reproductive effects in female rats exposed orally to 446–1,078 mg/kg bw/day from 2 weeks prior to mating through lactation. However, in a second study, the number of females conceiving litters was reduced following exposure to 1,000 mg/kg bw/day by gavage. The data do not permit clear delineation as to whether this was male- or female-related, although clear evidence of testicular toxicity was seen. The Expert Panel notes that the database does not allow for a complete evaluation of reproductive effects due to the lack of a multigeneration study that exposes animals during the development of the reproductive system. Lower NOAELs may be observed in studies with late gestational exposure and complete postnatal examination of the male reproductive system.

The Expert Panel believes the database is sufficient to judge that oral exposure to BBP can cause reproductive toxicity in adult rats and developmental toxicity in rats and mice. These data are assumed to be relevant to humans. The Panel is not confident that the lowest dose at which developmental toxicity, specifically effects on the developing male reproductive tract, has been established.

Lastly, the Panel is aware of studies performed at CDC using urine from human subjects. Results of these studies were given in an oral presentation in Copenhagen, Denmark, in May, 2000. MBuP values in the urine of women of child-bearing age were among the higher values. Such data, when published, should serve to improve our ability to assess phthalate exposure in the general population.

5.3 Expert Panel Conclusions

BBP is used in the manufacture of vinyl tile and PVC to make food conveyor belts, carpet tile, tarps, weather stripping, and, to a limited extent, vinyl gloves and adhesives. BBP can be released into the environment during production, incorporation into products, use, and disposal.

The best estimate of exposure to the general public is 2 µg/kg bw/day from food in adults, with exposures to infants and children possibly up to three-fold higher, with negligible exposures from infant formula, dermal absorption, drinking water, or soil intake. Occupational exposure is estimated at 286 µg/kg bw/workday.

Median indoor air levels (from 1 study of 125 southern California homes) were 0.034–0.035 ng/m^3, outdoor ambient air levels from 65 of these homes were 5.3–6.7 ng/m^3 for the 90^{th} percentile, and below the estimated detection limit of 0.051 ng/ m^3 for the median BBP level. The Expert Panel has low-to-moderate confidence in the completeness of the exposure database from which these estimates were made, based on the range of values provided by different sources for the same route of exposure and on the age of the data available for exposures for food and food packaging.

With regard to developmental and reproductive toxicity, the database is sufficient to judge that oral exposure to BBP can cause developmental toxicity in rats and mice, and reproductive toxicity in rats. The current database is insufficient to fully characterize the potential hazard. The lowest NOAELs identified by the Panel for developmental toxicity were 182 mg/kg bw/day in CD-1 mice and 185 mg/kg/day in Wistar rats. Given the low exposures to adults and the high dose designated as the NOAEL, the Panel agrees that there is an adequate database to provide negligible concern for male reproductive effects from adult exposure. There is not an adequate database to determine NOAELs/LOAELs for male or female reproductive effects from perinatal exposure. BBP and DBP have a common metabolite, MBuP, and the Panel noted that orally-administered DBP causes male reproductive tract malformations at 100 mg/kg bw/day (LOAEL). Data gaps did not permit the Panel to ascribe a level of concern for postnatal consequences from perinatal exposure to BBP. Multigeneration studies now in progress include endocrine-sensitive endpoints and should provide a robust dataset from which to determine the LOAEL/NOAEL and allow subsequent assignment of the level of confidence in these values, and of the level of concern.

5.4 Critical Data Needs

Critical data needs are discussed under two categories: experimental studies and human exposure.

Experimental Studies

1) Multigeneration study. There is a priority need for a multigenerational study that evaluates effects on reproductive development, fertility, and reproductive system structures, including endocrine sensitive parameters, with continuous exposure across multiple generations. Female reproductive effects need to be evaluated explicitly.

The Expert Panel is aware that a two-generation study under current testing guidelines and with evaluation of endocrine-sensitive endpoints in rats was recently completed in Japan and that a similar study is underway in the United States. It is likely that data needs cited in 1) above would be fulfilled by the results from these studies.

Human Exposure

1) No studies of humans were found. Occupationally-exposed cohorts might be located, but would be of limited utility if the major exposure source is food. Priority should be given to studies on occupational exposures and general population indoor exposures from BBP-releasing materials.

2) Better exposure data. The Panel is aware of emerging data for human exposure (by analysis of urinary phthalate metabolites from a human reference population) that may alter existing exposure estimates, particularly for women of child-bearing age.

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7.0 WEB TABLES