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

DI *n* BUTYL PHTHALATE

OCTOBER, 2000 NTP-CERHR-DBP-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.
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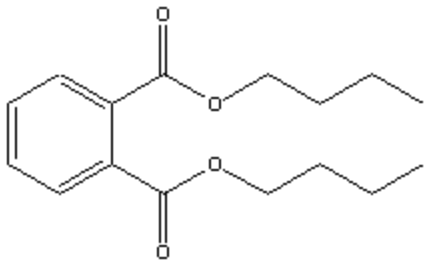
Di-n-Butyl Phthalate

1.0 CHEMISTRY, USAGE, AND EXPOSURE

1.1 Chemistry

Figure 1: Chemical Structure of Di-n-Butyl Phthalate



Di-n-butyl phthalate (DBP) (CAS RN 84-74-2) is produced through the reaction of n-butanol with phthalic anhydride (*1*).

Table 1: Physicochemical Properties of DBP

Property	**Value**
Chemical Formula	$C_{16}H_{22}O_4$
Molecular Weight	278.35
Vapor Pressure	2.7×10^{-5} mmHg at 25 ^{o}C
Melting Point	-35 ^{o}C
Boiling Point	340 ^{o}C
Specific Gravity	1.042
Solubility in Water	Slight: 11.2 mg/L
Log K_{ow}	4.45

(*2*)

1.2 Exposure and Usage

Overview

According to the American Chemistry Council (ACC, formerly CMA) (*1*), DBP is used mainly as a coalescing aid in latex adhesives. DBP is also used as a plasticizer in cellulose plastics and as a solvent for dyes. Although there was limited use of DBP in poly-vinyl chloride (PVC) plastics during the 1970's and 1980's, it is not currently used as a plasticizer in PVC. Release of DBP to the environment can occur during its production and also during the incorporation of the phthalate into plastics, adhesives, or dyes. Because DBP 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 intended for consumption by humans or livestock and can thus enter the food supply.

General Population Exposure

Exposure of the general population to DBP has been estimated by at least four authoritative sources: the International Program on Chemical Safety (*3*), the UK Ministry of Agriculture, Fisheries, and Food (MAFF) (*4, 5*), Health Canada (*6*), and the US Agency of Toxic Substances and Disease Registry (*7*). Levels of DBP in exposure media, assumptions used in exposure calculations, and estimated exposure levels are detailed in Table 2 (*3*), Table 3 (*7*), and Table 4 (*6*)**.**

Table 2: IPCS Exposure Estimates for Adults

	Ambient Air	Indoor Air	Drinking Water	Food
DBP Concentration in Media	0.0045–0.0062 μg/m^3	0.420 μg/m^3	<1.0 μg/L	Various levels in a Canadian market basket survey. (See text)
Assumptions	22 m^3 inhaled/day; 64 kg bw; 4/24 hours outdoors	22 m^3 inhaled/day; 64 kg bw; 20/24 hours indoors	1.4 L/day intake; 64 kg bw	Various intake rates for different food types; 64 kg bw
Estimated Doses μg/kg bw/day	0.00026–0.00036	0.120	<0.02	7

(*3*)

Table 3: ATSDR Exposure Estimates for Adults

	Ambient Air	Drinking Water	Fish
DBP Concentration in Media	0.003–0.006 μg/m^3	0.2 μg/L	78–200 μg/kg
Assumed Intake Rate	20 m^3/day/70 kg adult	2 L/day/70 kg adult	6.5 g/day/70 kg adult
Assumed Absorption Fraction	0.5	0.9	0.9
Estimated Dose (μg/kg bw/day)	0.0005–0.0009	0.005	0.007–0.02

(*7*)

Table 4: Health Canada DBP Exposure Estimates

	ESTIMATED INTAKE DBP (μg/kg bw/day)				
Substrate/Medium	0.0–0.5 years old	0.5–4 years old	5–11 years old	12–19 years old	20–70 years old
Ambient Air*	0.00030	0.00040	0.00041	0.00038	0.00034
Indoor Air	0.68	0.91	0.1	0.87	0.78
Drinking Water	0.11	0.062	0.033	0.022	0.021
Food	1.6	4.1	3.2	1.4	1.1
Soil*	0.0070	0.0054	0.0018	0.00049	0.00040
Total Estimated Intake	2.4	5.0	4.3	2.3	1.9

* Value represents the upper range of the estimates. (*6*)

As noted in exposure estimates by the IPCS, Health Canada, and ATSDR, the largest source of DBP exposure to the general population is food. Sources of DBP in food include environmental uptake during crop cultivation or migration from processing equipment or packaging materials. IPCS (*3*) and Health Canada (*6*) conducted more comprehensive exposure estimates. Both exposure estimates were based on a 1986 Canadian market-basket survey of 98 different food types. Foods reported to contain DBP included butter (1.5 mg/kg), margarine (0.64 mg/kg), freshwater fish (0.5 mg/kg), cereal products (0–0.62 mg/kg),

baked potatoes (0.63 mg/kg), bananas (0.12 mg/kg), coleslaw (0.11 mg/kg), gelatin (0.09 mg/kg), and white sugar (0.2 mg/kg). DBP exposure through food intake in adults was estimated at 7 μg/kg bw/day by IPCS (*3*) and at 1.9 μg/kg bw/day by Health Canada (*6*). DBP exposures in children were also estimated by Health Canada by applying appropriate assumptions such as intake rates of different food types per age group. Estimated DBP exposure levels from food ranged from 2.3 μg/kg bw/day in children aged 12–19 years to 5.0 μg/kg bw/day in children aged 6 months to 4 years.

MAFF (*4*) estimated adult DBP exposure through dietary intake based on a 1993 survey of fatty foods in the UK. DBP was detected in carcass meat (0.09 mg/kg), poultry (0.2 mg/kg), eggs (0.1 mg/kg), and milk (0.003 mg/kg). 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 DBP intakes were estimated at 13 μg DBP/person/day and 31 μg DBP/person/day, respectively. Specific details describing the calculations and assumptions used were not provided. Using the IPCS-assumed (*3*) adult body weight of 64 kg, the exposure values were converted to 0.20–0.48 μg/kg bw/day.

MAFF also addressed DBP exposure in infants resulting from the consumption of infant formula. A survey published in 1996 reported DBP levels of 0.08–0.4 mg/kg in infant formulas purchased in the UK, while a later survey reported DBP levels of <0.05–0.09 mg/kg (*5, 8*). It is speculated that the drop in DBP concentration occurred because infant formula manufacturers were urged to reduce phthalate levels after MAFF published the results of the 1996 survey. Exposure levels were estimated for infants based on the results from the 1998 survey using assumed body weights of 2.5–3.5 kg at birth and 7.5 kg at 6 months of age. Formula intake rates were determined from manufacturer instructions. Exposure levels for infants were estimated at 2.4 μg/kg bw/day at birth and 1.4 μg/kg bw/day at 6 months of age. Infants in the US are likely exposed to lower levels of DBP through formula than are infants in the UK. In a survey of infant formulas conducted in 1996, DBP levels in the US were approximately 10-fold lower than concentrations measured in the UK and ranged from <5 to 11 ppb (<0.005 to 0.011 mg/kg) (*9*). DBP has also been reported in baby food and breast milk samples collected from Germany and Japan; average values were within ranges reported by MAFF. DBP was measured in 7 German baby food samples (average 0.033 mg/kg), 8 baby formulas (<0.2–0.9 mg/kg; average 0.042 mg/kg), and in the breast milk of 5 mothers from Germany (average 0.035 mg/kg) and 3 from Japan (0.02–0.08 mg/kg). The time period when these samples were collected was not specified (*1*).

In their estimates of dietary exposure, ATSDR (*7*) only considered fish intake because at that time it was the only food source for which reliable data were available. The dietary estimate of 0.007–0.02 μg/kg bw/day was based on DBP levels of 78–200 μg/kg that were reported for fish in studies published between 1973 and 1987.

Levels of DBP in drinking water were estimated to be minimal. DBP exposure to adults through drinking water was estimated at 0.02 μg/kg bw/day by IPCS (*3*) and Health Canada (*6*) based upon a survey of drinking water supplies in Ontario, Canada. Health Canada also estimated DBP exposures through drinking water intake in children and those values ranged from 0.022 μg/kg bw/day in children aged 12–19 years to 0.11 μg/kg bw/day in infants aged 0–6 months. Adult DBP exposure through drinking water was estimated by ATSDR (*7*) at 0.005 μg/kg bw/day. The value was based on a survey of drinking water in 10 unspecified cities prior to 1986.

Mouthing of toys is another potential source of oral phthalate exposure in children. However, use of DBP in toys appears to be rare. In an analysis of 17 plastic toys, DBP was only detected in 1 polyvinyl chloride doll's head at 0.01% by weight (*10*).

Although off-gassing from building materials has been reported as a potential source of DBP exposure through inhalation, exposure has been postulated to be minimal because of the low vapor pressure of DBP. The available data, though minimal, support this view. IPCS (*3*) estimated that adults are exposed to 0.120 µg/kg bw/day through inhalation of indoor air. The estimate was based on the mean air concentration of DBP measured within 125 homes in California in 1990. Health Canada also estimated indoor inhalation exposure to DBP based on a survey of DBP air levels in 9 homes in Montreal (reported in 1985). Exposure to adults was estimated at 0.78 µg/kg bw/day and exposures in children ranged from 0.68 µg/kg bw/day in 0–6 month-old infants to 1.1 µg/kg bw/day in 5–11 year-old children. Exposures to DBP through ambient air was also estimated by IPCS (*3*) and Health Canada (*6*); the values were roughly 2–3 orders of magnitude lower than the indoor air estimates.

Dermal contact with products containing DBP is possible, but absorption through skin is most likely minimal. Studies in rats have demonstrated that absorption of DBP through skin is fairly slow (*11*). An *in vitro* study conducted with rat and human skin has demonstrated that human skin is much less permeable to DBP than is rat skin (*12*).

Caution is required to interpret exposure data for the general population. IPCS has emphasized that dietary intake can vary widely depending on the types of food eaten and the types of material in which the foods are packaged. In addition, the majority of data used to estimate exposure levels was collected 15–20 years ago and may not reflect current exposure levels. Lastly, exposures in children may be higher due to non-dietary intake through mouthing of DBP-containing objects.

Medical Exposure

According to IPCS (*3*), a DBP level of 5 mg/gram was measured in plastic tubing used for oral/nasal feeding. There are no other known uses of DBP in medical equipment.

Occupational Exposure

Exposure in occupational settings can occur through skin contact and by inhalation of vapors and dust. Phthalates are manufactured within closed systems, but workers can be exposed 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. In a limited number of surveys, DBP levels in US plants have ranged from concentrations below the detection limit (0.01–0.02 mg/m^3) to 0.08 mg/m^3 (*3*). OSHA established a permissible exposure limit of 5 mg/m^3 for DBP. Following a review of six studies, the ACC has estimated exposure to DBP in the workplace based upon an assumed level of 1 mg/m^3 during the production of phthalates (*1*). Exposure levels during the incorporation of DBP into plastics are not known. 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 estimate was 143 µg/kg bw/workday for workers employed in phthalate manufacturing. The maximum exposure, by regulation, would be five-fold greater. As stated in the General Exposure section, absorption of DBP through skin is expected to be minimal.

Conclusion

Exposure estimates varied between authoritative bodies. However, in all cases it was evident that food was the primary source of exposure to DBP. ATSDR only considered fish intake, and their exposure estimate therefore provides no information on total dietary exposure. The dietary exposure estimate by MAFF is approximately one order of magnitude lower than estimates by IPCS and Health Canada. 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 DBP levels in foods from different countries; and changing DBP levels in food over time. Table 5 lists the dietary DBP estimates calculated by the different agencies for infants and adults.

Table 5: Comparison of DBP Dietary Estimates

Agency	Exposure in Infants (0–6 months) (µg/kg bw/day)	Exposure in Adults (µg/kg bw/day)
IPCS (*3*)	N/A	7
MAFF (*4, 5, 8*)	1.4–2.4	0.2–0.48
ATSDR (*7*)	N/A	0.007–0.02
Health Canada (*6*)	1.6	1.1

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

There were no human data located for Expert Panel review.

2.1.2 Experimental Animal Data

Multiple evaluations are available for assessing the effects of oral exposure to DBP. A few inhalation and dermal evaluations have also been conducted; these studies are primarily in rats with a few assessments in mice, rabbits, hamsters, and guinea pigs.

Acute studies

The oral LD_{50} for DBP appears to be between 8,000 and 20,000 mg/kg bw in rats (*3*) and the 90-day dermal LD_{50} is 4,200 mg/kg bw in rabbits. Slight irritation was observed in rabbit dermal occlusion studies at 520 mg/kg bw.

Repeat-dose studies

In a 3-month sub-chronic study, 6-week-old Wistar rats, 10 of each sex per dose, were fed a diet containing 0, 400, 2,000 or 10,000 ppm DBP (*13*) (Table WEB-1). In addition to developing a toxicological profile of DBP, a stated purpose of the study was to evaluate possible neurological or testicular toxicity. A battery of standard hematological and clinical chemistry parameters (including thyroid function) was evaluated at points approximately halfway through and at the end of the study. Cyanide insensitive palmityl-CoA oxidation (PCoA) was also determined as a measure of peroxisome proliferation. Urinalyses were performed at the midpoint and at the end of the study. Neurological function, using the EPA functional observation battery, was assessed prior to DBP administration, and on days 34, 59, and 90 of the study.

Dietary consumption was not a factor in the study; nominal daily doses were calculated to be 27 (M) and 33 (F) mg/kg bw/day, 142 (M) and 162 (F) mg/kg bw/day, and 688 (M) and 816 (F) mg/kg bw/day for the three dose groups. Effects were observed only in the high-dose group, 688 (M), and 816 (F) mg/kg bw/day. Statistically significant increases in liver and kidney to body weight ratios were observed in the absence of body weight changes in females. Histologically, a decrease in lipid deposition was noted in hepatocytes; this effect was possibly due to peroxisome-related enzyme increases in the liver. An increase in PCoA activity was confirmed. Serum triglycerides and triiodothyronine were both decreased. RBC, hemoglobin, and hematocrit were transiently decreased in males. No histological effects on testes appropriately preserved in Bouin's fixative were observed.

Neurological function was assessed at three time points during the study and no effects were observed. A LOAEL was observed at 688 (M) and 816 (F) mg/kg bw/day based on multiple impacts and a NOAEL was determined at 142 (M) and 162 (F) mg/kg bw/day.

Marsman (*14*) reported two 13-week, sub-chronic NTP studies using male and female F344 rats. One of the studies was of traditional design; 5–6 week-old rats were exposed to either control or one of four test diets. In the second study, rats placed in a standard sub-chronic design were born and reared by mothers exposed to 10,000 ppm DBP during pregnancy and nursing; at weaning, they were further exposed to a 10,000 ppm DBP diet until 8 weeks of age.

In the standard study, 10 F344 rats/sex were exposed to DBP in their diet for 13 weeks starting at 5–6 weeks of age (Table WEB-2). The dietary levels were 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm (M: 0, 176, 359, 720, 1,540, and 2,964 mg/kg bw/day; F: 0, 177, 356, 712, 1,413, and 2,943 mg/kg bw/day). At the end of the study the rats were killed and necropsied with extensive tissue examination (testes preserved in 10% neutral buffered formalin), hematology and clinical chemistry, sperm morphology, and vaginal cytology parameters were evaluated. Zinc and testosterone levels were measured in sera and testes of all males. An increase in serum albumin was observed in exposed males at 176 mg/kg bw/day, the lowest dose tested. No other effects were seen in either sex at this dose. Adverse effects in males seen at the next highest dose (359 mg/kg bw/day) were evidenced by a decrease in hemoglobin and erythrocyte counts. Severity of the hematological effects, seen only in males, progressed in a dose-response manner at all other doses. Platelets and serum albumin were increased, as were liver and kidney organ to body weight ratios. An increase in PCoA activity was seen in both sexes, and an increase in bile acid was seen in females. Decreases in body weight occurred in males at the 720 mg/kg bw/day dose, the third highest out of 5 treatment levels. Males exposed to 359 mg/kg bw/day and males and females exposed to 712–720 mg/kg bw/day had increased liver and kidney organ to body weight ratios. Hepatic lesions in males and females and testicular lesions were first noted at 712-720 mg/kg bw/day. Testicular lesions consisted of focal seminiferous tubule atrophy in 4/10 males. The chemistry changes noted at the next lower dose (356–359 mg/kg bw/day) continued at this dose (712–720 mg/kg bw/day) with the addition of increases in alkaline phosphatase activity. The histologic hepatic lesions persisted and testicular lesions increased in severity at the higher doses with all males of that dose group affected. Hypospermia of the epididymis was observed at the two highest doses. Decreases in testicular organ weight ratios, testicular zinc, and testosterone were not observed until the 1,540 (M) mg/kg bw/day exposure level. Peroxisome proliferation was noted histologically at the highest dose tested (2,964 [M] and 2,963 [F] mg/kg bw/day). Good dose-response data was available for almost all parameters in this study. A NOAEL of 176 mg/kg bw/day was identified by the Expert Panel.

In the second NTP sub-chronic study, F344/N rats were born and reared by mothers exposed to 10,000 ppm DBP in diet throughout prenatal development and lactation; the weaned rats were then fed a 10,000 ppm diet until 8 weeks of age (*14*) (Table WEB 3). At that time, the male rats, 10 per sex per group, were placed on 1 of 5 diets for an additional 13 weeks that contained 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm

DBP (M: 0, 138, 279, 571, 1,262, or 2,495 mg/kg bw/day; F: 0, 147, 294, 593, 1,182, or 2,445 mg/kg bw/day) (*14*). The sub-chronic exposure doses and the protocols for histopathology, hematology, and chemistry were the same as the NTP sub-chronic study discussed above. The authors concluded that developmental exposure to DBP resulted in neither increased sensitivity nor resistance to DBP exposure during adulthood (compare results in Tables WEB-2 and WEB-3). The Expert Panel notes that there were significant increases in organ to body weight ratios for kidney and liver in females and in testes at the lowest exposure group, 138 (M) and 147 (F) mg/kg bw/day. Such findings were not observed at this dose level in the other sub-chronic study.

The NTP also conducted a sub-chronic study in 6-week-old $B6C3F_1$ mice where 10 mice per sex were fed DBP in the diet for 13 weeks at levels of 0, 1250, 2,500, 5,000, 10,000, and 20,000 ppm (M: 0, 163, 353, 812, 1,601, and 3,689 mg/kg bw/day; F: 0, 238, 486, 971, 2,137, and 4,278 mg/kg bw/day (*14*) (Table WEB-4). Experimental design in this study was similar to the 13-week sub-chronic study in rats. There were no clinical signs related to exposure and all mice survived until the end of the study. Decreases in body weight gain were observed in both sexes fed levels of 812 mg/kg bw/day or higher. Increases in absolute and relative kidney weight were seen in all treated female groups, but absolute kidney weight was decreased in high-dose males. There was no report of histological change in the kidney nor did weights increase with increasing dose. The liver was the only organ identified as a site of DBP toxicity by the study authors. Relative liver weights were increased at doses of 812 mg/kg bw/day and higher. Cytoplasmic alterations consisting of fine eosinophilic granules, more intensely-staining cytoplasm, and increased lipofuschin were observed at the 2 highest doses in males (1,601 and 3,869 mg/kg bw/day) and at the highest dose in females (4,278 mg/kg bw/day). A reduced hematocrit level was observed in high-dose females. Based on decreased body weight gain, the NOAEL is 353 mg/kg bw/day in males. A LOAEL based on increased kidney weight in females is 238 mg/kg bw/day, the lowest dose tested according to the Expert Panel.

In a series of three identical experiments, Walseth and Nilsen (*15*) examined lung and liver effects in groups of five male Sprague-Dawley rats. The rats were exposed for 6 hours/day for 5 days to DBP vapors at 0, 0.5, 2.5, or 7.0 ppm (0, 5.7, 28.4, and 79.5 mg/m^3 as calculated by authors). There were no effects on lung or liver weights. In the lung, there were dose-related decreases in microsomal cytochrome P-450 and cytochrome c-reductase levels in the two highest dose groups. There were no dose-related changes in liver cytochrome levels. A significant decrease in serum levels of alanine aminotranferase (ALAT) and significant increases in serum aspartate aminotranferase and albumin levels were observed, but the authors indicated that there was no evidence of liver cell damage. The authors concluded that the lung is the main target organ following inhalation exposure to DBP.

2.2 Toxicokinetics

Phthalate Moiety

Absorption

Humans: Dermal. In an *in vitro* study, human skin absorption rate was reported as 0.07 $\mu g/cm^2$/hour (*12*) which was considered "slow."

Humans: Oral. DBP was detected in blood from humans following ingestion of foodstuffs containing DBP (*3*). Background levels of DBP in human blood were much higher following exposure. Unfortunately, the authors measured only the parent compound so there is no estimate of total DBP equivalents absorbed in this

study. Similarly, levels of DBP in human adipose tissue were studied (*16*); again total DBP equivalents were not calculated.

Rodents: Dermal. Dermal absorption of DBP was studied in Fischer 344 rats by applying 30–40 mg/kg radiolabeled DBP to the skin (administration site occluded) and measuring the radioactivity in urine (*11*). Approximately 10–12% of the dose was excreted in urine per day with approximately 60% of the dose excreted within 1 week. Thirty-three percent of the dose was present at the application site 1 week following treatment.

Rodents: Oral. The extent of intestinal absorption of phthalate esters has been estimated by monitoring urinary excretion of the parent compounds or their metabolites after orally administering a known amount of the radiolabeled compound. Greater than 90% of radioactivity following an oral dose of DBP in rats is recovered in the urine within 2 days, indicating nearly complete intestinal absorption of this compound over a range of administered doses (*17*). This is consistent with the general observation that dialkyl phthalate esters are well absorbed following oral dosing. It is generally accepted that orally-ingested phthalate diesters are quantitatively hydrolyzed by gut lipases and absorbed almost entirely as the corresponding monoester.

Biotransformation

Humans. In a study comparing the relative rates of monohydrolysis of DBP by rat, baboon, and human gut preparations, Lake et al. (*18*) demonstrated that these species possess similar intrinsic lipase activity. Rates observed in human intestinal preparations were similar enough to the other species to expect that human intestinal metabolism of DBP would result in absorption of the monoester similarly to rats. The activity of pancreatic lipase was not assessed, so the quantitative relationships of this study to *in vivo* exposure cannot be accurately determined (*18*).

Rodents. Dialkyl phthalates including DBP were found to be metabolized to the monoesters by enzymes present in many tissues. It is generally accepted that orally-ingested phthalate diesters are quantitatively hydrolyzed by lipases in the wall of the small intestine and pancreatic lipases and not by gut flora. Absorption occurs almost entirely as the corresponding monoester (*19*).

Metabolites of DBP include monobutylphthalate, monobutylphthalate glucuronide, o-phthalic acid and oxidized monobutylphthalate glucuronide metabolites (*17*).

Distribution

Humans. No human data were located for Expert Panel review.

Rodents. DBP is rapidly cleared following oral or intravenous (IV) administration. There is little or no bioaccumulation observed. Radioactivity associated with DBP administration can be found in the GI tract and excretory organs of the liver and kidney, and in fat. Liver, kidney, and the GI tract probably accumulate the phthalate esters as a mechanism of excretion and not as depots (*20*). One week following dermal treatment of Fischer 344 rats with 30–40 mg/kg radiolabeled DBP, no tissues examined contained more than 2% of the administered dose (*11*).

Pregnant Rodents. Saillenfait et al. (*21*) studied metabolism and placental transfer of ^{14}C-DBP, administered by gavage on gestation day (gd) 14 at 500 or 1,500 mg/kg to Sprague-Dawley rats. Radioactivity peaked followed by a rapid decline in all tissues within 1–2 hours of administration. Maternal plasma had the highest peak concentration; all tissue levels were less than 7% of peak concentrations by 24

hours. Fifty-five percent and 29% of a 500 mg/kg ^{14}C dose were detected in urine and feces respectively in 24-hour samples; there was a slight increase to about 60% in urine at 48 hours, whereas fecal values did not change. Radioactivity in placenta, embryo, and amniotic fluid were 0.3, 0.15, and 0.2% of the administered dose, respectively. Concentrations in placenta and embryo did not exceed 30 and 21% of maternal plasma levels. The 1,500 mg/kg dose indicated slower absorption from the gastrointestinal tract; total fecal radioactivity was not affected, although there was lower excretion in urine over 48 hours. In maternal plasma, placental, and embryonic tissues, monobutyl phthalate (MBuP) and its glucuronide represented most of the DBP-derived activity. MBuP Levels ranged from 50 to 95%, dependent upon the time after administration when samples were taken. In contrast, unchanged DBP accounted for less than 1%. The authors speculate that the lower levels of MBuP glucuronide in embryonic tissues compared to those in maternal plasma could reflect limited placental transfer or limited ability to conjugate this substrate. Levels of radioactivity in placenta and embryos associated with DBP administration were approximately 65% of the levels found in maternal serum and there was no bioaccumulation of radioactivity observed in the embryonic tissues. DBP, MBuP, and MBuP-glucuronide were present in embryonic tissues at levels lower than were found in maternal plasma. MBuP accounted for most of the radioactivity recovered in maternal plasma, placenta, and embryos, which is consistent with the hypothesis that MBuP is the ultimate teratogenic species *in vivo*.

Distribution following IV exposure produces a different distribution pattern than that observed following oral administration. Since DBP is not in direct contact with gut esterases, metabolism to the monoester is slowed. This produces more DBP-associated radioactivity to distribute to lungs and blood in addition to liver and kidney. Radioactivity was detectable in adipose tissue 7 days after IV exposure (*22*). The difference between the oral and IV distribution probably reflects a higher concentration of parent DBP reaching adipose tissue following IV exposure, which would be expected to distribute to lipophilic tissues such as adipose tissue.

Excretion

Humans. No human data were located for Expert Panel review.

Rodents. The primary route of MBuP, the major DBP metabolite, elimination in rodents and humans is urinary excretion. The monobutylphthalate glucuronide appears to be the primary metabolite identified in rat urine (*23*). MBuP is excreted into the bile (about 45%), but only about 5% is eliminated in the feces, indicating that efficient enterohepatic recirculation occurs (*17*). Biliary metabolites of DBP include monobutylphthalate, monobutylphthalate glucuronide, and oxidized monobutylphthalate glucuronide metabolites (*17*). Following dermal exposure of rats to DBP, urine was the primary route of excretion with the excretion rate remaining nearly constant at 10–12% of the dose excreted per day (*11*).

Mice are known to excrete higher amounts of glucuronidated phthalate ester metabolites than rats and primates excrete higher levels of glucuronidated phthalate ester metabolites than mice (*24*). There appears to be little retention of DBP or MBuP in tissues of rats treated with DBP for 12 weeks (*20*).

Models

A physiologically-based pharmacokinetic (PBPK) model of the tissue distribution of DBP and its monoester metabolite, MBuP, in rats administered DBP by various routes has been developed by Keys et al. (*25*). The model is based on an earlier model developed by the same group for DEHP and its metabolite, MEHP (*26*). It includes a combined perfusion-limited and pH trapping mechanism for uptake of MBuP into tissues, and it provides a valuable tool for extrapolations of tissue doses among various routes and rates of exposure. With modification, the model can be used to extrapolate doses to target tissues among various species and ages and between genders and gravid vs non-gravid females. The model allows estimation of the internal dose to

specific target tissues for the evaluation of risk, rather than using total exposure or total internal dose as a risk estimate.

Side Chain-associated Toxicokinetics (butanol)

Butanol, a metabolite of DBP, is a primary alcohol that is easily oxidized to butyric acid (n-butanoic acid) by alcohol dehydrogenase and aldehyde dehydrogenase. Further metabolism by oxidation pathways converts butyric acid into acetyl-CoA conjugates in intermediary metabolism pathways with no toxicological importance (*27*).

2.3 Genetic Toxicity

DBP has tested negative or marginally positive in gene mutation and chromosomal aberration studies. The ASTDR (*7*) concluded that DBP may be weakly mutagenic. The significance of these findings is not known because *in vivo* genotoxicity studies have not been conducted. The Woodward et al. (*28*) review concluded that the evidence indicates that DBP is not directly genotoxic, but noted it does cause increases in sister chromatid exchanges and small increases in the incidence of gaps and breaks. However, the effect does not appear to be dose-related (*29*). IPCS (*3*) reviewed a number of mutagenic and related endpoints for DBP and concluded that the weight of the evidence indicated that DBP is not genotoxic. DBP was positive in the L5178Y mouse lymphoma assay in the presence, but not in the absence, of an Aroclor-induced rat liver activation system (S9) (*30*). The authors conclude that the positive activity was likely the result of *in vitro* metabolism of the DBP to an aldehyde, and therefore, that the results may not represent any real potential for *in vivo* genotoxicity. DBP is not mutagenic in the Salmonella/mammalian microsome mutagenicity assay (*31*), and was negative in the Balb/3T3 cell transformation assay (*30*).

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

A number of studies have evaluated DBP for both prenatal and postnatal developmental toxicity; the vast majority of studies have been performed in the rat using the oral route of exposure. In most cases, the doses were high (> 0.5% in diet; > 500 mg/kg bw/day), and the number of animals per dose group was small (10–15).

3.2.1 Prenatal Development

DBP

Results from a set of investigations in mice have been reported by Shiota et al. (*32*) and Shiota and Nishimura (*33*) (WEB Table 5). They evaluated the effects of oral exposure to DBP in concentrations of 0, 0.05, 0.1, 0.2, 0.4, and 1.0% in the diet. On the day a cervical plug was observed (gd 0), female ICR-JCL mice commenced eating the DBP diet until they were killed on gd 18. Using food consumption data, the authors calculated mean daily intake of DBP to be 0, 80, 180, 350, 660, and 2,100 mg/kg bw/day. Six-to-nine litters were examined per dose group, except that 15 litters were examined from the highest dose group. Food intake levels were not affected in pregnant dams. Maternal weight gain was significantly reduced at the high dose (2,100 mg/kg bw/day), but the effect may have been secondary to increased fetal loss. Resorptions (prenatal mortality) were significantly increased (98.4%) in the high-dose group. At this dose, malformations in 2/3 surviving fetuses (increase not statistically significant) were limited to neural tube defects (exencephaly and spina bifida, to which murine species are predisposed). Delayed ossification was observed at all dose levels as indicated by a reduction in the number of ossified coccygia in treated fetuses (n=9.4, 5.1, 4.5, 6.0, and 2.6 in the control to 660 mg/kg bw/day groups). Reduced fetal body weight was observed at the two highest doses. Because ossification was delayed at all dose levels, a developmental NOAEL could not be identified for this study and, therefore, a LOAEL of 80 mg/kg bw/day was selected by the Expert Panel. However, the authors stated that "the maximum non-embryotoxic dose" was 370 mg/kg bw/day. The maternal NOAEL and LOAEL were identified as 660 and 2,100 mg/kg bw/day, respectively.

Ema et al. (*34-36*) used Wistar rats to evaluate the developmental toxicity of DBP by exposure through gavage and feed. In all studies, dams were sacrificed on gd 20–21 and examined for implantation sites. Fetuses were weighed and examined for external, skeletal, and visceral malformations. In one Ema (*34*) study, 12 dams/group were gavaged with 0, 500, 630, 750, or 1,000 mg/kg bw/day (0, 1.80, 2.27, 2.70, or 3.60 mmol/kg bw/day) on gd 7–15 (Table WEB 6). Gestational weight gain was reduced in dams of the 630 mg/kg bw/day group and adjusted weight gain (dam weight not including gravid uterus) was reduced in dams exposed to 750 mg/kg bw/day and higher. Complete resorptions occurred in 2/12, 10/12, and 9/9 litters of the 630, 750, and 1,000 mg/kg bw/day dose groups, respectively, thus resulting in decreased live fetuses/litter. Fetal weight was reduced in groups exposed to 630 mg/kg bw/day and higher. External malformations, consisting entirely of cleft palate, were increased in the 750 mg/kg bw/day group. Maternal and developmental NOAELs and LOAELs of 500 and 630 mg/kg bw/day, respectively, were identified.

Another study conducted by Ema et al. (*36*) is of particular interest because it examines additional endpoints including anogenital distance and testicular descent (Table WEB 7). In this study, 11 dams/group were fed diets containing 0, 0.5, 1.0, or 2.0% DBP on gd 11–21. Authors estimated daily intake rates of 0, 331, 555, and 661 mg/kg bw/day for the control to high-dose groups, respectively. Maternal gestational and corrected weight gain were reduced in dams exposed to 555 mg/kg bw/day and higher and were accompanied by a reduction in food intake. Fetal weight was reduced and the incidence of external malformations (cleft palate) and skeletal malformations (fused sternebrae) were increased in the 661 mg/kg bw/day dose group. Reduced anogenital distance and increased incidence of undescended testes were observed in male fetuses exposed to 555 and 661 mg/kg bw/day. The maternal and developmental NOAEL and LOAEL of 331 and 555 mg/kg bw/day, respectively, were identified for this study.

The two remaining studies by Ema et al. (*35, 37*) focused on the time- and dose-dependency of DBP developmental toxicity. In the studies, groups of 10–13 pregnant rats were gavaged with 0, 750, 1,000, 1,250, or 1,500 mg/kg bw/day on gd 7–9, 10–12, or 13–15. Resorptions were increased in all dose groups at all time points. All dams treated with 1,500 mg/kg bw/day experienced complete litter resorptions. However, the types and frequencies of malformations varied according to the exposure time course.

Treatment on gd 10–12 did not result in an increased malformation rate. Treatment with doses of 750 mg/kg bw/day and higher on gd 7–9 resulted in increased skeletal malformations (fusion or absence of vertebral arches and ribs). Administration of 750 mg/kg bw/day and higher on gd 13–15 resulted in the greatest incidence of teratogenicity, including increased external malformations (cleft palate) and skeletal malformations (fusion of sternebrae).

Saillenfait et al. (*21*) exposed Sprague-Dawley rats (27 per group) to a single administration of DBP by gavage on gd 14 at 0, 500, 1,000, 1,500, or 2,000 mg/kg body weight. Increased resorptions at 1,500 and 2,000 mg/kg and reduced fetal body weights at 2,000 mg/kg were observed. Skeletal variations were also increased at these doses. Key aspects of the paper were studies on metabolism and placental transfer of ^{14}C-DBP, administered by gavage on gd 14 at 500 or 1,500 mg/kg. The toxicokinetic data are presented in Section 2.2. The authors concluded that their data support the view that MBuP may be the proximate toxicant.

Developmental effects were also noted in reproductive toxicity studies, which are discussed in detail under Section 4. In a continuous-breeding study, two generations of Sprague Dawley rats were exposed to 0, 80, 385, or 794 mg/kg bw/day through diet during a 98-day mating period (*38*). Maternal effects were only observed in the high-dose group and included a decrease in body weight for both generations and increased liver and kidney weights in F_0 dams. Developmental effects included a reduction in litter size in all dose groups and in live pup weight in the two highest doses of F_1 rats. F_2 pups in all treatment groups experienced a reduction in body weight. A developmental LOAEL of 80 mg/kg bw/day and a maternal NOAEL of 385 mg/kg bw/day were identified.

A similar continuous-breeding study was conducted in one generation of CD-1 mice treated with 0, 53, 525, and 1,750 mg/kg bw/day in diet (*39, 40*). Fetal effects that were observed only at the highest dose included reductions in litter size, live pups/litter, and pup weight. The developmental NOAEL was identified as 525 mg/kg bw/day, but a maternal NOAEL could not be identified because necropsies were only conducted in the high-dose group. In a multigeneration reproductive study, Long Evans Hooded rats were treated with 0, 250, or 500 mg/kg bw/day DBP by gavage from the time they were weanlings through the time that they nursed their own litters (*41*). Maternal toxicity was not reported. Developmental effects included malformations in reproductive organs, kidneys, and eyes in F_1 rats and reductions in F_2 litter size in all dose groups. The developmental LOAEL was identified as 250 mg/kg bw/day.

<u>MBuP</u>

The prenatal developmental effects of administering MBuP by gavage in the Wistar rat were reported (*42, 43*). The Expert Panel noted that some of the doses used in these studies were equimolar equivalents to doses used in earlier studies with DBP (described above). Ema et al. (*42*) studied doses of 0, 250, 500, and 625 mg/kg bw/day (0, 1.13, 2.25, or 2.80 mmol/kg bw/day) on gd 7–15. They observed maternal toxicity at the two highest doses expressed as reduced weight gain and feed consumption. Also, at these doses there were significant increases in post-implantation loss/litter and decreases in live fetuses/litter and fetal body weight/litter. Fetal malformations were increased, with cleft palate, deformed vertebral column, and dilated renal pelves the predominant findings. A maternal and developmental NOAEL and LOAEL of 250 and 500 mg MBuP/kg bw/day, respectively, were identified for this study.

Ema et al. (*43*) then followed up with evaluation of stage specificity studies by administering MBuP at doses of 0, 500, 625, or 750 mg/kg bw/day on gd 7–9, 10–12, or 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 at the 500 and 750 mg/kg bw/day doses on gd 7–9 and 13–15. Increased skeletal malformations were observed at 500, 625, and 750 mg/kg bw/day on gd 7–9 and at 625 and 750 mg/kg bw/day on gd 13–15 (deformed cervical vertebrae were predominant on gd 7–9).

Cleft palate and fused sternebrae were observed on gd 13–15. These results are consistent with the findings for DBP and imply that MBuP (and/or subsequent metabolites) may account for the developmental toxicity (embryolethality and malformations) for DBP.

3.2.2 Postnatal Development

DBP

Marsman et al. (*14*) exposed F344/N rats and B6C3F_1 mice to high dietary concentrations of DBP during gestation and lactation. Both species were exposed to 0, 1,250, 2,500, 5,000, 7,500, 10,000, and 20,000 ppm. Dosages in mg/kg bw/day were estimated by using average values from 2 NTP studies that included a food intake rate of 14.8 g/day and a body weight of 203.71 g for rats and a food intake rate of 7.18 g/day and body weight of 39.63 g for mice (*44-46*). The dosages are listed in Web Tables 8 and 9. After weaning on pnd 21, up to 10 F_1 pups/group were fed a diet with a DBP concentration identical to that fed to their dams fed for an additional 4 weeks. Author-calculated doses for pups were: 143, 284, 579, 879, and 1,165 mg/kg bw/day for male rats; 133, 275, 500, 836, and 1,104 mg/kg bw/day for female rats; 199, 437, 750, 1,286, and 3,804 mg/kg bw/day for male mice; and 170, 399, 714, and 1,060 mg/kg bw/day for female mice. Complete necropsies were performed on one rat and one mouse pup of each sex per litter at weaning and on all pups at the end of the 4-week post-weaning dietary exposure. Organ weights were obtained on major organs, including testis. Histopathological examination was performed on a broad array of tissues from all animals in the control and highest exposure group. In addition, the epididymis of rats from the 2,500, 5,000 and 7,500 ppm groups were studied.

For the rats (Table WEB-8), gestational index was reduced (fewer live litters) at 5,000 and 20,000 ppm, and gestational length was reduced at 5,000 ppm. Litter size and postnatal survival were reduced at 10,000 and 20,000 ppm. All F_1 pups died by pnd 1 in the 20,000 ppm group. Male pup body weights were reduced during lactation in dose groups receiving 7,500 ppm and higher. In the post-weaning period, relative liver and kidney weights were increased in female offspring exposed to ≥2,500 and ≥5,000 ppm (275 and 500 mg/kg bw/day), respectively. Increased liver and kidney to body weight ratios were observed in males of all dose groups. Reduced relative testis weights were observed at the highest dose. Mild-to-marked hypospermia was seen in all males at the 879 and 1,165 mg/kg bw/day doses and in 4/10 males of the 579 mg/kg bw/day dose group. There were no histopathological lesions observed in liver or kidney. Acquisition of vaginal patency and preputial separation were not assessed. Based on increased liver and kidney to body weight ratios in all treated males, no NOAEL was identified.

For B6C3F_1 mice (Table WEB-9), length of gestation was increased at 2,500 ppm and higher with 75 and 95% of litters lost at 10,000 and 20,000 ppm. Decreases were observed in litter size and pup body weights at 2,500, 7,500, and 10,000 ppm. In the F_1 post-weanling phase, males exhibited increased relative liver weights (one surviving male pup at 10,000 ppm exhibited hepatic lesions), and females exhibited increased relative kidney weights at 1,250 ppm (170–199 mg/kg bw/day) and higher. Except for liver lesions in the male at 10,000 ppm, no histpathological changes were observed, including in the testis. No NOAEL was identified.

Taking note of the Wine et al. (*38*) continuous-breeding study results (see Section 4), Mylchreest et al. (*47*) followed up the study using comparable dose levels (Table WEB 10). However, three important changes in experimental design were introduced: 1) shortening the exposure period to include only gestation and lactation; 2) using gavage (with corn oil) to control exposure more closely; and 3) including more sensitive endpoints of reproductive development, such as markers of sexual maturation. Thus, pregnant CD rats (10 per group) were administered DBP by gavage at 0, 250, 500, or 750 mg/kg bw/day from gd 3 until pnd 20.

At birth, pups were counted, sexed, weighed, and examined for signs of toxicity. Sexual maturity was assessed by observing age of vaginal opening and preputial separation in females and males, respectively. Estrous cycles were assessed in females for 2 weeks. The F_1 rats were sacrificed at 100–105 days of age. Necropsies were conducted on all males and up to three females per litter. A histological examination of sex organs was conducted on all rats with lesions and up to two unaffected rats per litter. Testes were preserved in Bouin's fixative.

Maternal body weight gain was comparable to controls throughout the dosing period. At 750 mg/kg bw/day, the number of live pups per litter at birth was decreased and maternal effects on pregnancy and postimplantation loss are likely to have occurred. Anogenital distance was decreased at birth in the male offspring at 500 and 750 mg/kg bw/day. The epididymis was absent or underdeveloped in 0, 9, 50, and 71% of adult offspring (100 days old) at 0, 250, 500, and 750 mg/kg bw/day, respectively, and was associated with testicular atrophy and widespread testicular germ cell loss. Hypospadias occurred in 0, 3, 21, and 43% of males, and ectopic or absent testes in 0, 3, 6, and 29% of males at 0, 250, 500, and 750 mg/kg bw/day, respectively. Absence of prostate gland and seminal vesicles as well as small testes and seminal vesicles were noted at low incidence in the 500 and 750 mg/kg bw/day dose groups. Dilated renal pelves, frequently involving the right kidney, were observed in all DBP dose groups. Vaginal opening and estrous cyclicity were not affected in the female offspring, although low incidences of reproductive tract malformations, mainly involving development of the uterus, were observed in 2 rats and 1 rat at the 500 and 750 mg/kg bw/day doses, respectively.

In the Mylchreest et al. 1998 study (*47*), all exposed groups showed adverse effects on male reproductive tract structure and indices of puberty. Based on this, the LOAEL in this study is 250 mg/kg bw/day/day. Based on the relationship between testis weight/histopathology and sperm production, the relationships between sperm numbers and fertility (*48*), and the number of major malformations of the reproductive tract, it is expected that at least the high- and mid-dose animals would be sub-fertile. The Panel's confidence in the quality of the study is high.

In a subsequent study, Mylchreest (*49*) reduced DBP exposure to just late gestation (gd 12–21) and compared the effects of DBP to the pharmacological androgen receptor antagonist, flutamide (Table WEB-11). Pregnant CD rats received DBP at 0, 100, 250, or 500 mg/kg bw/day by gavage with corn oil (n =10) or flutamide at 100 mg/kg bw/day (n =5) on gd 12–21. Males were killed at approximately 100 days of age and females at 25–30 days of age. In F_1 males, DBP (500 mg/kg bw/day) and flutamide caused hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of the seminiferous epithelium, and interstitial cell hyperplasia of the testis. Agenesis of the epididymis was also observed at 250 mg/kg bw/day. Flutamide and DBP (250 and 500 mg/kg bw/day) also caused retained thoracic nipples and decreased anogenital distance. Interstitial cell adenoma occurred at 500 mg/kg bw/day in two males from the same litter. The only effect seen at 100 mg/kg bw/day was delayed preputial separation. The low incidence of DBP-induced intra-abdominal testes contrasted with the high incidence of inguinal testes seen with flutamide. Thus, the prenatal period is sensitive for the reproductive toxicity of DBP. Uterine and vaginal development in female offspring was not affected by DBP treatment. There were no signs of maternal toxicity with the exception of a 16% body weight loss at the time of birth and complete fetal mortality in 1 dam of the 500 mg/kg bw/day group. In addition, testicular focal interstitial cell hyperplasia and an adenoma (in 1 male) were observed in males at 500 mg/kg bw/day at 3 months of age. A LOAEL of 100 mg/kg bw/day was established in this study, based on delay in preputial separation at all dose levels. A NOAEL was not established.

To identify a NOAEL for DBP-induced developmental toxicity, Mylchreest et al. (*50*) gavaged 19–20 Sprague-Dawley CD rats/group with 0, 0.5, 5, 50, or 100 mg/kg bw/day and 11 Sprague-Dawley CD rats with 500 mg/kg bw/day in corn oil on gd 12–21 (Table WEB 12). Dams delivered and pups were weighed

and examined at birth. After the pups were weaned, dams were killed and implantation sites and organ weights were evaluated. Pups were weighed weekly and examined for sexual maturation. When pups reached puberty they were killed and organ weights were determined. The testes and epididymides were preserved in Bouin's solution and examined histologically.

There was no evidence of maternal toxicity at any dose. In male pups, the incidence of retained aereolas or nipples was increased at the 100 and 500 mg/kg doses (31% of rats in 16/20 litters and 90% of rats in 11/11 litters, respectively). Malformations observed in the highest dose group included: hypospadias (9% of rats in 4/11 litters); and agenesis of the epididymis (36% of rats in 9/11 litters), vas deferens (28% of rats in 9/11 litters), and prostate (1/58 rats). Reduced testis, epididymis, prostate, and levator muscle weight and reduced anogenital distance in males were also observed at the high dose. Histological effects in high-dose males included interstitial cell hyperplasia (35% of rats in 3/5 litters), adenoma (1/23 rats), and seminiferous tubule degeneration (56% of rats in 3/5 litters). The single case of seminiferous tubule degeneration in the 100 mg/kg bw/day group was considered equivocal because the lesion does occur spontaneously in a small number of Sprague-Dawley rats. In female offspring, the age of vaginal opening and reproductive organ weight and histology were unaffected. A developmental NOAEL and LOAEL of 50 and 100 mg/kg bw/day, respectively, and a maternal NOAEL of 500 mg/kg bw/day, were identified for this study.

The qualitative findings of Mylchreest et al. (*47*, *49*, *50*) were confirmed by Gray et al. (*41*) who gavaged 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3 with corn oil vehicle or DBP at 500 mg/kg bw/day, and groups of 4–6 Long Evans Hooded rats with 0 or 500 mg/kg bw/day on gd 16–19.

Gray et al. (*41*) also compared the effects of DBP at 500 mg/kg bw/day and an equimolar concentration of 750 mg/kg bw/day DEHP administered by gavage to 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3 (WEB Table 13). The male F_1 pups were evaluated for sexual maturation and were then killed and necropsied at 5 months of age. Organ weights were measured and a histological examination of reproductive organs (preserved in Bouin's) was conducted. The presence or absence of maternal toxicity was not described. Effects in F_1 males are summarized in Table 6 and included reduced anogenital distance, and increases in percent areolas and nipples at birth, numbers of areolas and nipples at birth and adulthood, hypospadias, and testicular and epididymal atrophy or agenesis. A decrease in weight for prostates, epididymides, testes, penis, and the levator ani muscle was also observed in the treated rats. None of the control pups were found to have nipple development, malformations, or testicular degeneration. DEHP and DBP exposure resulted in effects that were qualitatively similar. Several males from DEHP-treated dams also had hemorrhagic testes. The authors stated that DEHP was considerably more toxic to the male reproductive system than DBP.

Table 6: Comparison of Reproductive Effects following *in Utero* Exposure to Equimolar Concentrations of DEHP (750 mg/kg bw) and DBP (500 mg/kg bw) in Sprague Dawley Rats

Effect	Control	Chemical	
		DEHP	DBP
Anogenital distance (mm)	3.7±0.09	2.45±0.11*	2.79±0.09*
Areolas at birth (%)	0	88±12	55±14
Number of areolas at birth	2.7±0.75	8.4±15	2.7±0.75
Retained nipples at birth	0	8.1±1.4*	2.2±0.8*
Number of nipples at necropsy	0	8.1±1.4*	2.2±0.8*
Hypospadias (%)	0	67±14	6.2±6.2
Vaginal pouch (%)	0	45±17	0
Ventral prostate agenesis (%)	0	14±14	0
Testicular & epididymal atrophy or agenesis (%)	0	90±10	45.8±12

*Statistically significant.
(*41*)

In an abstract, DBP was reported to have been evaluated for developmental toxicity in amphibian and non-rodent mammalian test systems (*51*). *Xenopus laeris* (African clawed toad) tadpoles were exposed to 0 (n=14) or 10 (n=52) ppm DBP beginning at 2 weeks of age (stage 52) through complete metamorphosis (stage 66), with mortality and time to complete metamorphosis monitored weekly. Mortality at 10 ppm was 85% in week 1 (0% in controls) and 92% in week 16 (28% in controls). Seventy-five percent of the controls were metamorphosed by week 12 with 100% by week 14; none of the treated tadpoles completed metamorphosis until week 16. The authors concluded that DBP or its metabolite(s) may disrupt thyroid hormone cascade, since metamorphosis, a thyroid hormone-dependent event, is affected at 10 ppm. The same group administered DBP in corn syrup at 0 or 400 ppm/kg body weight to pregnant Dutch belted rabbits, 6 does/group, on gd 15–30. Does were allowed to litter and male pups were monitored until 12 weeks of age. At 12 weeks of age, body, testes, and epididymides weights were unaffected, but accessory gland weights and anogenital distance were lower in treated male offspring. In addition, analogously to male rats effects, one treated rabbit had undescended testes, ambiguous external genitalia, hypospadias, and was missing (agenesis of) the prostate and bulbourethral glands. The authors concluded that DBP disrupts androgen-dependent developmental events and is consistent with anti-androgenic effects of DBP observed in rodents after perinatal exposure.

MBuP

Imajima et al. (*52*) 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 based on actual rat body weights) (Table WEB 17). Male offspring were evaluated on gd 20 and on pnd 30–40 to determine the position of the testes. In control males, all the 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, all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent on gd 20. On pnd 30–40, MBuP-exposed males exhibited cryptorchidism (22 of 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 trans-abdominal migration of the testis.

The summary for Section 3 is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

The relationship between either human sperm density or total number of sperm and DBP concentration in the cellular fraction of ejaculates was studied in a group of unselected college students (*53*). A negative correlation between DBP concentration and the studied sperm indices was found. The authors point out that there was no reason to believe that any of the students examined had been exposed to phthalate esters other than at ambient levels in the environment. However, the use of this study to support a causal relationship to DBP exposure is limited because subjects' characteristics and other potential risk factors that could confound or modify the observed association were not taken into account by the authors.

4.2 Experimental Animal Toxicity

Approximately 20 studies were reviewed in the evaluation of the reproductive toxicity of DBP. Collectively, these studies predominantly used rodents, and built on the original observation that DBP produced testicular atrophy in a sub-acute toxicity study (*54*). The literature contains numerous redundant studies, usually at high doses (e.g., 2 g/kg, usually in rats), all of which show similar effects on the testis. For example, Gray et al. (*55*) reported on the testicular effects of DBP in the adult rat, mouse, guinea pig, and hamster. In these studies, DBP was administered by gavage for 7 or 9 days at doses of 2,000 or 3,000 mg/kg bw/day. Severe effects were seen on testis weight with histopathological damage (reduction in spermatids and spermatogonia) affecting almost all tubules. Mouse testis was less severely affected and no effects were observed in hamsters. The monoester of DBP was also essentially without effect in the hamster. As discussed in Section 2.1.2 of this monograph, sub-chronic oral exposure of adult F344 rats resulted in testicular lesions at doses of 720 mg/kg bw/day and higher (*14*). A second study (*14*) demonstrated that exposure to DBP during gestation and lactation did not increase sensitivity in rats exposed to DBP for 3 months during adulthood. Sub-chronic studies in $B6C3F_1$ mice at doses up to 3,689 mg/kg bw/day did not cause histological or organ weight changes in the testes.

A number of more specific studies in the rat have attempted to investigate the mode of action of DBP using *in vivo* and *in vitro* protocols. The papers summarized here illustrate important facets of DBP-induced reproductive effects.

The key study for the quantitative assessment of the reproductive toxicity of DBP is reported by Wine et al. (*38*) (Table WEB 14). CD Sprague Dawley rats, 10 weeks old at the start of exposure, were used for continuous-breeding phase and cross-over mating studies. There were 20 breeding pairs in each treated dose group, and 40 pairs in the control group. DBP was mixed with feed to levels of 0, 0.1, 0.5 and 1.0% (w/w); this yielded calculated doses of 0, 52, 256, and 509 mg/kg bw/day for males and 0, 80, 385, and 794 mg/kg bw/day for females. Following a 7-day premating period, the rats were housed as breeding pairs for 14 weeks. Litters were removed immediately after birth. Endpoints in-life included clinical signs, parental body weight and food consumption, fertility (numbers of pairs producing a litter/total number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex ratio, and pup body weights within 24 hours of birth.

In the F_0 generation there was no effect on the overall fertility of the breeding pairs (i.e., the ability to produce litters with at least one live pup); all produced approximately five litters. There was clear indication that DBP, when administered in the diet, affected total number of live pups per litter in all treated groups (reduced by ~ 8–17%) and live pup weights in the 256–385 and 509–794 mg/kg bw/day groups by 6–12 %.

A cross-over mating study was conducted between the high-dose treatment group and the controls. The percent of pairs mating, becoming pregnant, and delivering a litter was unaffected, as was litter size, although adjusted live pup weight was reduced in litters from treated females. At F_0 necropsy, there were no gross or histopathologic effects in the reproductive tracts of treated animals. Epididymal sperm count, testicular spermatid number, and estrous cycle length were not affected by DBP treatment in the F_0 animals. Systemic effects in the F_0 rats included decreased body weight in females and increased liver and kidney to body weight ratios in both sexes of the high-dose group.

The final F_1 litters following the continuous F_0 breeding phase were weaned and raised to sexual maturity (pnd 88) and received the same dose in feed as their parents. Upon reaching sexual maturity, 20 non-sibling F_1 males and females within the same treatment group were housed in pairs for 1 week and then housed individually until delivery of an F_2 litter.

F_1 pup weight was significantly reduced in the high-dose group on pnd 0, 14, and 21. During rearing, three high-dose males were found to have small and malformed prepuces and/or penises and were without palpable testes. Mating, pregnancy, and fertility were significantly lower in the high-dose F_1 group with only 1 of 20 pairings resulting in a litter. While litter size was unaffected, F_2 pup weight was reduced in all treatment groups. All dose groups were killed and necropsied, at which point the body weights of the high-dose animals were 8–14 % lower than controls, but unchanged at other dose levels. For males only, kidney to body weight ratio increased at the 256–509 mg/kg bw/day levels and liver to body weight ratio was increased at the highest level. The relative weights of the ventral prostate and seminal vesicles and the absolute weight of the right testis were decreased in the F_1 males from the high-dose group. There were no effects on the ovary of F_1 females. Epididymal sperm count and testicular spermatid count was significantly reduced in the high-dose F_1 males. Histologic analysis was only performed on selected males (n=10) from the control, mid-, and high-dose groups (the solution used to preserve testes is not clear). Widespread seminiferous tubular degeneration was noted in 1/10 controls, 3/10 in the mid-dose group, and 8/10 in the high-dose group. The high-dose group also exhibited interstitial cell hyperplasia. Five of ten high-dose males also had underdeveloped or defective epididymides. No ovarian or uterine lesions were noted in F_1 females and there was no effect on ante-mortem estrous cyclicity.

In Wine et al. (*38*), the F_1 high-dose group had a high rate of infertility, the middle dose had fewer (F_0 mating) and lighter pups (F_0 and F_1 matings), while the low-dose animals had fewer pups (F_0 mating) and lighter pups (F_1 mating). Thus, a NOAEL was not established. The LOAEL was 52–80 mg/kg bw/day based on reductions in F_0 litter size and F_2 pup weight. The Expert Panel's confidence in the quality of the study is high, and our confidence is also high that these doses correctly represent the LOAEL.

A multigeneration reproductive study was conducted to assess effects of DBP exposure in Long Evans Hooded rats (*41*) (WEB Table 15). Weanling male and female rats of the parental (F_0) generation (10–12/sex/group) were gavaged daily with DBP in corn oil through puberty, adulthood, mating, gestation, and lactation. Females received 0, 250, or 500 mg/kg bw/day; male rats received 0, 250, 500, or 1,000 mg/kg bw/day. Sexual maturation and estrous cycles of the F_0 were evaluated. Treated rats were mated with untreated controls. When the F_1 litters were weaned, the parental rats were killed and necropsied. Implantation sites, serum hormone levels, organ weights, and testicular histology were evaluated.

A delay in puberty was observed in all treated F_0 males based on the age of preputial separation (42.6, 43.4, and 44.4 days from low to high-dose group vs 39.6 days in control group). Fertility was reduced in F_0 males and females in the 500 mg/kg bw/day group. Infertility in F_0 males was apparently due to testicular atrophy and reduced sperm counts. F_0 females in the 500 mg/kg bw/day group cycled and mated successfully, but experienced an increased incidence of mid-term abortion. Malformations were significantly increased in F_1

pups from the 250 and 500 mg/kg bw/day groups. Types of malformations included low numbers of hypospadias, abdominal testes, anophthalmia, uterus unicornous, and renal agenesis.

The F_1 pups were not treated with DBP after weaning. Four to eighteen pairs of F_1 pups from treated dams were selected for continuous mating within dose groups for 11 cycles and the remaining F_1 pups were necropsied. The F_2 pups born during the continuous breeding phase were counted and discarded. Fecundity was reduced in F_1 rats from treated dams and the number of F_2 pups born was reduced in breeding pairs from the 250 and 500 mg/kg bw/day groups. At necropsy, a non-significant reduction in caudal sperm counts (19%) and a significant reduction in caudal sperm levels (34%) were noted in F_1 males from the 250 and 500 mg/kg bw/day groups, respectively.

The study by Gray et al. (*41*) is somewhat limited because many endpoints and details of their experimental methods were not reported.

In Lamb et al. (*39*) and Reel et al. (*40*) (Table WEB 16), DBP was one of four phthalate esters compared using the Continuous Breeding protocol in CD-1 mice; the same basic protocol as reported in Wine et al. (*38*). Male and female CD-1 mice, 20 pairs/treatment group and 40 pairs in control, were fed a diet with DBP at 0 300, 3,000, or 10,000 ppm (doses of 53, 525, and 1,750 mg/kg bw/day as reported by Reel et al. (*40*)) for 7 days prior to and during a 98-day cohabitation period. Litters were removed immediately after birth. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair and of live pups per litter, pup weight, and offspring survival. Testes were fixed in Bouin's solution for histological evaluation. DBP exposure reduced litter size, numbers of litters per pair, number of fertile pairs, live pups per litter, and proportion of pups born alive in the high-dose group. These effect were not seen at lower dose levels. A crossover mating trial demonstrated that female, but not male, mice were affected by DBP, as shown by significant decreases in the percentage of fertile pairs, the number of live pups per litter, the proportion of pups born alive, and live pup weight. Only the control and high-dose F_0 DBP groups were necropsied. There were no effects on sperm parameters in the males, although body weight was significantly decreased (8%) and liver to body weight ratio significantly increased (11%). For females, liver to body weight ratio was significantly increased (19%) and relative uterine weight significantly decreased (28%), but there was no effect on estrous cycles. No treatment-related gross or histological lesions were noted. A second generation was not evaluated.

In Lamb et al. (*39*), the high-dose group was subfertile and the middle-dose and the low-dose groups were functionally unaffected. Thus, the NOAEL was calculated at 525 mg/kg bw/day, based on reductions in litter size and in proportions of pairs having litters. The mid- and low-dose groups were not necropsied or evaluated for reproductive development or performance. For these reasons, the Expert Panel has moderate-to-low confidence that these doses correctly represent the LOAEL and NOAEL. Confidence in the quality of the data reported is high.

<u>Mode of Action</u>

The Expert Panel believes that data from studies with DEHP are relevant to a consideration of the mechanism by which DBP causes adverse effects. It is well understood that DEHP produces a range of hepatic effects in rats (induction of peroxisomes; increased Cyp4A1; PCoA) including hepatic tumors. The induction of these effects in rats is believed due to activation of PPAR-alpha. In PPAR-knockout mice, administration of DEHP does not result in the induction of hepatic effects or tumors unlike the wild-type control animals. In humans, PPAR-alpha is activated upstream of different enzymes from those noted in the rat. Recently, an IARC review of the cancer issue led them to conclude that DEHP rat tumor data was of limited relevance to human risk.

In studies with DEHP, a genetically-modified strain of mouse (the PPAR-alpha knockout mouse) cannot activate PPAR-alpha, but is susceptible to phthalate-induced developmental toxicity and testicular toxicity. This mouse does express PPAR-gamma in the testis which can be activated by MEHP (*56*). PPAR-gamma may conceivably play a role in the reproductive toxicity of phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. Other members of the PPAR family (beta and gamma) have not been extensively studied with regard to activation by phthalates.

Finally, the guinea pig, a non-responding species to the peroxisomal proliferating effects of DBP, is susceptible to the testicular effects of this phthalate.

Gray et al. (*55*) investigated the reason for the lack of testicular lesions in hamsters orally administered DBP and MBuP at doses exceeding those that produced testicular lesions in rats. Using ^{14}C-labelled DBP and monobutyl ester (MBuP), it was determined that intestinal esterase activities were similar in the two species and that the principal metabolite in the rat and hamster was MBuP glucuronide (*23*) However, the levels of unconjugated MBuP in urine were 3–4 fold higher in the rat. Finding that the activity of testicular beta-glucuronidase was significantly higher in the rat than the hamster, the authors speculated that the testicular damage might be associated with greater concentrations of unconjugated MBuP, the putative toxicant.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (*57-59*). It is known that some Sertoli cell functions are mediated by follicle stimulating hormone (FSH) interaction with membrane bound receptors. Lloyd and Foster (*60*) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (*61*).

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, di-n-hexyl phthalate (DnHP), and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (*62*) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young versus mature rats. Further, *in vitro* studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (*63*). Lloyd and Foster (*60*) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats, but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals.

The Panel was not able to reach agreement that interfering with FSH signaling function was the accepted mode or mechanism of action.

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 (*64*), rainbow trout hepatic cytosol (*65*), recombinant human ERs (rhER) overexpressed in SF9 insect cells using the baculovirus system (*66, 67*) and rainbow trout ERs expressed in yeast . Triated E2 was used in the tissue cytosol binding assays while a high affinity fluorescent E2 derivative was used in the rhER binding assays. DBP exhibited no or weak activity in *in vitro* assays that measured binding of phthalates to estrogen receptors (*64, 65, 68*). The assays did not include the addition of esterases or lipases to metabolize DBP to its monoester.

Selected phthalate esters have been examined in a number of in vitro gene expression assays systems. The assays have used stably transfected cells (*64*), transiently transfected cells (*64, 65*), yeast based assays (*64, 68-70*) and vitellogenin induction in rainbow trout hepatocyte cultures (*68*). DBP was weakly active in an

assay of estrogen-induced gene expression, but its metabolite MBuP was inactive (*70*). There was no synergism in estrogenic response with DBP and other phthalates (*70, 71*).

In vivo assays demonstrated that DBP does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*64*) and prepubertal mice (*69*). Uterine permeability was not affected following the subcutaneous injection of DBP (*71*). Malformations in reproductive organs and effects on androgen-related endpoints of male rats exposed to DBP or MBuP during prenatal development suggest antiandrogenic activity by DBP and MBuP (*41, 49, 50, 52*).

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

The major use of DBP is as a coalescing aid in latex adhesive. It is also used as a plasticizer for cellulose plastics and as a solvent for dyes. DBP is not used as a plasticizer for PVC plastics (*1*).

Several authoritative estimates of human exposure, described in Section 1, have been published since 1990. All estimates place total DBP exposure in the general population at less than 10 µg/kg bw/day and were consistent in identifying food as the major exposure source. In addition to food, general human exposure occurs primarily through indoor air followed by drinking water, soil, and ambient air. Infants and young children may have higher exposures than adults, primarily because of dietary differences and possible mouthing of DBP-containing household articles (not limited to toys). Using reasonable assumptions and data from surveillance and food surveys, Health Canada (*6*) estimated total exposures of 2.4, 5.0, 4.3, 2.3, and 1.9 µg/kg bw/day for humans aged 0–0.5, 0.5–4, 5–11, 12–19, and 20–70 years, respectively. Discrepancies in food exposure estimates may be due to inherent variability of food eaten by individuals based on age, sex, ethnicity, time of sampling, and geographical locations.

DBP was found in infant formula, but amounts vary internationally and seem to be falling (*5, 9*). The most recent estimate of DBP exposure from infant formula to a newborn in the UK is 2.4 µg/kg bw/day (*5*) and is the same as the Health Canada total exposure estimate. DBP has been found in 1 of 17 European children's toys at a very low level (0.01% by weight) (*10*). Use of DBP in plastic nasogastric tubing has also been reported (*3*). Occupational exposure during phthalate manufacture is estimated at 143 µg/kg bw/day. Exposures in other occupational settings have not been estimated.

5.1.1.1 Utility of Data to the CERHR Evaluation

DBP exposures resulting from contact with various media (e.g., food, drinking water, and air) have been estimated by several authoritative sources. Limitations in the dataset include the fact that most of the data used in calculations were 15–20 years old and may not reflect current exposure. Further, the majority of data was collected in Europe and Canada and may not accurately reflect US patterns. Data from Health Canada were selected for use since they provide age-based exposure estimates.

5.1.2 General Biological and Toxicological Data

Toxicity. The Expert Panel had to rely on animal toxicity data in its evaluation of general biology and toxicity. DBP is not acutely toxic to rodents with the oral LD_{50} given in grams per kilogram (g/kg) quantities. There are sufficient data to establish that DBP in the diet is toxic to adult rats and mice at repeated daily doses of ~350 mg/kg bw/day and higher (*14*). The liver and testes are consistently found to be target organs with the hematopoetic system also affected in some strains of rats and at higher doses in mice. Testicular lesions were observed at doses of 720 mg/kg bw/day and higher in adult rats (*14*). DBP increases liver to body weight and kidney to body weight ratios. These effects are consistent with effects seen with other phthalates. Indications of peroxisome proliferation, such as elevated levels of PCoA oxidation, were consistently observed. The lowest repeated dose NOAEL in rats was observed in males exposed through diet to 142 mg/kg bw/day (*13*). The corresponding NOAEL in male mice was 353 mg/kg bw/day (*14*). Chronic carcinogenicity studies were not available for review.

Table 7: Summaries of NOAELs and LOAELs and Major Effects in General Toxicity Studies

Protocol and DBP Doses (mg/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects	Major Effects at Higher Doses
3-month repeat dose dietary study in Wistar rats. 6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 27, 142, 688; F: 0, 33, 162, 816. (*13*)	M: 142 F: 162	M: 688; F: 816 ↑Liver and kidney weight (F). Peroxisomal proliferation. Histological liver changes. ↓Thyroid hormone. Anemia (M). No testicular lesions.	No higher doses in study.
13-week repeat-dose dietary study in F344 rats. 5–6 weeks old at start of study, 10 rats/sex/group. Doses – M: 0, 176, 359, 720, 1,540, 2,964 F: 0, 177, 356, 712, 1,413, 2,943. (*14*)	M: 176 F: 177	M: 359; F: 356 ↑ Liver and kidney weights (M). Peroxisomal proliferation. Anemia (M).	↑ Liver and kidney weights. Hepatic lesions. Changes in liver enzyme activity. Peroxisomal proliferation. Testicular lesions. Hypospermia. ↓Testes weight. ↓ Testicular testosterone levels. Anemia (M).
13-week repeat-dose dietary study in $B6C3F_1$ mice. 6 weeks-old at start of study, 10 mice/sex/group. Doses – M: 0, 163, 353, 812, 1,601, 3,689 F: 0, 238, 486, 971, 2,137, 4,278. (*14*)	M: 353 F: None	M: 812 F: 238 ↑ Kidney weight (F) (No dose response or histological changes). ↑ Liver weight (M). ↓Body weight gain (M).	↑ Kidney weight (F) (No dose response or histological changes). ↑ Liver weight. ↓Body weight gain. Mild histological liver effects. No testicular lesions.

Toxicokinetics. There are no conclusive *in vivo* toxicokinetic data in humans. Orally-administered DBP in rodents is rapidly hydrolyzed to the monoester, MBuP, by pancreatic lipases secreted into the small intestine. The monoester is rapidly absorbed from the gut, widely distributed in tissues, and is rapidly excreted in urine, mainly as a glucuronide. No studies are available on the absorption of orally-administered DBP in primates. Thus, it is not known whether DBP is more poorly hydrolyzed and absorbed in the gut of primates compared to rats, as has been observed with other phthalates. Rodent studies indicate there is no bioaccumulation of absorbed DBP or its metabolites (including testes and prostate tissue). *In vitro* human and rat skin were compared for their absorption of DBP; and human skin was found to be much less permeable than rat skin (*12*). In rats, dermal absorption of DBP as identified by urinary excretion of metabolites is 10–12% of the 30–40 mg/kg dose per day (*11*).

Rats treated with ^{14}C-DBP on gd 14 showed concentrations of radioactivity in placenta and fetuses that were approximately 65% of the levels in maternal serum. MBuP was the major metabolite found in both maternal and embryonic tissues (*21*).

A PBPK model of the tissue distribution of DBP and MBuP in rats has been developed by Keys et al. (*25*); the model includes diffusion limitations and pH trapping as mechanisms of uptake of MBuP into tissue. A model has been derived to extrapolate rodent data to predicted values in humans. The model does not contain parameters for estimating fetal or pediatric values.

Genetic Toxicity. IPCS (*3*) reviewed a number of mutagenicity and related endpoints for DBP and concluded that the weight of the evidence indicated that DBP is not genotoxic.

5.1.2.1 Utility of Data to the CERHR Evaluation

The oral subchronic studies in rats and mice are adequate for the evaluation of general toxicity induced by DBP. Some studies were conducted according to GLP standards and relevant exposure routes were utilized. Small group numbers, used in some studies, are of limited concern considering the reproducibility of effects between studies. Adult rodents were tested for DBP-induced testicular lesions. Sections 3 and 4 of this document address studies where the male rodent reproductive tract was exposed to DBP during prenatal and postnatal development. The examination of hepatic effects was adequate and included an evaluation of peroxisome proliferation in rodents.

There are acceptable toxicokinetic data for DBP, consisting of absorption, distribution, metabolism, and excretion, following oral and dermal exposure in the rat. The human data available are of very limited utility. *In vitro* comparisons of DBP metabolism suggest that effects observed in rodents are relevant to humans.

5.1.3 Developmental Toxicity

The are no data on the developmental toxicity of DBP in humans. The most complete description of effects characterizing key aspects of the developmental toxicity of DBP is contained in a series of publications by Ema et al. (*34-36*) and Mylchreest et al. (*47, 49, 50*). Ema et al. (*42*) characterized the prenatal developmental toxicity of DBP in Wistar rats and subsequently demonstrated that the metabolite MBuP caused developmental toxicity similar to DBP. These effects were produced at approximately equimolar concentrations. For example, a maternal and development NOAEL and LOAEL of 500 and 630 mg/kg bw/day (1.80 and 2.27 mmol/kg bw/day), respectively, were identified for DBP following gavage of Wistar rats on gd 7–15 (*34*). Using a similar experimental design, a maternal and developmental NOAEL and LOAEL for MBuP of 250 and 500 mg/kg bw/day (1.13 and 2.25 mmol/kg bw/day), respectively, were

identified (*42*). Similar fetal effects in these studies included increased prenatal mortality, decreased fetal weight, and cleft palate. Dose and time dependency studies with DBP and MBuP resulted in similar findings and are described in Section 3.2.1.

The most complete prenatal exposure study by Ema et al. from the perspective of group size and development of the male reproductive tract established a maternal and fetal NOAEL and LOAEL of 331 and 555 mg/kg bw/day, respectively, in Wistar rats fed DBP-dosed diets on gd 11–21 (*36*). Developmental effects at higher doses (≥555 mg/kg bw/day) included decreased fetal weight, cleft palates, fused sternebrae, reduced anogenital distance in males, and cryptorchidism.

A group of studies from Mylchreest et al. looked at postnatal effects following *in utero* exposure to DBP (*47, 49, 50*). CD rats were gavaged with DBP from gd 3 to pnd 20 or gd 12–21. Delayed preputial separation and retained nipples were observed at doses as low as 100 mg/kg bw/day. Effects noted at doses of 250 mg/kg bw/day or higher were consistent between studies and included hypospadias, agenesis of epididymides or seminal vesicles, cryptorchidism, decreased anogenital distance in males, and/or a low incidence of interstitial adenomas. A NOAEL of 50 mg/kg bw/day was identified. The three Mylchreest studies (*47, 49, 50*) exposed animals during the appropriate window of development, analyzed the tissues appropriately, and combined them with other indices of puberty and reproductive development. The concordance in dose-response to the Wine et al. (*38*) study is good.

The role of the monoester metabolite of DBP in developmental toxicity was elucidated by Saillenfait et al. (*21*), who gavaged Sprague-Dawley rats with 500 or 1,500 mg/kg of radiolabeled DBP/kg bw/day on gd 14. They demonstrated radioactivity in placentas and embryos at levels of 21–30% of those measured in maternal plasma. The majority of the radioactivity was associated with MBuP and its glucuronide. Postnatal effects following *in utero* exposure to the DBP metabolite MBuP were studied in WKA rats that were gavaged with 300 mg MBuP/day (~1,000 mg/kg bw/day) on gd 15–18 (*52*). Testes descent was reduced on both gd 20 and pnd 30–40. Although only one dose was administered, the findings are consistent with those observed in DBP developmental toxicity studies conducted by Ema et al. (*36*) and Mylchreest et al. (*47, 49, 50*), thus supporting the hypothesis that MBuP is responsible for effects associated with DBP exposure.

The hallmark of developmental toxicity in the mouse following oral exposure to DBP appears to be primarily systemic toxicity and death. In a study with ICR mice exposed to diet containing DBP on gd 0–18, Shiota et al. (*32, 33*) reported a 98% incidence of fetal mortality at 2,100 mg/kg bw/day. Fetal body weight was reduced at 660 mg/kg bw/day. The authors stated that the maximum non-embryotoxic dose was 370 mg/kg bw/day. However, the Expert Panel noted that delayed ossification occurred at all dose levels, and selected the lowest dose, 80 mg/kg bw/day, as a LOAEL. These data are from groups with small sample size and have not been replicated. In a continuous breeding protocol with CD-1 mice, Lamb et al. (*39*) observed a decrease in the number of pups, live pups per litter, and pup weight in dams that consumed a dose of 1,750 mg/kg bw/day in the diet. The developmental NOAEL was identified as 525 mg/kg bw/day. Effects of *in utero* and lactational exposure to DBP were studied in $B6C3F_1$ mice where Marsman et al. (*14*) reported that length of gestation was increased at 2,500 ppm (454 mg/kg bw/day) and higher. Seventy-five and ninety-five percent of litters were lost at 10,000 (1,816 mg/kg bw/day) and 20,000 (3,632 mg/kg bw/day) ppm. Decreases were observed in litter size and pup body weights at 2,500, 7,500, and 10,000 ppm. The Expert Panel is not confident that these three studies fully assessed DBP developmental toxicity, including reproductive function, due to limitations in study design that include small group size, failure to perform necropsies in critical dose groups, and failure to assess appropriate landmarks of maturation.

NOAELs and LOAELs for the key developmental toxicity studies for DBP are listed in Table 8. The Ema et al. (*36*) study examined the most sensitive prenatal endpoints and allows for a comparison between

maternal and developmental toxicity. The Ema et al. (*34*) study of DBP was also included to allow comparison with the study of its metabolite, MBuP (*42*) that was evaluated according to the same protocol. The Mylchreest et al. (*50*) study is considered key because it examined the most sensitive endpoints at the lowest doses.

Table 8: Summaries of NOAELs and LOAELs and Major Effects in Key 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 studies in Wistar rats. 11–12/group received DBP (0, 500, 630, 750, or 1,000 mg/kg bw/day) or MBuP (0, 250, 500, or 625 mg/kg bw/day) on gd 7–15 by gavage. In a third study rats were treated by diet with 0, 331, 555, or 661 mg/kg bw/day on gd 11–21. Fetuses were evaluated late in gestation. (*34, 36, 42*)	<u>DBP Gavage:</u> Maternal: 500 Fetal: 500 (1.80 mmol/kg bw/day)	<u>DBP Gavage:</u> 630 (2.27 mmol/kg bw/day) ↓ Weight gain.	<u>DBP Gavage:</u> 630 (2.27 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight.	<u>DBP Gavage:</u> ↑ Prenatal mortality. ↓ Fetal weight. ↑ External malformations
	<u>MBuP Gavage:</u> Maternal: 250 Fetal: 250 (1.13 mmol/kg bw/day)	<u>MBuP Gavage:</u> 500 (2.25 mmol/kg bw/day) ↓ Weight gain.	<u>MBuP Gavage:</u> 500 (2.25 mmol/kg bw/day) ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.	<u>MBuP Gavage:</u> ↑ Prenatal mortality. ↓ Fetal weight. ↑ External and skeletal malformations. ↑ Visceral variations.
	<u>DBP Diet:</u> Maternal: 331 Fetal: 331	<u>DBP Diet:</u> 555 ↓ Weight gain.	<u>DBP Diet:</u> 555 ↓ Anogenital distance in males. ↑ Fetuses with undescended testes.	<u>DBP Diet:</u> ↓ Fetal weight. ↑ External and skeletal malformations. ↓ Anogenital distance in males. ↑ Fetuses with undescended testes.
Prenatal gavage study with postnatal evaluation in CD rats. 11–22 per group received 0, 0.5, 5, 50, 100 or 500 mg/kg bw/day on gd 12–21. Pups were evaluated until puberty. (*50*)	Maternal: 500 Developmental: 50	None	100 Retained aereolas and nipples in males.	Retained aereolas and nipples in males. Testicular lesions and adenoma. Malformations of reproductive organ. ↓ Reproductive organ weights. ↓ Anogenital distance in males.

Prenatal dietary study in ICR-JCL mice. 6–15 dams per treated group received 0, 80, 180, 350, 660, and 2,100 mg/kg bw/day on gd 0–18. Dams and pups examined late in gestation. (*32*, *33*)	Maternal: 660 Developmental: None	2,100 ↓ Body weight gain.	80* Delayed ossification (number of ossified coccygia from control to 660 mg/kg bw/day group: 9.4, 5.1, 4.5, 6.0, 2.6).	Delayed ossification. ↑ Prenatal mortality. ↓ Fetal weight. ↑ Neural tube defects.

* Effect level selected by Expert Panel differs from that of the study authors

5.1.3.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of developmental toxicity. Studies examined effects following dosing of dams through portions of or the entire period of pregnancy. Fetuses were evaluated for prenatal malformations and postnatal effects. Evaluations included an examination of reproductive organs and androgen-regulated endpoints, which are thought to be the most sensitive indicators of phthalate-induced toxicity. Prenatal effects following prenatal exposure to MBuP were also examined. A second rodent species, the mouse, was examined in a prenatal exposure and effect study. Based on the limited parameters examined in the mouse it is not possible to compare sensitivity in rats and mice.

5.1.4 Reproductive Toxicity

Human Data

The relationship of human sperm density and total number of sperm to DBP concentration was studied in a group of unselected college students (*53*). A negative correlation was found between sperm density and DBP concentration in the cellular fraction of ejaculate of non-occupationally exposed subjects, but the data are of little value to the Expert Panel due to the insufficient evidence for a causal relationship of sperm characteristics to DBP levels.

Experimental Animal Studies

Reproductive studies have been performed primarily in the rat and, to a lesser extent, the mouse. There are single reports of studies in guinea pigs and hamsters (*55*). Collectively, the data are sufficient to show that oral exposure to DBP can cause reproductive toxicity in male rats, mice, and guinea pigs. In contrast, the hamster failed to show testicular effects. Data that characterize effects on female reproduction are not as complete and detailed interpretation is therefore less certain. The data do indicate a decrease in female fertility in mice and rats.

Females. The Lamb et al. (*39*) data from a continuous breeding study in mice clearly show adult female functional effects at 1,750 mg/kg bw/day. The limited examination of the lower dose groups (necropsies were not performed) precludes the setting of a reliable NOAEL. The continuous-breeding study by Wine et al. (*38*) in F344 rats did not show specific deficits in female parameters; however, the data do not rule out that decreases in litter size at all doses may have a female component. In contrast, Gray et al. (*41*) reported that fertility in female Long Evans rats was reduced following treatment with 500 mg/kg bw/day from weaning through puberty, gestation, and lactation. The effect was apparently due to an increase in mid-term abortions. The F_1 female pups in this study were also mated and experienced a reduction in fecundity at doses of 250 mg/kg bw/day and higher. Thus, clear effects on female reproduction are seen in rats at doses

of 250 mg/kg bw/day (LOAEL) and in mice at higher doses. NOAELs can not be established with any confidence.

Males. Data from the Wine et al. (*38*) continuous breeding study clearly show functional and structural reproductive effects in male Sprague-Dawley rats. In the F_0 generation there was clear indication that DBP, when administered in the diet, affected total number of pups per litter in all treated groups. The F_1 high-dose group had malformations of the reproductive tract and a high rate of infertility. Dose related increases in seminiferous tubular degeneration were seen at the 256 and 509 mg/kg bw/day doses. The LOAEL was 52–80 mg/kg bw/day based on reductions in F_0 litter size. Thus, a reproductive NOAEL was not established.

A delay in preputial separation was observed in Long Evans rats exposed at the lowest dose of 250 mg/kg bw/day by gavage from the time they were weaned until the litters they sired were weaned in the Gray et al. study (*41*). At higher doses (500–1,000 mg/kg bw/day), reductions in sperm counts and fertility and testicular lesions were also observed. The F_1 offspring exposed to DBP only during gestation and lactation experienced a reduction in sperm counts.

Three studies by Mylchreest et al. (*47, 49, 50*), presented in Sections 3.2.2 and 5.1.3 of this document, indicated that the range of male structural abnormalities in the Wine et al. (*38*) study could be reproduced with a much shorter dosing regime. Mylchreest et al. (*49, 50*) also detected a significant increase in testicular Leydig cell hyperplasia and a low incidence of Leydig cell adenomas in ~3-month-old animals following only a late gestational exposure (gd 12–21) of 500 mg/kg bw/day. Wine et al. (*38*) dosed for 14 weeks with DBP in the diet, whereas Mylchreest et al. (*50*) exposed pregnant rats by gavage during gd 12–21. A NOAEL was established at 50 mg/kg bw/day in the Mylchreest et al. (*50*) study.

The existing data show consistent effects (testicular pathology, reduced sperm numbers, effects on reproductive tract development), and are sufficient to conclude that DBP is a reproductive and developmental toxicant in male rats at doses of 100 mg/kg bw/day and higher. Treatment of rat weanlings with 250 mg/kg bw/day resulted in delayed puberty and doses of 500 mg/kg bw/day induced testicular lesions. In general toxicity studies (Section 2.1.2), testicular lesions were observed in adult rats (6 weeks old) treated with 720 mg/kg bw/day, but not in adult mice treated with up to 3,689 mg/kg bw/day for 3 months (*14*). Histological changes in testes of 4–6 week-old mice and guinea pigs of a similar nature have also been observed following administration of a single high dose (2,000 mg/kg bw/day) for 7–9 days, but hamsters were unaffected. The overall effects on the testes indicate an age sensitivity with fetal sensitivity >pubertal sensitivity> adult sensitivity in male rats to the action of DBP.

The responses that occur at the lowest doses appear to involve the development of the reproductive system. These responses were seen with some consistency in the studies by Mylchreest et al. (*47, 49*) and Wine et al. (*38*). The report by Reel et al. (*40*) and the paper by Lamb et al. (*39*) did not report on measures of reproductive system development. However, they are consistent with the Mylchreest et al. and Wine et al. papers in that they show reproductive toxicity under oral (dietary) exposure, and do so in a second species, the mouse.

Mode of Action

Gray et al. (*55*) investigated the reason for the lack of testicular lesions in hamsters administered DBP and MBuP orally at doses exceeding those that produced testicular lesions in rats. Using ^{14}C-labelled DBP and MBuP, it was determined that intestinal esterase activities were similar in the two species and that the principal metabolite in the rat and hamster was MBuP glucuronide (*23*). However, the levels of unconjugated MBuP in urine were 3–to 4-fold higher in the rat. Finding that the activity of testicular beta-

glucuronidase was significantly higher in the rat than the hamster, the authors speculated that the testicular damage might be associated with greater concentrations of MBuP, the putative toxicant.

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (*57-59*). It is known that some Sertoli cell functions are mediated by FSH interaction with membrane bound receptors. Lloyd and Foster (*60*) demonstrated that MEHP disturbs FSH interaction with the FSH receptor. Further studies with MEHP using primary rat Sertoli cell cultures revealed that the monoester of DEHP inhibited FSH-stimulated cAMP accumulation. The MEHP-induced inhibition was specific for FSH (*61*).

Factors affecting increased sensitivity to phthalate-induced testicular toxicity in young animals were studied for DBP, DEHP, DnHP, and dipentyl phthalate. The monoester derivatives of DBP and DEHP have been shown to cause similar testicular effects. Sjoberg et al. (*62*) demonstrated that gavage treatment with DEHP resulted in greater absorption of MEHP, and hence, a greater systemic dose to young versus mature rats. Further, *in vitro* studies did not find that FSH-stimulated cAMP accumulation and lactate secretion were age related (*63*). Lloyd and Foster (*60*) noted that initiation of spermatogenesis was dependent on FSH interaction with the Sertoli cell in young rats but was not necessary for maintenance of spermatogenesis in adults. Their experiment in Sertoli cell cultures demonstrated that MEHP interferes with FSH interaction at the receptor level and provided a hypothesis for increased sensitivity to testicular toxicity in young animals. The Panel was not able to reach agreement that interfering with the FSH-signaling function was the accepted mode or mechanism of action.

The Expert Panel believes that data from studies with DEHP are relevant to a consideration of mechanism for DBP-induced toxicity. It is well understood that DEHP produces a range of hepatic effects in rats (induction of peroxisomes; increased Cyp4A1; PCoA) including hepatic tumors. The induction of these effects in rats is believed due to activation of PPAR-alpha. In genetically altered mice who do not express PPAR, administration of DEHP does not result in the induction of hepatic effects or tumors unlike the wild-type control animals. In humans, PPAR-alpha is activated upstream of different enzymes from those noted in the rat. Recently, an IARC review of the cancer issue led them to conclude that DEHP rat tumor data was of limited relevance to human risk.

In studies with DEHP, a genetically modified strain of mouse (the PPAR-alpha knockout mouse) cannot activate PPAR-alpha, but is susceptible to phthalate-induced developmental toxicity and testicular toxicity. This mouse does express PPAR-gamma in the testis which can be activated by MEHP (*56*). PPAR-gamma may conceivably play a role in the reproductive toxicity of phthalates. PPAR-gamma has been found in human testis, ovary, placenta, and embryo. Other members of the PPAR family (beta and gamma) have not been extensively studied with regard to activation by phthalates.

Finally, the guinea pig, a non-responding species to the peroxisomal-proliferating effects of DBP, is susceptible to the testicular effects of this phthalate.

Imajima et al. (*52*) suggests that the active metabolite for reproductive effects due to gestational exposure is MBuP. This pattern of effects induced in rodents by late gestational exposure (gd 12–21) is 'anti-androgenic,' in that flutamide mimics these effects (*49*); however, DBP/MBuP does not bind to the androgen receptor (*72*). In pubertal and adult rodents, the Sertoli cell is the likely cellular target for testicular injury mediated by the monoester (*63, 73*).

DBP exhibited no or weak activity in *in vitro* assays that assess estrogenicity (*64, 65, 68, 70*). The assays did not include the addition of esterases or lipases to metabolize DBP to its monoester. However, the DBP metabolite MBuP was determined to be inactive in one assay (*70*). There was no synergism in estrogenic response with DBP and other phthalates (*70, 71*). DBP was inactive in rodent *in vivo* assays that measure

endpoints such as increases in uterine wet weight, vaginal epithelial cell cornification, or uterine permeability (*64, 69, 71*). Malformations in reproductive organs and effects on androgen-mediated endpoints in male rats exposed to DBP or MBuP during prenatal development suggest antiandrogenic activity by DBP and MBuP (*41, 49, 50, 52*).

Table 9: Summaries of NOAELs and LOAELs and Major Effects in Reproductive Toxicity Studies

Protocol & Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day) and Effects		Reproductive Effects Observed at Higher Dose Levels
		Reproductive	Systemic	
Dietary continuous breeding protocol with crossover breeding and evaluation of second generation in Sprague-Dawley rats. 20 pairs per group were treated at doses of M: 0, 52, 256, or 509 mg/kg bw/day; F: 0, 80, 385, or 794 mg/kg bw/day during a 14 week mating period. (*38*)	Reproductive: None Systemic: 256 (M); 385 (F)	M: 52; F: 80 ↓ F_1 live litter size. ↓ F_2 pup weight.	M: 509; F: 794 ↓Body weight gain in F_0 females and F_1 males and females. ↑ Liver and kidney weight in F_0 males and females and F_1 males.	↑ Malformed reproductive organs in F_1 males. ↓ Mating, pregnancy, and fertility in F_1. ↓ Reproductive organ weights in F_1 males. ↑ Testicular lesions in F_1 males. ↓ Sperm counts in F_1. ↓ F_1 litter size. ↑ F_1 pup mortality. ↓ F_1 and F_2 pup weight.
Dietary continuous-breeding protocol with crossover mating in CD-1 mice. 20 pairs per group were treated with 0, 53, 525, and 1,750 mg/kg bw/day during a 14-week mating period. (*39, 40*)	Reproductive: (M): 525 (F): 525 Systemic: Not known because only high-dose group was necropsied.	M: Can't determine F: 1,750 ↓ Fertility in F_0 females. ↓ Uterine weight in F_0. ↓ Live pups/litter. No effects on sperm in F_0.	1,750 ↓ Bodyweight in males. ↑ Liver weight.	No higher doses.
Multigeneration-reproductive study in Long Evans Hooded rats. 10–12 pairs per group were treated by gavage from weaning throughout puberty, adulthood, mating, and lactation with 0, 250 or 500 mg/kg bw/day. Males were also dosed with 1,000 mg/kg bw/day. F_1 rats were not treated following weaning. (*41*)	Reproductive: None Systemic: Not reported	250 Delayed puberty in F_0 males. ↓ Sperm production in F_1 males (non-significant). ↓ Fecundity in F_1. ↑ Malformations in F_1 reproductive organs. ↓ F_2 litter size.	Not reported.	Delayed puberty in F_0 males. ↓ Fertility in F_0 males and females. ↑ Midterm abortion in F_0 females. ↑ Testicular lesions in F_0 males. ↓ Sperm production in F_0 and F_1 males. ↓ Fecundity in F_1. ↑ Malformations in F_1 reproductive organs. ↓ F_2 litter size.

5.1.4.1 Utility of Data to the CERHR Evaluation

The data in rats are adequate for an assessment of reproductive toxicity as several studies are available that evaluate both structure and reproductive function. Transgenerational effects were examined in many of the studies. Animals were treated during gestational development, during lactation, and at weaning, thus ensuring that the most sensitive age for reproductive effects was assessed. The evaluation included

androgen-regulated endpoints that are believed to be the most sensitive indicators of DBP effects. Reproductive organs were preserved in Bouin's fixative, a method that reduces histological artifacts. Although studies in other species are not as detailed, they do allow for limited comparisons of interspecies sensitivity.

5.2 Integrated Evaluation

DBP is used as a coalescing aid in latex adhesives, as a plasticizer in cellulose plastics, and as a solvent for dyes. General human exposure occurs primarily through food. All estimates place total DBP exposure in the general population at less than 10 µg/kg bw/day. Although infants and young children may have higher exposures than adults, primarily because of different dietary patterns, estimates of their exposure remain at approximately 10 µg/kg bw/day, with the possible exception of non-dietary intake through mouthing of phthalate-containing objects. Workplace exposure at phthalate production facilities is estimated to be below 143 µg/kg bw/day. Exposure levels during incorporation of DBP in plastics are not known.

Following oral exposure to rodents and humans, DBP is quickly metabolized in the small intestine to mono-n-butyl phthalate, MbuP, and n-butyl alcohol. Several investigators have postulated that it is the monoester that is of toxicological interest. The Panel finds logic and data to support this view. Absorption of the monoester into blood occurs in both rats and humans. Although data for DBP is not available for humans or primates, it is reasonable to assume that MBuP would be rapidly glucuronidated and excreted in the urine in a manner analogous to DEHP in humans. The toxicokinetic data indicate that no tissue bioaccumulation would be expected via the oral or dermal route.

There are no data on the developmental or reproductive toxicity of DBP in humans. There are data in rats and mice that show oral exposure to DBP causes developmental toxicity. The developing male reproductive system is most sensitive to the formation of structural and functional abnormalities, with effects seen in rats whose mothers were exposed to 100 mg/kg bw/day during pregnancy. The NOAEL for male reproductive system developmental effects in rats is 50 mg/kg bw/day. Breeding studies provide a good indication of the potential for adverse functional reproductive effects from DBP exposure. Moreover, it is apparent that DBP testicular exposure late in gestation can induce Leydig cell hyperplasia and a low incidence of Leydig cell adenoma. Traditional teratogenicity protocols that evaluate fetuses just prior to birth were not effective in detecting these effects on the developing male reproductive system. While a series of three recent studies have replicated and characterized the male reproductive system effects in rats, studies of similar design have not been performed in other species. The Panel is confident that these studies in rats correctly characterize the effects based on replication, good dose response, and full reporting of study results. As a default assumption, these data in rats are assumed relevant to a prediction of hazard to humans.

The Expert Panel notes that the male reproductive system is a sensitive target organ for effects in rodent studies where exposure is confined to the adult phase of life. Data in several species, including rat, mouse, and guinea pig, show such effects. The Panel also notes that studies in the hamster, although limited, do not show effects on the testes.

There are indications that oral exposure of females during the adult phase of life impairs functional reproductive performance in rats at doses of 250 mg/kg bw/day and higher. There is also a report that exposure to similar doses during gestation and nursing may impair fertility in female offspring. However, the data are not of the scope and quality for the Expert Panel to confidently characterize these effects.

Data indicate that the monoester of DBP, MBuP, is the principal toxicant. Studies suggest that an antiandrogenic mechanism appears to be responsible for the most sensitive endpoints observed in

developing males rats (e.g., anogenital distance, nipple retention, preputial separation). It is not currently known whether the target for DBP is similar or different for gestational versus postnatal exposures.

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 populations.

5.3 Expert Panel Conclusions

DBP is used as a coalescing aid in latex adhesives, as a plasticizer in cellulose plastics, and as a solvent for dyes. The best estimate for exposures from all sources to the general population is 2–10 µg/kg bw/day. There is significant uncertainty in the exposure database based on the age of many of the values/studies. The Expert Panel has high confidence in the available studies to characterize reproductive and developmental toxicity based upon a strong database containing studies in multiple species using conventional and investigative study designs. When administered via the oral route, DBP elicits malformations of the male rat reproductive tract via a disturbance of the androgen status: a mode of action relevant for human reproductive development. This antiandrogenic mechanism occurs via effects on testosterone biosynthesis and not androgen receptor antagonism. DBP is a testicular toxicant in three species of young adult laboratory animals in high dose (>1,000 mg/kg bw/day), sub-acute oral exposure studies. In the rat, there is a life-stage sensitivity for testicular toxicity with the fetus most sensitive, pubertal less sensitive, and adult least sensitive. Adult female functional reproductive toxicity (decreases in fertility) has been noted in rats; however, the data do not permit confident characterization of dose-effects below 250 mg/kg bw/day. The Expert Panel has negligible concern for adult reproductive toxicity.

DBP is developmentally toxic to both rats and mice by the oral routes; it induces structural malformations. A confident NOAEL of 50 mg/kg bw/day by the oral route has been established in the rat. Data from which to confidently establish a LOAEL/NOAEL in the mouse are uncertain. The Expert Panel has minimal concern about effects to human development and development of the reproductive system from current estimated exposure to DBP. A modified dietary multigeneration study is available, but did not establish a NOAEL. The LOAEL (M:52 F:80 mg/kg bw/day) is based on decreases in litter size and pup weight.

5.4 Critical Data Needs

The multigeneration study for DBP in rodents, with support from other studies that incorporated more modern endpoints including developmental landmarks, indicates no immediate data gaps. The potential effects of DBP on female rats warrant further investigation.

Although there are no critical data needs, studies in the following areas would increase understanding about reproductive and developmental effects that occur following DBP exposures.

There is a need to extend the current PBPK model for DBP to include parameters for pregnant women and their fetuses.

There is a need to find out how broad or narrow the window of prenatal exposure is that results in postnatal male effects. The known current window in rats, 12–20 days, is still quite wide from a rodent ontogenesis perspective. Greater precision as to size of the window of sensitivity may be relevant to estimating the temporal bounds of human sensitivity.

Much of the recent focus on reproductive toxicity of phthalate esters has focused on the ability of certain esters to induce effects on reproductive development. Significant primate data exist to support the view that the high blood levels of monoester necessary to achieve adult testicular toxicity in rodents will not occur in humans. Appropriate exposure to monoester in blood from diester exposure could be achieved such that experiments could be conducted in primates to elucidate species sensitivity for equivalent exposures. This would require exposure of pregnant animals during the critical window of development of the reproductive system for the species studied, followed by an examination of reproductive development in the resulting offspring. Such a study would indicate if there is a species sensitivity. In the absence of such a study, the rodent data must be considered relevant and critical for human risk examinations

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7.0 WEB TABLES