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CONSUMER PRODUCT SAFETY COMMISSION
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Memorandum

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THROUGH : Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences
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SUBJECT : Toxicity Review of Di-*n*-butyl Phthalate*

The following memo provides the U.S. Consumer Product Safety Commission (CPSC) Health Sciences' staff assessment of the potential toxicity associated with di-*n*-butyl Phthalate.

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition 15 USC 1262 (f)(1)(A). First, it must be toxic under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial illness or injury during or as a result of reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards under the FHSA (CPSC, 1992; summarized at 16 CFR 1500.135)

The FHSA addresses both acute and chronic hazards. While the FHSA does not require manufacturers to perform any specific battery of toxicological tests to assess the potential risk of chronic health hazards, the manufacturer is required to label a product appropriately according to the requirements of the FHSA. The first step in the risk assessment process is hazard identification, that is, a review of the available toxicity data for the chemical under consideration and a determination of whether the chemical is considered "toxic" under the FHSA. Chronic toxicity data (including carcinogenicity, neurotoxicity, and reproductive and developmental toxicity) are assessed by the CPSC staff using guidelines issued by the Commission (CPSC, 1992). If it is concluded that a substance is toxic under the FHSA due to chronic toxicity, then a quantitative assessment of exposure and risk is performed to evaluate whether the chemical may be considered a "hazardous substance" under the FHSA.

* These comments are those of the CPSC staff, have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

This memo represents the first step in the risk assessment process; that is, the hazard identification step.

Toxicity Review for Di-*n*-butyl Phthalate
(Dibutyl Phthalate or DBP)

Introduction

Di-*n*-butyl phthalate (DBP) is a manmade phthalic ester often added to hard plastics to make them softer, such as cellulose and some polyvinyl chloride (PVC) plastics. In addition, it is used in the making of adhesives, dyes, lacquers, personal care products, cosmetics, and more (ATSDR, 2001). DBP is produced by the reaction of *n*-butanol with phthalic anhydride. Since DBP is not bound to the final product, through its production and incorporation into products, DBP can be released into the environment (CERHR, 2003). Therefore, DBP has become ubiquitous in the environment, and can now be found in food, water, and air. The major metabolite for DBP is monobutyl phthalate (MBP). However, this metabolite is also considered one of the major metabolites for another phthalate, benzyl butyl phthalate (Ema, 1995).

DBP's potential risk to human health has been, and continues to be, investigated. Researchers have evaluated its developmental and reproductive effects, as well as sensitization, systemic effects, and genetic toxicity. Based on studies by the National Toxicology Program (NTP), the International Programme on Chemical Safety (IPCS) determined a tolerable daily intake value of 66 µg/kg body weight per day (bw/d) for DBP (IPCS, 1997; CERHR, 2003).

Physicochemical Properties

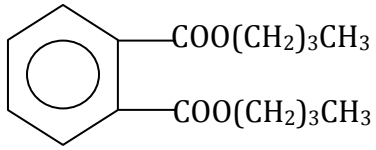

Table 1: Physicochemical Properties

Identification	**Information**
Chemical Name	1,2-Benzenedicarboxylic acid, dibutyl ester
CAS Number	84-74-2
Chemical Formula	$C_{16}H_{22}O_4$
Molecular Weight	278.34
Physical State	Oily liquid
Color	Colorless to faint yellow
Odor	Slight ester-like
Melting Point	-35°C
Boiling Point	340°C
Vapor Pressure	$2.7x10^{-5}$mm Hg
Solubility	Water: 11.2 mg/L Organic solvents: very soluble
Log K_{ow}	4.45
Flashpoint	157°C
Incompatibilities	Liquid chlorine

ATSDR, 2001; NICNAS, 2008

Toxicokinetics

Fennell et al. (2004) studied the pharmacokinetics of the major metabolites of DBP in pregnant Sprague Dawley rats. In a pilot study, pregnant female rats were dosed at 100 mg/kg ^{14}C-DBP by gavage on gestational day (gd) 20; virgin rats were similarly dosed on the same day. Samples were collected at 24 hours for the pregnant rats and 2 hours for the virgin rats. In virgin rats, the majority (85%) of the dose was excreted within 24 hours, with 77% in the urine, 7% in the feces, and 0.008% remaining in the carcass. Using data from Saillenfait et al. (1998), 2 hours is thought to be the time of peak blood concentration. In the pregnant rats, Fennell et al. found similar concentrations of radioactivity in the plasma and carcass (329 and 357 µM, respectively). The fetal plasma concentration is approximately half that of the maternal plasma concentration (182 and 329 µM, respectively). Analysis of the virgin rat urine by HPLC showed a peak identified as MBP at 2 hours, which accounted for approximately 18% of the ^{14}C label in the urine, and a peak identified as MBP-glucuronide, accounting for 61% of the ^{14}C label in the urine. The maternal and fetal plasma were analyzed by shielded hydrophobic phase column mass spectrometry. Metabolites found in the urine and plasma were phthalic acid (1.6-2.6% urine; 0.7% plasma), MBP (12-31% urine; 77.1% plasma), MBP-glucuronide (MBP-G) (53-69% urine; 19% plasma), mono-n-hydroxybutylphthalate (8-9% urine; 1.7% plasma), monobutanoic phthtalic acid (0.8-0.9% urine; 0.6% plasma), mono-n-hydroxybutylphthalate glucuronide (2.5-2.9% urine; 0.2% plasma), and monobutanoic phthalic acid glucuronide or mono-1-hydroxybutan-2-one phthalic acid glucuronide (0.5-1.0% urine; none detected in plasma). Parent DBP was not detectable in the urine or plasma.

Fennell et al. (2004) also studied the pharmacokinetics of DBP in pregnant Sprague Dawley rats given a single dose (50, 100, or 250 mg/kg) of DBP by gavage on gd 20, and analyzed maternal and fetal plasma and amniotic fluid samples by HPLC. The authors found MBP and MBP-G in the maternal and fetal plasma for all doses, with MBP being the major metabolite. The maximum concentration (C_{max}) in plasma for MBP was 3-4 fold higher than the C_{max} for MBP-G. The half-life for the maternal plasma MBP was similar in all doses (2.75-2.94 hours), as was the half-life of MBP-G (2.89-3.52 hours). For the fetal plasma, C_{max} for MBP ranged from 40% to 65% of maternal C_{max}. C_{max} for MBP-G was 30% to 110% of the maternal C_{max}. The time to maximum concentration for both MBP and MBP-G was achieved later in the fetal plasma than the maternal plasma at 0.5-3hours versus 0.5-1hr, respectively. In the amniotic fluid, MBP reached its maximum at 4 hours for all doses and had a half-life of about 6 hours for the 100 and 250 mg/kg doses. MBP-G reached its maximum at 8 hours for all doses and only decreased slightly by 24 hours.

In addition, Kremer et al. (2005), using 24 pregnant Sprague Dawley rats, administered 10, 30, or 50 mg MBP/kg body weight (bw) by intravenous injection on gd 19, showed that serum levels of MBP were decreased by 80% within 2 hours and MBP-G was noted in the blood within 5 minutes. MBP levels returned to background levels in maternal serum by 24 hours. However, both MBP and MBP-G were higher in the fetus at 24 hours. The half-life of maternal MBP-G was found to be about 2 hours, and C_{max} increased with dose and was non-linear.

Elsisi et al. (1989) found that after applying a single dose of 157 μmol/kg ^{14}C-DBP to the shaved backs of male F344 rats, and covering the site with a perforated cap, dermal absorption resulted in a constant rate of urinary excretion of 10-12% of dose in 24 hours for the 7 days urine samples were collected. In addition, at the end of the 7 days between 0.41 and 1.4% of the dose was collected from other tissues.

Through an *in vitro* absorption study of DBP through human and rat skin, Scott et al. (1987) showed that human skin is less permeable than rat skin. Steady state absorption rates were 0.07 and 9.33 μg/cm^2/hr, respectively. The rat skin studies were done in 8 hours, while the human skin studies were done in 30 hours. In addition, they calculated damage ratios to determine irreversible alterations to permeability. Damage ratios are calculated by dividing the water permeability factor at the beginning of the experiment by the water permeability factor at the end of the experiment to determine alterations in the barrier properties of the epidermal membranes. The authors found large increases in permeability in the rat skin after contact with DBP. These increases were not found in the human skin.

Exposure

The major source of DBP exposure to the general population is reported to be through food (CERHR, 2003; IPCS, 1997). Exposure also occurs through indoor air, drinking water, soil, and ambient air. Total exposure to the general population is estimated to be less than 10 μg/kg bw/d (CERHR, 2003). Using data from surveillance and food surveys, The Center for the Evaluation of Risks to Human Reproduction (CERHR) (2003) reported that Health Canada estimated total exposures of 2.4, 5.0, 4.3, 2.3, and 1.9 μg/kg bw/d for ages 0-0.5, 0.5-4, 5-11, 12-19, and 20-70 years, respectively.

Some researchers have evaluated the correlation between phthalates and the indoor/outdoor air environment. Otake et al. (2004) concluded that the concentration of phthalates is 100 – 1000 times higher in indoor air compared to outdoor air. They also found a significant correlation between DBP and DEHP. This suggested that these two phthalates have the same emission profiles or come from the same products. To determine the concentrations of phthalates, the authors collected 27 indoor air samples from homes in the Tokyo Metropolitan area. Each air sample was collected by passing air through glass tubes containing charcoal granules, for 3 days. The samples were then quantified by gas chromatograph/mass spectrometer and gas chromatograph/flame photometric detection. Using the dietary intake estimate of 7μg/kg/day, established by the World Health Organization (1997), the authors estimated that the maximum inhalation exposure is approximately 30% of the dietary intake, or 20% of the total intake of DBP. They further estimated DBP exposure due to indoor air concentrations to be about 136 μg/day.

Occupational exposures have also been reported. An acute case of exposure of a healthy male worker accidentally ingesting 10 g DBP reported delayed nausea, vomiting, and dizziness, followed by headache, pain and irritation of the eyes, lacrimation, photophobia and conjunctivitis (Sandmeyer et al., 1981; as reported in IPCS, 1997). Urine was dark yellow with sediment and numerous erythrocytes and leukocytes with moderate

oxalate crystals were noted. The worker started recovering within 2 weeks, with complete recovery after 1 month (Sandmeyer et al., 1981; as reported in IPCS, 1997).

In another study, it was reported that workers were exposed to DBP above the Maximum Allowable Concentration of 0.5 mg/m^3, however quantitative data on exposure was not given (Aldyreva et al., 1975; as reported in IPCS, 1997). Of 189 women given gynecological examination, 33% were normal, 33% showed deviations of the uterus, and 34% were undisclosed. A decrease in pregnancies and birth in the women exposed to DBP, as well as estrogen/progesterone cyclicity abnormalities were noted. However, inadequate documentation of exposure was provided, making it difficult to draw significant conclusions (Aldyreva et al., 1975; as reported in IPCS, 1997).

Infants and children may have higher exposures than adults, possibly due to dietary differences and mouthing behaviors (CERHR, 2003). Koch et al. (2005) suggests that if children and adults took up the same amount of phthalates, children would have an increased amount of phthalate metabolite in their urine and would receive an increased dose compared to the adult due to body weight. To address this question, Koch conducted a voluntary study in a southern Germany nursery school. Morning urine voids were taken from children, school teachers, and parents. Urine samples were analyzed for metabolites using a multi-dimensional liquid chromatography tandem mass spectrometry method. It was found that MBP, the primary metabolite for DBP, was higher in the children when compared with the adults. The median urinary MBP concentration for the children was about 1.5 times higher than for the adults. The authors approximate that an infant would receive a seven times greater dose than an adult with respect to body weight. Creatinine is produced by the body at a consistent rate based on muscle mass. Since creatinine excretion is approximately proportional to muscle mass, the authors think this creatinine adjustment could compensate for the differences in children and adult mass. With this adjustment, the internal exposure of children to MBP was estimated to be about twice as high as for adults. The maximum values for internal exposure, after removing a child on medication that may contain a coating with DBP, was 517 µg/g for the children and 149 µg/g for the adults, which is about 3.5 times the maximum concentration of the adults. After reviewing the answers from the questionnaire regarding use of skin care or body care products, the authors concluded that the use of skin care or body care products may have a significant influence on DBP exposure of children (Koch, 2005).

Non-voluntary, high dose exposures to phthalates found in coatings of medications or use of medical devices, such as tubing, are of concern. In a case report, Hauser et al. (2004) showed that chronic use of medication with DBP increased the urinary metabolite MBP in the patient. DBP exposure in the Hauser et al. (2004) study was found to approach the lowest no observed adverse effect level (NOAEL) in animal studies of 50 mg/kg/day (Mylchreest, 2002). The patient's concentration of MBP was two orders of magnitude higher than the US population 95th percentile of men in the NHANES 1999 – 2000 data set. Previously, the highest estimated DBP exposure in the US population (Kohn, 2000; Blount, 2000; David, 2000) was more than two orders of magnitude less than the lowest NOAEL in animal studies (Hauser, 2004). Hauser et al. (2004) concluded that concentrations of levels

approaching a NOAEL of 50 mg/kg/day can possibly contribute to the testicular dysfunction reported to be associated with DBP exposure in other studies.

Hazard Identification

In evaluating toxicity data, staff applies the definition for toxicity in the regulations (16 CFR 1500.3 (c)(2)(ii))and chronic hazards guidelines (CPSC, 1992) promulgated under the FHSA (15 U.S.C. 1261-1278). A substance or mixture is classified as "known to be toxic" in humans only if there is sufficient evidence in humans, and is regarded as "probably toxic" if there is either limited evidence in humans, or sufficient evidence in animals (Table 2). If a substance is "known to be toxic" or "probably toxic" in humans it is considered "toxic" under the FHSA. If a substance is "possibly toxic", it would not be considered "toxic" under the FHSA.

Table 2: Classification of Chronic Hazards under the FHSA

Evidence	Human studies	Animal studies
Sufficient evidence	Known [a]	Probable [a]
Limited evidence	Probable [a]	Possible
Inadequate evidence	Possible	---

[a] Considered "toxic" under the FHSA

Acceptable daily intakes values (ADI's) are calculated when a given chemical is considered "toxic" due to chronic effects and sufficient toxicity information is available. The ADI is the amount of a chemical that one may be exposed to on a daily basis without posing a significant risk of health effects to consumers. In some cases insufficient data is available to calculate an ADI.

Systemic Effects

Acute toxicity of DBP in mice and rats is low (IPCS, 1997). The oral lethal dose in 50% of the study animals (LD_{50}) is between 8,000 and 20,000 mg/kg bw in rats. The intraperitoneal (IP) LD_{50} ranged from 4,000 to 7,000 mg/kg bw in rats and 3,000 to 6,000 mg/kg bw in mice. The dermal LD_{50} in rabbits is greater than 4000 mg/kg bw. MBP may be more acutely toxic than DBP with an IP LD_{50} of 1000 mg/kg in mice (Chambon et al., 1971; as reported in IPCS, 1997).

Several studies of dietary administration of DBP have been reviewed. Pregnant IGS rats (6-8/group) were fed a diet containing 0, 20, 200, 2000, or 10,000 parts per million (ppm) DBP from gd 15 to postnatal day (pnd) 21(Lee, 2004). Authors noted significantly reduced body weight gains in dams in the 20 and 10,000 ppm groups from gd 15-20 ($p<0.05$). Male and female offspring were examined during pre-puberty (pnd 21) and puberty through adult stages (pnd 27-postnatal week (pnw) 20) for developmental and systemic effects. At pnd 21 both sexes showed a significant increase in liver weights ($p<0.01$) and brain weights ($p<0.05$) for males at the 10,000 ppm dose. Although not

statistically significant, there was an increase in female brain weights at pnd 21, and an increase in female kidney weights at pnw 20. At pnw 11, males had a decrease in kidney weight at 10,000 ppm. There was an increase in pituitary weights at pnw 11 at 200 and 2000 ppm in male offspring, while a statistically significant decrease at 10,000 ppm was seen in female offspring ($p<0.01$). This decrease in pituitary weight was sustained at pnw 20 in females. There were an insufficient number of male animals at pnw 20 to determine if reduced pituitary weights were sustained. A NOAEL was not established. However, Lee et al. concluded that the lowest observed adverse effect level (LOAEL) was 20 ppm (1.5-3.0 mg/kg/d) due to reduced maternal weight gains.

In a study by NTP (1995), 5 to 6 week F344/N male and female rats (10/sex/group) were fed 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm (dose equivalent to 176, 359, 720, 1540, and 2964 mg/kg-day for males and 177, 356, 712, 1413, and 2943 mg/kg-day for females) DBP in their diet for 13 weeks. The rats were evaluated at the end of the study for systemic, reproductive, and histological effects. A significant ($p\leq0.01$) decrease in male body weights was seen starting at 10000 ppm, and in female rats starting at 20,000 ppm. In addition, male rats showed a significant ($p\leq0.01$) increase in liver and kidney weights starting at 5000 ppm, and female rats showed an increase at 10,000 ppm. Male rats showed a decrease in testes weights starting at 20,000 ppm. Histology revealed hepatic lesions starting at 10,000 ppm for both male and female rats, with peroxisomal proliferation at the highest dose. The male rats also showed testicular lesions at 10,000 ppm. This study also included hematology and blood chemistry. The male rats had decreased hemoglobin and red blood cells, and an increase in platelets at 5000 ppm. Both male and female rats showed an increase in mixed cell volume at 10,000 ppm and 20,000 ppm, but only the male rats showed a significant ($p\leq0.01$) increase at 40,000 ppm. There were also increases in albumin, bile acids, and alkaline phosphatase starting at 2500 ppm in the male rats and 5000 ppm in the female rats. In addition, there was a decrease in triglycerides, cholesterol, and total protein levels starting at 10,000 ppm in the male rats and 20,000 ppm in the female rats. Based on the findings, the authors determined a NOAEL of 2500 ppm.

Concurrent to the study described above, 6 week old $B6C3F_1$ mice (10/sex/group) were fed 0, 1250, 2500, 5000, 10,000, or 20,000 ppm (dose equivalent to 163, 353, 812, 1601, and 3689 mg/kg-day for males and 238, 486, 971, 2137, and 4278 mg/kg-day for females) DBP in their diet for 13 weeks. The mice were evaluated at the end of the study. As in the previous study in rats, the authors found decreased body weights and an increase in kidney weights in female mice starting at 1250 ppm and increased liver weights for both male and female mice starting at 5000 ppm. Statistically significant ($p\leq0.01$) liver lesions in male mice were noted at 10,000 ppm and both male and female mice at 20,000 ppm. No other organs were affected. Based on decreases in body weights, a NOAEL of 2500 ppm was established in male mice. A LOAEL of 1250 ppm was established for female mice based on the increase in kidney weights.

NTP (1995) also conducted a sub-chronic study where F344/N rats (10/sex/group) were fed 0 or 10,000 ppm DBP during prenatal development and lactation. All weaned rats were fed 10,000 ppm until 8 weeks old. The rats were then fed diets containing 0, 2500,

5000, 10,000, 20,000, or 40,000 ppm (dose equivalent to 138, 279, 571, 1262, and 2495 mg/kg-day for males and 147, 294, 593, 1182, and 2445 mg/kg-day for females) DBP for another 13 weeks. The authors noted a statistically significant increase in liver ($p \leq 0.01$) and kidney ($p \leq 0.05$) weight starting at 2500 ppm for female and 5000 ppm for male rats. Male rats that were exposed to DBP prenatally also had decreased testes weights with and without additional DBP exposures after weaning. Histology showed testicular lesions in the male rats, and hepatic lesions in both male and female rats starting at 10,000 ppm. The authors concluded that prenatal exposure did not increase the susceptibility of rats to DBP exposure during adulthood.

Table 3: Systemic endpoints (NTP, 1995) (modified from CERHR, 2003)*

Dose(mg/kg-day)(male/female)	163/238	353/486	812/971	1601/2137	3689/4278	
$B6C3F_1$ Mice	↑kidney weight (F)	↑kidney weight (F)	↑liver weight ↓body weight ↑kidney weight (F)	↑liver weight ↓body weight ↑kidney weight (F) Liver lesions (M)	↑liver weight ↓body weight ↑kidney weight (F) Liver lesions	
Dose(mg/kg-day)(male/female)	In utero exposure only	138/147	279/294	571/593	1262/1182	2495/2445
F344/N Rats	↑body weight gain ↑testes weight ↓testosterone	↑kidney weight(F) ↑liver weight(F) ↑Testes weight	↑kidney weight ↑liver weight ↑Testes weight	↓male and female body weight gain ↑kidney weight ↑liver weight ↑testes weight Hepatic/test. lesions ↓hemaglobin Red blood cells, platelets (M)	↑kidney weight ↑liver weight ↓Testes weight ↓body weight gain Hepatic/test. lesions ↓sperm counts/ hypospermia of epid.	↑kidney weight ↑liver weight ↓Testes weight ↓body weight gain Hepatic lesions Peroxisomal proliferation Testicular lesions ↓sperm counts/ hypospermia of epid. ↓hemaglobin, Red blood cells, platelets(M)

*Statistically significant changes reported

Jiang et al. (2007) compared hypospadic, a malformation where the urethral opening is not at the top of the penis, and non-hypospadic male Sprague Dawley rats, 10 rats/group. Pregnant rats were dosed by gastric intubation with 0, 250, 500, 750, or 1000 mg/kg-day DBP from gd 14 to gd 18. Rats with hypospadia showed a decrease in liver, kidney, prostate, testes, and epididymis weight at 500 and 750 mg/kg-day when compared to non-hypospadic rats in the same dose group. The same organ weights of the non-hypospadiac rats were also significantly decreased compared to controls ($p<0.05$). In addition, adrenal and pituitary glands were statistically significantly ($p<0.05$) increased

starting at 500 mg/kg-day in the hypospadic and non-hypospadic rats compared to controls. It was concluded that rats showing hypospadia were more severely affected by DBP exposure than those rats not showing hypospadia from the same litter (Jiang, 2007).

One inhalation exposure study was reviewed. In this study male Sprague Dawley rats (6/group) were exposed 6 hours/day for 5 days to 0, 0.5, 2.5, or 7.0 ppm DBP vapor (Walseth, 1984; as reported in CERHR, 2003). They found a decrease in microsomal cytochrome P-450 and cytochrome c-reductase at 2.5 and 7.0 ppm in the lung. No significant changes in the liver were noted and no significant changes in lung or liver weights were seen. Blood serum alanine aminotransferase and aspartate aminotransferase were decreased, and albumin was increased. It was concluded that the lung was the target organ during inhalation exposure of DBP (Walseth, 1984; as reported in CERHR, 2003).

In a one year study by Smith et al. (1953; as reported in NICNAS, 2008), rats were fed 0.01, 0.05, 0.25, and 1.25% DBP (equivalent to 5, 25, 125, and 625 mg/kg-day). In the first week, there was a 50% mortality rate for the 625 mg/kg-day dose. No abnormal clinical signs, hematology, pathology, or histopathology were noted at any of the other doses. The specifics of this study are very limited and some parameters were not evaluated. A NOAEL of 125 mg/kg-day, and a LOAEL of 625 mg/kg-day were established.

In summary, studies show that DBP is not acutely toxic. In the subchronic studies, authors found reduced body weights and increased liver and kidney weights. One study using hypospadic rats showing decreased liver and kidney weights was inconsistent with the others. Exposure to DBP through inhalation resulted in no significant changes in the liver or lung weights. However, there were decreases in microsomal cytochrome P-450 and cytochrome c-reductase. This could result in alterations in metabolism pathways. One chronic study reviewed showed a 50% mortality rate at the highest dose, but no other clinical signs. Therefore, DBP is systemically toxic. However, under the FHSA DBP would not be considered acutely toxic because there is a lack of human and animal evidence showing acute toxicity.

Irritation/Allergic Response

There have been some studies correlating DBP exposure and allergic response and pulmonary function. Using the third National Health and Nutrition Examination Survey (NHANES), Hoppin et al. (2004) determined that in males, but not females, an increase in MBP, the primary metabolite of DBP, correlated to a decrease in pulmonary function. The study consisted of 140 women and 100 men with urine samples, pulmonary function data, and medical and smoking histories examined. Forced vital capacity (FVC), forced expiratory volume at 1 second (FEV_1), peak expiratory flow (PEF), and maximum mid-expiratory flow (MMEF) were used to determine pulmonary function. Statistically significant ($p<0.05$) decreases in FVC, FEV_1, and PEF were associated with an increase in MBP. Removing male smokers sustained the significant decrease in FVC. With no adjustment for creatinine, the association between MBP and PEF became statistically insignificant.

Phthalates are found in indoor air and household dust, and are believed to be associated with allergic symptoms of the airway, nose, and skin. In 2008, Kolarik et al. examined the association between phthalates in dust and allergic diseases among Bulgarian children. Dust samples were collected from the bedrooms of 102 children between the ages of 2-7 with symptoms of wheezing, rhinitis, and/or eczema within the previous 12 months. Eighty-two non-symptomatic children were used as controls. DBP was found in all samples that were collected; however the authors did not find an association between DBP and allergic disease in their sample population.

In summary, Hoppin et al. (2004) found an association between MBP and significant decreases in pulmonary function in males participating in the NHANES study. Males showed a decrease in FVC, FEV_1, and PEF. When smokers were removed from the data set, only the decrease in FVC associated with MBP levels remained significant. Kolarik et al. (2008) did not find an association between DBP found in indoor air dust and allergic diseases in children ages 2-7.

Sensitization

No sensitization was found in guinea-pig maximization studies or rabbit patch tests (as reported in NICNAS, 2008).

Dermatitis has been reported in 2 women after use of an antiperspirant containing DBP (Calnan, 1975; Sneddon, 1972; as reported in NICNAS, 2008). Both women showed a positive response to a patch test containing DBP, but not to the other constituents of the antiperspirant.

Husain et al. (1975) reported an individual with eczema under a plastic watch strap on both the left and right wrists. When patch tested, the individual was positive for 5% DBP, as well as 20% colophony, 1% paratertiary butylphenol formaldehyde resin, and the plastic watch strap. It is unknown what the content of the watch strap was (as reported in IPCS, 1995).

Through several studies, cosmetic products were patch tested on over 159 individuals. The cosmetics included nail polish with 6% or 9% DBP and deodorant with 4.5% DBP. None of the subjects showed signs of sensitization (as reported in NICNAS, 2008; IPCS, 1997).

A short case report by Chowdhury and Statham (2002), indicates that DBP in personal care products can cause allergic contact dermatitis and sensitization. A 65 year old man, after using the anti-itch cream Timodine, showed an acute erythemic rash. The cream contains DBP; however, the concentration of DBP in the cream was not reported. The subject did not have a family history of atopy or occupational history of exposure to DBP. After conducting a patch test with Timodine cream and/or the components of the cream, the subject showed a positive reaction to 5% DBP, as well the cream its self, containing benzalkonium chloride, Myroxylon Pereirae resin, and a fragrance mix. Similar

reports have been made for plastic watch straps, spray antiperspirants and deodorants, and Timodine cream.

In summary, the data for DBP sensitization is inconsistent. The human data is mixed. None of the animal data indicated that DBP is a sensitizer. There is not sufficient data to conclude that DBP is a strong sensitizer under the FSHA.

Endocrine Effects

Urine samples from adult men recruited from Massachusetts General Hospital between 1999 and 2003 were tested for phthalate metabolites, including MBP, and reproductive hormones (Duty et al. 2005). Concentrations for testosterone and inhibin B, a member of the transforming growth factor-β family secreted by the Sertoli cells in males (Bernard et al., 2001; Skinner et al., 1989), closely approximated normality. However, there was a slight positive association between those with elevated MBP and elevated inhibin B when linear regression models were applied. The range of concentration of follicle stimulating hormone (FSH) was increased compared to the reference ranges for the hospital. Ninety percent of those men with increased FSH had at least one abnormal sperm parameter. Overall, 25% of the men had sperm concentrations less than $20x10^6$/ml (previously associated with altered inhibin B and FSH (Jensen et al., 1997)), 50% had motility below the WHO reference range (ranges not given), and 35% had less than 4% sperm with normal morphology. The Second International Reference Preparation (WHO 71/223) was used as the reference standard for the hormone analysis. These findings are unexpected since for both men and women, in general, inhibins inhibit FSH. Reduced FSH leads to a reduction of testosterone in men through a feedback loop between FSH and testosterone (Skinner et al., 1989). The authors admit that the changes in hormones did not show the expected pattern, and it is unclear if these changes are physiologically relevant or the product of conducting multiple comparisons (Duty et al. 2005).

In another study, serum and spot urine samples were collected from Taiwanese pregnant women in their second trimester to investigate associations between thyroid hormones and phthalate monoesters (Huang et al. 2007). Although reference ranges for estrodiol and thyroid hormones for pregnant women were not reported, the authors reported that more than 90% of the women had triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) thyroid hormones within the reference values for the general population. The authors found that the median level of freeT_4 (FT_4) for the pregnant women were at the lowest level of the general population, suggesting that approximately 50% of the participating women had a slight insufficiency in T_4 hormone levels, indicative of hypothyroidism. Analysis of the data using Spearman correlation coefficients resulted in significant ($p<0.05$) positive associations between estradiol and progesterone, T_3 and T_4, and T_4 and FT_4. Significant ($p<0.05$) negative correlations were found between T_4 and MBP, and FT_4 and MBP. Other correlations found were increased age and decreased T_3 and T_4 levels, increased body mass index (BMI) and increased urinary MBP (Huang et al. 2007).

Main et al. (2006) studied phthalates, including DBP, in human breast milk and its association with altered endogenous reproductive hormones in three month old infants.

Using samples from a prospective Danish-Finnish cohort study on cryptorchidism from 1997-2001, they found no correlation between monoester and cryptorchidism. There was a significant positive correlation between MBP and the sex hormone binding globulin ($p=0.01$) and MBP and the LH:free testosterone ratio ($p=0.006$). Free testosterone was significantly negatively correlated with MBP ($p=0.033$) with a negative change of 15% with a 10 fold increase in MBP.

Studies show that phthalates weakly bind to receptors (Zacharewski, 1998). Using an estrogen receptor (ER) competitive ligand binding assay, and mammalian and yeast based gene expression assays, Zacharewski et al. (1998) showed that DBP weakly competed with estradiol (E2) for the ER. DBP also showed 37% activity in a transiently transfected MCF-7 Gal-4 human ER construct at 10μM, where E2 is 100% at 10nM. DBP did not show any estrogenic activity *in vivo* when uterine wet weights and vaginal cell conification of ovariectomized Sprague Dawley rats orally treated with 20, 200, or 2000 mg/kg DBP dose were assessed.

In summary, DBP metabolite, MBP, was associated with increases in inhibin B and FSH in men tested at Massachusetts General Hospital. The increased hormones were associated with reduced sperm parameters, such as reduced sperm concentration, motility, and abnormal morphology (Duty et al., 2005). In addition, increased MBP was associated with decreased thyroid hormones T_4 and FT_4 in pregnant women when compared to general population reference values. However, no reference values for thyroid hormones in pregnant women were found. Main et al. (2006) found a correlation between MBP and increased sex hormone binding globulin and the LH:free testosterone ratio, and MBP and decreases in free testosterone in a prospective Danish-Finnish cohort study on cryptorchidism. However, no correlation was found between MBP and cryptorchidism. It was previously found that DBP can weakly bind and activate the estrogen receptor *in vitro*, but the relevance of binding is unknown at this time. No estrogenic activity was found *in vivo*. This data does not provide sufficient evidence to consider DBP toxic to the endocrine system.

Reproductive Effects

Human Data

Several attempts have been made to connect human reproductive effects to phthalate exposure. Jönsson et al. (2005) collected urine, sperm, and semen samples from men undergoing military medical examinations. Sperm concentration, motility, and integrity; semen volume; epididymal and prostate function; and serum reproductive hormones were evaluated. For those whose urine tested positive for monobutyl phthalate (MBP), a metabolite of DBP, no association between DBP exposure and the reproductive endpoints were found.

In Shanghai, semen from men ages 23 to 48 was collected from the Shanghai Institute of Planned Parenthood Research (Zhang, 2006). All men were out patients, but it is unclear if any of the men had a previous reproductive history. DBP concentration was

measured and semen quality was evaluated. Zhang reported no correlation between DBP concentration in the semen and sperm concentration or viability. The authors noted a positive correlation between liquefied time of semen (the amount of time it takes for the semen to become liquid at room temperature) and DBP concentration, and a negative correlation coefficient associated with semen quality and DBP concentration. The author stated that the negative correlation coefficient suggests that phthalates could affect sperm motility.

One study on the effects of DBP on female reproduction was reviewed. Blood samples were collected from infertile women with endometriosis and those without endometriosis, but having other causes of infertility (Reddy, 2006). In addition, blood samples were collected from fertile women with no history of gynecological disorders. DBP was measured by gas chromatography. There was a significant increase of DBP in infertile women with endometriosis compared to infertile women without endometriosis and fertile women ($p<0.05$). There was no significant difference in phthalate concentration between the infertile women without endometriosis and the fertile women. The authors concluded that higher serum DBP concentrations may be associated with increased endometriosis in women (Reddy, 2006).

Animal Data

Male Reproduction

Several studies were reviewed addressing the reproductive toxicity of DBP in male rats. Mahood et al. (2007) evaluated fetal and adult end points in Wistar rats after *in utero* DBP exposure of 0, 4, 20, 100, or 500 mg/kg from gd 13.5 to 20.5 or 21.5 through maternal oral gavage. A dose dependent decrease in the fertility of the male offspring was noted starting at the 20 mg/kg dose when offspring were housed for one week with proven fertile females. At 500 mg/kg, infertility was statistically significant ($p=0.03$). Ninety percent of the animals exposed to 500mg/kg DBP showed cryptorchidism, the absence of one or both testes from the scrotum, and a significant decrease in testicular weight at gd 21.5 and adulthood. Testicular testosterone levels were significantly decreased in gd 21.5 animals with 100 and 500 mg/kg exposures ($p<0.05$ and $p<0.001$, respectively). In gd 21.5 testis sections, the authors noted an increase in occurrence of multinucleated gonocytes starting at 20 mg/kg, with significance achieved at 100 mg/kg ($p<0.001$), a decrease in Leydig cell number, and an increase in Leydig cell size at the 100 and 500 mg/kg doses. These fetal endpoints suggest abnormal development of the testis. Focal dysgenesis in adult rats was statistically significant at 500 mg/kg dose ($p=0.029$), although focal dysgenesis was also noted with the 100 mg/kg dose. Focal dysgenesis was defined as malformed seminiferous tubules with intratubular Leydig cells and immature sertoli cells in testis with no other malformations. The authors concluded that the fetal endpoints were the most sensitive to DBP effects. Since infertility and cryptorchidism were only significantly increased at the highest dose, they were insensitive end points for the use of investigating lower dose effects of DBP exposure in fetal life. A NOAEL of 20 mg/kg/d was established (Mahood, 2007).

Female Reproduction

While it has been generally thought that female reproduction is less sensitive to phthalate treatment, recent studies have shown that DBP may actually have a significant effect on female reproduction. In a two part study, Gray et al. (2006) evaluated the effect of chronic exposure on fertility. In the first study, Long Evans hooded 21 day old female rats were administered an oral dose of DBP [0 (n=12) or 500 mg/kg/day (n=8)] for the duration of the study (gd 13 of third pregnancy). Females were examined for vaginal opening daily, and then estrous cyclicity was evaluated daily by vaginal smears. At day 83, each female rat was mated for 14 days to treated male rats. Litters were then counted and weighed at birth and postnatal day 15, when they were euthanized. The same female rats were then mated to untreated male rats, after a 30 day recovery period. Litters were euthanized at weaning (day 21). The same 12 female rats were mated for a third time, to untreated male rats. At gd 13, the 12 female rats were euthanized and necropsied, fetuses counted, and serum collected for progesterone analysis. DBP did not affect maturation, estrous cyclicity, or percentage of females mating or pregnant. However, the results did indicate that there was a significant decrease in the number of live pups delivered by treated females in both pregnancy 1 and 2 ($p<0.05$). The presence of blood in the vaginal lavages of some of the females suggested mid-pregnancy losses. At mid-pregnancy necropsies during the third pregnancy, researchers found no reduction of implantations in the DBP treated females, but did view a decrease in the percentage of viable fetuses. This is consistent with the reduced litter sizes of the first two pregnancies.

In the second study, 24 day old female rats (n=12-13) were orally dosed with 0, 250, 500, or 1000 mg/kg/day DBP five days a week until day 110, and then they were dosed 7 days a week until euthanization during the second pregnancy (Gray, 2006). On the first day of proestrus, female rats were mated for 24 hours to untreated male rats. Pups were counted and weighed at days 1, 5, and 15 before euthanization. The female rats were re-mated to untreated male rats for 24 hours. At gd 13, rats were euthanized by CO_2, serum was collected for hormone analysis, organ weights evaluated, fetuses counted, and stimulated ovary hormone evaluated *ex vivo*. Chronic exposure to DBP did not affect female rat growth or ability to mate. Results did show that 42% and 8% of treated females receiving 500 and 1000 mg/kg/day DBP were fertile when compared to 92% for untreated female rats. Litter sizes were also significantly reduced at these doses when compared to control animals ($p<0.01$). A large number of the pregnant females in the 500 and 1000 mg/kg/day treated group did not produce live pups and presented consistent pregnancy-like vaginal lavages with detectable blood at mid-pregnancy. During necropsy at gd 13, uterine weights ($p<0.05$, $p<0.05$) and the number of live fetuses ($p<0.01$, $p<0.01$) and total number of fetuses ($p<0.01$, $p<0.05$) were significantly reduced at the two highest doses of DBP (500 and 1000 mg/kg/d). The ovaries showed visible hemorrhagic corpora lutea, and the serum progesterone was significantly reduced at 1000 mg/kg/day (to 25%, $p>0.1$). When ovaries of females with live fetuses were stimulated *ex vivo*, the 500 and 1000 mg/kg/day dose groups had significantly reduced ($p<0.001$) progesterone production and

increased estradiol production. Ovaries from female rats with no live fetuses had low progesterone production similar to those seen in non-pregnant rats (Gray, 2006).

From these two studies, the authors concluded that DBP can cause a negative effect on female fertility. Also, the F1 generation is more sensitive to phthalate reproductive toxicity than the F0 generation. The authors concluded that the effect of phthalate exposure on female reproduction was previously over shadowed by phthalate effects on male reproduction because effect on pregnancy is not seen with shorter term studies. In addition, in standard testing treated females are mated with treated males. As a result of no obvious changes in females, it may have been assumed that infertility was due to the altered male reproductive tract development induced by phthalate exposure. The authors did not establish a NOAEL or LOAEL, but did note that their effects were seen below the previous NOAEL of 600 mg/kg/d set by McKee et al. (2004) (Gray, 2006).

Although it has been shown that DBP treatment does not reduce the ability of female rats to mate, Lee et al. (2006) did show that female Wistar rats exposed to 20, 200, 2000, or 10,000 ppm DBP from gd 15 to weaning showed decreased lordosis, the position female rats assume to allow male mounting, at all doses.

NTP, reported by Wine et al. (1997), evaluated the generational reproductive toxicity of DBP in male and female Sprague Dawley rats (~20/group) in a continuous breeding study (in conjunction with a crossover mating study). Male and female rats were fed a diet of 0, 0.1, 0.5, or 1% DBP, yielding average daily intakes of 52, 256, and 509 mg/kg for males and 80, 385, and 794 mg/kg for females through five litters (F1A-F1E) until the last litter (F1E) was weaned. The final litter (F1E) was given the same diet as their parents until necropsy, and was mated to non-sibling F1 males or females around day 88 for 7 days. F2 litters were evaluated after delivery and necropsied. The first four F1 litters were evaluated at birth and euthanized. The same F0 animals participated in the one week crossover study consisting of control male and female pairs, control males and 1% treated females, and 1% treated male and control females. In the crossover study, the litters were evaluated at birth and euthanized, and estrous cyclicity of F0 females was evaluated for 12 days by vaginal lavage. Male and female F0 rats were necropsied.

During the continuous breeding portion of the study, there was a significant decrease in body weight gain ($p<0.001$) with the high dose treatment. No other clinical signs of toxicity were seen, and the fertility and average number of litters per pair was not affected. However, a dose dependent reduction in live pups per litter was seen, as was weight reduction in live pups in the middle and high dose groups. Dam weights were significantly decreased during lactation for the final litter, by 7% at pnd 21 for the low dose treatment; by 6% at pnd 14 and 21 for the middle dose treatment; and by 10% at all time points for the high dose treatment. The F1E litters were fed and mated as previously stated. Male and female pups from the 1% treatment had weights that were reduced 10-15% compared to control animals. A significant decrease in mating, pregnancy rate and fertility rate in the high dose treatment group ($p<0.05$ for each parameter) was noticed: one litter was born to 20 breeding pairs in the high dose group compared to 19 litters born to 20 breeding pairs in the control group. In addition, a reduction in F2 live pup weights

was also seen. At necropsy, F1 males showed a significant increase in liver weight in the 1% group ($p<0.05$) and an increase in kidney weight in the middle and high dose groups ($p<0.05$ for each dose). Females showed no significant change in liver or kidney weights. F1 ventral prostate, seminal vesicles and right testis weights were all decreased in the 1% dose group, while the female ovaries were unchanged. In addition, epididymal sperm concentrations were decreased by 51% in the 1% treated male, and both total spermatid heads per testis and per gram of testis were decreased. The treated males also showed degeneration of seminiferous tubules in the middle and high doses, interstitial cell hyperplasia at the high dose, and under developed or defective epididymides in the high dose group (Wine, 1997).

In the crossover study, there was no significant difference in mating, pregnancy, or fertility rate, but a significant decrease in live pup weight for the control male and the 1% female group ($p<0.05$). Treated female rats weighed approximately 12% less than control rats, while male rat weights were unchanged. No other clinical signs of toxicity were seen. At necropsy of the F0 after the crossover study, liver and kidney weights were significantly increased in both sexes at the high dose ($p<0.05$). Gross structures of the ovaries and testes were not affected. Sperm appeared to be unaffected, and estrous cyclicity appeared unchanged (Wine, 1997).

The authors concluded that DBP shows mild reproductive toxicity in F0 rats, but the F1 rats have severely damaged reproductive function. It is also thought that the F0 females are more sensitive than the males to the systemic effects of DBP due to their reduced body weight at the 1% dose, compared to no change in the male body weight. In addition, the authors suggest that DBP is a developmental toxicant due to the reduced live pup weights seen at the 0.5 and 1% doses during the continuous breeding, crossover study (in the F1 litters), and that exposure during development rendered the animals much more sensitive to developmental toxicity compared to animals exposed in adulthood only. A NOAEL of 256 and 385 mg/kg-day for male and female rats was established by NTP due to the systemic effects seen (Wine, 1997).

Table 4: Continuous breeding reproductive endpoints* (Wine, 1997) (modified from CERHR, 2003)

# of rats	40	20	19	20
Dose (mg/kg-day)(male/female)	**0**	**52/80**	**256/385**	**509/794**
		↓live pups	↓live pups ↓pup weight	↓live pups ↓pup weight ↑liver/kidney:body weight
F_1 final litter (F1E)				
# of rats	20	20	20	20
		↓F_2 pup weight	↑kidney:body weight (M) ↑degen. of seminiferous tubules ↓F_2 pup weight	↓mating, pregnancy, fertility indices ↓sperm count ↑degen. Of seminiferous tubules, interstitial cell hyperplasia, underdev. Epididymis, malformed penises and prepuces ↓prostate/seminal vesicle:body weight ↓testes weight ↓male and female body weight ↑liver/kidney:body weight (M) ↓F_2 pup weight

*Statistically significant changes reported

In summary, the studies reviewed did not show a significant relationship between DBP exposure and reproductive health in humans. MBP is not an exclusive metabolite for DBP. Therefore, it cannot be concluded that DBP caused effects seen. Reddy et al. demonstrated an association between DBP concentrations and endometriosis. Animal studies showed a decrease in mating, fertility, and pregnancy rates, and a decrease in spermatid concentration in the males. The studies show that infertility may be due to effects seen in females as well as males. Based on these studies, CPSC staff considers DBP a reproductive toxicant under the FHSA.

Developmental Effects

The developmental effects of the *ortho*-di-alkylphthalate's (*o*-DAP) have been well-studied in animals. A thorough review of the developmental effects of o-DAP's in general is beyond the scope of this review. Briefly, perinatal exposure to certain phthalates is

associated with the "phthalate syndrome" in rats, which encompasses a range of effects on the development of the male genitourinary system including reduced anogenital distance (AGD), nipple retention, undescended testes, testicular atrophy, testicular histopathology, underdeveloped gubernacular cords, and hypospadias (reviewed in Foster et al. 2001; Foster 2006; Howdeshell et al. 2008). These effects persist into adulthood, even in the absence of further exposure (Barlow et al. 2004; compare McIntyre et al. 2001). The effects are mainly due to the inhibition of testosterone synthesis (Mylchreest et al. 1998; Foster et al. 2001; Gray et al. 2000; Parks et al. 2000), along with reduced expression of insulin-like hormone 3 gene (insl3) (Wilson et al. 2004). The specific cellular and molecular targets of o-DAP's are unknown (Howdeshell et al. 2008).

Human Data

One study was reviewed on the association of prenatal phthalate exposure, including DBP, and the decrease of male infant anogenital distance (AGD). Swan et al. (2005) used data from the Study of Future Families from 1999-2002. The study participants were pregnant women at least 18 years of age who were seen at one of the four study clinics. Their pregnancies ended in a live birth, and their babies were 2-36 months of age at the time of examination. Urine samples were collected from the mothers during mid-gestation, and physical examinations were done on the infants, as well as post natal maternal and infant urine samples collected. There were no obvious genital malformations or grossly abnormal parameters. Of all the male infants tested, 86.6% of them had normal or normal-retractile testes. Eighty-five, out of 214 mothers, were then tested for phthalate metabolites, including MBP, the main metabolites for DBP, and a major metabolite of benzyl butyl phthalate. Prenatal, post-natal and infant urine samples were evaluated in those 85 mothers and infants. There was a significant inverse relationship between MBP and the male anogenital index (AGI), a modification of AGD where AGD is divided by the weight of the child. Swan further grouped the examined infants based on their AGI; short AGI (n=25), those who fell below the 25th percentile, long AGI (n=17), those who were at or above the 75th percentile, and those in between were considered intermediate (n=43). Those in the short AGI group, on average, had an AGI 18.3% shorter than predicted by the regression model used. In addition, there was a significant increase of incompletely descended testicles (20, 9.5, and 5.9% for short, intermediate, and long ADI, respectively) when short ADI was compared to all other boys. Other significant correlations were the proportion of boys with scrotum categorized as small or indistinguishable from surrounding tissue, penile volume, and penile volume/weight. The authors concluded that the AGD is shortened and testicular descent impaired by prenatal maternal exposure to phthalates. However, it is unclear the source of the phthalates.

The Swan et al. study has raised questions about the non-conventional use of the AGI in humans and statistical methods used. There is no standardized range for a normal AGD or AGI. Several scientists have reviewed the data presented by Swan et al. and have not been able to follow statistical logic or reproduce significance and associations presented. NIH and CERHR (draft dated October 1, 2005) were unable to validate the findings reported (Butterworth, 2005). In addition, BASF outlined nine specific questions

regarding methodology, the results, and interpretation of data (Ott, 2005). Due to these questions and concerns, CPSC staff is unable to draw conclusions from this study.

Animal Data

There are many studies showing diverse developmental toxicities with *in utero* exposure to phthalates. Pregnant Sprague Dawley rats (20/group) were orally dosed with 0, 50, 250, or 500 mg/kg-day from gd 1 – pnd 21. Zhang et al. (2004) determined that the NOAEL for developmental and reproductive toxicity for DBP is 50 mg/kg-day. The endpoints for establishing this were a dose related decrease in birth weights, decreased AGD in male pups on pnd4, a significant ($p<0.01$) decrease in right epididymis and prostate weights, and increased liver weight when normalized to body weight. They also found reduced epididymal sperm motility and a dose dependent decrease in sperm production at pnd70.

The Zhang et al. study confirms the results of Mylchreest et al. (2000) who previously established a developmental toxicity NOAEL for DBP of 50 mg/kg/d after a 10 day, gd 12 – 21, oral administration of 0, 0.5, 5, 50, 100(19-20/group), or 500 mg/kg/d (n=11) DBP in Sprague Dawley rats. Mylchreest et al. showed a decrease in AGD at the highest dose on pnd 14 and nipple retention with the 100 and 500 mg/kg/d doses. In addition, 5 of the 58 rats in the highest dose had hypospadia. Other reproductive tract malformations included malformed epididymis in 40% of the animals and malformed vas deferens in 28% of the animals given the highest dose. In males, significantly ($p<0.05$) smaller epididymides, dorsolateral prostate, and levator ani-bulbocavernosus muscle were noted at the 500 mg/kg/d dose, and decreased testes weights at all doses. In females there were no treatment changes or altered gross morphology for any dose. The authors also noted testicular lesions at the 500 mg/kg/d dose. In addition to the NOAEL (50 mg/kg/d) established by this study, the authors also established a LOAEL of 100 mg/kg/d and a calculated reference dose of 500 µg/kg/d (calculated as NOAEL divided by 100 for uncertainty factors). The authors concluded from their current and previous work, that fetal testis and upper Wolffian duct are more sensitive to DBP toxicity than the urogenital sinus and genital tubercle.

Carruthers & Foster (2005) concluded that gestational day (gd) 16 – 18 is the critical window for male reproductive tract development. Using pregnant Sprague Dawley rats (n=11/group) treated on either gds 14 and 15, 15 and 16, 16 and 17, 17 and 18, 18 and 19, or 19 and 20 with 0 (n=18) or 500 mg/kg/d DBP, they reported a permanent decrease in male AGD when rats were exposed on gd 15 and 16 or gd 18 and 19. The AGD was also reduced with exposures on gd 16 and 17 or gd 17 and 18, but the reduction was no longer seen by pnd 90. Those individual rats exposed on gd 15 and 16, 16 and 17, 17 and 18, 18 and 19, or 19 and 20 showed a significant increase in areola and nipple presentation at pnd 13. Those males dosed on gd 16 and 17 retained the areola and nipple presentation through pnd 90. A significant number of epididymal and testes malformations were seen in males exposed on gd 17 and 18 ($p<0.05$). It was also shown that males exposed on gd 15 and 16 show significantly more seminal vesicle malformations ($p<0.05$), and those exposed on gd 19 and 20 showed malformations in the coagulating gland. Terminal organ weights

were collected at pnd 90 – 100. The authors reported that epididymis and testes weights were significantly increased in those rats exposed on gd 16 and 17 ($p<0.05$), and seminal vesicle weights significantly decreased in rat exposed on gd 15 and 16 ($p<0.05$). Histology revealed testicular abnormalities in all the dose groups, with those exposed on gd 15 and 16 showing severe abnormalities.

Other studies have also shown male and female delayed maturation, altered female estrous cyclicity, increased Leydig cell clustering and multinucleated gonocytes, and decreased testosterone (Salazar, 2004; Lee, 2006; Mahood, 2007; Lee, 2004; Jiang, 2007; Mylchreest, 2000).

One rabbit study was reviewed. Dutch Belted rabbits were exposed on gd 15-29 or pnw 4-12 or post pubertally for 12 weeks with 0 or 400 mg/kg/d DBP (Higuchi, 2003). Male rabbits were examined at week 6, 12, and 25. The authors reported that 1 out of 17 rabbits exposed to 400 mg/kg/d from gd 12 to 29 had hypospadia, hypoplastic prostate, and cryptorchid testes with carcinoma *in situ*-like cells at 12 weeks. Other rabbits in this group had a significant decrease in testes ($p<0.05$) and accessory sex gland weights ($p<0.01$) at week 12. This was persistent through week 25, except for the testis weights, which was not different than controls. Those rabbits exposed in adolescence, week 4 – 12, showed similar decreases in sex organ weights at 12 weeks as the *in utero* exposed rabbits. These weights returned to control-like by 25 weeks. In the adolescent exposed group, one rabbit was unilaterally cryptorchid. Animals treated postpubertally, at 6 – 8 months of age, for 12 weeks showed no change in sex organ weight. However, the thyroid gland weight was significantly increased ($p<0.05$). All three treatment groups, *in utero*, adolescent, and postpubertal, showed a significant decrease in the amount of morphologically normal sperm ($p<0.05$, $p<0.05$, and $p<0.01$, respectively). Those exposed *in utero* also have a decrease in ejaculate volume, sperm concentration, and total sperm per ejaculate. In the testes the degree of germinal epithelial loss was significantly higher in all treated groups ($p<0.05$ for all groups). The two rabbits with undesended testes showed atypical germ cells. The authors note that these morphological changes seen in the testes resemble carcinoma *in situ* reported in humans. One out of six rabbits in the postpubertal exposed group also presented with atypical germ cells. However, these were found in the seminiferous epithelium. The authors concluded that there were more pronounced effects in the *in utero* exposed animals, such as a permanent effect on the accessory sex glands, and that exposure to DBP *in utero* or during adolescence is a more sensitive time frame for toxicological effects to the reproductive system than exposure in adults (Higuchi, 2003).

In summary, DBP causes decreased AGD in males in animal studies. MBP is not an exclusive metabolite for DBP. Therefore, conclusions on DBP's effect on human AGD cannot be made. In addition, due the uncertainties related to the Swan study, no conclusion in humans can be drawn at this time. The animal studies show an increased incidence of undecended testes, hypospadias, reproductive organ malformations, and nipple retention in males. In females, the data shows altered estrogen cyclicity and delayed maturation. The data is sufficient to consider DBP developmentally toxic under the FHSA.

Genotoxicity and Carcinogenicity

Recently, several investigators have published papers on the ability of phthalates to alter gene expression. Using high density oligonucleotide DNA microarrays, Gwinn et al. (2007) showed altered expression in genes of interest in reproductive toxicity, signal transduction, protein processing, immune response, cell proliferation, organogenesis, and oncogenes after 5 or 10 hours of DBP treatment in four strains of normal human mammary epithelial cells. Inhibin A was increased in all the cell strains. Upon confirmation with Reverse Transcription Polymerase Chain Reaction (RTPCR), the microarray results were most likely not specific for inhibin A and included inhibin B. It was previously shown that knockout mice that were deficient in inhibins developed ovarian or testicular tumors. Knockout mice have been used to evaluate the role of inhibins in reproductive toxicology. Mitotic arrest deficient 2 gene (MAD2) is also altered in all cell strains. It is involved in the spindle assembly checkpoint mechanism. MAD2 binds with tumor necrosis factor α convertase (TACE) and MDC9, both of which are implicated in fertilization, sperm migration, myoblast fusion and other developmental processes. The presence of MAD2 has been implicated as a key causal agent in carcinogenesis studies. DNA cystine-5 methyltransferase (DNMT) was also decreased in the Gwinn et al. study. DNMT catalyzes DNA methylation. Mice deficient in DNMT have shown increased pre-implantation loss, and decreases in DNMT have led to increased disruption of embryo development and infertility in female mice. Decreases in placental growth factor (PIGF), a member of the vascular endothelial growth factor family (VEGF), may be related to preeclampsia. At shorter exposures to DBP, Gwinn et al. suggests there may be an increased risk of preeclampsia. However, at longer exposures, there is an increase in the gene expression. Therefore, DBP could provide a protective level of PIGF.

It has been previously suggested that phthalates cause DNA damage, having an impact on tumorigenesis and cancer outcomes (Kleinsasser, 2000; Anderson, 1999). In an *ex vivo* study using oropharyngeal mucosa biopsies from tonsillectomy patients with and without tumors, mucosa and lymphocyte cells were incubated for 60 minutes with 354 µmol/ml DBP or DiBP, an alternate isoform of DBP, and analyzed using the Comet assay. It was found that DBP caused genotoxicity in both cell types, showing a significant ($p<0.001$) difference from the negative control (Kleinsasser, 2001).

Ryu et al. (2007), using GeneFishing PCR on total RNA that was isolated from Sprague Dawley male rats orally administered 250, 500, or 750 mg/kg/day DBP for 30 days, saw that, not only were the testes weights in the 500 and 750 mg/kg/day rats significantly reduced, but also that 56 differentially expressed genes were seen in the 750 mg/kg/day dosed rat testes. The known genes were involved in xenobiotic metabolism, testis development, sperm maturation, steroidogenesis, and immune response, as well as the up regulation of peroxisome proliferation and lipid homeostasis genes. Using RTPCR, they found that the LDHA and Spag4 genes were significantly increased, and the PBR gene was significantly decreased in a dose dependent manner. They also found that at the

highest dose, 750 mg/kg/day, steroidogenic related genes SR-B1, StAR, P450scc and Cyp17 were significantly increased, while CYP19 was significantly decreased at 250 and 750 mg/kg/day DBP. Because these genes may play a significant role in cholesterol transport and steroidogenic pathways, testosterone levels were examined by RIA. Serum testosterone levels showed a decrease in all DBP treatment groups, but none were statistically significant. Ryu et al. also evaluated the expression of TR-α1, AR and ERβ proteins using western blot analysis and RTPCR. They found that the expression of TR-α1 was dose dependently increased, while AR and ERβ were significantly decreased in the 500 and 750 mg/kg/day exposure groups. In addition, protein expression of PPARγ was significantly increased at the highest dose, while RXR-γ remained unchanged. All reported statistically significant findings were $p<0.05$, as stated by authors. The authors concluded that DBP can significantly affect the testicular gene expression profiles involved in steroidogenesis and spermatogenesis affecting testicular growth and morphogenesis.

The granulin (grn) precursor gene and p130 gene were previously identified as sex steroid regulated genes in the rat hypothalamus that may be involved in sexual differentiation of the rat brain. In an effort to correlate serum sex steroid level and hypothalamic gene expression, Lee et al. (2006) used pregnant Wistar rats that were fed 20, 200, 2000, or 10,000 ppm DBP from gd 15 to weaning. On pnd 7, serum testosterone and estrodiol levels and gene expression of grn and p130 were evaluated. DBP (2,000 ppm) decreased estradiol in female rats, but the serum concentration of testosterone was unaffected and estradiol was unaffected at the other concentrations. At pnd 7, female pups showed an increase in grn gene expression with 2000 and 10,000 ppm doses, but grn expression was unchanged in male rats at these doses. The p130 gene expression was increased at the lower doses (20 and 200 ppm) in male rats, and was unaffected in female rats. The authors conclude that the increase in grn expression in female rats may be due to the DBP estrogenic properties, and the p130 gene increase in male rats may be due to DBP's mild androgenic properties due to the non-dose dependent nature of the increases.

No carcinogenicity studies related to DBP exposure were found. In addition, studies have shown a change in gene expression with exposure to DBP. However, there is insufficient data to determine that DBP is mutagenic.

Discussion

DBP is a phthalate ester that has become ubiquitous in our environment. While food intake is the main source of exposure, exposure can occur through indoor air, water, and soil. Exposure to DBP resulted in decreased body weights and increased liver and kidney weights in animals. However, there was one study that showed decreased liver and kidney weights. Inhalation exposure to DBP resulted in no change in lung, liver, or kidney weights; however, there were decreases in serum alanine aminotransferase and aspartate aminotransferase, and increases in serum albumin. Studies reviewed did not indicate that DBP was acutely toxic, but the chronic studies showed 50% mortality at the highest dose with no other clinical signs at the other doses. Given this data, DBP can be considered systemically toxic. However, the data do not support a conclusion that DBP is acutely toxic under the FHSA.

The studies reviewed provided evidence that DBP can be considered toxic to reproductive systems under the FHSA. The studies showed that DBP reduced fertility, mating, and pregnancy rates, as well as reduced sperm concentrations in male rats. NTP did an extensive continuous breeding and cross over study which showed that reduced reproduction may be due to the reproductive effects of DBP on female rats. This conclusion is in agreement with the Gray et al. study showing increased post implantation loss at mid-pregnancy in female rats. In addition, reduced progesterone was found in exposed female rats. Mahood et al. showed reduced fertility, as well as reduced sperm concentration and developmental malformations, when *in utero* exposed male rats were mated with proven fertile female rats. Reduced fertility was found at 20 mg/kg/d, providing a NOAEL of 20 mg/kg/d. This NOAEL was the lowest found, and shows male fertility to be the most sensitive endpoint. Therefore, an acceptable daily intake (ADI) of 0.2 mg/kg/d has been calculated for DBP (calculated from the NOAEL divided by safety factor of 100 for species difference and sensitive population).

The human data by Swan et al. showed an association between MBP and anogenital distance (AGD) in male infants. However, MBP is not an exclusive metabolite of DBP; therefore, it cannot be concluded that the MBP levels are due to the levels of DBP. Also, due to the questions raised about the design of the study and calculations used, additional work is needed before conclusions are able to be drawn from this study. The animal data showed an increased incidence of hypospadia, undescended testes, epididymis and testes malformations, and nipple retention. The critical window for male rat reproductive tract development was gd 16-18. In a rabbit study, two rabbits had a malformed penis, undescended testes, hypospadia, and altered epididymis and testis. These studies provide sufficient evidence that DBP can be considered developmentally toxic under the FHSA.

DBP may induce health effects through altering hormones. Duty et al. found that men with increased MBP showed normal inhibin B and increased FSH hormones. These findings were unexpected since inhibins generally inhibit FSH production. These results could be due to multiple comparisons. In a study that evaluated pregnant women's thyroid hormones, the authors found a negative correlation between MBP and T_4 and FT_4. Another study showed a positive correlation between MBP in human breast milk and sex hormone binding globulin and LH:free testosterone ratio in male infants. However, the authors were not able to show a correlation between an incidence of cryptorchidism and MBP. *In vitro* studies have shown that DBP weakly competes with estradiol for the estrogen receptor and activates the receptor. In *in vivo* studies, however, no estrogenic activity was seen.

Phthalates are found in indoor air and household dust. They have been associated with increased allergic symptoms and decreased airway function. An increase in MBP was correlated to a decrease in the respiratory function parameters. When smokers were removed from the data set, FVC remained significantly decreased. In one study, children diagnosed with wheezing and eczema within the previous year showed a non-significant increase in DBP levels in their environment.

The human studies on sensitization were inconsistent. Some showed sensitization to DBP by patch test after exposure to a consumer product. Animal testing did not show sensitization. This evidence is not sufficient to consider DBP a strong sensitizer.

Studies were reviewed showing DBP can alter expression of genes that are important in sex hormone synthesis and reproductive organ development. In addition, one study was reviewed showing DBP can cause DNA damage in cultured mucosa and lymphocyte cells isolated from tonsillectomy patients. No carcinogenicity studies related to DBP exposure were found. There is insufficient data to demonstrate that DBP to be carcinogenic under the FHSA.

To be considered a "hazardous substance" under the FHSA, the substance must first present one or more of the following hazards: it must be toxic, corrosive, flammable, an irritant or a strong sensitizer, or generate pressure through decomposition, heat, or in other ways; and second it must have the potential to cause substantial personal injury or illness during or as a result of any customary or reasonably foreseeable handling or use, including reasonably foreseeable ingestion by a child.

Following the definitions set forth by the FHSA, DBP can be considered toxic. However, the determination of potential injury or illness cannot be concluded at this time. Products would be considered 'hazardous' under the FHSA if oral exposure during 'reasonably foreseeable handling and use' were to exceed the ADI of 0.2 mg/kg/day DBP, which was calculated on the most sensitive endpoint found, reproductive and developmental effects.

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Appendix 1: NOAEL and LOAEL w/endpoints

Citation	Model	Route/Dose	NOAEL	LOAEL & Endpoint
Mahood et al. (2007)	Wistar Rats	Gavage 0, 4, 20, 100, and 500 mg/kg/d gd13.5-21.5	20mg/kg/d	100mg/kg/d *↓ testicular testosterone *↑ multinuceated gonocytes *↑ leydig cell cluster size
Lee et al. (2004)	IGS Rats	Dietary 0, 20, 200, 2000, and 10,000 ppm gd15-pnd21	NR	1.5-3.0mg/kg/d *(f) mammary gland hypoplasia *(f)↓pituitary weight *(m) testicular lesions *(m)↑pituitary weight
Zhang et al. (2004)	Sprague-Dawley Rats	Gavage 0, 50, 250, and 500mg/kg-day gd1-pnd21	50mg/kg-day	NR
Mylchreest et al. (2000)	Sprague-Dawley Rats	Gavage 0, 0.5, 5, 50, 100, and 500mg/kg/d gd12-21	50mg/kg/d	100mg/kg/d *↓Anogenical distance *retention of areola and nipples *↓testis weights *reproductive organ malformations
Patel et al. (2001)	Sprague-Dawley Rats	Dietary 1, 4, 10, 100, 1000, 10,000ppm	(m)60mg/kg-day (f)600mg/kg-day	
Ema et al. (2000)	Wistar Rats	0, 250, 500, 750, 1000, 1250, 1500mg/kg/d Gd0-8, 12-14, 18-20	(M) 500mg/kg/d	(M)750mg/kg/d *postimplantational loss 1000mg/kg/d *male reproductive malformations
Wine et al. (1997)	VAF Crl:CD BR SD albino rats	Dietary (m)0, 52, 256, 509mg/kg-day (f)0, 80, 385, 794mg/kg-day Cross generational		(M) (m)52mg/kg-day (f)80mg/kg-day *↓litter size *↓pup weight
Mylchreest et al. (2002)	Sprague Dawley Rats	0, 500mg/kg/d Gd12-21		500mg/kg/bw/d *testicular atrophy *↓testicular testosterone *Leydig cell hyperplasia
Lehmann et al. (2004)	Sprague Dawley rats	Gavage 0, 0.1, 1, 10, 30, 50, 100, 50mg/kg/day Gd12-19	30mg/kg/d	50mg/kg/d *↓testosterone

Kim et al. (2004)	Sprague Dawley Rats	Subcutaneus 0, 250, 500, 1000mg/kg-d Pnd 5-14	(m)500mg/kg-day	(m)1000mg/kg-day *↓testes & accessory organ wt.
Lamb et al. (1987)	CD-1 mice	Dietary 0, 40, 420, 1410 mg/kg-day	420mg/kg-day	1410mg/kg-day *(m)↓growth *(f)↑liver wt *↓fertile pairs *↓#of litters/pair *↓#of live pups/litter
Salazar et al. (2004)	Long Evans rats	Dietary 0, 12, 50 mg/kg-day (may be incorrect by author); 0, 40, 166mg/kg-day (NICNAS)	50/166mg/kg-day	12/40 mg/kg-day *↓testes wt *↓pup wt
Gray et al. (2006)	LE hooded rats	Gavage 0, 250, 500, 1000 mg/kg-day	(M)500mg/kg-day 250mg/kg-day	(M)1000mg/kg-day *↑liver wt 500mg/kg-day *↓#live pups *↓ovarian hormone production
Mylchreest et al. (1998)	Sprague Dawley rats	Gavage 0, 250, 500, 750mg/kg-day Gd3-pnd20		(M)250mg/kg-day *↓body wt gain *seminiferous tubules atrophy *hypospadias, underdev. epididymis
Higuchi et al. (2003)	Dutch belted rabbits	Gavage 0, 400mg/kg-day Pnw4-12 Pnw6-8	(M)400mg/kg-day	400mg/kg-day *testis pathology *↓%normal sperm *↓sex organ wts *↓sperm conc. *↓testosterone level
Wolf et al. (1999)	Sprague Dawley rats	Gavage 0, 500mg/kg-day Gd14-pnd3		500mg/kg-day *↓sex organ wts *↓AGD *retained nipples
Hamano et al. (1977)	ICR-JCL mice	Diet 0, 10, 100, 400 mg/kg-day Gd1-18	(M)100mg/kg-day	(M)400mg/kg-day *↑kidney wt *↓#live pups *↑malformations
Shitoa et al. (1980)	ICL-ICR mice	Diet 0, 80, 180, 350, 660, 1200 mg/kg-day Gd1-18	(M)660mg/kg-day 350mg/kg-day	(M)2100mg/kg-day *↓bw gain 660mg/kg-day *↑resorption *↓fetal wt
Ema et al. (1993)	Wistar rats	Gavage 0, 500, 630, 750, 1000mg/kg-day Gd7-15	(M)500mg/kg-day	(M)630mg/kg-day *↓body wt gain *↑resorptions

Ema et al. (1994)	Wistar Rats	Gavage 0, 750, 1000, 1500mg/kg-day gd 7-9,10-12,13-15		750mg/kg-day *↑post implantation loss *↑malformations
Sallenfait et al. (1998)	Sprague Dawley rats	Gavage 0, 500, 1000, 1500, 2000mg/kg-day Gd14	(M)1000mg/kg-day 500mg/kg-day	(M)1500mg/kg-day *↓wt gain 1000mg/kg-day *↑skeletal malformations
Nikonorow et al (1973)	Wistar rats	Gavage 0, 120, 600mg/kg-day Gd0-21	120mg/kg-day	600mg/kg-day *↑resorption *↓# fetuses *↓fetus wt
Mylchreest et al. (1999)	CD rats	Gavage 0, 100, 250, 500mg/kg-day Gd12-21	(M)500mg/kg-day	100mg/kg-day *delayed preputial separation
Ema et al. (1998)	Wistar rats	Diet 0, 331, 555, 661mg/kg-day Gd11-21	(M)331mg/kg-day	(M)555mg/kg-day *↓bw gain *↓food consumption *↑cryptorchidism *↓AGD
Ema et al. (1997)	Wistar rats	Diet 0, 895mg/kg-day Gd0-11		(M)895mg/kg-day *↓bw gain *↓food consumption *↑resorptions
Ema et al. (1997)	Unknown rats	Gavage 0, 1500mg/kg-day Gd6-16		(M)1500mg/kg-day *↑post implantation loss (except gd7&11) *↑fetus malformations gd8-9&15
Barlow et al. (2004)	CRL:CD(SD) BR rats	Gavage 0, 100, 500mg/kg-day Gd12-21	100mg/kg-day	500mg/kg-day *testicular lesions *germ cell degeneration 100mg/kg-day *areolae retention pnd13
Carruthers & Foster (2005)	SD rats	Gavage 0, 500mg/kg-day Gd14-15, 15-16, 16-17, 17-18, 18-19, 19-20		500mg/kg-day *↓AGD gd15-16, 18-19 *areolae/nipple retention gd16-17 *epididymal malformation gd16-17 *small testes gd16-17
BASF (1992)	Wistar rats	Dietary (m)0, 27, 142, 688mg/kg-day (f)0, 33, 162, 816mg/kg-day 3months	(m)142mg/kg-day (f)162mg/kg-day	(m)688mg/kg-day (f)816mg/kg-day *peroxisomal proliferation *liver histology *↑liver & kidney wt (f) *↓thyroid hormone *anemia (m)

Marsman (1995)	F344 rats	Dietary (m)0, 176, 359, 720, 1540, 2964mg/kg-day (f)0, 177, 356, 712, 1413, 2943mg/kg-day 13 weeks	(m)176mg/kg-day (f)177mg/kg-day	(m)359mg/kg-day (f)356mg/kg-day *↑liver & kidney wt (m) *peroxisomal proliferation *anemia (m)
Marsman (1995)	$B6C3F_1$ mice	Dietary (m)0, 163, 353, 812, 1601, 3689 mg/kg-day (f)0, 238, 486, 971, 2137, 4278,mg/kg-day 13 weeks	(m)353mg/kg-day	(m)812 mg/kg-day (f)238 mg/kg-day *↑kidney wt (f) *↑liver wt (m) *↓bw gain
Ema et al. (1995)	Wistar rats	Gavage (MBP) 0, 250, 500, 625mg/kg-day Gd7-15	(M)250mg/kg-day	(M)500mg/kg-day *↓bw gain *↑prenatal mortality *↓fetal wt *↑malformations *↑visceral variations

*data collected from CERHR (2000), NICNAS (2008), ATSDR (2001), EPA (2005), and referenced articles

Appendix 2: Reproductive and Developmental Critical Effects

Citation	Model/Dose	Critical Effects
Reproductive		
Gray et al. (2006)	Rats – oral 0, 250, 500, 1000mg/kg/d	↓%fertility
Mahood et al. (2007)	Rats – oral 0, 4, 20, 100, 500mg/kg/d Gd13.5-20.5 or 21.5	↓fertility
Jiang et al. (2007)	Rats – gastric intubation 0, 250, 500, 750, 1000mg/kg-day Gd14-18	↓live births ↓pup gestational days
Ema et al. (2000)	Rats – gastric intubation 0, 250, 500, 750, 1000, 1250, 1500mg/kg Gd0-8	↓implantations & live births
Zhang et al. (2004)	Rats – gavage 0, 50, 250, 500mg/kg-day Gd1-pnd21	↓sperm count
Lee et al. (2004)	Rats – diet 0, 20, 200, 2000, 10000ppm Gd15-pnd21	Alt. estrous cyclicity ↓sperm counts
Salazar et al. (2004)	Rats – diet 0, 12, 50mg/kg rat/d 2 months	↓live births
Reddy et al. (2006)	Infertile human women	↑endometriosis
Lee et al. (2006)	Rats – diet 20, 200, 2000, 10000ppm Gd15-pnd21	↓lorodosis ↓#ejaculations
Wine et al. (1997)	CD Rats – diet (m)52, 256, 509 (f)80, 385, 794mg/kg-day Continuous breeding	↓live pups/litter ↓pup wt ↑liver and kidney wts ↓fertility ↓sperm count ↑malformed penises ↑underdeveloped epididymis
Gray et al. (1999)	LE Hooded – gavage 0, 250, 500, 1000mg/kg-day Multigenerational to F2	↑malformations ↓fertility ↑testicular atrophy ↓sperm production ↑midterm abortions ↓fecundity ↓#of live pups
Developmental		
Johnson et al. (2008)	Orl Rats – oral 0, 50, 100, 200mg/kg-day Gd12 and 21	↓testes wt. ↑ cryptorchidism
Mahood et al. (2007)	Rats – oral 0, 4, 20, 100, 500mg/kg-day Gd13-20.5 or 21/5	↓testes wt. ↑ cryptorchidism

Jiang et al. (2007)	Rats – gastric intubation 0, 250, 500, 750, 1000mg/kg-day Gd14-18	↑hypospadia ↑ cryptorchidism ↓AGD ↓reproductive organ wt
Carruthers & Foster	Rats – oral 500mg/kg-day Gd14-20	↓AGD ↑nipple retention ↑reproduction organ malformations
Zhang et al. (2004)	Rats – gavage 0, 50, 250, 500mg/kg-day Gd1-pnd21	↓AGD ↑ cryptorchidism ↑underdeveloped reproductive organs
McKinnell et al. (2005)	Rats – oral 0, 500mg/kg-day Gd13.5-21.5	↑ cryptorchidism
Salazar et al. (2004)	Rats – diet 0, 12, 50mg/kg rat/d 2 months	↓testes wt Delayed maturation
Mylchreest et al. (2000)	Sprague Dawley Rats – gavage 0, 0.5, 5, 50, 100, 500mg/kg-day Gd12-21	↓AGD ↑nipple retention ↓reproductive organ wt ↑reproduction organ malformations
Lee et al. (2004)	Rats – diet 0, 20, 200, 2000, 10000ppm Gd15-pnd21	↑nipple retention ↑hypospadia ↓testes wt.
Swan et al. (2005)	Paired human male infants and mothers	↓AGI ↑ cryptorchidism
Lee et al. (2006)	Rats – diet 20, 200, 2000, 10000ppm Gd15-pnd21	↓AGD ↑AGD (females)
Shiota et al. (1980, 1982)	Mice – diet 0, 80, 180, 350, 660, 2100mg/kg-day Gd0-18	↑resorptions ↓fetal wt Neural tube defects Delayed ossification
Ema et al. (1993)	Wistar Rats – gavage 0, 500, 630, 750, 1000mg/kg-day Gd7-15	↑complete resorptions ↓live fetuses/litter ↓fetal wt ↑external malformations
Ema et al. (1998)	Wistar Rats – diet 0, 331, 555, 661mg/kg-day Gd11-21	↓AGD in males ↑undescended testes ↓fetal wt ↑external and skeletal malformations
Marsman et al. (1995)	F344/N Rats – diet 0, 92, 184, 368, 551, 736, 1472mg/kg-day Gd0-weaning F1 pups - diet (f)133, 275, 500, 836, 1104 (m)143, 284, 579, 879, 1165 Pnd28 for 4 weeks	↓fetal wt ↓pups/litter Complete pup mortality ↑kidney and liver wts ↑hypospermia ↓testis wt

Mylchreest et al. (1998)	CD rats – gavage 0, 250, 500, 750mg/kg-day Gd3-weaning	↑hypospadia ↑underdeveloped sex organs ↓AGD ↓kidney wt
Mylchreest et al. (1999)	CD rats – gavage 0, 100, 250, 500mg/kg-day Gd12-21	↓AGD ↑nipple retention ↑hypospadia ↑underdeveloped sex organs ↑testicular and epididymal lesions ↑interstitial adenoma ↑undescended testes
Gray et al. (1999)	Sprague Dawley – gavage 0, 500mg/kg-day Gd14-pnd3 LE Hooded Rats – gavage 0, 500mg/kg-day Gd16-19	↓AGD ↑areolas/nipple retention ↑hypospadia Testicular and epididymal atrophy/agenesis ↓sex organ wt
Imajima et al. (1997)	Wistar King A rats – gavage 0, 1000mg/kg-day Gd15-18	↓testicular descent and undescended testes

*data collected from CERHR (2000), NICNAS (2008), ATSDR (2001), EPA (2005), and referenced articles

Appendix 3: LD_{50}

Model/Route	**LD_{50}**	**Citation**
Rat – oral	8000 mg/kg bw	Smith 1953
Rat – oral	6300 mg/kg bw	BASF 1961
Mouse – oral	4840 mg/kg bw	BIBRA 1987
Rabbit – dermal	>20000 mg/kg bw	Clayton et al. 1994
Rabbit – dermal	>20000 mg kg bw	RTECS 1993
Rat – inhalation	≥15.68 mg/L/4hr	Greenough et al. 1981
Mouse – IV	720 mg/kg/bw	RTECS 1993
Rat – IM	>8000 mg/kg bw	Smith 1953
Mouse – IP	3400 – 4000 mg/kg bw	BASF 1961
Mouse – IP	3400 – 4000 mg/kg bw	Calley et al. 1966
Mouse – IP	3400 – 4000 mg/kg bw	Lawrence et al. 1975
Rat – IP	3178 mg/kg bw	Singh et al. 1958
Rat – IP	4200 mg/kg bw	BASF 1958
Mouse – SC	20800 mg/kg bw	RTECS 1993

*as sited in NICNAS (2008)