Human Health Hazard Assessment

Dibutyl Phthalate (DBP)
(CAS No. 84-74-2)

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INTRODUCTION

This review of dibutyl phthalate (DBP) is a health hazard assessment only. For this assessment, two key reviews on DBP prepared by the European Chemicals Bureau (ECB, 2004) and the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2003) were consulted. Information from these reviews was supplemented with relevant studies from more recent literature surveys conducted up to September 2006.
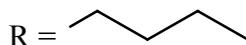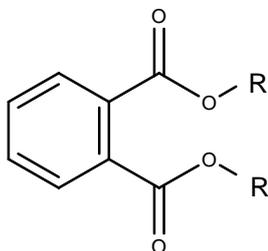
References not marked with an asterisk were examined for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also noted in this assessment and marked with an asterisk.

Hazard information from this assessment is published also in the form of a hazard compendium providing a comparative analysis of key toxicity endpoints for 25 phthalates (NICNAS, 2007).

1. IDENTITY

1.1 Identification of the Substance

CAS Number: 84-74-2
Chemical Name: 1,2-Benzenedicarboxylic acid, dibutyl ester
Common Name: Dibutyl phthalate (DBP)
Molecular Formula: $C_{16}H_{22}O_4$
Structural Formula:

Molecular Weight: 278.34
Synonyms: DBP (ester); Di-n-butylphthalat; Phthalic acid, dibutyl ester; Bis-n-butyl phthalate; Butyl phthalate; Dibutyl o-phthalate; Di(n-butyl) 1,2-benzenedicarboxylate; n-Butyl phthalate; Phthalic acid di-n-butyl ester
Purity/Impurities/Additives: Purity: ≥ 99% w/w
                                Impurity: ca. 0.01% w/w butan-1-ol
                                          ca. 0.01% w/w butyl benzoate
                                Additives: none

1.2 Physicochemical Properties

Table 1: Summary of physicochemical properties

Property	Value
Physical state	Oily liquid
Melting point	-69°C
Boiling point	340°C (101.3 kPa)
Density	1045 kg/m^3 (20°C)
Vapour pressure	9.7 ± 3.3 x 10^{-6} kPa (25°C)
Water solubility	0.01 g/L (20°C)
Partition coefficient n-octanol/water (log Kow)	4.57
Henry's law constant	(8.83 – 45) x10^{-7} atm.m^3/mole
Flash point	157°C

Source: ATSDR (2001); ECB (2004)

2. USES

DBP is used as a plasticiser in resins and polymers. DBP is also used as a softener in adhesives, lacquers, varnishes and printing inks. The ubiquity of DBP in consumer products is demonstrated by its wide usage in cosmetics: a perfume solvent and fixative, a suspension agent for solids in aerosols, a lubricant for aerosol valves, an antifoamer, a skin emollient and a plasticiser in nail polish and fingernail elongators (ECB, 2004).

In Australia, DBP is mainly imported as finished products or mixtures. The chemical is used industrially for automotive repair and assembly (in adhesives), mining and construction coatings (e.g. sealants; clear wood and waterproofing coatings, protection for marine structures and vessels), explosives, rocket propellants, in textiles and leather treatments and as a plasticiser in nitrocellulose lacquers, elastomers, rubber, and epoxy products. Screen printing inks also contain DBP. Downstream products include safety glass, resins, adhesives, sealants, fragrance bases for household, personal care and cosmetic products, children's toys, exercise balls, hoses and rubber sheets.

3. HUMAN HEALTH HAZARD

3.1 Toxicokinetics

Previous Evaluations

Absorption and excretion

DBP was readily absorbed from the gastrointestinal tract in oral studies in rats and hamsters given ^{14}C-DBP (dose not given). Between 63 and ≥90% of the dose was excreted in urine within 48 h (Foster et al., 1983*; Tanaka et al., 1978*; Williams & Blanchfield, 1975*). Faecal excretion was low (1.0-8.2%) (Tanaka et al., 1978*).

Tomita et al. (1977*) reported oral absorption of DBP in humans after detecting increases (cf. controls) in blood levels in 13 individuals who had ingested food contaminated with DBP from plastic packaging.

After dermal application (with occlusion) of 43.7 mg/kg bw/d (157 μmol/kg bw/d) ^{14}C-DBP in ethanol to the clipped skin of male F344 rats, 10-12% of the administered dose/day was excreted in urine for a sum of ca. 60% over 7 days (length of application not mentioned). One percent of the dose was excreted in faeces over 24 h for a sum of ca. 12% over 7 days (Bronaugh et al., 1982*; Elsisi et al., 1989*).

Scott et al. (1987*) demonstrated a slower absorption of DBP by human skin (2.40 μg/cm^2/h) than by rat skin (93.35 μg/cm^2/h) in an *in vitro* study with undiluted DBP.

In a placental transfer study, pregnant Sprague-Dawley rats received an oral dose of 500 or 1500 mg ^{14}C-DBP/kg bw/d on gestational day (GD) 14. Maternal and foetal tissues were collected at intervals from 0.5 to 48 h. Radioactivity in embryonic tissues was <0.12 - 0.15% of the dose. Radioactivity in the placenta and embryo was less than or equal to one-third of that in maternal plasma. No accumulation of radioactivity was observed in maternal or embryonic tissues. DBP and its metabolites, mono-n-butyl phthalate (MBP) and MBP-glucuronide, were shown to rapidly transfer to embryonic tissues but at levels that were consistently lower than those in maternal plasma. Most of the radioactivity recovered in maternal plasma, placenta and embryo was attributed to MBP with intact DBP present at low levels (Saillenfait et al., 1998*).

Kaneshima et al. (1978*) reported a recovery of 4.5% of the dose in bile collected 6 h after a single oral dose of 500 mg ^{14}C-DBP/kg bw/d in 50% ethanol was administered to male rats.

Tanaka et al. (1978*) reported 32.2% and 56.7% dose recovery over 3 days in the bile of 2 rats dosed with a single oral dose of 60 mg ^{14}C-DBP/kg bw/d. DBP and MBP were the main products in the bile (ratio 1:1). However, it is likely the DBP was reabsorbed from bile and then ultimately excreted in urine.

Distribution

No significant retention was seen in any organ after male Wistar rats were dosed with 0.27 or 2.31 g ^{14}C-DBP/kg bw/d in corn oil (tissue distribution was similar at both dose levels). The highest activity was recorded in the kidneys (0.66%) and the lowest was recorded in the brain (0.03%), 4 h after administration. Radioactivity was detected at 0.4% of the dose in the blood, at both dose levels, after 24 hours. Less than 0.01% was detected in all tissues after 48 h (Williams & Blanchfield, 1975*).

Tanaka et al. (1978*) determined retention in 14 different tissues after administering 60 mg ^{14}C-DBP/kg bw/d (in DMSO) orally to rats. No retention was seen in brain, heart, lung, spleen, testicles, prostate and thymus at 24 h after administration and low amounts were detected in the following tissues: liver (0.06%), kidneys (0.02%), muscle (0.3%), adipose tissue (0.7%), intestines (1.53%), stomach (0.01%) and blood (0.02%).

Wistar rats (24 males) received ground rat chow with 2% corn oil and 0.1% DBP for 12 weeks, with the controls receiving ground rat chow with 2% corn oil. Eight treated rats and 4 controls were terminated at 4, 8 and 12 weeks. No significant accumulation was detected in any tissue (Williams and Blanchfield, 1975*).

A week after dermal application (with occlusion) of 43.7 mg/kg bw/d (157 μmol/kg bw/d) ^{14}C-DBP in ethanol to the clipped skin of male F344 rats, tissue permeation was as follows: adipose tissue (0.41%), skin (1.4%), muscle (1.1%), all other tissues (<0.5%). A third of the dose remained at the site of application (Elsisi et al., 1989*).

Kawano (1980a*) performed a study in rats to measure organ distribution of DBP after inhalation. Highest concentrations of DBP were found in the brain after daily exposure for

both 3 and 6 months (maximum 0.54 – 1.46 mg/kg), at both dose levels. Accumulation in other organs was less marked.

Biotransformation

After oral administration of DBP to rats, mono-n-butyl phthalate (MBP), MBP glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones and carboxylates) and a small amount of phthalic acid were detected (Albro & Moore, 1974*; Foster et al., 1983*; Tanaka et al., 1978*; Williams & Blanchfield, 1975*).

After oral administration of 2 g DBP/kg bw/d in rats and hamsters, 37.6% and 52.5% of the dose, respectively, was recovered as MBP-glucuronide and 14.4% and 3.5%, respectively, as unconjugated MBP, in urine (Foster et al., 1983*).

In vitro studies with liver homogenates (rat, baboon, and ferret), kidney homogenates (rat), and intestinal cell preparations (rat, baboon, ferret, man) showed hydrolysis of DBP to MBP (Lake et al., 1977*; Rowland et al., 1977*; Tanaka et al., 1978*; White et al., 1980*).

A rat liver microsomal fraction demonstrated rapid hydrolysis of DBP to MBP (73% within 2 h). Intestinal mucosal cell preparations of rat, baboon, ferret and also, human preparations were shown to be able to hydrolyse DBP to MBP. Rate of hydrolysis by rat GIT contents was the greatest with small intestine contents and slower with caecal and stomach contents. Phthalate diester hydrolase activity decreased in the order baboon>rat>ferret (Lake et al., 1977*; Rowland et al., 1977*).

An *in vitro* study using an everted gut sac preparation from rat small intestine showed only 4.5% of DBP crossed the intestinal mucosa, with the remainder being hydrolysed by esterases in the mucosal epithelium, before it reached the serosal perfusion solution. Inhibition of esterases reduced DBP hydrolysis but also significantly reduced DBP absorption; MBP absorption was unaffected (White et al., 1980*).

Data not Reported in Previous Evaluations

The pharmacokinetics of MBP and MBP glucuronide were not influenced by chemical (parent DBP vs metabolite MBP), vehicle (oil vs aqueous), dose level (10-50 mg/kg bw MBP vs 50-250 or 500-1500 mg/kg bw DBP), and route of exposure (oral vs i.v.) (Kremer et al., 2005). Following i.v. dosing with MBP (10, 30, or 50 mg/kg bw/d) on GD 19 in pregnant dams, MBP was metabolised to MBP glucuronide within 5 min, and MBP and MBP glucuronide disappeared from maternal and foetal plasma within 24 h (Kremer et al., 2005).

Conclusion

In laboratory animals (rats and hamsters), DBP is rapidly absorbed and excreted after oral administration, with ≥ 90% excreted in urine within 24-48 h. Faecal excretion is limited. DBP is also excreted in bile and consequently enters the enterohepatic circulation. No significant accumulation in tissues was seen in laboratory animals after oral and dermal exposure. Limited data suggest some accumulation in tissues following inhalation exposure.

DBP is also absorbed in humans after oral exposure.

DBP is mostly hydrolysed to MBP and its analogue alcohol prior to absorption by the small intestines (DBP hydrolysis can also occur in liver and kidneys). Metabolites in urine include MBP, MBP-glucuronide, various ω- and ω-1-oxidation products of MBP (more polar ketones and carboxylates) and a small amount of phthalic acid.

No data on biotransformation after dermal or inhalation exposure are available.

DBP was found to be absorbed dermally in rats with ca. 60% of the dose being excreted in urine within a week; faecal excretion was ca. 12%. The *in vitro* flux rate for DBP across rat skin was ca. 40-times faster than across human skin. Absorption data from inhalational exposure are not available.

Placental transfer studies revealed DBP and its metabolites, MBP and MBP-glucuronide, rapidly transferred to embryonic tissues but were at levels consistently lower (1/3) than those in maternal plasma.

3.2 Acute Toxicity

Previous Evaluations

Oral studies in rats and mice established LD50 values of 4840 to 5289 mg/kg bw for mice and 6300 to 8000 mg/kg bw/d for rats. None of these studies were performed under GLP conditions.

LD50 values of >20000 mg/kg bw/d were determined in dermal studies in rabbits. No further information was available and no indication of GLP-compliance was given (Clayton & Clayton, 1994*).

Inhalational studies revealed a 2-h LC50 value of 25 mg/L in mice. Decreased respiration, ataxia, pareses, hind leg paralysis and marked irritation of eye and upper respiratory mucous membranes were observed.

Mice exposed to 0.25 mg/L for 2 h and cats exposed to 1 mg/L for 5.5 h both displayed nasal mucous membrane irritation. Salivation, restlessness and languor were seen in cats exposed to 11 mg/L. These symptoms abated on cessation of treatment.

Sprague-Dawley rats (5/sex/dose) were exposed to 12.45, 15.68 and 16.27 mg DBP/L of air for 4 hours, and observed for 14 days, in a GLP compliant study. Controls were air exposed. The corresponding respirable fraction was 64.4%, 56.9%, and 59.9%, respectively. The LC50 was estimated to be ≥15.68 mg/L/4h. A reduction in respiratory rate was seen at 15.68 mg/L. Excessive grooming in surviving animals led to persistent poor coat condition throughout the study. Macroscopy of the lungs revealed the following anomalies: white foci in all lobes in 1 male and 1 female rat at 15.68 mg/L, dark red regions in 2 female rat at 12.50 mg/L, and 1 male and 1 female rat at 16.27 mg/L (Greenough et al., 1981*).

A Russian report cited in RTECS (1993a*) reported a LC50 of 4.25 mg/L in the rat but no further information was provided and the study was not GLP compliant.

A summary of acute studies is shown in Table 3 (all studies except Grenough et al. (1981) were not GLP compliant).

Table 3. Acute animal toxicity studies

Study	Species	Results (LD50/LC50)	References
Oral	Rat	8000 mg/kg bw	Smith, 1953*
		6300 mg/kg/bw	BASF, 1961*
	Mouse	4840 mg/kg/bw	BIBRA, 1987*
Dermal	Rabbit	>20000 mg/kg/bw	Clayton & Clayton, 1994*
			RTECS, 1993b*
Inhalation	Rat	≥15.68 mg/L/4h	Greenough et al., 1981*
Other routes			
i.v.	Mouse	720 mg/kg/bw	RTECS, 1993c*
i.m.	Rat	>8000 mg/kg/bw	Smith, 1953*
i.p.	Mouse	3400 – 4000 mg/kg/bw	BASF, 1961*
			Calley et al., 1966*
			Lawrence et al., 1975*
i.p.	Rat	3178 mg/kg/bw	Singh et al., 1972*
i.p.	Rat	ca. 4200 mg/kg/bw	BASF, 1958*
s.c.	Mouse	20800 mg/kg/bw	RTECS, 1993d*

Human studies

Cagianut (1954*) reported the following symptoms in a man following accidental ingestion of 10 g DBP: nausea, vomiting, dizziness, lacrimation, photophobia and eye pain. Keratitis erosiva of the cornea was noticed. Urinalysis revealed pathological leucocyte counts, oxalate crystals and microhaematuria. A 14-day mydriatic and antibiotic course resulted in recovery.

Data not Reported in Previous Evaluations

No data.

Conclusion

The oral LD50 value for rats is 6300 – 8000 mg/kg bw/d, the dermal LD50 for rabbits is >20000 mg/kg bw/d and the inhalational LC50 (4 h) for rats is ≥15.68 mg/L.

3.3 Irritation

Skin Irritation

Previous Evaluations

A study in rabbits with undiluted DBP (OECD Guideline 404) revealed slight erythema in 2/3 animals, immediately after exposure and 24 h after initiation. No oedema was seen. Erythema disappeared 48 h after commencing the study. DBP was not considered a skin irritant (BASF, 1990a*).

Greenough et al. (1981*) reported mild reactions 24 h after 0.5 mL undiluted Vestinol C (DBP trade name) was applied to intact and abraded skin of rabbits. 10% laurylsulphate was the positive control. No reaction was observed after 72 h at any treatment site. The irritation index was calculated as 0.54/8.

A study cited in NTP-CERHR Report on DBP (CERHR, 2003) reported minor irritation in rabbit dermal occlusion studies at 520 mg/kg bw/d.

Data not Reported in Previous Evaluations

No data.

Conclusion

DBP causes minimal skin irritation in rabbits.

Eye Irritation

Previous Evaluations

In a study in rabbits with undiluted DBP (OECD Guideline 405), prominent conjunctival redness was observed after 1 h and 24 h in all animals which reduced in severity after 48 h. Normality was attained after 72 h. DBP was not considered to an eye irritant (BASF, 1990b*).

Undiluted 0.1 mL Vestinol C (trade name of DBP) was applied to the eyes of rabbits (3/sex) and the eyes were not rinsed. Mild redness in 3/6 animals and extremely mild redness in 3/6 animals was seen after 1 h. After 24 h, very mild redness was observed in 2/6 animals. Iris or corneal reactions were not seen. The irritation index was calculated as 0.11/110. DBP was not considered an eye irritant (Greenough et al., 1981*).

Data not Reported in Previous Evaluations

No data.

Conclusion

DBP causes minimal eye irritation in rabbits.

Respiratory Irritation

Previous Evaluations

Irritation of nasal mucous membranes was seen in cats after 5.5 h exposure to 1 mg DBP/L (1000 mg/m^3) and in mice after 2 h exposure to 0.25 mg/L (250 mg/m^3). No additional data were available (BIBRA, 1987*; BUA, 1987*).

Wistar rats were head-nose exposed to DBP (liquid aerosol) for 6 h/d, 5 d/w for 4 weeks. At the maximum exposure of 509 mg/m^3, red crust at the snouts was seen after cessation of daily treatment in 4/10 animals (effect was prominent between days 13-27). Recovery occurred 18 h after treatment. Histopathology revealed a dose-related increase in mucous cell hyperplasia in the nasal cavity (levels II, III and IV) and in squamoid metaplasia in the larynx (level I), at all dose levels (1.18, 5.57 or 509 mg/m^3). No inflammation or epithelial changes were seen in the nasal cavity (Gamer et al., 2000*).

Data not Reported in Previous Evaluations

No data.

Conclusion

DBP caused nasal mucous membrane irritation in mice after inhalational exposure of 0.25 mg/L for 2 h. Red crust formation of snouts in rats was seen after repeated exposure to 0.5 mg DBP/L (approx. 509 mg DBP/m^3). Concentrations above 0.001 mg/L (approx. 1.18 mg/m^3) caused local histopathological changes in nasal cavity and larynx but no inflammation.

3.4 Sensitisation

Previous Evaluations

No sensitisation was observed in 2 guinea-pig maximisation studies performed according to OECD Guideline 406 and a GLP-approved FDA recommended method, respectively (BASF, 1990c*; Greenough et al., 1981*).

No sensitisation was seen in a non-GLP repeated patch test in rabbits (BASF, 1957*).

Human Studies

Oliewiecki et al. (1991*) reported recurrent 'ear infections' from a hearing aid in a 71-year-old woman. Dermatitis resulted in areas in contact with spectacle frames (behind the ears and on temples). Patch tests with 5% DBP, 5% dimethyl phthalate (DMP) and 5% diethyl phthalate (DEP) in petrolatum solvent gave positive results in each case. Less positive reactions were seen with scrapings from the spectacle frame or hearing aid.

Calnan (1975*) and Sneddon (1972*) reported dermatitis of the axillae in 2 women after use of antiperspirant spray containing DBP. Both women gave positive responses when patch tested with DBP, but not to other constituents of the spray.

Schulsinger and Mollgaard (1980*) found 1/1532 positive reaction after routine patch testing with phthalate ester mixture (2% DMP, 2% DEP and 2% DBP in petrolatum).

Patch tests were done with cosmetics (nail polish with 6 or 9% DBP or deodorant with 4.5% DBP) or 5% DBP in petrolatum, on 13 to 159 people in 11 different studies, including 48 h closed patch tests, modified maximisation tests, (modified) repeated insult patch tests, 21 d cumulative irritancy tests, prophetic patch tests and controlled use studies (2 d or 4 w long). In 9/11 studies, no irritation, contact sensitisation or photosensitisation were seen. In 2/11 studies with 9% nail polish and 4.5% deodorant, with 13 and 12 persons respectively, minor irritation was seen. The subjects received twenty one 23-24 h lasting patches on the same side of the back (no further data available) (Anonymous, 1985* cited in ECB, 2004).

Data not Reported in Previous Evaluations

No data.

Conclusion

DBP did not display skin sensitising properties in 2 maximization tests in guinea pigs. Human data available are limited and ambiguous.

3.5 Repeated Dose Toxicity

Previous Evaluations

<u>Oral</u>

Several studies of general toxicity and specific effects on peroxisome proliferation were available. The results of oral studies are compiled in Table 4 and key studies are summarised below.

Table 4. *Summary of oral repeated dose toxicity studies in animals*

Repeated dose toxicity	*Administration mode and duration*	*Species*	*Results*	*References*
1. Oral (general toxicity)	Diet, 86 or 90 days	Mouse	LOAEL 500 mg/kg bw/d, degeneration of parenchyma	Ota et al., 1973*; 1974*
	Diet, 13 weeks	Mouse	NOAEL (males) ca. 353 mg/kg bw/d. LOAEL (males) ca. 812 mg/kg bw/d, ↓ body weight gain, ↑ rel. liver weights and testis zinc levels. LOAEL (females) ca. 238 mg/kg bw/d, ↑ kidney weights.	NTP, 1995*
	Diet, 34-36 days	Rat	LOAEL ca. 250 mg/kg bw/d, ↓ body weight gain.	Murakami et al., 1986*
	Diet, 90 days	Rat	NOAEL ca. 152 mg/kg bw/d. LOAEL ca. 752 mg/kg bw/d, ↑ liver and kidney weights, haematological and clinical effects, and histopathological changes in liver.	Schilling et al., 1992*
	Gavage, 3 months	Rat	LOAEL 120 mg/kg bw/d, ↑ rel. liver weights.	Nikonorow at el., 1973*
	Diet, 13 weeks	Rat	NOAEL ca. 177 mg/kg bw/d. LOAEL ca. 357 mg/kg bw/d, ↑ liver and kidney weights, haematological and clinical effects.	NTP, 1995*
	Diet, 4 weeks		LOAEL ca. 51.5 mg/kg bw, ↑ liver weights.	BIBRA, 1990*
2. Oral (peroxisome proliferation)	Diet, 1 year	Rat	NOAEL ca. 62.5 mg/kg bw/d (only dose tested).	Nikonorow at el., 1973*
	Diet, 1 year	Rat	NOAEL ca. 125 mg/kg bw/d. LOAEL ca. 625 mg/kg bw/d, ↓ survival rates.	Smith, 1953*
	Diet, 2 weeks	Rat	NOAEL 200 mg/kg, ↑ activity of LAH-11 and LAH-12.	Jansen et al., 1993*
	Diet, 3 weeks	Rat	LOAEL ca. 600 mg/kg bw/d, ↑ activity of PCoA, LAH-11 and LAH-12, and ↑ liver weights.	Barber et al., 1987*; BIBRA, 1986*

	Diet, 4 weeks	Rat	NOAEL ca. 104 mg/kg bw/d, ↑ activity of PcoA. LOAEL ca. 51.5 mg/kg bw/d, ↑ liver weights.	BIBRA, 1990*
	Diet, 3 months	Rat	NOAEL ca. 152 mg/kg bw/d. LOAEL ca. 752 mg/kg bw/d, ↑ peroxisome proliferation.	Kaufmann, 1992*
3. Oral (testicular effects)	Diet, 15 days	Rat	LOAEL 250 mg/kg bw/d, histopathological and testicular effects	Srivastava et al., 1990*

↑ = increased, ↓ = decreased
LAH-11 and LAH-12 = 11- and 12-lauric acid hydroxylase (indicators for peroxisome proliferation)
PCoA = cyanide-insensitive palmitoyl-CoA oxidase activity (indicator for peroxisome proliferation)
Adapted from ECB (2004).

Ota et al. (1973*; 1974*) reported necrosis and notable vacuolar degeneration of hepatocytes and cysts, and degeneration of renal tubular epithelium after administering 2.5% DBP in the diet to mice (ca. 5000 mg/kg bw/d – high dose group). Parenchymal degeneration and minor histopathological changes in the liver were seen at the low dose group of ca. 500 mg/kg bw/d.

In a 13-week dietary study in B6C3F1 mice (10/sex/dose) at 0, 0.125, 0.25, 0.5, 1.0 or 2.0% (equivalent to 0, 163, 353, 812, 1601 and 3689 mg/kg bw/d in males and 0, 238, 486, 971, 2137 and 4278 mg/kg bw/d for females), the following findings were reported:

- Significant decrease in growth at 0.5% and above in both sexes.
- Significant increase in relative liver weight at 0.5% and above, significant increase in absolute and relative kidney weight in females at all dose levels (not significant at 2.0%) and significant decrease in epididymal weights in males.
- Significant decreased haematocrit (Hct) count in females at 2.0%.
- Hepatocellular cytoplasmic alterations (indicative of glycogen depletion) were revealed in males at 1.0% and above, and in females at 2%. Peroxisome proliferation was observed in hepatocytes at 2.0%. Lipofuscin accumulation was seen in the liver at 1.0% and above.
- Serum testosterone levels were generally higher in treated groups reaching significance at 0.125% only. Testicular zinc concentrations were significantly higher at 0.5% and above.
- Significant increase in spermatid heads/g of testis at 2.0%.

The NOAEL in males was established to be 353 mg/kg bw/d and LOAEL was 812 mg/kg bw/d based on changes in growth, liver weight and testicular zinc levels. A NOAEL could not be established in females because of organ weight changes (kidney) at all dose levels (NTP, 1995*).

In a 13-week dietary study in F344/N rats (10/sex/dose) at 0, 0.25, 0.5, 1.0, 2.0 or 4.0% DBP (equivalent to 0, 176, 359, 720, 1540 and 2964 mg/kg bw/d for males and 0, 178, 356, 712, 1413, 2943 mg/kg bw/d for females) the following effects were reported:

- A statistically significant decrease in growth was observed in males at ≥1.0% and females at ≥2.0% levels. Emaciation resulted from decreased food consumption in all animals at 4.0%.
- Increase in relative liver and kidney weights (males at ≥0.5% and females at ≥1.0%), and decrease in testes weight (males at ≥2.0% level; statistically significant).
- Haemoglobin (Hb) values and erythrocyte counts were decreased significantly in males at ≥ 0.5%. Hct values were decreased at ≥0.5 % levels but were statistically significant only

at ≥2.0%. Blood platelet counts were elevated to statistically significant levels in males at ≥0.5%. Nucleated RBC levels were statistically significantly increased in all animals at 4.0%.

- Cholesterol concentrations decreased statistically significantly in both sexes at ≥2.0%. Triglyceride levels decreased in a dose-relate fashion and were statistically significantly in males at all dose levels and in females at ≥1.0%. Statistically significant increases in serum alkaline phosphatase (males at ≥2.0% and females at ≥1.0%) and bile acid concentration (males at ≥2.0% and females at ≥0.5%) were seen. PCoA activity was elevated in both sexes in a dose-related manner at ≥0.5%.
- Hepatocellular cytoplasmic alterations (indicative of glycogen depletion) were revealed in both sexes at ≥1.0%. Minor eosinophilic granulation and peroxisome proliferation were seen at 4.0% level. Dose-related germinal epithelium degradation was seen at ≥1.0% levels with complete loss at 4.0%.
- Statistically significant decreases were seen in testicular Zn and serum testosterone levels (at ≥ 2.0%) and serum Zn levels (at 4.0% levels). Lipofuscin accumulation was seen at ≥ 1.0%.
- Statistically significant decreases (at 2%) in spermatid heads/testis and per g of testis, epididymal motility and number of epididymal spermatozoa per g epididymis.

The NOAEL in this study was established at the 0.25% level (equivalent to 177 mg/kg bw/d) and a LOAEL of 5% based on perturbations in haematological parameters and organ weight changes (NTP, 1995*).

A NOAEL of 152 mg/kg bw/d for changes in haematological and clinical chemistry parameters was determined in a 3-month dietary toxicity study in Wistar rats performed according to OECD Guideline 408. At 752 mg/kg bw/d, changes in the following parameters were observed: haematology (decreased Hb, Hct and erythrocyte counts) and clinical chemistry (decreased triglyceride levels, increased serum glucose and albumin levels). Statistically significant increases were seen in the activity of cyanide-insensitive palmitoyl-CoA oxidase (PCoA – indicator for peroxisome proliferation), and liver and kidney weights. T3 levels decreased significantly. Histopathology revealed a reduction or absence of lipid deposition in hepatocytes at 752 mg/kg bw/d. No effects on the testes were observed (Schilling et al., 1992*).

The following studies were not performed according to any guideline nor were they GLP compliant.

In a dietary study, Wistar rats (5 males/dose) were given 0.5% and 5% DBP in the diet (equivalent to 250 and 2500 mg/kg bw/d, respectively) over 34-36 days. Decreased body weight gains were seen at both dose levels and were statistically significant at 5%. Various clinical parameters showed statistically significant changes at 5%. Microsomal hepatic changes were seen at both dose levels. Peroxisome proliferation was observed at both dose levels but was more pronounced at 5%. The LOAEL was considered to be at 0.5% (250mg/kg bw/d). However, the study showed several limitations (nature of changes and magnitude were not reported) (Murakami et al., 1986*).

In a 3-month gavage study performed by Nikonorow et al. (1973*), Wistar rats (10/sex/group) received 120 or 1200 mg/kg bw/d DBP. A statistically significant increase in liver weight was seen at all doses. The LOAEL was 120 mg/kg bw/d.

The same authors performed a 12-month dietary study in Wistar rats (20/sex/group) at 0 or 0.125% DBP (62.5 mg/kg bw/d). 10% and 15% mortality were seen in the controls and treated groups, respectively. Clinical signs, pathological and haematological parameters were all normal. The NOAEL was 62.5 mg/kg bw/d. However, the study had only one dose group amongst other limitations.

In a one-year dietary study by Smith (1953*), rats (10 males/dose) were given 1.25, 0.25, 0.05 and 0.01% DBP (equivalent to 625, 125, 25 and 5 mg/kg bw/d). Mortality was 50% at the 1.25% level during the first week. No anomalies in clinical signs, haematology, pathology or histopathology were seen at the other dose levels. The NOAEL was 125 mg/kg bw/d. However, this study was extremely limited (rat species not identified, organ weights not determined, clinical chemistry parameters not evaluated).

Peroxisome proliferation

Several phthalate esters have been linked with peroxisome proliferation in rodents. The following studies examined enzyme changes and histopathological alterations suggestive of peroxisome proliferation.

In a 2-week dietary study, male Wistar rats were given 20, 60, 200, 600 and 2000 mg DBP/kg of diet (equivalent to 1.1, 5.2, 19.9, 60.6 and 212.5 mg/kg bw/d). The NOAEL was 60.6 mg/kg bw/d for PCoA activity and 19.9 mg/kg bw/d for LAH-11 and LAH-12 (11- and 12-lauric acid hydroxylase, respectively) induction. Therefore, the overall NOAEL for induction of peroxisome-associated enzymes was 19.9 mg/kg bw/d (Jansen et al., 1993*).

Male and female F344 rats were given 0.6, 1.2 and 2.5% DBP in the diet (ca. 600, 1200 and 2100 mg/kg bw/d) in a 3-week dietary study. The lowest dose (ca. 600 mg/kg bw/d) resulted in increased activities of peroxisome associated enzymes (PCoA, LAH-11 and LAH-12), increased liver weights and decreased serum triglyceride and cholesterol levels. A NOAEL could not be established (Barber et al., 1987*; BIBRA, 1986*).

Male F344 rats were given 0.05, 0.1, 0.5, 1 and 2.5% DBP in the diet (equivalent to 51.5, 104, 515, 1040 and 2600 mg/kg bw/d) in a 4-week dietary study. Liver weights were statistically significantly increased at all doses, in a dose-related manner. The NOAEL for PCoA activity was 104 mg/kg bw/d (BIBRA, 1990*).

Wistar rats (3/sex/group) received 400, 2000 or 10000 mg DBP/kg in the diet (ca. 30, 152 and 752 mg/kg bw/d) in a 3-month dietary toxicity study. The frequency and severity of peroxisome proliferation was measured by histochemical analysis of the number and size of peroxisomes. The NOAEL for peroxisome proliferation was ca. 152 mg/kg bw/d (Kaufmann, 1992*).

Testicular effects

The following studies have specifically examined testicular effects of DBP.

Repeated oral exposure to DBP resulted in distinct testicular changes in rats. The lowest dose level of 250 mg/kg bw/d caused changes in testicular enzymes associated with spermatogenic cell atrophy. Histopathology revealed testicular degeneration in 5% of tubules at this dose (Srivastava at al., 1990*).

At ≥500 mg/kg bw/d, the following effects were seen in several studies: decreased weight of testes and accessory sex glands, spermatocyte depletion, seminiferous tubule degeneration, decrease in testicular zinc and serum testosterone levels, increase in testicular testosterone levels and urinary zinc excretion (Cater et al., 1977*; Gray et al., 1982*, 1983*; Oishi & Hiraga, 1980a*; Srivastava et al., 1990*).

Repeated oral exposure caused severe testicular changes in guinea-pigs at 2000 mg/kg bw/d (Gray et al., 1982*).

Mice and hamsters were less susceptible to testicular effects. Oral administration of 2000 mg/kg bw/d by gavage to mice for 9 days or 2% DBP in the diet (ca. 2400 mg/kg bw/d) for 7 days resulted in minor testicular changes (Gray et al., 1982*; Oishi & Hiraga, 1980b*). Studies in hamsters did not reveal any testicular effects at 2000 or 3000 mg/kg bw/d orally for 9 days or 500 mg/kg bw/d orally for 35 days (Gray et al., 1982*; 1983*). Oral administration of 1000 mg/kg bw/d for 35 days produced marked effects. Species-related differences in testicular toxicity appear to be related to differences in free monobutyl phthalate (MBP) concentrations (MBP is a metabolite of DBP and is known to cause testicular changes in the rat) (Foster et al., 1981*, 1983*; Oishi & Hiraga, 1980c*; Tanaka et al., 1978*; Zhou et al., 1990*).

Dermal

Slight irritation and dermatitis were reported in a 90-day dermal study in rabbits by Lehman (1955*). The animals received dermal applications of 0.5, 1.0, 2.0 or 4.0 mL DBP/kg bw/d to the clipped intact skin. Slight renal damage was seen at 4.0 mL/kg bw/d. This study had severe limitations and was poorly documented (strain of rabbits not identified, number and sex of animals, duration of daily application, dose levels at which effects were seen).

Inhalation

Sprague-Dawley rats (15 males/dose) were exposed 6 h/d to 0, 0.5, 2.5 and 7.0 ppm DBP (ca. 0, 6, 28 and 80 mg/m^3) in a 5-day inhalation study. Body, lung and liver weights were unaffected. Microsomal cytochrome P-450 (Cyt. P-450) levels were markedly affected in the lung at 28 mg/m^3 and above (unaffected in the liver). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity and serum albumin levels were statistically significantly increased at 80 mg/m^3. Serum alkaline phosphate (SAP) activity and serum total protein levels remained normal (Walseth & Nilson, 1984*; 1986*).

Wistar rats (5/sex/dose) were head-nose exposed 6 h/d, 5 d/w, for 4 weeks, to 0, 1.18, 5.57, 49.3 or 509 mg DBP/m^3 of air as liquid aerosol in an inhalation study performed according to OECD Guideline no. 412 and 407 (for clinical and neurofunctional examinations and pathology). There were no mortalities. Red crust formation at the snouts (recovery within 18 hours) was seen at cessation of daily exposure at 509 mg/m^3 in a maximum of 4 animals from days 13-27.

Apart from a statistically significant increase in rearing of males at 49.3 mg/m^3 from a functional observation battery, no treatment-related findings were shown by open-field observations, home cage observations, sensorimotor/reflex tests or motor activity measurements.

Statistically significant decreases in food, water consumption and food efficiency were seen intermittently in only one sex (not specified) and did not show a dose-relationship. These changes were considered minor. No significant deviation of mean body weights was seen. Haematology, clinical chemistry and urinalysis parameters were normal with the exception of a statistically significant decrease in serum sodium levels in females at 509 mg/m^3. However, this was considered minor given the effect being sex-limited.

Absolute lung weights and testes were significantly changed at the lower doses. However, these effects were considered incidental as they were not dose- related.

Histopathology revealed a dose-dependant increase in incidence of mucosal cell hyperplasia in the nasal cavity. The severity ranged from grade 1 (minimal) to grade 2 (slight). Inflammation and epithelial abnormalities were absent. A dose-related increase in incidence of squamoid metaplasia (minimal degree) was seen (0/1/3/4/5 males and 0/1/3/5/4 females at 0, 1.18, 5.57, 49.3 and 509 mg/m^3, respectively).

No systemic effects (including neurotoxicity) were seen at up to and including the highest dose of 509 mg/m^3. Since dose-dependent changes were localised in the nasal cavity and can be considered adaptive, the systemic NOAEC was established as 509 mg/m^3. The LOAEC for local adaptive effects in upper respiratory tract was 1.18 mg/m^3 (Gamer et al., 2000*).

In another study, male Wistar rats (11-14/sex/dose) were exposed to 0.5 or 50 mg DBP mist/m^3 for 6 months, 6 d/w, 6 h/d (3 h for Saturday). Growth was reduced and elevated relative brain, kidney, lung and testes weights were observed at 50 mg/m^3 (only significant for brain and lung weight). Absolute weights were not provided. Haematology revealed decreased levels of lymphocytes and elevated neutrophil counts at both doses without a dose-response. Clinical chemistry revealed changes in certain parameters (mild increases in ALT, AST and SAP activities, serum glucose and triglyceride levels; decrease in serum cholesterol) at both doses at random time points (not dose-related). Gross and histopathology examinations were not performed. The NOAEL in this study was 0.5 mg/m^3 (Kawano, 1980b*).

Human studies

Workers (147) involved in the manufacture of artificial leather and exposed chronically to phthalates (mostly DBP and higher phthalates but also traces of adipates, sebacates and tricresylphosphate) were investigated for toxicity in a study by Milkov et al. (1973*). Forty seven workers experienced polyneuritis (frequency increasing with period of exposure) and 22 had neurofunctional disturbance. Vestibular and olfactory receptor excitability and cutaneous sensitivity were found to be reduced. Ambient vapour or aerosol levels of the plasticizers at the workplace were 1.7-60 mg/m^3. No control group was available.

Male workers involved in the production of phthalate esters, including DBP, were investigated for neurological symptoms in a cross sectional study. Twenty three workers were exposed to phthalates, 6 to phthalic anhydride and 9 to alcohols. Mean phthalate concentration varied from 1-5 mg/m^3 with a maximum of 61 mg/m^3. Polyneuropathy was observed in 12/23 subjects exposed to phthalates and bilateral painful decreased sensitivity of skin or senses of the hands and feet was observed in 7 of these 23 subjects (3 showed reduced sense of vibrations). In the alcohol exposed group, 2/9 showed sensory neuropathy and 1/6 showed hyporeflexia in the anhydride exposed group (Gilioli et al., 1978*).

Human studies for neurological symptoms showed severe limitations (lack of controls, small population size, lack of sufficient protocol documentation and results and contaminated exposure). Therefore, these studies are unsuitable for assessment of the neurotoxicological potential of DBP to humans.

Data not Reported in Previous Evaluations

No data.

Conclusion

Human studies are considered inadequate because of their limitations.

In animals, the oral NOAEL for general toxicity was 152 mg/kg bw/d in a 3-month dietary study in rats (performed according to current OECD guidelines). The LOAEL was 752 mg/kg bw/d based on changes in haematological and clinical chemistry parameters (increases in palmitoyl-CoA oxidase activity, and decreases in T3 levels), increased liver and kidney weights and histopathological changes (decrease or absence of hepatocyte lipid deposition). No testicular or neurological changes were seen at this dose. However, in a separate study, 250 mg/kg bw/d appeared to be the lowest effect level in rats with regard to testicular toxicity.

In a separate study, the NOAEL with regards to peroxisome proliferation in rats was 19.9 mg/kg bw/d.

In a 4-week inhalational study in rats performed according to OECD guidelines, the NOAEC was 509 mg/m^3 for systemic effects including neurological effects. No LOAEC for systemic toxicity was established. A LOAEC was established at 1.18 mg/m^3 for local adaptive effects in the upper respiratory tract. For these effects, no NOAEL was established.

Data were inadequate to establish a NOAEL for repeated dermal exposure.

3.6 Genetic Toxicity

Previous Evaluations

The mutagenicity of DBP was evaluated in a battery of *in vitro* tests (with and without metabolic activation) which included gene mutation assays (*S. typhimurium, E. coli, S. cerevisiae*), mouse lymphoma assay (L5178Y TK+/- and L5178 TK+/-), cytogenetic assay (chromosomal aberrations with chinese hamster lung cells and sister chromatic exchange rates in chinese hamster ovary cells) and DNA-repair tests (*E. coli* and *B. subtilis*).

The majority of the tests yielded negative results except for the following: an equivocal result in a bacterial gene mutation assay in *S. typhimurium* (TA100) in the absence of metabolic activation (S9 fraction), weak positive increases at cytotoxic doses in another gene mutation assay (TA100) in the absence of S9 fraction and a positive result in a mouse lymphoma assay in the absence of S9 fraction.

In vivo studies included gene mutation tests (sex-linked recessive lethal test in Drosophila) and chromosomal aberration tests (micronucleus assay in NMRI and B6C3F1 mice). All tests were negative for genotoxicity.

Data not Reported in Previous Evaluations

Kleinsasser et al. (2000a) reported, using an *in vitro* Comet assay, that DNA damage (single-strand breaks) was significantly induced by both DBP (354μmol/ml) and DIBP (354μmol/ml) in 70 human oropharyngeal and nasal mucosa samples, as compared to the negative control (DMSO). The effect of DIBP was more pronounced than that of DBP.

In further work, Kleinsasser et al. (2000b) found that DBP and DIBP were more genotoxic than N-nitrosodiethylamine and benzo[a]pyrene, in terms of inducing strand breaks in DNA, in both blood lymphocytes and normal mucosal cells from the oropharynx or larynx of 60 human patients with head and neck cancer.

Conclusion

DBP induced DNA damage (single-strand breaks) in an *in vitro* Comet assay. However, these results were not confirmed by the *in vitro* chromosomal aberrations, direct DNA damage and more importantly, the *in vivo* sex-linked gene mutation and chromosomal aberrations studies. Therefore the validity of the *in vitro* DNA damage results is questionable. DBP was also shown to be negative in the majority of bacterial, yeast and mammalian mutation studies.

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

3.7 Carcinogenicity

Previous Evaluations

No cell transformation was induced in BALB/3T3 cells in the absence of metabolic activation (Litton Bionetics, 1985*).

Data not Reported in Previous Evaluations

A phthalate ester mixture containing 21.9% DBP was tested in an *in vitro* mammalian C3H/10T1/2 cell transformation assay. The mixture did not induce cell transformation at doses ranging from 0.0195 to 0.0025 μl/ml (Nuodex, 1982).

DBP was also tested on Balb/c-3T3 mouse cells (Barber et al., 2000). With an exposure period of 72 hours and incubation over 4 weeks, DBP did not induce statistically significant increases in transforming activity with concentrations up to 82 nl/ml.

Using PPARα-null mice, Lapinskas et al. (2005) recently showed that expression of PPARα is necessary for DBP induced liver effects (hepatomegaly and induction of fatty acid metabolising enzymes).

No *in vivo* carcinogenicity studies were available for assessment.

Conclusion

Data are insufficient to determine the carcinogenic potential of DBP.

3.8 Reproductive Toxicity

Traditional hazard assessments consider reproductive toxicity separate from developmental toxicity. Reproductive toxicity is tested by exposing sexually mature adults to a chemical and examining the effects on the animal capacity to reproduce. Developmental toxicity is studied by exposing pregnant dams and looking for effects in the foetuses. However, these tests generally do not detect chemicals that induce effects that only appear postnatally. Thus, chemicals that affect the developing reproductive system following prenatal exposure may also affect sexual maturation or functional reproductive disorders that are only apparent at maturity. Developmental toxicity can therefore lead to reproductive toxicity and the two endpoints cannot be clearly distinguished.

The effects of DBP on reproductive endpoints have been tested in a variety of species including rats, mice and hamsters. Test procedures have repeat dose toxicity studies that dose adult animals for varying duration, two-generation studies, prenatal developmental toxicity studies (only the dam is dosed, study ends before parturition) and developmental/postnatal studies (dam is dosed during gestation and allowed to litter, study ends during weaning).

In this hazard assessment, data will be presented on the basis of test procedure. The effects on fertility and development will then be discussed separately in the conclusion. Reproductive and developmental toxicity data are summarised in Table 5.

3.8.1 Human Studies

Previous Evaluations

DBP was thought to induce hormonal changes leading to decreased fertility and vaginal cycle disruptions in a cross-sectional study in women (189) working in conditions involving DBP exposure. However, quantitative data were unavailable and the women were also exposed to other unknown compounds (Aldyreva et al., 1975*).

Data not Reported in Previous Evaluations

Duty et al. (2003) studied whether the general population levels of phthalate monoesters in urine were associated with altered semen quality. In this study 168 male partners, aged 20 to 54 years, of sub-fertile couples were recruited. The comparison group was men with all three semen parameters above the reference values. Eight urinary phthalate monoesters were measured in a single spot urine sample collected on the same day as the semen sample. The results from this study indicated that median monobutyl phthalate (MBP) levels (mean 15.7 ng/ml urine) were associated with sperm motility and sperm concentration below the reference values with odds ratio (95% confidence interval) of 2.37 (1.13 − 5.00) and 2.41 (0.80 − 7.23), respectively. A non-significant negative association between urine MBP levels and sperm velocity was observed (Duty et al., 2004). In a later study, Jonsson and colleagues studied semen parameters and urinary phthalate monoester levels in 234 military recruits (Jonsson et al., 2005). There were no significant associations between highest versus lowest urinary MBP quartile and any of the dependent variables. When human sperm suspensions were incubated with DBP (0, 0.4, 4, 40 mM) for up to 18 hours, the mean motility and

straight-line motion was dose-dependently decreased at doses higher than 0.4 mM (60% motility at 4 mM) (Fredricsson et al., 1993).

Breast milk samples were analysed for six different phthalate monoesters in a Danish–Finnish cohort study on cryptorchidism, gonadotropins, sex-hormone binding globulin, testosterone and inhibin B (Main et al., 2006). No association was found between MBP and cryptorchidism. However, MBP showed positive correlations with sex-hormone binding globulin and LH:free testosterone ratio and was negatively correlated with free testosterone.

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

Pan et al (2006) measured the gonadotropins and gonadal hormone levels of 74 male workers exposed to high levels of both DBP and DEHP. Urinary MBP and MEHP levels (normalized to creatinine) were significantly higher in exposed workers compared with controls. Free testosterone was significantly lower in exposed workers and was negatively correlated with MBP and MEHP concentrations in urine.

Laboratory animals

3.8.2 Repeat Dose Toxicity Studies

Previous Evaluations

In oral studies described in Section 3.5, repeated oral exposure to DBP resulted in distinct testicular changes in rats, with mice and hamsters less susceptible to testicular effects. The LOAEL was 250 mg/kg bw/d based on changes in testicular enzymes associated with spermatogenic cell atrophy and testicular degeneration in 5% of tubules following 15 days exposure (Srivastava at al., 1990*).

Data not Reported in Previous Evaluations

Kim et al. (2004) gave neonatal male rats subcutaneous injections of 0, 5, 10 or 20 mg/animal (ca. 250 – 1000 mg/kg bw) on postnatal days (PND) 5 to 14. Controls were given corn oil. Underdevelopment of reproductive organs (testes, seminal vesicles, ventral prostate and levator ani plus bulbocavernosus muscles) was seen at 20 mg when observed at PND 31. The effect persisted at PND 42 for all organs except for the ventral prostate. Histopathology revealed minor Leydig-cell hyperplasia in the interstitium of affected tubules. The study also looked at the changes in expression of androgen receptors (ARs), estrogen receptors (ERs) and steroidogenic factor-1 (SF-1) in testes. At 20 mg/animal, significant decreases in AR expression and significant inhibition of ER expression were seen. A dose-related increase in

expression of ER and SF-1 was seen on PND 31. The authors concluded that DBP disrupted AR or ER expression in early neonatal stages, leading to its antiandrogenic action. The NOAEL was 10 mg/animal (ca. 500 mg/kg bw/day) and the LOAEL was 20 mg/animal (1000 mg/kg bw/day) based on decreased male reproductive organ development.

3.8.3 Continuous Breeding Reproductive Toxicity Studies

Previous Evaluations

Doses of 0, 0.03, 0.3 and 1.0% DBP in the diet (ca. 0, 40, 420 and 1410 mg/kg bw/d) were administered to CD-1 mice (Lamb et al., 1987*; Morrissey et al., 1989*). Animals (20/sex/group) were treated over a week-long premating period, during a 98-day mating period (as pairs), and after mating until any litters delivered during this period were at least 21 days old. A week-long crossover mating trial was performed between F_0 animals of control and 1% dose groups.

Effects at the 1% level were as follows: significantly decreased growth in F_0 males; significantly increased liver weights in F_0 females; significant decreases in percentage of fertile pairs, number of litters/pair, number of live pups/litter and pups born alive. These effects were absent at the lower dose levels. In the crossover mating trial (using dosed females and control males), statistically significant decreases were seen in the percentage of fertile pairs, number of live pups/litter, pups born alive and live pup weight indicating that the effects were dam-mediated. The NOAEL for parental toxicity and embryotoxicity in this study was the 0.3% level (420 mg/kg bw/d).

3.8.4 One/Two-Generation Reproductive Toxicity Studies

Previous Evaluations

In a multigenerational study, Sprague Dawley rats (20/sex/group; 40/sex for controls) were given DBP at 0, 0.1, 0.5 and 1.0% in the diet (0, 52, 256 and 509 mg/kg bw/d for males and 0, 80, 385 and 794 mg/kg bw/d for females) (NTP, 1995*; Wine et al., 1997). Animals were treated during a 7-day premating period and a 112-day cohabitation period (pairwise mating). Mating pairs were separated on completion of the cohabitation period and further treated, and final litters delivered during this phase were maintained for a minimum of 21 days. A week long crossover mating trial was performed between F_0 animals of control and 1% dose groups.

The following effects were seen in F_0 animals during the continuous breeding phase: males (increased relative liver, kidney and right cauda epididymis weights at 1% level); females (reduction in growth, decreased body weight and increased relative liver and kidney weights at the 1% level). There was no difference in fertility, mating or pregnancy indices. The total number of live pups/litter was significantly decreased in a dose-related manner (significant from 0.5%). There was no effect on sperm parameters.

The following effects were seen in the F_1 generation males. At 0.5%, testicular atrophy in 1/20 animal and poor epididymal development in 1/20 animal at both 0.1 and 0.5% levels. At 1.0%, reduced body weight and relative weights of all reproductive organs, significantly increased relative liver and kidney weights, significantly decreased epididymal sperm count and testicular spermatid head count; poor epididymal development in 12/20 animals, testicular atrophy in 4/20 animals, cryptorchidism in 3/20 animals, impaired seminal vesicle

development in 4/20 animals and underdevelopment of prepuce or penis in 4/20 animals;. Histopathology revealed seminiferous tubule degeneration in 3/10 animals at the 0.5% level. At the 1.0% level, seminiferous tubule degeneration in 8/10 males, testicular interstitial cell hyperplasia in 7/10 animals, and vesiculitis with inspissated secretion was observed. In females, significantly reduced body weights and absolute ovary, liver and kidney weights at the 1% level were observed. Oestrous cyclicity or oestrous cycle length in F_1 females was unaffected at any dose.

Mating, pregnancy and fertility indices were all significantly reduced (30%, 5% and 17% respectively) for F_1 breeding pairs at 1% in the diet. Live pup weights were statistically significantly reduced at the $\geq 0.5\%$ level in the F_1 generation and at all dose levels in the F_2 generation. In the crossover mating trial, no effect on mating, pregnancy or fertility indices were seen but pup weight was significantly decreased when treated dams were mated with control males.

The effects on the F_1 generation were greater than that on the F_0 generation. The NOAEL for maternal toxicity was established at the 0.5% level (385 mg/kg bw/d). For fertility effects in the F_0 generation, the NOAEL was 0.5% (256 mg/kg bw/d for males; 385 mg/kg bw/d for females) and the LOAEL was 1% (509 mg/kg bw/d for males; 794 mg/kg bw/d for females) based on decrease in epididymis weight. Developmental effects (testicular atrophy) were observed at 0.5% level (256 mg/kg bw/d for males; 385 mg/kg bw/d for females). The finding of one male with poor epididymal development at the 0.1% level was considered incidental.

LE hooded rats (10-12 /sex/dose) were gavaged with 0, 250 or 500 mg DBP/kg bw/d in a continuous breeding study (Wolf et al., 1999). Treatment was from weaning, through puberty, young adulthood, mating and lactation in the P_0 generation. F_1 pups were untreated. A separate group of males received 1000 mg/kg bw/d. Treated P_0 animals were mated with untreated controls. F_1 animals were not treated. Sixteen F_1 animals/sex/group were chosen for fertility assessment for continuous mating over 11 breeding cycles.

In the P_0 generation, delayed puberty (preputial separation) occurred in males at all dose levels. DBP treatment did not accelerate the age at vaginal opening or cause persistent vaginal cornification (effects indicative of subchronic oestrogen exposure). Decreased fertility in both sexes (mated to untreated animals) at 500 mg/kg bw/d and in males at 1000 mg/kg bw/d were seen. Male infertility was attributed to testicular atrophy and decreased spermatogenesis. Treated females cycled and mated successfully but many females at 500 mg/kg bw/d aborted at mid-gestation.

In the F_1 generation (animals exposed in utero and via lactation from treated P_0 dams) the following effects were seen: urogenital malformations (including epididymal agenesis, hypospadias, ectopic testis, renal agenesis and uterine malformations), anophthalmia and decreased cauda epididymal sperm counts (F_1 males from 250 and 500 mg/kg bw/day groups). A dose-related decrease in fecundity was seen in the F_1 offspring (significantly fewer F_2 pups/litter) in similarly treated pairs under continuous breeding conditions. A LOAEL for fertility was 250 mg/kg bw/d based on decreased epididymal sperm counts. The developmental LOAEL was 250 mg/kg bw/d based on increased frequency of delayed puberty in F_0 and increased malformations in F_1 males.

In a GLP-compliant fertility study (IRDC, 1984*), male or female Charles River COBS CD rats were treated from days 60 and 14, respectively, prior to mating with diet containing DBP with a final dose of 0, 5, 50 or 500 mg/kg bw/d. Treatment continued through mating, gestation and lactation. F_1 weanlings were given either control diets or similar diets to the mothers, for a 49-day post-weaning period.

No effect on clinical signs, haematology or fertility was observed in treated males. Pathology revealed significant increases in absolute and relative liver and kidney weights at 500 mg/kg bw/d. Minor increases in relative kidney weights were also seen at 50 and 5 mg/kg bw/d but these were not dose-dependent. Histopathology of the kidneys was normal. No abnormalities were seen in reproductive performance, parturition, neonatal viability, newborn growth, organ weights and histopathology in weanlings.

No effect on clinical signs, haematology or fertility was seen in treated females. A reduction in growth was seen throughout treatment at 500 mg/kg bw/d (statistically significant at weeks 7, 9 and 11). Statistically significant increases were seen in kidney weights in treated females at 500 mg/kg bw/d but without histopathological changes. Depressions in pup weight at birth and pup growth were seen through lactation at 500 mg/kg bw/d. Reduced body weights of offspring were seen at all dose levels (occasionally significant but not dose-related) during the 7-week post-weaning period.

After the post-weaning period, there were slight decreases in testicular weights in weanlings treated at 500 mg/kg bw/d. Histopathology revealed testicular lesions (6/10 weanlings) at 500 mg/kg bw/d and 2/9 weanlings in rats whose mothers were fed 500 mg/kg bw/d and given control diet for the post-weaning period. The NOAEL for maternal toxicity and embryotoxicity was 50 mg/kg bw/d. The NOAEL for fertility was 50 mg/kg bw/d and the LOAEL was 500 mg/kg bw/day based on increased testicular lesions in F_1 males at weaning.

Female Long Evans rats were fed chow containing 0.6 g/kg or 2.5 g/kg (authors estimate: 0, 12 and 50 mg/kg bw/day) for 2 months prior to mating and throughout pregnancy and weaning (Salazar et al., 2004). Pups were sacrificed on PND 14 and PNW 12. At 12 mg/kg bw/d, decreased pup survival and significantly decreased pup weights were seen (p<0.01). At 50 mg/kg bw/d, pronounced decreases in the percent of pregnancies and significant decreases in pup weight (p<0.001) were seen. Decreased relative thymus and testes weights (at PND 14) and delayed vaginal opening and onset of first oestrous cycle in pups were seen at both treatment levels. Preputial separation was significantly delayed in the high dose group. A NOAEL could not be established due to decreased testes weight at the lowest dose tested (12 mg/kg bw/day). There is some doubt over the calculated dose in this study. An adult rat typically consumes 20g feed/day. This would be equivalent to 12 mg or 50 mg DBP/day not 12-50 mg/kg bw/day as stated in the paper. Assuming a 300g rat, the estimated doses would be 40 and 166 mg/kg bw/day respectively.

3.8.5 Developmental/Postnatal Toxicity Studies

Previous Evaluations

Pregnant SD rats were gavaged daily with 0 or 500 mg/kg bw/day DBP on GD14-PND3 (Wolf et al., 1999). Anogenital distance was reduced (significant after controlling for body weight), there was an increased frequency of retained thoracic nipples, hypospadias, and testicular and epididymal atrophy. Ventral prostate and testes weight was reduced.

Pregnant Sprague-Dawley CD rats (10/dose) received 0, 250, 500 or 750 mg DBP/kg bw/d in corn oil by gavage from GD 3 throughout pregnancy and lactation, until PND 20 (interruption of 2 days at parturition and on the following day) (Mylchreest et al., 1998). Dams were terminated on PND 21 (weaning) and pups on PND 100-105 (sexual maturity).

One female at 500 mg/kg bw/d and three females at 750 mg/kg bw/d were not pregnant. At 750 mg/kg bw/d, the following parameters were decreased: number of live births/ litter (significant decrease), body weight gains of dams and pup survival to weaning (significant decrease). There was no significant effect on number of implantations or pup weight.

The following effects were seen in male offspring: ≥250 mg/kg bw/d (hypospadias in 3, 21 and 43% offspring at 250, 500 and 750 mg/kg bw/d, respectively; underdeveloped/absent epididymis, frequently bilaterally, in 9, 50 and 70% offspring; atrophy of seminiferous tubules; increase in frequency of dilated renal pelvis); ≥500 mg/kg bw/d (decreased anogenital distance; dose-related increase in frequency of malformations of genitalia; seminiferous tubule underdevelopment/weight decrease in 16 and 32 % offspring at 500 and 750 mg/kg bw/d, respectively); and 750 mg/kg bw/d (significantly decreased mean kidney weights; decrease in mean prostate weight in 27% offspring).

The following effects were seen in female offspring: ≥500 mg/kg bw/d (absence of vaginal opening in 1/30 rats (1/8 litters) and 2/9 rats (1/4 litters) at 500 and 750 mg/kg bw/d, respectively; absence of patent vagina, uterus or left kidney in animal with no vaginal opening at 500 mg/kg bw/d; uterine horn abnormalities in 1 female at 500 and 750 mg/kg bw/d each). A NOAEL could not be established for this study. The LOAEL was 250mg/kg bw/day based on atrophy of seminiferous tubules and hypospadias.

Data not Reported in Previous Evaluations

Zhang and colleagues (2004) investigated the developmental toxicity of DBP in Sprague-Dawley male rats exposed to DBP in utero and during lactation. Pregnant rats were administered 0, 50, 250 or 500 mg/kg bw/d by gavage from GD 1 to PND 21. F_1 pups were examined on PND70. There was a dose-response decrease in birth weight at 250 and 500 mg/kg bw/d and significantly decreased number of live pups/litter at 500 mg/kg bw/d. In the F_1 generation, the following effects were seen: ≥250 mg/kg bw/d (significant decreased anogenital distance; increased frequency of testicular atrophy, underdeveloped/absent epididymis and cryptorchidism; decreased epididymal sperm motility (and sperm number at 500 mg/kg bw/d only), total sperm heads/g of testis, decreased epididymis weight). Histopathology revealed mild degeneration of seminiferous epithelium at 250 mg/kg bw/day that was more severe at 500 mg/kg bw/day. The NOAEL was established to be 50 mg/kg bw/d and the LOAEL was 250 mg/kg bw/day based on decreased pup weight and male reproductive tract malformations.

In a study investigating the developmental toxicity of DBP in both sexes, maternal CD rats were given a diet containing 0, 20, 200, 2000 and 10000 ppm (ca 1.5- 3 mg/kg bw/d, 14-29 mg/kg bw/d, 148-291 mg/kg bw/d or 712-1372 mg/kg bw/d respectively) from GD 15 to PND 21 (6-8/group) (Lee et al., 2004). Offspring were sacrificed on PND 21, postnatal week (PNW) 11 or 20. The following effects were seen in males: ≥20 ppm (minimal or slight reduction in testicular spermatocyte development on PND 21); ≥200 ppm (minimal reduction in testicular spermatocyte development on PND 21, increased relative pituitary weights but

not dose-related); ≥2000 ppm (aggregation of Leydig cells and decreased epididymal duct cross-sections at PND 21) and 10000 ppm (decreased neonatal anogenital distance, retention of nipples, decreased testes weight at PND 21 but not PNW 11; significant loss of testicular germ cells; increased percentages of luteinizing hormone (LH) positive cells, decrease in follicle-stimulating hormone (FSH) and prolactin producing cells in the anterior pituitary). Vacuolar degeneration of the alveolar cells of the mammary gland was observed at PNW 11 in male offspring from 20 ppm but there was no clear dose-response. The effects on spermatocyte development evaluated on PND 21 was graded as minimal or slight at 20 and 200 ppm and the degree of severity was only statistically significant at the two highest doses.

In females the following effects were seen: ≥20 ppm (hyperplasia of mammary alveolar bud on PND 21; vacuolar degeneration of mammary gland alveolar cells on PNW 11), ≥200 ppm (atrophy of alveolar cells of mammary gland on PNW 20, FSH fluctuations, decreased relative pituitary weight); ≥ 2000 (LH fluctuations) and 10000 ppm (slight delay in puberty onset, increased percentages of LH-positive cells, decrease in FSH and prolactin-producing cells, increase in incidence of females with extended diestrus). The significance of increased vacuolar degeneration in the alveolar cells of mammary glands to male fertility is unknown. The NOAEL was 200 ppm (14-29 mg/kg bw/day) based on significant reduction in testicular spermatocyte development, aggregations of Leydig cells and decreased epididymal duct crossection on PND 21 at 2000 ppm (148-291 mg/kg bw/day).

Higuchi et al. (2003) evaluated effects of DBP exposure in male rabbits in utero, during adolescence and after puberty. For in utero exposure, Dutch-Belted rabbits were dosed with 0 (corn oil; n=5) or 400 mg DBP/kg bw/d (n=8) from GD 15 to 29. In adolescent exposure, rabbit pups (n=11 representing 4 litters) were individually administered 400 mg DBP/kg bw/d from postnatal weeks 4-12. Male offspring were examined at 6, 12, and 25 weeks of age. For postpubertal exposure, 6-8 month old rabbits were ranked on body weight and pre-treatment seminal parameters then alternately assigned to 0 (n=6) or 400 mg DBP/kg bw/d (n=6) for 12 weeks. Body weights were recorded before treatment and weekly thereafter and serum samples were examined before and at the conclusion of treatment. The most pronounced reproductive effects were found in male rabbits exposed in utero. Male offspring in this group exhibited reduction in numbers of ejaculated sperm (43%; p<0.01), in weights of testes (23%; p<0.05 at 12 weeks), and in accessory sex glands weight (36%; p<0.01 and 27%; p<0.05 at 12 and 25 weeks respectively). Serum testosterone levels were reduced (32% at 6 weeks; p<0.05); a slight increase in histological alterations of the testis (p<0.05) and a doubling in the percentage (from 16 to 30%, p<0.01) of abnormal sperm; and 1/17 males manifesting hypospadias, hypoplastic prostate, and cryptorchid testes with carcinoma in situ-like cells. In the DBP group exposed during adolescence, basal serum testosterone levels were reduced at 6 weeks (p<0.01) while at 12 weeks, testosterone production *in vivo* failed to respond normally to a GnRH challenge. In addition, weight of accessory sex glands was reduced at 12 weeks but not at 25 weeks after a recovery period; there was a slight increase in the percentage of abnormal sperm in the ejaculate; and 1/11 males was unilaterally cryptorchid. In the DBP-treated groups, daily sperm production, epididymal sperm counts, mating ability, and weights of body and non-reproductive organs were unaffected.

3.8.6 Prenatal Developmental Toxicity Studies

Previous Evaluations

Pregnant LE Hooded rats were gavaged daily with 0 or 500 mg/kg bw/day DBP on GD14-19 (Wolf et al., 1999). Anogenital distance was reduced (significant after controlling for body weight) and there was an increased frequency of retained thoracic nipples. Seminal vesicle weight was reduced.

In a study by Hamano et al. (1977*) only a summary was available. ICR-JCL mice were given 0.005, 0.05 or 0.5% DBP in the diet (ca. 10, 100 and 400 mg/kg bw/d) from GD 1–18. At 0.5%, the following effects were seen: maternal toxicity (increased kidney weights), embryotoxicity (reduced number of live offspring), teratogenicity (significant increase in incidence of non-closing eyelids, encephalocele, cleft palates and spina bifida, and increased incidence of skeletal abnormalities). The NOAEL for maternal and foetal toxicity was 100 mg/kg bw/d.

ICL–ICR mice were dosed with 0.05, 0.1, 0.2, 0.4 or 1.0% DBP in the diet (ca. 80, 180, 350, 660 and 2100 mg/kg bw/d) during GD 1–18 (Shiota et al., 1980). Maternal growth was significantly reduced at 1.0%. Foetal mortality and the number of resorptions were higher at 0.1% and above (statistically significant at 1.0% but no dose-response). Foetal weights were depressed at all doses but significant at 0.4% and above. An increased incidence of skeletal variations (lumbar ribs) was seen at all dose levels (not statistically significant) and there was a significant reduction in number of ossified coccygia (dose-responsive and significant at all doses). In another study, in the Wistar rat on GD 21, the number of ossified sacrococcygeal vertebrae decreased with body weight (Chahoud & Paumgartten, 2005). It is therefore likely the decrease in ossified coccygia is an indirect effect of decreasing body weight in the DBP-exposed pups. The NOAEL for maternal toxicity was 0.4% (660 mg/kg bw/d). The NOAEL for foetal toxicity was 0.2% and the LOAEL was 0.4% based on decreased pup weight.

In a developmental study, Wistar rats were gavaged with 500, 630, 750 or 1000 mg/kg bw of DBP on GD 7-15 (Ema et al., 1993*). Maternal toxic effects included dose-related increases in incidence of reddish-brown staining of facial fur and piloerection, and dose-related decreases in maternal body weight gain (significant $\geq$ 630 mg/kg bw/d). Embryotoxic effects included increased incidence of resorptions (significant $\geq$ 630 mg/kg bw/d), and dead foetuses per litter and post implantation loss per litter; and increased malformations at $\geq$750 mg/kg bw/d (increased incidence of cleft palate). The NOAEL for maternal toxicity and fetotoxicity was 500 mg/kg bw/day.

Pregnant Wistar rats were gavaged with 750, 1000 or 1500 mg DBP/kg bw/d during GD 7-9, 10-12 or 13-15 in a follow-up study by Ema et al. (1994*). Dams were sacrificed on GD 20. At $\geq$750 mg/kg bw/d, post implantation loss was significantly increased at all dosing periods. At 750 and 1000 mg/kg bw/d, dose-related increases in number of external and skeletal malformations (cleft palate and fusion of sternebrae) were seen with treatment on GD 7-9 or GD 13-15 but not GD 10-12. A NOAEL could not be established.

Pregnant Sprague-Dawley rats were dosed once with 0, 500, 1000, 1500 or 2000 mg DBP/kg bw/d on GD 14 (Sallenfait et al., 1998*). Dams were terminated on GD 21. At $\geq$1000 mg/kg bw/d, higher incidences of skeletal variations were seen. At $\geq$1500 mg/kg bw/d, significantly decreased maternal body weight gain, increased incidence of resorptions and reduced foetal body weights were seen. Foetal mortality/ litter was elevated at 2000 mg/kg bw/d. There were no increases in post implantation losses. The NOAEL for developmental effects was 500 mg/kg bw/d.

Pregnant Wistar rats (10/dose) were dosed with 0, 120 or 600 mg DBP/kg bw/d in olive oil by gavage on GD 0-21 in a limited study by Nikonorow et al. (1973). At ≥120 mg/kg bw/d, placental weight was significantly reduced. Increases in number of resorptions and decreases in number of foetuses and foetal weight were significant at 600 mg/kg bw/d. The NOAEL for embryotoxicity was 120 mg/kg bw/d.

Pregnant rats were gavaged with 100, 250 or 500 mg/kg bw/day on GD 12-21 (Mylchreest et al., 1999). The following results were seen: ≥100 mg/kg bw/d (delayed preputial separation); ≥250 mg/kg bw/d (retained thoracic nipples and decreased anogenital distance); 500 mg/kg bw/d (hypospadias, cryptorchidism and degeneration of the seminiferous epithelium). No reproductive or developmental anomalies were detected in female pups. The NOAEL for development could not be established as delayed preputial separation was induced in the male pups at the lowest dose tested (100 mg/kg bw/day).

CD rats (19-20/group) were gavaged with 0, 0.5, 5, 50, 100 or 500 mg/kg bw/day DBP on GD 12-21 (Mylchreest et al., 2000). No effect on maternal body weight gain or food consumption was noted. At 100 mg/kg bw/day, there was increased seminiferous tubule atrophy and retained nipples (statistically significant). At 500 mg/kg bw/day, in addition there was also significantly decreased anogenital distance in male pups and increased frequency of male reproductive organ malformations (hypospadias, absent or partially developed epididymis), and decreased testes, prostate, epididymis and seminal vesicle weight at 110 days. The NOAEL for developmental effects was 50 mg/kg bw/day based on increased seminiferous tubule atrophy and retained nipples at 100 mg/kg bw/day.

Pregnant Wistar rats received 0, 0.5, 1.0 or 2.0 % DBP in the diet (ca. 0, 331, 555 or 661 mg/kg bw/d, respectively) from GD 11-21 (Ema et al., 1998*). Dams were terminated on GD 21. The following effects were seen: ≥1.0 % (dose-related significant decrease in body weight gain and food consumption in dams; increased number of males with cryptorchidism and decreased urogenital distance); 2% (significant decrease in foetal weights, increased incidence of foetuses with cleft palate and fusion of sternebrae). The NOAEL for maternal and foetal toxicity was 0.5% (ca. 331 mg/kg bw/d).

Data not Reported in Previous Evaluations

Female Sprague Dawley rats were gavaged with 0, 100 or 500 mg DBP/kg bw/day on GD 12-21 (Barlow et al., 2004). Male offspring were necropsied when 6, 12 or 18 months old. Anogenital distance was decreased at the highest dose and there was an increased incidence of areolae retention at both doses on PND 13 and at the highest dose only on PND 180. The incidence of testicular lesions was significantly higher at 500 mg/kg bw/d (effects included testicular atrophy and occasional enlargement with oedema) at all time points. Other effects included significantly higher incidence of malformed epididymides, absent vas deferens, malformed or absent seminal vesicles, decreased prostate size and hypospadias at the highest dose. Histopathology revealed testicular dysgenesis and germ cell degeneration at 500 mg/kg bw/d. The NOAEL could not be established and the LOAEL for foetal toxicity was 100 mg/kg bw/day.

SD rats were gavaged with 500 mg/kg bw/day on GD 14-15, 15-16, 16-17, 17-18, 18-19 or 19-20 (9-11/group) (Carruthers and Foster, 2005). Anogenital distance was decreased after exposure of GD 15-16 or GD 18-19; persistent areolar nipples was observed in male offspring following exposure on GD 16-17 and there was a significant increase in epididymal

malformations and small testes at GD 17-18 and a decrease in epididymal weights on GD 17-18.   The data suggest that GD 16-18 is the critical window for male reproductive tract development and this two-day gestational exposure is long enough to induce permanent developmental abnormalities.

Pregnant CD rats were gavaged with 500 mg/kg bw/day DBP on GD 12-21 (Mylchreest et al., 2002). Dams were killed on GD 14, 16, 18 or 21. At GD 16-21, Leydig cell hyperplasia was induced. Testicular testosterone was decreased on GD 18 and 21. Testis atrophy was induced together with enlarged seminiferous cords and contained multinucleated gonocytes was observed on GD 21.

3.8.7 Reproductive/Developmental Toxicity Studies

Data not Reported in Previous Evaluations

LE Hooded female rats (8-12/group) were gavaged with 0, 250, 500 or 1000 mg/kg bw/day DBP from weaning, through puberty, young adulthood then mated with untreated males. Dosing continued through mating, pregnancy and lactation (Gray et al., 2006). Liver weight was increased at 1000 mg/kg bw/day with no effect on body weight. The percentage of females delivering live pups was reduced by more than 50% at 500 mg/kg bw/d and by 90% at 1000 mg/kg bw/d in the absence of overt toxicity whereas the ages at vaginal opening and first estrous, estrous cyclicity and mating indices were not significantly affected. The crossover study using dosed males with untreated females also induced decreased fertility at 500 and 1000 mg/kg bw/day. On GD 13, prior to the stage when litters were being aborted, ex vivo ovarian hormone production was also significantly decreased at these dose levels. The NOAEL was 250 mg/kg bw/day and the LOAEL was 500 mg/kg bw/day based on decreased fertility in P_0 generation.

3.8.8 Mode of Action

Previous Evaluations

Two *in vitro* studies in human breast cancer cell lines (ZR-75 and MCF-7) were conducted by Jobling et al. (1995*). DBP was found to exert mitogenic effects on cell growth of ZR-75 cells (response was lower than that of β-estradiol and octylphenol). DBP also stimulated transcriptional activity of the oestrogen receptor.

DBP has been shown to have extremely weak estrogenic activity in recombinant yeast assay (Harris et al., 1997). DBP was a weak competitive agonist at the oestrogen receptor in an *in vitro* competitive ligand-binding assay and weakly induced oestrogen receptor-mediated gene expression in MCF-7 cells (Zacharewski et al., 1998). DBP did not induce estrogenic responses *in vivo* in uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998).

Data not Reported in Previous Evaluations

DBP has been shown to bind to human oestrogenic receptor *in vitro* (Nakai et al., 1999) but was negative for estrogenic activity in a yeast two-hybrid assay (Nishihara et al., 2000).

DBP demonstrated estrogenic activities in a human oestrogen receptor (ER) α (but not β) reporter gene assay in CHO-K1 cells transfected with expression vectors for human oestrogen

receptor ERα, ERβ and androgen receptor (AR) (Takeuchi et al., 2005) but had no binding affinity for the oestrogen receptor α or β *in vitro* (up to 10^{-5}M) (Toda et al., 2004). Expression of CaBP-9k mRNA (a gene highly regulated by 17-β estradiol) increased in 7 day old female SD rats following oral treatment with 600 mg/kg bw/day DBP for 3 days (Hong et al., 2005). DBP increased proliferation of human breast cancer MCF-C7 cells in one assay (Hong et al., 2005) but not another (Okubo et al., 2003). DBP also showed antiandrogenic activity in the hAR-transactivation assay (Takeuchi et al., 2005).

In utero, 500 mg/kg bw/day DBP from GD 12 to 19 in rats resulted in reduced testicular testosterone levels, dose-related reduction in expression of vital genes and proteins involved in cholesterol transport and steroidogenesis as well as increased mRNA expression of different members of the insulin-like growth factor family in the developing Wolffian ducts. There was also a variable decrease in androgen receptor protein in ductal epithelial cells on GD 19 (Lehmann et al., 2004; Bowman et al., 2005).

Orally administered DBP to the dam on GD 14-18 significantly reduced both ex vivo testosterone production and insl3 gene expression in rat foetal testes (Wilson et al., 2004).

MBP induced detachment of germ cells from a Sertoli cell monolayer *in vitro* but was 100 fold less potent than MEHP (Gray & Gangoli, 1986).

Conclusion

Effects On Fertility

The critical studies for reproductive toxicity were a two-generation reproduction study in rats (continuous breeding protocol; NTP, 1995*; Wine et al., 1997) and a prenatal/postnatal developmental toxicity study in rats (Lee et al., 2004). In these two studies, the LOAELs were based on testicular effects and were comparable. In the two-generation study, the NOAEL for fertility effects was 0.1% (52 mg/kg bw/d for males; 80 mg/kg bw/d for females) and the LOAEL was 0.5% level (256 mg/kg bw/d for males; 385 mg/kg bw/d for females) based on testicular atrophy in the F_1 males. In the single generation study, the NOAEL for fertility was 200 ppm (14-29 mg/kg bw/day) based on significantly increased reduction in spermatocyte development on PND 21 at 2000 ppm (148-291 mg/kg bw/day).

There is limited evidence in humans associating MBP, the principal metabolite of DBP, with effects on sperm motility *in vitro* and *in vivo* as well as positive correlations with sex-hormone binding globulin and LH:free testosterone ratio. However, a recent limited study suggests that at high exposure levels to DBP and DEHP, free testosterone levels are reduced in workers (Pan et al., 2006). MBP urine levels in mothers were negatively correlated with free testosterone and reduced anogenital index in boys. The relationship between MBP levels in urine and exposure to DBP was not established in the human studies.

Developmental Effects

Studies that began *in utero* and continued through weaning demonstrated that the effects on reproduction were increased or induced at a lower dose when exposure included the prenatal period (NTP, 1995*; Wine et al., 1997). Mating, pregnancy and fertility indices were all significantly reduced (30%, 5% and 17% respectively) for F_1 breeding pairs but were no

different to control values in F_0 generation. Sperm concentration and motility were unchanged in F_0 but were significantly reduced in F_1 males at 1% DBP in the diet.

Studies in which exposure occurred solely during the development stage induced similar effects. The most sensitive endpoint was effects on testicular morphology and reproductive maturation. Increased seminiferous tubule atrophy and retained nipples were seen at 100 mg/kg bw/day in rats exposed from GD 12-21 (Mylchreest et al., 2000). The same LOAEL was established in rats dosed over the same gestation period based on retained nipples (Barlow et al., 2004) and delayed preputial separation (Mylchreest et al., 1999). One study suggests that GD 16-18 is the critical window for male reproductive tract development and that a two-day gestational exposure was sufficient to induce permanent developmental abnormalities (Carruthers and Foster, 2005). At maternotoxic doses (> 500 mg/kg bw/day), there were an increased number of resorptions and malformations (including cleft palate, cryptorchidism, male reproduction organ malformations) and decreased foetal weight. The NOAEL for foetotoxicity is considered to be 50 mg/kg bw/day (Mylchreest et al., 2000). Gestational exposure to DBP induced a decrease in expression of insl3 in rat foetal testes (Wilson et al., 2004) perhaps explaining the increased incidence of cryptorchidism.

In some *in vitro* assays, DBP was weakly oestrogenic but this was not replicated by *in vivo* studies. Therefore no certainty can be associated with the oestrogenic activity observed *in vitro*. However these assays did not include esterases and lipases to metabolise DBP to its monoester. It has been suggested that the monoester is the proximate toxicant and it was shown *in vitro* that MBP induced detachment of germ cells from a Sertoli cell monolayer. Malformations in reproductive organs and effects on androgen-mediated endpoints in male rats exposed to DBP or MBP during prenatal development suggest antiandrogenic activity by DBP and MBP. There are *in vitro* data to support this hypothesis.

Table 5: Summary of reproductive and developmental studies on DBP

Study type	Route	Doses (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d) & endpoint	Reference
Repeat dose Studies					
SD rat 12 males/gp PND 5-14	SC	0, 5, 10, 20 mg/animal (0, 250, 500, 1000)	500	Fert: 1000, ↓ testes and accessory organ wt.	Kim et al., 2004
Continuous Breeding Studies					
CD-1 mice; 20/sex/gp	Diet	0, 0.03, 0.3, 1% (0, 40, 420, 1410)	Sys: 420 Fert: 420 Devp: 420	Sys: 1410, ↓ growth (m), ↑ liver wt (f). Fert: 1410: ↓ fertile pairs, ↓ no. of litters/pair. Devp: 1410, ↓ no. of live pups/litter.	Lamb et al., 1987; Morrissey et al., 1989*
Cross-over F_1			NE	Fert: 1410, ↓ fertile pairs. Devp: 1410, ↓ no. of live pups/litter, ↓ pups born alive, ↓ live pup wt (dosed f x control m),	Lamb et al., 1987; Morrissey et al., 1989*
One/Two-Generation Reproduction Studies					
Sprague Dawley rats 20/sex/gp;	Diet	0, 0.1, 0.5, 1% (0, 52, 256, 509 (m) 0, 80, 385, 794 (f))	Sys: 256 (m)/ 385 (f) Fert: 52 (m)/ 80 (f) Devp: NE	Sys: 509 (m)/ 794 (f), ↓ body wt. Fert: 509 (m)/ 794 (f), ↓ epididymis wt (F_0). Devp: 52 (m)/ 80 (f), ↓ no. liveborn; ↓ pup wt.	NTP, 1995*; Wine et al., 1997
Cross-over F_1		0, 1% (0, 509 (m) 0, 794 (f))	NE	Devp: 509 (m)/ 794 (f), ↓ pup wt (dosed f x control m).	
Charles River COBS CD rats	Diet	0, 5, 50, 500	Sys: 50 (F_0) Fert: 50 (F_1) Devp: 50 (F_1)	Sys: 500, ↑ liver, kidney wt. Fert: 500, testicular lesions (F_1). Devp: 500, ↓ pup wt at birth.	IRDC, 1984*
LE hooded rats 10-12/sex/gp	Gavage	0, 250, 500, 1000	Sys: NR Fert: NE Devp: NE	Sys: NR Dert: 250, ↓ fecundity, ↓ sperm count (F_1). Devp: 250, ↑ urogenital malformations.	Wolf et al., 1999
Long Evans rats 15/gp 2.5 m prior to mating – PND22	Diet	0, 0.6, 2.5 g/kg chow (0, 12, 50 (author's calculation may be incorrect); 0, 40, 166 (NICNAS calculation))	Sys: 50/166 Fert: NE Devp: NE	Sys: NE Fert: 12/40, ↓ testes wt (F_1). Devp: 12/40, ↓ pup wt.	Salazar et al., 2004
Reproductive/Developmental Studies					

LE hooded rats 10-12 /sex/dose	Gavage	0, 250, 500, 1000	Mat: 500 Devp: 250	Mat: 1000, ↑ liver wt. Devp: 500, ↓ no. liveborn, ↓ ovarian hormone production.	Gray et al., 2006
Developmental/Postnatal Studies					
Sprague-Dawley CD rats 10/dose GD 3 - PND 20	Gavage	0, 250, 500, 750	Mat: NE Fert (F_1): NE Devp: NE	Mat: 250, ↓ body wt gains of dams. Fert: 250, atrophy of seminiferous tubules. Devp: 250, hypospadias, underdeveloped/absent epididymis.	Mylchreest et al., 1998
Sprague-Dawley rats 20/gp GD 1 - PND 21	Gavage	0, 50, 250, 500	Mat: 500 Fert (F_1): 50 Devp: 50	Mat: NE Fert (F_1): 250, ↑ testicular atrophy, underdeveloped/absent epididymis, ↓ epididymal sperm motility, total sperm heads/g of testis, ↓ epididymis wt. Devp: 250, ↓ in birth wt, sign ↓ anogenital distance/body wt, cryptorchidism.	Zhang et al., 2004
CD (SD) IGS rats 6-8/gp GD 15 – PND 21	Diet	0, 20, 200, 2000, 10000 ppm (0, 1.5-3, 14-29, 148-291, 712-1372)	Mat: 148-291 Fert (F_1): 14-29 Devp: 14-29	Mat: 712-1372, ↓ body wt gain. Fert (F_1): 148-291, ↓ testicular spermatocyte development, ↓ epididymal duct cross-section. Devp: 148-291, testicular toxicity (F_1).	Lee et al., 2004
Dutch-belted rabbit 6/gp PNW 4-12 (8w) PNW 6-8 (12w)	Gavage	0, 400	Mat: 400 Fert: NE Devp: NE	Mat: NE Fert: 400, changes in testis pathology, ↓ % normal sperm. Devp: 400, ↓ testes wt, ↓ accessory sex organ wt, ↓ sperm concentration, ↓ testosterone level, changes in testis pathology.	Higuchi et al., 2003
Sprague Dawley rats 8/gp GD14-PND 3	Gavage	0, 500	Mat: NR Fert (F_1): NE Devp: NE	Mat: NR Fert (F_1): 500, ↓ prostate, testes, epididymis wt. Devp: 500, ↓ anogenital distance, retained nipples.	Wolf et al., 1999
Prenatal Developmental Studies					
ICR-JCL mice GD 1–18	Diet	0, 0.005, 0.05, 0.5% (0, 10, 100, 400)	Mat: 100 Devp: 100	Mat: 400, ↑ kidney wt. Devp: 400, ↓ no. liveborn, ↑ malformations.	Hamano et al., 1977*
8-15/gp ICL–ICR mice GD 1–18	Diet	0, 0.05, 0.1, 0.2, 0.4, 1% (0, 80, 180, 350, 660, 2100)	Mat: 660 Devp: 350	Mat: 2100, ↓ body wt gain. Devp: 660, ↑ resorptions, ↓ foetal wt.	Shiota et al., 1980*
Wistar rats 12/gp GD 7-15	Gavage	0, 500, 630, 750, 1000	Mat: 500 Devp: 500	Mat: 630, ↓ body wt gain. Devp: 630, ↑ resorptions.	Ema et al., 1993*

Wistar rats 10-12/gp GD 7-9, 10-12 or 13-15	Gavage	0, 750, 1000, 1500	Mat: NR Devp: NE	Mat: NR Devp: 750, ↑ post implantation loss; ↑ malformations.	Ema et al., 1994*
Sprague-Dawley rats GD 14	Gavage	0, 500, 1000, 1500, 2000	Mat: 1000 Devp: 500	Mat: 1500, ↓ wt gain. Devp: 1000, ↑ skeletal variation.	Sallenfait et al., 1998*
Wistar rats 10/dose GD 0-21	Gavage	0, 120, 600	Mat: NR Devp: 120	Mat: NR Devp: 600, ↑ resorptions, ↓ no. foetuses, ↓ foetal wt.	Nikonorow et al., 1973
CD Rats 10/gp GD 12-21	Gavage	0, 100, 250, 500	Mat: 500 Devp: NE	Mat: NE Devp: 100, delayed preputial separation.	Mylchreest et al., 1999
CD Rats 19-20/gp GD 12-21	Gavage	0, 0.5, 5, 50, 100, 500	Mat: 500 Fert (F_1): 100 Devp: 50	Mat: NE Fert (F_1): 500, ↑ seminiferous tubule atrophy. Devp: 100, retained nipples.	Mylchreest et al., 2000
Wistar rats 11/gp GD 11-21	Diet	0, 0.5, 1, 2% (0, 331, 555, 661)	Mat: 331 Devp: 331	Mat: 555, ↓ body wt gain and food consumption in dams. Devp: 555, ↑ cryptorchidism, ↓ anogenital distance.	Ema et al., 1998*
Wistar rats 10/gp GD 0-11	Diet	0, 2% (0, 895)	Mat: NE Devp: NE	Mat: 895, ↓ body wt gain and food intake Devp: 895, ↑ resorptions	Ema et al., 1997a*
Rat (strain unknown) GD 6-16	Gavage	0, 1500	Mat: NE Devp: NE	Mat: 1500, ↓ body wt gain Devp: 1500, sig ↑ post implantation loss at GD 6-16 (except GD 7 and 11), ↓ foetal wt at GD 6-11 and 15, sig ↑ foetuses with malformations at GD 8-9 and 15.	Ema et al., 1997b*
CRL:CD (SD) BR rats 10/dose GD12-21	Gavage	0, 100, 500	Mat: NR Fert (F_1): 100 Devp: NE	Mat: NR Fert (F_1): 500, testicular lesions, germ cell degeneration. Devp: 100, areolae retention at PND 13 (not PND 180).	Barlow et al., 2004
SD rats 9-11/gp GD 14-15, 15-16, 16-17, 17-18, 18-19, 19-20	Gavage	0, 500	Mat: NR Devp: NE	Mat: NR Devp: 500, ↓ anogenital distance at GD 15-16, GD 18-19, areolae nipple retention at GD16-17, epididymal malformations and small testes at GD 16-17.	Carruthers and Foster, 2005
SD rats 4/gp GD 16-19	Gavage	0, 500	Mat: NR Fert (F_1): NE Devp: NE	Mat: NR Fert (F_1): 500, ↓ seminal vesicle wt. Devp: 500, ↓ anogenital distance, retained nipples.	Wolf et al., 1999

| Dutch-belted rabbit 6/gp GD15-29 | Gavage | 0, 400 | Mat: 400 Fert (F$_1$): NE Devp: NE | Mat: NE Fert (F$_1$): 400, ↓ sperm, testes wt, accessory sex organ wt, testosterone level, changes in testis pathology. Devp: 400, testicular effects. | Higuchi et al., 2003 |

m: male, f: female; wt: weight; sig: significant; no: number of; gp: group

NE: not established; NR: not reported; Sys: systemic; Mat: maternal; Devp: development; Fert: fertility; GD: gestation day; PND: post natal day; PNW: post natal week

4. HAZARD CHARACTERISATION

Toxicity data for DBP were not available for all health endpoints. For endpoints with missing or incomplete data, information from structurally similar phthalates, where available, was used to extrapolate potential toxicity. Relevant read-across information was obtained from other NICNAS assessment reports for relevant phthalates and the NICNAS Phthalates Hazard Compendium (2007), which contains a comparative analysis of toxicity endpoints across 25 phthalates, including DBP.

DBP is rapidly absorbed and excreted after oral administration (in rats and hamsters), with $\geq$ 90% excreted in urine within 24-48 h. Faecal excretion is low (1.0–8.2%). DBP was found to be absorbed dermally in rats with ca. 60% being excreted in urine within a week. In *in vitro* dermal studies, DBP is absorbed faster in rats than in humans. DBP has been shown to be absorbed in humans after oral exposure. Absorption data from inhalational exposure were not available. DBP is also excreted in bile and subsequently enters the enterohepatic circulation. No significant accumulation in tissues occurs in laboratory animals after oral and dermal exposure.

DBP is mostly hydrolysed to MBP and corresponding alcohol following absorption by the small intestines. No data on biotransformation after dermal or inhalational exposure are available.

Placental transfer studies revealed DBP and its metabolites, MBP and MBP-glucuronide, are transferred to embryonic tissues in rats. Most of the radioactivity recovered in maternal plasma, placenta and embryo was attributed to MBP, with intact DBP present at low levels only.

DBP has low acute oral, dermal and inhalational toxicity. The acute oral LD50 value in rats is 6300 – 8000 mg/kg, the dermal LD50 in rabbits is >20000 mg/kg and the inhalational LC50 (4h) in rats is $\geq$ 15.68 mg/L.

DBP causes minimal skin, eye and respiratory irritant effects in animals. DBP did not display skin sensitising properties in two maximization tests that were GLP-compliant and human data are limited and conflicting.

DBP induced DNA damage (single-strand breaks) in *in vitro* Comet Assay. However, *in vitro* studies, including chromosomal aberrations, DNA repair and more importantly, the *in vivo* studies, including sex-linked gene mutation and chromosomal aberrations, were all negative. DBP was also shown negative in majority bacterial, yeast and mammalian mutation studies. Based on all data available and on a weight-of-evidence basis, DBP is considered to be non-genotoxic in both somatic and germ cells.

The NOAEL for general toxicity is 152 mg/kg bw/d and the LOAEL is 752 mg/kg bw/d for changes in haematological and clinical chemistry parameters, enzymes, liver and kidney weights, based on a 3-month oral study in rats. No testicular or neurological changes were seen at this dose level. However, in other studies testicular effects have been observed at 250 mg/kg bw/d in rats.

The NOAEL with regard to peroxisome proliferation is 19.9 mg/kg bw/d for increased activity of peroxisome associated enzymes. Humans are considered to be less susceptible for this effect, and hence DBP-induced hepatomegaly in rodents, may be not relevant for humans.

The available dermal exposure studies are unsuitable for establishing a NOAEL. Based on a 4-week study in rats, the NOAEC for inhalational exposure is 509 mg DBP/m^3 for systemic effects.

No adequate animal or human carcinogenicity studies are available.

There are no human reproductive or developmental data for DBP. There is limited evidence in humans associating MBP (the principal metabolite) with effects on sperm motility *in vitro* and *in vivo* as well as positive correlations with sex-hormone binding globulin and LH:free testosterone ratio. MBP urine levels in mothers were negatively correlated with free testosterone and reduced anogenital index in boys.

The critical animal studies for reproductive toxicity are a two-generation reproduction study (continuous breeding protocol; NTP, 1995*; Wine et al., 1997) and a single generation study (Lee et al., 2004) in rats. In these two studies, the LOAELs are both based on testicular effects and are comparable. In the two-generation study, the NOAEL for fertility and development effects was 0.1% (52 mg/kg bw/d for males; 80 mg/kg bw/d for females), with a LOAEL based on testicular atrophy at the 0.5% level in the F_1 generation (256 mg/kg bw/d for males; 385 mg/kg bw/d for females). In a single generation study (Lee et al., 2004), the NOAEL for fertility and development is 200 ppm (14-29 mg/kg bw/day), with LOAEL based on significantly increased reduction in spermatocyte development on PND 21 at 2000 ppm (148-291 mg/kg bw/day) in males exposed to DBP from GD 15 to PND 21.

Studies in which exposure occurred only during development induced similar effects. The most sensitive endpoint is effects on testicular morphology and reproductive maturation. The lowest NOAEL is 50 mg/kg bw/day in rats exposed on GD 12-21, and LOAEL based on increased seminiferous tubule atrophy and retained nipples at 100 mg/kg bw/day (Mylchreest et al., 2000). Other studies suggest that GD 16-18 is the critical window for male reproductive tract development and a two-day gestational exposure is long enough to induce permanent developmental abnormalities (Carruthers and Foster, 2005). At maternotoxic doses (> 500 mg/kg bw/day), there is increased number of resorptions and malformations (including cleft palate, cryptorchidism, male reproduction organ malformations) and decreased foetal weight.

The reproductive and developmental effects of DBP are similar to other phthalates with C4-6 carbon backbones (NICNAS, 2007). These phthalates are collectively known as 'transitional' phthalates. Transitional phthalates which have been tested all demonstrated effects on male reproductive organs, most notably decreased testes weight. Transitional phthalates also induce a recognisable pattern of malformations in offspring including decreased anogenital distance, delayed preputial separation and retained thoracic nipples in male pups. At high doses, hypospadias and cryptorchidism are induced, as well as increased frequency of supernumerary ribs.

In some *in vitro* assays, DBP was weakly oestrogenic but this was not replicated by *in vivo* studies. DBP also demonstrated antiandrogenic activity *in vitro*.

5. HUMAN HEALTH HAZARD SUMMARY TABLE

Phthalate	*Acute Toxicity*	*Irritation & Sensitisation*	*Repeated Dose Toxicity*	*Genetic Toxicity*	*Carcinogenicity*	*Fertility*	*Developmental Toxicity*
Dibutyl phthalate (DBP)	Oral Rat: LD50 = 6300 – 8000 mg/kg bw Dermal Rabbit: LD50 >20000 mg/kg bw Inhalation Rat: LC50 ≥15.68 mg/L/4h	Skin irritation: minimal effect Eye irritation: minimal effect Respiratory irritation: minimal effect Skin sensitisation: negative	Oral Rat: NOAEL = 152 mg/kg bw/d LOAEL = 752 mg/kg bw/d, ↑ liver and kidney weight; ↓ hepatocellular lipid deposition; ↑ palmitoyl-CoA oxidase activity and ↓ T3 levels. Inhalation Rat: NOAEC = 509 mg/m3, systemic effects. LOAEC = 1.18 mg/m3, local effects in upper respiratory system. High doses: liver, kidney, testes effects. PP noted.	*In vitro* Negative in majority mutagenicity and cytogenicity tests. *In vivo* Negative in sex-linked gene mutation and chromosomal aberration assays.	*In vitro* Negative in cell transformation assays. *In vivo* No data.	Rat: NOAEL = 256-385 (m-f) mg/kg bw/day LOAEL = 509-794 (m-f) mg/kg bw/day ↓ epididymis weight (F0)	Two generation study Rat: NOAEL = 52-80 (m-f) mg/kg bw/d LOAEL = 256-385 (m-f) mg/kg bw/d ↓ F1 sperm counts ↑ F1 testes atrophy Gestation studies Rat: NOAEL = 50 mg/kg bw/d LOAEL = 100 mg/kg bw/d, seminiferous tubule atrophy, retained nipples

PP: peroxisome proliferation; m-f: male-female; ↑: increase; ↓: decrease.

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7. ROBUST STUDY SUMMARIES

Developmental Toxicity/ Teratogenicity

Test substance	Di-n-butyl phthalate (DBP)
Species	Long Evans (LE) rats, Source: Charles River Breeding Laboratory, Michigan
Route of administration.	Gavage
Exposure period	Study 1: 21 days of age to GD13 of the third pregnancy
	Study 2: 24 days of age to GD13 of the second pregnancy
Study Duration	From weaning, through puberty, young adulthood, mating and three pregnancies
Frequency of treatment	Study 1: 7 days/week
	Study 2: 5 days/week until 110 days old, then 7 days/week
Doses	Study 1: 0 and 500 mg/kg bw/d (20 females/dose)
	Study 2: 0, 250, 500 and 1000 mg/kg bw/d (12-13 females/dose)
Control group	Vehicle only (corn oil)
NOAEL maternal toxicity	1000
NOAEL teratogenicity	250
Guidelines	Not specified
GLP	Not indicated
Method	Pregnant LE rats were treated by gavage with 500 mg DBP/kg bw/d (Study 1) and 250, 500 and 1000 mg DBP/kg bw/d from weaning, through puberty, young adulthood, mating and two or three pregnancies (Study 2). Controls received corn oil (vehicle).
	Study 1: Females were examined daily for vaginal opening and estrous cyclicity via vaginal smears. At 83 days of age, females were mated with treated males, and F1a litters were counted and weighed at birth and 15 days of age, then pups were euthanised. After a 30 day recovery period, the females (150 days of age) were mated for a second time with untreated male rats. The F1b litters were randomly reduced when possible to 4 males and 4 females per litter at birth and pups were euthanised at weaning. Production of an F1c began when the P0 females were mated with untreated males at 200 days of age. On GD13, prior to the stage, the females were necropsied and the numbers of live and dead foetus were counted and serum taken for progesterone analysis by radioimmunoassay.
	Study 2: On the day a proestrus vaginal smear was detected a female was placed with an untreated male rat for 24 h. P0 dams began delivering the F1a litters at 140 days of age, and the pups were counted and weighted at 1, 5, and 15 days of age and then euthanised. After weaning of the F1a pups, P0 dams were remated to control males for 24 h on the day of proestrus. On day 13 of the second pregnancy, the females were necropsied and the numbers of live and dead foetus were counted, organs weighed, and serum taken for progesterone, testosterone and estradiol analyses by radioimmunoassay. In animals with viable foetus, ovaries were cultured for P4, E2 and T hormonal analyses.
Result	Study 1: DBP at 500 mg/kg bw/d did not affect growth, puberty, estrous cyclicity from 30 to 80 days of age, or pregnancy percentages. In the DBP group, the numbers of females delivering

live F1a and F1b pups and the percent of viable foetuses in the third mating were reduced compared to controls but the number of implantations was not reduced. DBP treated females displayed significantly reduced serum progesterone levels.

Study 2: DBP at up to 1000 mg/kg bw/d did not affect growth, viability, or the ability to mate with a control male. However, for the F1a mating only 42% and 8% of the females were fertile in 500 and 1000 mg DBP groups, respectively, versus 92% of controls. The litter numbers were reduced to 1.7 and 0.1 pups in 500 and 1000 mg DBP groups respectively, versus 11.4 pups in the control group. Many of the 500 and 1000 mg DBP treated females that were pregnant, but did not deliver live pups, displayed a constant leucocytic, pregnancy-like vaginal lavage for 21-29 days in duration and blood was detected in the vagina at or after mid-pregnancy. On GD13 of the second pregnancy, gravid uterine weight and numbers of foetuses were reduced in 500 and 1000 mg DBP groups. Total ovarian progesterone production was decreased at 500 and 1000 mg/kg bw/day while total estradiol production was increased.

Conclusion	Chronic administration of DBP from weaning, through puberty, young adulthood, mating and pregnancy had adverse effects on female rat fecundity and fertility in the P0 generation at 500 and 1000 mg/kg bw/d. The effects of DBP at 500 mg/kg bw/d in female rats is equal to that seen in similarly treated male rats mated to untreated females.
Reliability	Reliable
Reference	Gray LE Jr, Laskey J, & Ostby J (2006) Chronic di-n-butyl phthalate exposure in rats reduces Fertility and alters ovarian function during pregnancy in female Long Evans hooded rats. Toxicol Sci, 93:189-195.

Developmental Toxicity/ Teratogenicity

Test substance	Di-n-butyl phthalate (DBP) (99.5% pure)
Species	Sprague-Dawley rats, 20 females/dose, Source-Shangai SIPPR-BK Experimental Animal Co. Ltd
Route of admin.	Gavage
Exposure period	Gestation day 1 to postnatal day 21
Study Duration	Mating to postnatal day 70
Frequency of treatment	Daily
Doses	0, 50, 250 and 500 mg/kg bw/d
Control group	Vehicle only (corn oil and Tween-80)
NOAEL maternal toxicity	500
NOAEL teratogenicity	50
Guidelines	OECD guidelines no. 414 (Prenatal development toxicity study), no. 421 (Reproductive/developmental toxicity screening test)
GLP	Not indicated
Method	Proestrous virgin female rats were mated with proven-fertile male rats overnight and pregnancy was detected by presence of sperm in vaginal smears. Pregnant rats were gavaged with 0, 50, 250 and 500 mg DBP/kg bw/d from GD 1 to PND 21. Controls received corn oil and Tween-80 (vehicle). Clinical signs were recorded daily (body weight was recorded weekly). The number of pups, sex and body weight of live pups was recorded on PND 1. Pup weight was recorded weekly. Dams and female pups were sacrificed at weaning (PND 21) and necropsied. Male pups were sacrificed on PNDs 14, 21 and 70 and necropsied.
Result	The no. of live/pups per litter was statistically significantly reduced at 500 mg/kg bw/d (p<0.01). Live pup weight at birth was significantly reduced in both sexes at 250 and 500 mg/kg bw/d; the decrease was slightly dose-related. Decreased anogenital distances were seen in male pups (statistically significant at 250 and 500 mg/kg bw/d) on PND 4. Significant decreases in relative prostate weight were seen at 250 mg/kg bw/d. Liver, kidneys and right relative epididymis weight were significantly reduced at 500 mg/kg bw/d. Necropsy revealed testes retention and underdeveloped epididymides in 2/20 pups at the highest dose on PND 21. The following effects were seen in male pups after completion of dosing on PND 70: 250 mg/kg bw/d (testicular atrophy and underdeveloped epididymides in 1/20 pups, decreased percent motile sperm, decreased number of sperm heads, mild degeneration of seminiferous epithelium) and 500 mg/kg bw/d (testicular atrophy and underdeveloped epididymides in 6/20 and 5/20 pups, respectively; absence of epididymides in 1/20 pups, more severe degeneration of seminiferous epithelium)). Dose-related increase in degeneration of the seminiferous epithelium and incidence of testes lesions.
Conclusion	Marked treatment-related effects were seen in male reproductive organs and their development. DBP also impaired parameters such as number of live pups/litter and pup body weight.
Reliability	Reliable
Reference	Zhang Y, Jiang X, & Chen B (2004) Reproductive and developmental toxicity in F₁ Sprague-Dawley male rats exposed to di-n-butyl phthalate *in utero* and during lactation and determination of its NOAEL. Reprod Toxicol, 18: 669-676.

Developmental Toxicity/ Teratogenicity

Test substance	Di-n-butyl phthalate (DBP) (>98% pure)
Species	CD(SD)IGS rats, 6-8 females/dose, bw-320 to 330 g, Source-Charles River Japan Inc.
Route of admin.	Orally in the diet
Exposure period	Gestation day 15 to postnatal week 20
Study Duration	Mating to postnatal day 35
Frequency of treatm.	In the diet
Doses	0, 20, 200, 2000 and 10000 ppm
Control group	Soy-free diet only
NOAEL maternal tox.	10000 ppm for maternal toxicity
NOAEL teratogen.	A NOAEL could not be established due to decreased spermatocyte development at the lowest dose
Guidelines	Not mentioned
GLP	Not mentioned
Method	Dams were dosed with soy-free diets containing DBP at concentrations of 0, 20, 200, 2000 and 10000 ppm from GD 15 until PND 21. Body weight and food intake were measured on GD 15 and 20, and on PNDs 2, 10 and 21. Pups were weaned on PND 21 and grouped as follows: 8 m and 8 f per group for prepubertal necropsy, 8-10 m and 8 f per group for necropsy at postnatal week 11 and 8-10 animals/sex for necropsy at postnatal week 20. Immunochemistry was performed on the pituitaries of all offspring.
Result	A slight-minimal decrease in testicular spermatocyte development at 20 ppm onwards was seen on PND 21. Degeneration and atrophy of mammary gland alveoli in males was seen at 20 ppm onwards but there was no dose-response. Male ratio of pups at birth was decreased at 2000 ppm and 10000 ppm, compared to control. Anogenital distance was reduced on PND 2 and areolar nipple retention in males was increased at 10000 ppm. The increase in nipple retention showed a dose response. An increased percentage of luteinizing hormone (LH) positive cells, and decrease in follicle stimulating hormone (FSH) and prolactin-producing cells in both sexes were seen at 10000 ppm at PND 21.
Conclusion	DBP affected female sexual development involving pituitary function at the lowest dose. Male testicular toxicity was reversible but mammary gland toxicological effects were apparent at even the lowest dose.
Reliability	Reliable
Reference	Lee KY, Shibutani M, Takagi H, Kato N, Takigami S, Uneyama C, & Hirose M (2004) Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. Toxicology, 203: 221-238.

Developmental Toxicity/ Teratogenicity

Test substance	Di-n-butyl phthalate (DBP) (purity not given)
Species	Long Evans rats, 15 females/dose, age; 2-months old
Route of admin.	Orally in the diet
Exposure period	2 and a half months prior to mating and during pregnancy for dams, PNDs 1-22 for pups.
Study Duration	12 weeks
Frequency of treatment	In the diet
Doses	0, 0.61 and 2.5 g/kg chow (equivalent to 12 and 50 mg/kg bw/d, respectively) (Conversions done by authors)
Control group	Soya-free rat chow only
NOAEL maternal toxicity	0.61 g/kg chow (12 mg/kg bw/d) for decreased body weight
NOAEL teratogenicity	A NOAEL could not be established due to decreased pup and testes weights at the lowest dose
Guidelines	Not mentioned
GLP	Not mentioned
Method	Female rats were dosed with 0.61 and 2.5 g DBP/kg rat chow for 2.5 months. Males received the control diet. Animals were mated and continued to receive the same diet. Pups were weaned on PND 22 and divided into 3 groups according to the chow their mothers had received. Animal weights were recorded weekly. Six males from each group were sacrificed on PND 14 and necropsied.
Result	Maternal weight gain (not significant) and % pregnancy were both lower than controls at both treated levels. Pup weights were significantly decreased in treated groups as compared to controls at PND 2 but not PND 6 ($p < 0.001$). Testes weights were significantly decreased at both treated levels but no dose-response was evident. Significantly delayed vaginal opening ($p < 0.001$) and onset of first oestrus cycle ($p < 0.05$) in pups were observed at 2.5 g/kg chow. Preputial separation was delayed in males born of mothers treated with 2.5 g/kg chow.
Conclusion	Oral intake of DBP by mothers during pregnancy had adverse effects on the reproductive development of male pups.
Reliability	Reliable
Reference	Salazar V, Castillo C, Ariznavarreta C, Campon R, & Tresguerres JA (2004) Effect of oral intake of dibutyl phthalate on reproductive parameters of Long Evans rats and pre-pubertal development of their offspring. Toxicology, 205: 131-137.

Developmental Toxicity/ Teratogenicity

Test substance	Di-n-butyl phthalate (DBP) (purity not given)
Species	CRL:CD(SD)BR rats, 10 females/dose, Source-Charles River Breeding Laboratory (Raleigh, NC)
Route of administration	Gavage
Exposure period	Gestation day 12 to 21
Study Duration	Mating to postnatal day 540
Frequency of treatment	Daily
Doses	100 and 500 mg/kg bw/d (10 dams per group, 3 replicates)
Control group	Corn oil vehicle only
NOAEL maternal toxicity	Could not be established
NOAEL teratogenicity	A NOAEL could not be established due to significantly increased areolae retention
Guidelines	US Federal guidelines (National Research Council, 1996)
GLP	Not mentioned
Method	Dams were gavaged with 100 or 500 mg DBP/kg bw/d in corn oil on GDs 10 to 21. Controls received corn oil vehicle only. Body weights were recorded daily. Male pups were weaned on postnatal day 21. Dams and female offspring were sacrificed on the same day. Male offspring were necropsied when 6, 12 or 18 months old. The anogenital distance (AGD) and number of areolae were measured on postnatal days 1 and 13, respectively.
Result	AGD was significantly reduced in pups whose mothers had been exposed to the highest dose. Areolae retention was significantly higher at both treated levels at PND13 but only at the highest dose when examined at PND 180. The incidence of testicular lesions was significantly higher at 500 mg/kg bw/d (effects included testicular atrophy and occasional enlargement with oedema). Other effects included significantly higher incidence of malformed epididymides, absent vas deferens, malformed or absent seminal vesicles, decreased prostate size and hypospodias. Histopathology revealed testicular dysgenesis and germ cell degeneration at 500 mg/kg bw/d. Significantly early mortality was seen at 500 mg/kg bw/d and was attributed to urinary tract obstructions (linked to nephrolithiasis).
Conclusion	DBP caused adverse developmental effects in male pups born of exposed mothers including malformed or missing reproductive organs, germ cell degeneration, and significantly decreased AGDs and increased areolae retention.
Reliability	Reliable
Reference	Barlow NJ, McIntyre BS, & Foster PM (2004) Male reproductive tract lesions at 6, 12 and 18 months of age following *in utero* exposure to di(n-butyl) phthalate. Toxicol Pathol, 32: 79-90.

Other studies

Test substance	Di-n-butyl phthalate (DBP) (purity not provided)
Species	Sprague-Dawley rats, 5 females/dose, Source: Charles River Laboratories Inc
Route of administration	Gavage
Exposure period	Gestation day 12 to 19
Study Duration	Mating to gestation day 19
Frequency of treatment	Daily from gestation day 1 to 19
Doses	0.1, 1, 10, 50, 100 or 500 mg/kg bw/d
Control group	Corn oil
NOAEL maternal toxicity	A NOAEL could not be established due to a decrease in gene expression at the lowest dose
NOAEL teratogenicity	10 mg/kg bw/d for decreased intratesticular testosterone levels
Guidelines	Not mentioned
GLP	Not mentioned
Method	Pregnant rats were gavaged with 0.1, 1, 10, 50, 100 or 500 mg/kg bw/d. Controls were given corn oil vehicle only. Body weights were recorded on GD 4 and daily during exposure. Animals were sacrificed on GD 19. Foetuses were extracted, weighed, sacrificed and necropsied. Changes in gene and protein expression were measured by RT-PCR and Western analysis. Foetal testicular testosterone concentrations were quantified by radioimmunoassay.
Result	A dose-related decrease in expression of genes responsible for cholesterol transport and steriodogenesis was seen after immunochemistry. Radioimmunoassay revealed decreased intratesticular testosterone levels at 50 mg/kg bw/d and above.
Conclusion	DBP impaired gene-expression and reduced intratesticular testosterone levels in offspring in a dose-related manner after maternal exposure.
Reliability	Reliable
Reference	Lehmann KP, Phillips S, Sar M, Foster PM, & Gaido KW (2004) Dose-dependant alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. Toxicol Sci, 81: 60-68.