Human Health Hazard Assessment

Diethylhexyl Phthalate (DEHP)
(CAS No. 117-81-7)

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INTRODUCTION

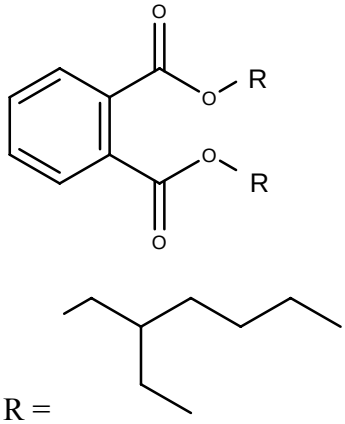
This review of diethylhexyl phthalate (DEHP) is a health hazard assessment only. For this assessment, key reviews on DEHP prepared by the European Chemicals Bureau (ECB, 2006), the Centre for the Evaluation of Risks to Human Reproduction (CERHR, 2005) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2002) 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:	117-81-7
Chemical Name:	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
Common Name:	Diethylhexyl phthalate (DEHP)
Molecular Formula:	$C_{24}H_{38}O_4$
Structural Formula:	
	R =
Molecular Weight:	390.56
Synonyms:	1,2-Benzenedicarboxylic acid, bis(2-ethylhexylester); Phthalic acid, bis(2-ethylhexyl)ester; Bis(2-ethylhexyl) 1,2-benzenedicarboxylate; Bis(2-ethylhexyl) o-phthalate; Bis(2-ethylhexyl)phthalate; Di-2-ethylhexyl-phthalate; Ethylhexyl phthalate; Dioctyl Phthalate; Di(isooctyl) phthalate; Octyl phthalate; DOP
Purity/Impurities/Additives:	Purity: ≥ 99.7% w/w Impurity: other phthalates Additives: none

1.2 Physico-Chemical Properties

Table 1: Summary of physico-chemical properties

Property	***Value***
Physical state	Oily liquid
Melting point	-47˚C
Boiling point	384˚C
Density	984 kg/m^3 (20˚C)
Vapour pressure	1.33 x10^{-8} kPa (25˚C)
Water solubility	4.1 x 10^{-5} g/L (25˚C)
Partition coefficient n-octanol/water (log Kow)	7.50
Henry's law constant	1.71 x 10^{-5} atm.m^3/mole (25˚C)
Flash point	196˚C

Source: ATSDR (2002)

2. USES

DEHP is the one of the most extensively used phthalates worldwide. In the USA, approximately 97% of DEHP is used as a plasticiser in PVC (ATSDR, 2002). In the European Union (EU), DEHP use represents around half of the total volume of phthalates used as plasticisers (ECB, 2006). DEHP-containing PVC is used in a variety of consumer products eg. toys, building material such as flooring and roofing, electrical cables, surface coatings, automotive components, furniture, shoes and boots, outdoor and rainwear. DEHP is also used as a plasticiser in medical devices such as blood bags and dialysis equipment.

In Australia, DEHP is used in flooring, waterproofing materials, cable sheathing/insulation, PVC labels, surface repair resin moulds, epoxy and polyurethane products, rubber components in automotive brake assemblies and hot melt adhesives for automotive assembly and repair. The chemical is also used in fragrance bases for perfumery and cosmetic products. Some businesses note phasing out of the chemical in these latter applications following the ban on cosmetics use in the EU.

3. HUMAN HEALTH HAZARD

3.1 Toxicokinetics

Previous Evaluations

The toxicokinetics of DEHP has been reviewed extensively. Toxicokinetic studies in experimental animals have been performed for the oral, inhalation, dermal and parenteral routes of exposure with the majority of toxicokinetic studies performed in rats by the oral route. There are a limited number of studies on the toxicokinetics of DEHP in humans. The following information is sourced from ECB (2006) and ATSDR (2002).

Oral

The first step in the absorption and metabolism of DEHP is hydrolysis by lipases to MEHP and 2-ethylhexanol (2-EH). Lipases are found in all tissues (intestinal mucosa, liver, kidney, lungs, skin, pancreas and adipose tissues) but especially in the pancreas (Albro, 1986*), correlating with the particularly rapid metabolism of DEHP in the intestine. Whereas unhydrolyzed DEHP can be absorbed, absorption in the intestine is increased following hydrolysis to MEHP. The extent of absorption in rats, non-human primates and humans is up to approximately 50% for doses up to 200 mg/kg bw. At higher doses, absorption in non-

human primates is dose-limited, in contrast to rodents (Albro et al., 1982*; Rhodes et al., 1983*). This species difference is reflected in differences in the activity of DEHP-metabolizing enzymes. The rate of MEHP formation from DEHP substrate differed by up to 357-fold among species, being highest in CD-1 mice, next in Sprague–Dawley rats and lowest in marmosets in all organs measured (liver, lungs, kidneys, and small intestine) (Ito et al., 2005).

Radiolabel studies show the liver, kidney, testes and blood as the main sites of distribution following orally administered DEHP in rats and monkeys (Rhodes et al., 1986*). There is no evidence of accumulation of DEHP or metabolites in animal tissues.

Metabolic pathways for DEHP involve a number of reactions. Following esteratic or hydrolytic cleavage of DEHP resulting in the formation of MEHP and 2-EH, MEHP is further metabolised via oxidative reactions resulting in the formation of numerous metabolites and a small amount of phthalic acid. Some of these oxidized derivatives are then conjugated with glucuronic acid prior to urinary excretion (Albro, 1986*, Astill, 1989*). The phthalaic acid remains undegraded. Oxidation of 2-EH primarily yields 2-ethylhexanoic acid and several keto acid derivatives, which are also excreted in the urine.

Orally administered DEHP results in excretion of metabolites and minimal quantities of the parent compound predominantly via urine and faeces. In rats, excretion following low administered doses of DEHP occurs mostly via the urine, whereas in monkeys, excretion of similar doses occurs mostly via faeces. A recent human study noted that most (75%) of orally administered DEHP was eliminated as metabolites via urine within 2 days (Koch et al., 2005). In rats and mice, elimination is rapid with 85-90% of a dose of radiolabelled DEHP being excreted via urine and faeces in the first 24 hours. In monkeys, the 24 hour excretion rate was lower at 50-80% (Astill, 1989*).

Limited human data from autopsies have indicated the presence of DEHP in adipose tissues (EPA 1989). However, it is suggested that this may be an artifact from contamination of biological samples during tissue processing.

Dermal

The absorption of DEHP via the skin has been reported to be low. No human *in vivo* dermal studies were found. However, Barber et al (1992*) and Scott et al (1987) compared *in vitro* absorption of DEHP through rat and human skin and found that permeability to DEHP was considerably greater for rat skin compared to human skin.

Dermal absorption was low in two studies conducted in rats (Melnick et al., 1987*; Elsisi et al., 1989). In these studies, 95 or 86% of the applied dose remained at the site of application after 5 or 7 days, respectively. Several studies in different species calculated the cumulative amount detected in excreta and tissues following dermal exposure: excluding the dosed skin, 6.5% in rat (Elsisi et al., 1989), 9% in rat (Melnick et al., 1987*), 26% in guinea pig (Ng et al., 1992*) and including the skin bound dose 9.7-18.9% in guinea pig (Chu et al., 1996*). One study determined the percutaneous absorption rate for DEHP in PVC applied to rat skin to be 0.24 $\mu g/cm^2/h$ (Deisinger et al., 1998*).

Inhalation

Absorption of DEHP occurs via the respiratory tract but quantitative data following inhalation exposure of animals or humans have not been published. In rats exposed to an aerosol of radioactively labeled DEHP, the major route of elimination was the urine (General Motors, 1982a*, b*).

Case studies of patients and workers have been published indicating absorption of DEHP through the lungs. DEHP is absorbed by infants undergoing respiratory therapy with plasticised medical devices (Roth et al. 1988*). MEHP and three other main metabolites were identified from workers exposed to DEHP by inhalation in several studies (Liss et al. 1985*; Dirven et al., 1993a*, b*). Considerable human inter-individual variations in percentages of detected unmetabolised MEHP were reported.

Parenteral

DEHP exposures through the parenteral route bypass intestinal lipases, so the amounts of intact DEHP in organs and tissues would be expected to be higher with this exposure route. This is evident in data from human studies of exchange transfusions and haemodialysis where initially there is more DEHP than MEHP in the blood (Pollack et al., 1985a*,b*; Sjoberg et al., 1985a*). DEHP levels then decline rapidly with a half-life of 10 hours (Sjoberg et al., 1985a*) and MEHP levels increase until the time-averaged concentrations are roughly equal (Pollack et al., 1985b*).

Similar results were seen in animal studies. Following arterial injection, DEHP was rapidly cleared from the blood (half-life of 15 hours) (Pollack et al., 1985a*). In mice intravenously injected with labelled DEHP, radioactivity rapidly accumulated in the gall bladder, intestine, urinary bladder, liver, kidney and brown fat (Lindgren et al., 1982*). Prolonged high levels in gall bladder and intestine after 24 hours suggest that secretion by the liver via bile is a major elimination route in mice. Following intravenous administration to marmosets, approximately 40% of the dose was excreted in urine and approximately 20% in the faeces (cumulative excretion) (Rhodes et al., 1983*). Around 28% remained in the lungs 7 days after administration with minimal levels in other tissues. Residual lung activity was postulated by these authors as reflecting insoluble emulsion entrapped within alveolar capillaries (Rhodes et al., 1986*).

Data not Reported in Previous Evaluations

No data.

Conclusion

DEHP is rapidly absorbed from the gastrointestinal tract following oral administration. In contrast, absorption of DEHP via the skin is low. Case studies of patients and workers indicate absorption of DEHP via inhalation and the parenteral route. The extent of absorption of oral doses up to 200 mg/kg bw/d in rats, primates and humans is up to approximately 50%. Studies in rats and monkeys show the liver, kidney, testes and blood as the main sites of distribution following orally administered DEHP. However, DEHP and metabolites do not to accumulate in tissues.

The first metabolic step is the hydrolysis of DEHP to MEHP and 2-EH by tissue lipases. MEHP is further metabolised via oxidative reactions resulting in the formation of numerous metabolites and a small amount of phthalic acid. Elimination of metabolites and minimal

quantities of the parent DEHP occurs mostly via urine and faeces. A recent human study noted that 75% of orally administered DEHP was eliminated as metabolites via urine within 2 days.

3.2 Acute Toxicity

Previous Evaluations

The acute toxicity of a single dose of DEHP has been evaluated in a number of species using oral, dermal, inhalation and intravenous routes of administration. LD_{50} values derived from these studies are shown in the Table 2.

Table 2. Acute animal toxicity studies

Study	*Species*	*Results (LD50/LC50)*	*Reference*
Oral	Rat	30600 mg/kg bw	Shibko & Blumenthal, 1973
	Rat	>20000 mg/kg bw	NTP, 1982*
	Rat	>40000 mg/kg bw	Nuodex, 1981a*
	Mouse	>20000 mg/kg bw	NTP, 1982*
	Mouse	>9860 mg/kg bw	Nuodex, 1981b*
	Guinea pig	26000 mg/kg bw	Krauskop, 1973*
	Rabbit	34000 mg/kg bw	Shaffer et al., 1945*
Dermal	Rabbit	24750 mg/kg bw	ATSDR, 2002
Inhalation (4 h)	Rat	>10.62 mg/L	Hüls, 1981*
Intravenous	Rat	250 mg/kg bw	Schmidt et al., 1975*; Rubin & Chang, 1978*
	Mouse	1060 mg/kg bw	Health Canada, 2002
Intraperitoneal	Rat	5675 mg/kg bw	Shaffer et al., 1945*
	Mouse	2800 mg/kg bw	Lawrence et al., 1975*; Woodward et al., 1986*

Source: ECB (2006)

Human studies

Shaffer et al (1945*) reported 2 adult male subjects who swallowed DEHP as single doses of 5 g and 10 g. No symptoms resulted from the 5 g dose while the ingestion of 10 g caused mild gastric disturbances and "moderate catharsis". Assuming 70 kg body weight, this equates to a dose of 140 mg/kg.

Data not Reported in Previous Evaluations

No data.

Conclusion

DEHP has low acute oral, dermal and inhalation toxicity. Intravenous and intraperitoneal administration of DEHP results in higher acute toxicity than oral or dermal administration.

3.3 Irritation

Skin Irritation

Previous Evaluations

In a human study, an unidentified quantity of DEHP applied to the skin of 23 humans for a 7-day period did not show any adverse effects (Shaffer et al., 1945*).

Two skin irritation studies in rabbits were performed according to OECD guideline 404 (BASF, 1986*; Hüls, 1987*) and another irritation study in rabbits was performed according to FDA recommended methods (Hüls, 1981*). In the first study, no erythema or oedema was observed (BASF, 1986*). In the second, very slight erythema was observed in all rabbits that persisted for 48 hours (Hüls, 1987*). In one rabbit, this progressed to a well-defined erythema. All reactions were reversible. The report concluded that DEHP was a slight skin irritant. The third earlier study reported that DEHP caused mild to moderate skin irritation at 24 hours after application in an unknown number of animals. Reactions were reversible (Hüls, 1981*).

Data not Reported in Previous Evaluations

No data.

Conclusion

DEHP causes minimal skin irritation in rabbits. Limited data suggest no irritant effects in humans.

Eye Irritation

Previous Evaluations

Two eye irritation studies in rabbits were performed according to OECD guideline 404 (BASF, 1986*; Hüls, 1987*) and another eye irritation study in rabbits was performed according to FDA recommended methods (Hüls, 1981*).

No reaction was observed in the cornea or iris in any of the studies. All three studies reported mild conjuctival redness after one hour. In the earlier study, mild conjunctival redness was observed in 5 eyes, 1 hour after dosing and in three eyes, 24 hours after application (Huls, 1981*). Where reported, all reactions resolved at later timepoints.

Data not Reported in Previous Evaluations

No data.

Conclusion

DEHP causes minimal eye irritation in rabbits.

Respiratory Irritation

Previous Evaluations

No studies specifically addressing this issue have been found. One acute toxicity study included examination of the lungs. Exposure to 10 mg/L DEHP for 4 hours induced dark red

foci and patches in the lungs of 19/31 rats (Hüls, 1981*; Klimisch et al., 1992*). It is unknown if these effects were reversible.

There are no adequate human data. In a case report of 3 preterm infants artificially ventilated with PVC respiratory tubes, an unusual lung disorder resembling hyaline membrane disease was observed in 2 infants, whilst the third died (Roth et al., 1988*). The authors assumed that these findings were causally related to the exposure to high doses of DEHP released from the PVC tubes.

Data not Reported in Previous Evaluations

No data.

Conclusion

Data are insufficient to determine the respiratory irritant potential of DEHP.

3.4 Sensitisation

Skin Sensitisation

Previous Evaluations

In a human study (Shaffer et al., 1945*), 23 human subjects were subjected to patch tests (skin of the back) where DEHP was left in contact for 7 days and reapplied on the same spots 10 days later. There was no erythema or any other reaction at any time following application of undiluted DEHP.

Two skin sensitisation studies in guinea pigs, using the Magnusson-Kligman maximization test protocol (Hüls, 1981*) and the Buehler test protocol (Exxon, 1994*), reported no positive reactions.

Data not Reported in Previous Evaluations

No data.

Conclusion

DEHP was not a skin sensitiser in a Magnusson-Kligman maximization test and a Buehler test in guinea pigs. Limited data indicate no sensitisation reactions in humans.

Respiratory Sensitisation

Previous Evaluations

A case control study was performed to assess the link between interior surface materials in the home and the development of bronchial obstruction (as an indicator for development of asthma**)** during the first 2 years of life (Jaakkola et al., 1999*). The results showed that the risk of bronchial obstruction was greater in the presence of PVC in the floor, but not wall, materials. The risk of bronchial obstruction increased in relation to the amount of plasticizer-emitting materials in the home.

In an earlier study by the same group (Oie et al., 1997), DEHP was predominant amongst different phthalates detected in sedimented dust samples (69% of total phthalate) and suspended particulate matter samples (52% of total phthalate) taken from dwellings.

An *in vitro* animal study provides limited support linking DEHP with respiratory hyperresponsiveness. MEHP (but not DEHP) provoked reversible hyperresponsiveness to methacholine in rat tracheal tissue (Doelman et al., 1990*). The authors concluded that only continous exposure to DEHP might cause bronchial hyperresponsiveness.

Data not Reported in Previous Evaluations

No data.

Conclusion

Human studies indicate correlations between the risk of bronchial obstruction and plasticiser-emitting components of the indoor environment. However, there is currently insufficient evidence supporting a causal relationship between respiratory effects and DEHP. Data are insufficient to determine the respiratory sensitization potential of DEHP.

3.5 Repeated Dose Toxicity

Previous Evaluations

The toxicity of DEHP following repeated exposures has been evaluated exhaustively in many animal species, both over short-term and chronic periods (ECB, 2006). The studies are summarised in Appendix A. Only key studies are detailed below.

Oral

DEHP has been tested via the oral route in many studies particularly in the rat but also in the mouse and marmoset monkey. Liver hypertrophy, increased liver weights and peroxisome proliferation were noted in most of the repeated dose studies. In a well performed 104-week dietary study in F-344 rats (70/sex/group) fed DEHP at 0, 100, 500, 2500 or 12,500 ppm (0, 5.8-7.3, 28.9-36.1, 146.6-181.7 and 789-938.5 mg/kg bw/d males-females respectively), hepatotoxicity (significant increase in serum albumin, absolute and/or relative liver weights and peroxisome proliferation) was observed in both sexes at 2500 ppm (146.6-181.7 mg/kg bw/d) and above (Moore, 1996*).

As well as liver effects, testicular effects such as decreased weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis were evident in most of the repeated dose studies. A NOAEL of 50 ppm (3.7 mg/kg bw/d) for testicular effects was identified from a 13-week rat study conducted according to OECD guidelines and GLP (Poon et al., 1997). In this study, Sprague-Dawley rats (10/sex/dose) were fed DEHP at doses of 0, 5, 50, 500 or 5000 ppm (0, 0.4-0.4, 3.7-4.2, 37.6-42.2 or 375.2-419.3 mg/kg bw/d, males-females respectively). A LOAEL of 500 ppm (37.6 mg/kg bw/d) was established based on an increased incidence of Sertoli cell vacuolation. Significantly decreased absolute and relative testicular weights, mild to moderate seminiferous tubule atrophy and Sertoli cell vacuolation were observed at higher doses.

Increases in kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy in the kidney were also observed in studies. The majority of these changes were seen in both sexes in different species in studies of varying durations. In chronic studies in rats and mice (Moore, 1996*; Moore, 1997*), there was no indication that these DEHP-related changes in the kidney were reversible upon cessation of exposure. From the 104-week rat dietary study of Moore (1996*), a NOAEL for kidney effects was established at 500 ppm (28.9 mg/kg bw/d for males and 36.1 mg/kg bw/d in females). The LOAEL in this study was 146.6 mg/kg bw/d (males) and 181.7 mg/kg bw/d (females), based on increased absolute and relative kidney weights.

Dermal

In the only available dermal study, 0.2 mL of 10, 30, 50, or 100% DEHP in olive oil was administered percutaneously to mice for one month (Watari et al., 1978*). Macroscopically, the liver was greatly enlarged. Inflammatory signs were observed in the peritoneum in the two highest dose groups. Hepatic cells showed atrophied nuclei and frequently contained fat droplets. The authors concluded that DEHP is absorbed and accumulates in the liver. This study was considered to have several limitations (ECB, 2006).

Inhalation

Four inhalation studies in experimental animals are identified. In the first study, rats were exposed up to 1000 mg/m^3, 6 hours per day, 5 days per week for 4 weeks (BASF, 1990*; Klimisch et al., 1992*). In the highest dose group, there was a significant increase in relative lung weights in male rats accompanied by foam cell (macrophage) proliferation and thickening of the alveolar septi. Liver weights were slightly increased but unusually, this was not accompanied by peroxisome proliferation that had been reported in a similar range-finding study conducted earlier by BASF (Merkle et al., 1988*). No testicular toxicity was detected histologically.

A poorly described study of male mice (20 animals) exposed to air saturated with vapours of DEHP (purity not specified) for 2 hours per day, 3 days per week, for 4-16 weeks failed to reveal consistent abnormalities which could be attributed to inhalation of DEHP (Lawrence et al., 1975*). No further data were available.

The only long-term inhalation study available was on hamsters (Schmezer et al., 1988*). However, only a single, very low dose (continuous inhalation of 15 μg/m^3 for 23 months) was used. No signs of any toxicological effects were reported.

Parenteral

Several published reports were found on the effects of DEHP administered intravenously (IV) to animals (Jacobson et al., 1977*; Sjoberg et al., 1985b*; Greener et al., 1987*; Baxter Healthcare Corporation, 2000*; Cammack et al., 2003).

Jacobson et al (1977*) studied hepatic effects in 6-month-old rhesus monkeys receiving transfusions of plasma from DEHP-plasticised bags over a 6-month or 1 year period. The average total exposures to DEHP for the groups of monkeys transfused weekly for one year were: Group 1 (plasma stored at 20°C): 3 monkeys at a mean dose of 27 mg/kg bw; Group 2 (plasma stored at 4°C): 2 monkeys at a mean dose of 8 mg/kg bw; Group 3 (transfused biweekly for 6 months, platelet poor plasma stored at 22°C): 2 monkeys at a mean dose of

32 mg/kg bw; Group 4 (untransfused control group): 3 monkeys and Group 5 (platelet-rich plasma stored for 48 hours at 22 °C in polyethylene blood bags): 2 monkeys. Three of the seven PVC-transfused monkeys showed some impairment of hepatic perfusion and four out of seven monkeys demonstrated abnormal sulfobromo-phthalein clearance indicative of subclinical liver disease. Six out of the seven had abnormal liver histology (aggregates of inflammatory cells, hepatocyte degeneration, and multi- and bi-nucleated giant cells) upon completion of transfusion period that persisted in three of the five surviving monkeys throughout the follow-up period of 26 months. None of the five control animals had abnormal liver histology. The results of this study are confounded by the small sample size, inconsistent responses in the two groups that received the largest (and similar) doses; use of pooled plasma to re-transfuse into the monkeys and appearance of a tuberculosis outbreak in the monkey colony which might have contributed to the hepatic effects. The authors conceded that the observed hepatic effects were mild and could be attributed to background effects.

Sprague Dawley rats (5-6/group) were cannulated and 3 hour infusions of 0, 5, 50, or 500 mg/kg DEHP were performed daily every other day for a total of six infusions over 12 days (Sjoberg et al., 1985b*). This was equivalent to time-weighted average doses of 2.5, 25, and 250 mg/kg bw/d. The DEHP was emulsified with egg yolk phosphatides and administered in a glycerol solution. Animals were sacrificed 2-3 hours after the last infusion. The results showed a dose-related decrease in body weight gain, an increase in relative liver weight at the middle and highest doses but no change in clinical chemistry parameters. Liver and kidney histology appeared unchanged except for an increase in hepatic peroxisomes. There was no change in the relative weight of the reproductive organs but transmission electron microscopic examination revealed slight enlargements of the smooth endoplasmic reticulum in Sertoli cells at the highest dose in three of five rats. The NOAEL was 25 mg/kg bw/d, with hepatic changes at 250 mg/kg bw/d.

Rhodes et al (1986*) reported an intraperitoneal marmoset study. Five marmosets were dosed with 1 g/kg bw/d in corn oil for 14 days. There was no indication of the length of time reported between the last dose and necroscopy. At necropsy, blood was taken for toxicokinetic studies, a gross examination was made and selected tissues were subject to microscopic examination. The marmoset data was considered by CERHR (2005) to be confusing and poorly reported: a single set of bar graphs is presented, while two studies were performed. The authors state that organ weights were not changed in marmosets at 1 g/kg bw/d IP but provided no data. Based on histology and biochemical measures, peroxisomes were not induced in marmosets. The authors presented no histological findings of testes. This limits the study as testicular pathology is the most sensitive endpoint at this exposure duration, and poor histology could well mean that lesions could go undetected.

Cammack et al (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3- to 5-day old) rats. Rats were injected interperitoneally with 0, 60, 300 or 600 mg/kg bw/d. A second group of animals was dosed for 21 days then held for a recovery period until 90 days of age. Terminal body weight was significantly less in the high dose group only. At the end of the 21-day dosing period, mean liver weight was increased and mean testes weight was decreased in the two higher dose groups. Testicular atrophy was observed in all animals in the 300 and 600 mg/kg bw/d treatment groups. The NOAEL in the study was 60 mg/kg bw/d.

Human studies

Three preterm infants artificially ventilated through PVC respiratory tubes, developed unusual lung disorders resembling hyaline membrane disease during the fourth week of life. One infant died two weeks after birth; the other two were healthy at follow-up 20 months later. DEHP was detected in the lung after autopsy of the infant who died. The estimated inhalation exposure in the three infants ranged between 1 - 4200 μg/h based on the concentrations of DEHP in the condensate collected from the water traps of the respirator tubing. However, this is likely to be an over-estimate as infants were not exposed to the condensate. DEHP, but not MEHP, could be demonstrated in urine samples (Roth et al., 1988*).

A morbidity study was carried out on a group of workers (97 men and 4 women) employed in a German plant producing DEHP (Thiess et al., 1978c*). The average exposure period was 12 years (range: 4 months to 35 years). DEHP levels measured in ambient air were generally low (0.001-0.004 ppm, ~ 0.016-0.064 mg/m^3). Higher levels up to 0.01 ppm (~0.16 mg/m^3) were measured near the chemical reactor. Blood lipids, serum activities of liver enzymes and routine haematological tests were normal, and no excess of any pathological condition was found. There was no referent group.

A mortality study of 221 workers exposed to DEHP in the same plant was also conducted (Thiess et al., 1978b*). Eight deaths occurred in the cohort compared with expected values of 15.9 and 17.0 for city and country workers, respectively. The cohort size was small and follow-up was short.

Three separate studies reported the incidence of polyneuropathy in workers exposed to phthlate esters (including DEHP). Milkov et al (1973*) conducted a morbidity study in the USSR on 147 workers at a PVC-processing plant. The workers were exposed to a mixture of phthalates, including DEHP as a minor constituent. The total phthalate air concentrations recorded varied between 1.7 and 66 mg/m^3. Polyneuropathy was evident in 47 workers (32%); the incidence increased with length of employment. Vestibular abnormalities were evident in 63 workers (78%) of 81 workers specifically examined. No referent group was included in the study.

In a cross-sectional study, symptoms and signs of polyneuropathy were reported in 12 out of 23 workers at a phthalate production plant in Italy (Gilioli et al., 1978*). The workers were exposed to a mixture of phthalates, including DEHP, but also to a lesser degree, to the corresponding alcohols and to phthalic anhydride. Total phthalate air concentrations varied between 1 and 60 mg/m^3. No referent group was included in the study.

In a study involving a Swedish PVC-processing factory, 54 male workers were examined for anomalous peripheral nervous system symptoms and clinical signs (Nielsen et al., 1985*). The workers were exposed mainly to DEHP, di-isodecyl phthalate, and butylbenzyl phthalate. They were divided into three groups of approximately equal size and mean phthalate exposures of 0.1, 0.2, or 0.7 mg/m^3. Peripheral nervous system symptoms and signs displayed were not related to the level of exposure.

Data not Reported in Previous Evaluations

McKee et al., (2004) describes a study reported as an abstract (Tomonari et al., 2003) of marmoset monkeys given DEHP orally (gavage) at doses of 0, 100, 500 or 2500 mg/kg bw/d for 65 weeks. At all doses tested, DEHP had no effects on liver and testicular weights.

Weights of the other accessory male reproductive organs in the monkey were similarly unaffected by treatment. Microscopic examinations did not reveal any testicular lesions and there were no differences in sperm counts. No differences were observed in testicular 3-beta hydroxysteroid dehydrogenase levels or peroxisome proliferator-activated receptor mRNE expression between control and DEHP-treated animals. The NOAEL from this study was 2500 mg/kg bw/d.

A subsequent report by Tomonari et al (2006) describes 90-115 day old marmoset monkeys (5-6/sex/group) given DEHP at 0, 100, 500 or 2500 mg/kg bw by gavage for 65-weeks. Blood samples were taken throughout the study and analysed for DEHP, MEHP, zinc and testicular enzyme activity. At the end of the study, the liver and primary and secondary sex organs were weighed and examined histologically. Peroxisomal enzyme activities were measured in liver samples. There were no treatment-related changes in body weights, liver weights or male reproductive organ weights. Absolute and relative uterine weights were increased significantly at 500 mg/kg bw/d and absolute and relative ovarian weights were increased significantly at 500 and 2500 mg/kg bw/d. These increases were not dose related. There were no microscopic changes in male gonads, secondary organs, Leydig, Sertoli or spermatogenic cells. No increases in hepatic peroxisomal enzyme activities were noted. The NOAEL was 2500 mg/kg bw/d.

Kurahashi et al (2005) exposed 4 week old male Wistar rats (4/group) to doses of DEHP at 0, 5 or 25 mg/m^3, 6h per day, for 4 or 8 weeks. There were no differences in body or testes weights. Seminal vesicle weight was reduced after 8 weeks but not 4 weeks exposure to both doses. Histological examination showed no significant pathological changes in the testes after 4 or 8 weeks exposure to either dose. The study did not show a dose-response and included a small sample size.

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

Conclusion

The toxicity of DEHP has been evaluated in a number of animal species, both short-term (few weeks) and life-time studies by several routes of exposure. The most pronounced findings included effects on the liver (hepatomegaly, peroxisome proliferation), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). Reproductive endpoints have been assessed particularly in later studies and will be described in greater detail in Section 3.8.

For hepatotoxicity, a well-conducted 104 week rat dietary study noted significant increases in serum albumin, absolute and/or relative liver weights and peroxisome proliferation in both sexes at 2500 ppm (146.6-181.7 mg/kg bw/d) and above (Moore, 1996*). The NOAEL for these effects was 28.9-36.1 mg/kg bw/d. Oral and inhalation studies in monkeys failed to elicit similar hepatotrophic effects at doses up to 2500 mg/kg bw/d, the maximum dose tested (Tomonari et al., 2003; 2006).

Kidney effects were also observed consistently following DEHP administration. From the 104-week rat dietary study of Moore (1996*), a LOAEL was established at 146.6-181.7

mg/kg bw/d, based on increased absolute and relative kidney weights. Mineralization of renal papilla, tubule cell pigmentation and chronic progressive nephropathy was observed at higher doses. The NOAEL for kidney effects was 500 ppm (28.9-36.1 mg/kg bw/d).

In addition to liver and kidney effects, testicular toxicity manifesting as decreased weights, testicular atrophy, increased bilateral aspermatogenesis, immature or abnormal sperm forms, seminiferous tubular degeneration, Sertoli cell vacuolation or complete loss of spermatogenesis were evident in repeated dose studies. In a well-conducted 13-week rat dietary study, a LOAEL of 500 ppm (37.6 mg/kg bw/d) was established based on an increased incidence of Sertoli cell vacuolation (Poon et al., 1997). Significantly decreased absolute and relative testicular weights, mild to moderate seminiferous tubule atrophy and Sertoli cell vacuolation were observed at higher doses. The NOAEL was 50 ppm (3.7 mg/kg bw/d).

3.6 Genetic Toxicity

Previous Evaluations

The genotoxicity of DEHP has been reviewed extensively (IARC, 2000; ECB, 2006). DEHP has been tested in a variety of short-term genotoxicity assays with predominantly negative results. In 15 published Ames tests, all results were negative. The maximum concentration used was 14,700 μg/plate. Two studies in fungi were negative, failing to show any evidence of mutation or recombination events. Primary DNE damage, mutation, sister chromatid exchange or chromosomal aberrations were not induced in cultured mammalian cells.

Results were generally negative in *in vivo* (mouse, rat and *Drosophila melanogaster*) studies testing DEHP and its main metabolites MEHP and 2-EH. Low levels of mutation but not DNE damage were induced in somatic cells of *Drosophila melanogaster*. Gene mutations were not induced *in vivo* in the liver of dosed mice and there was no evidence of chromosomal aberrations in mice or rats *in vivo*.

Human studies

The frequency of chromosomal aberrations in blood lymphocytes were investigated in ten workers employed from 10-30 years in a DEHP production plant in Germany (Thiess & Fleig, 1978*). Exposure levels ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3). There was no evidence of increased frequencies of chromosome aberrations in the exposed workers. The small number of workers examined and low levels of exposure limits this study.

Data not Reported in Previous Evaluations

No data.

Conclusion

Based on weight-of-evidence, DEHP is considered to be non-genotoxic.

3.7 Carcinogenicity

Previous Evaluations

Numerous studies on the carcinogenicity and mechanisms of carcinogenicity of DEHP have been performed *in vivo* and *in vitro*. Several *in vitro* mammalian cell transformation assays have been performed on DEHP and metabolites. Some, but not all, report positive transforming potential in the presence or absence of metabolic activation.

ECB (2006) summarises several *in vivo* tumour initiation/promotion experiments in rats and mice where the measured effect was the number and/or volume of altered liver cell foci. Overall, data indicate that DEHP has no tumour initiating activity, a positive promoting activity in mouse liver and a weak or no promoting activity in rat liver.

In a carcinogenicity study, B6C3F1 mice (70/sex/group) received DEHP in the diet at concentrations of 0, 100, 500, 1500, or 6000 ppm (M/F: 0/0, 19.2/23.8, 98.5/116.8, 292.2/354.2, or 1266.1/1458.2 mg/kg/d) for 104 weeks (Moore, 1997*). In an additional recovery group, mice were dosed with 6000 ppm of DEHP for 78 weeks, followed by a 26-week recovery period. Significantly increased incidences of hepatocellular adenomas and carcinomas were observed at 1500 ppm and 6000 ppm in male mice. In these two high dose groups, induction of peroxisome proliferation but not hepatocellular proliferation was more pronounced in both sexes. In the 6000 ppm recovery group, the incidence of hepatocellular adenomas, but not carcinomas, was less than in the 6000 ppm group. Non-tumour endpoints are described in Section 3.5.1. The LOAEL for tumour induction (hepatocellular neoplasms in male mice) in this study was 1500 ppm (292 mg/kg bw/d). The NOAEL was 500 ppm (98 mg/kg bw/d).

In a chronic/carcinogenicity study, F-344 rats (70/sex/group) received DEHP in the diet at doses of 0, 100, 500, 2500, or 12500 ppm (M/F: 0/0, 5.8/7.3, 28.9/36.1, 146.6/181.7, or 789/938.5 mg/kg bw/d) for 104 weeks (Moore, 1996*). In an additional recovery group, rats (55/sex/group) were administered 12500 ppm DEHP for 78 weeks, followed by a 26-week recovery period. Increases in hepatocellular adenomas and mononuclear cell leukaemia (MCL) in males at 2500 ppm and above and hepatocellular carcinomas in males and females at 12500 ppm, were observed. However, the incidence of hepatocellular adenomas/carcinomas was decreased in recovery animals at 12500 ppm (2-week recovery period), compared with the same dose group at the end of the dosing period. Peroxisome proliferation was induced from 2500 ppm. Effects on the liver, kidney and testis induced at 2500 ppm and above are described in Section 3.5.1. The LOAEL for tumour induction (hepatocellular neoplasms and MCL in male rats) was 2500 ppm (147 mg/kg b.w. per day for males). The NOAEL was 500 ppm (28.9 mg/kg bw/d, males).

The carcinogenicity of DEHP was tested in rats and mice in the US National Toxicology Program (NTP) in 1982-1983 (Kluwe et al., 1982*; NTP, 1982*; Kluwe et al., 1983*). F-344 rats and B6C3F1 mice were fed diets containing 0, 6000 or 12000 ppm (rats), and 0, 3000 or 6000 ppm (mice) DEHP for 103 weeks. This corresponded to a daily DEHP intake of 0, 322, and 674 mg/kg bw/d for male rats; 0, 394, and 774 mg/kg bw/d for female rats; 0, 672 and 1,325 mg/kg bw/d for male mice, and 0, 799 and 1,821 mg/kg bw/d for female mice. There was a dose-dependent increased incidence of hepatocellular carcinomas in male and females rats, with the increase statistically significant in females at the highest dose. The combined incidence of rats with hepatocellular carcinomas or neoplastic nodules was significantly greater than controls for females at both doses and for high dose males. In mice, a dose-related trend for hepatocellular carcinomas was observed for both sexes, with a significant increase in females at both doses and in high dose males. The incidence of

hepatocellular carcinomas or adenomas when combined was dose-related, with a significant increase in both sexes at both doses. The LOAEL for tumour induction in rats and mice was 6000 ppm (320 mg/kg bw/d for male rats) and 3000 ppm (670 mg/kg bw/d for male mice) respectively. No NOAEL could be identified for either species.

DEHP was administered in the diet at 0, 600, 1897, and 6000 mg/kg to male Sprague-Dawley rats beginning at an age of 90–110 days and continuing for the remaining lifetime of the animals (up to 159 weeks) (Berger, 1995; Voss et al., 2005). DEHP dose levels were 0, 30, 95, and 300 mg/kg bw/d. Significantly increased incidence of hepatocellular adenomas and carcinomas were observed at the highest dose. The percentage of benign Leydig cell tumors in the highest dose group was almost twice as high as the percentage in the control group (28.3% versus 16.4%). There was a significant dose-related trend in incidence of hepatic neoplasms and Leydig cell tumours. Leydig cell tumours have not been reported in previous studies in Sprague-Dawley rats, most likely due to late appearances outside the normal observation ranges of carcinogenicity studies (Voss et al., 2005).

The only inhalation study available is on Syrian golden hamsters continuously exposed to 15 $\mu g/m^3$ of DEHP by inhalation for 23 months (Schmezer et al., 1988*). There was no significant increase in tumour incidence.

Human studies

In a prospective cohort mortality study, eight deaths (including one carcinoma of the pancreas and one bladder papilloma) were observed (expected values of 15.9 and 17.0 from the city and county data, respectively) among 221 workers exposed to DEHP for periods of 3 months to 24 years (average 11.5 years) (Thiess et al., 1978b*). No information about exposure levels is given in the report, however in two other reports by the same group, exposure levels in the plant ranged from 0.0006 to 0.01 ppm (0.01-0.16 mg/m^3) are given (Thiess & Fleig, 1978*; Thiess et al., 1978a*).

Occupational exposure to polyvinyl chloride (PVC) and other products in the plastics industry were assessed in a case-control study on testicular cancer using self-administered questionnaires (148 cases and 315 controls) (Hardell et al., 1997*). An increased risk was observed for exposure to PVC (an increased odds ratio of 6.6; 95% confidence interval, 1.4-32), but not for other types of plastics.

Data not Reported in Previous Evaluations

Hardell's earlier study was followed up by a larger case-control study taken from the Swedish Cancer Registry during 1993-1997 (Hardell et al., 2004). A total of 791 matched pairs completed a questionnaire regarding exposure. Overall exposure to PVC plastics gave an odds ratio (OR) of 1.35 (confidence interval = 1.06-1.71). No dose-response relationships were found. The results do not support their previous study. There was no clear association with testicular cancer and exposure to PVC.

Conclusion

The results show that DEHP is carcinogenic in rats and mice. A statistically significant increase in the incidence of hepatocellular tumours with a dose-response relationship was observed in rats and mice of both sexes and a significant dose-related increase in the

incidence of Leydig cell tumours was observed in male rats. It was also noted that low doses did not cause hepatocellular tumours, which suggests a threshold for this effect.

Leydig cell tumours have been reported in only one study in Sprague–Dawley rats at doses that have been used in two other studies (using F-344 rats). It has been argued that the differing results are the consequence of the high spontaneous incidence of Leydig cell tumors in Fischer 344 rats compared to Sprague–Dawley rats.

In a 104-week rat study, an increased incidence of mononuclear cell leukaemia (MCL) was also noted. The relevance of MCL is unknown, but it was only seen in one of two rat studies and in neither of the two mouse studies. Moreover, this tumour type is well known to occur spontaneously with high incidence in the F344 rat strain used in the study.

The LOAEL and the NOAEL for tumour induction in rats (both liver tumours and MCL) were established as 2500 ppm (146.6 mg/kg bw/d for males) and 500 ppm (28.9 mg/kg bw/d for males) respectively (Moore, 1996*). In mice, the LOAEL and the NOAEL for induction of liver tumour were 1500 ppm (292 mg/kg bw/d for males) and 500 ppm (98 mg/kg bw/d for males) respectively (Moore, 1997*).

A single human case-control study suggested an increased risk of testicular cancer from DEHP in the PVC industry. However, a larger follow-up study did not support these findings. Overall, data are inadequate to determine the carcinogenicity of DEHP in humans.

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 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.

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 data are summarised in Appendix B.

3.8.1 Human Studies

Previous Evaluations

The following information was summarised from FDA (2002), ATSDR (2002), CERHR (2005) and ECB (2006). The studies are summarised in Appendix B.

There are limited data available on the reproductive toxicity of DEHP or its major metabolites in humans. Several studies associating adult male MEHP levels and various reproductive endpoints are described below.

Modigh et al (2002*) evaluated time-to-pregnancy in the partners of men potentially exposed occupationally to DEHP by inhalation. Median time-to-pregnancy was 3.0 months in the unexposed group, 2.25 months in the low exposure group (< 0.1 mg/m^3), and 2.0 months in the high-exposure group (0.1-0.2 mg/m^3). The authors concluded that there was no evidence of a DEHP-associated prolongation in time-to-pregnancy, although they recognized that there were few highly exposed men in their sample.

In a series of related human studies, spot urinary MEHP and semen and sperm motion parameters and sperm DNE damage were evaluated (Duty et al., 2003a; Duty et al., 2003b; Duty et al., 2004; Duty et al., 2005). The relationship between serum concentrations of reproductive hormones and MEHP urine concentrations was also assessed. Subjects included more than 150 men attending a clinic as part of a fertility evaluation. There were no significant associations between abnormal semen parameters, serum testosterone, sperm DNE damage and MEHP urine concentration above or below the group median. Jonsson et al. (2005) also studied semen parameters and urinary phthalate monoester levels in 234 military recruits. There were no significant associations between highest versus lowest urinary MEHP quartile and any of the dependent variables.

Cobellis et al (2003*) measured DEHP and MEHP concentrations in the plasma and peritoneal fluid of 35 women identified by laparoscopy as having endometriosis. There was no difference in the proportion of surgical patients compared to control women with detectable DEHP or MEHP (91.4% compared to 92.6% respectively). There was a significant difference in the median concentration of DEHP in the patients compared to control women (0.57 μg/mL compared to a control value of 0.18 μg/mL) but no difference in median MEHP concentration.

The human studies are limited but are consistent in that they do not identify any significant associations between MEHP and adverse semen parameters, hormone levels, time-to-pregnancy, or infertility diagnosis.

There have been several studies in humans where development of the male reproductive system and estimates of DEHP exposure during pregnancy or early childhood have been evaluated.

Cord blood samples were collected from 84 consecutive newborns (including a set of twins) delivered at an Italian hospital (Latini et al., 2003). DEHP and/or MEHP were detected in 74 of 84 cord blood samples with a mean (range) DEHP cord blood serum concentrations of 1.19 (0–4.71) μg/mL and MEHP of 0.52 (0 – 2.94 μg/mL). Mean gestational age, but no other parameter, was significantly lower in MEHP-positive neonates (38.16 weeks) versus MEHP-negative neonates (39.35 weeks). However, the levels measured in blood were unusually high compared to other studies.

Main et al (2006) reported phthalate concentrations in pooled milk samples collected 1-3 months after birth from 65 Finnish and 65 Danish women as part of a study of cryptorchidism and hormone levels in male children. Phthalate monoesters mono-methyl phthalate (mMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), MEHP and mono-isononyl phthalate (MiNP) were measured in milk and gonadotropins, sex-hormone binding globulin (SHBG), testosterone, and inhibin B were measured in the serum of breast fed boys. Cryptorchidism was identified in 62 of the 130

children of these women. However, there was no significant association between milk phthalate concentrations and cryptorchidism.

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). There was no significant association between maternal urinary MEHP concentration and infant anogenital index (AGI) (i.e. anogenital distance normalized for body weight). However, urinary concentrations of four other phthalate metabolites MEP, MBP, MBzP and monoisobutyl phthalate (MiBP) were inversely related to AGI. This study has been criticised by McEwen et al. (2006) from the Cosmetic and Fragrance Associations of America and Europe. They suggested that AGD is more likely to be proportional to height rather than weight and that maternal phthalate urinary concentrations were not 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).

Rais-Bahrami et al (2004*) examined onset of puberty and sexual maturity parameters in 14 to 16-year-old adolescents (13 males and 6 females) who had been subjected to extracorporeal membrane oxygenation (ECMO) as neonates. Pubertal development was normal. Thyroid, liver and kidney function, LH, FSH, testosterone and 17β-estradiol levels were normal for this stage of pubertal development.

Colon et al (2000) compared blood phthalate levels in 41 premature thelarche patients and 35 controls. There was a statistically significant difference in average blood DEHP levels. DEHP was detected in 25 of the samples from premature thelarche patients at a mean concentration of 450 μg/L (187 - 2098 μg/L); MEHP concentration ranged from 6.3 to 38 μg/L. DEHP was detected in 5 of 35 blood samples from control patients at at a mean concentration of 70 μg/L (276–719 μg/L). The reported levels in the control group are unusually high compared with the background MEHP concentration in urine in the normal population (mean 4.27, range 3.80–4.79 μg/L; Silva et al., 2004) and may reflect patient exposure to medical procedures within the hospital.

Data not Reported in Previous Evaluations

Pan et al (2006) measured the gonadotropins and gonadal hormone levels of 74 male workers exposed to elevated levels of DBP and DEHP in a PVC factory. Urinary MBP and MEHP levels (normalized to creatine) were significantly higher in exposed workers compared with controls (MBP 644.3 μg/g versus 129.6 μg/g; MEHP 565.7 μg/g versus 5.7 μg/g). Free testosterone was significantly lower in exposed workers (8.4 μg/g) versus control workers (9.7 μg/g) and was negatively correlated with MBP and MEHP.

Laboratory Animals

The effects of DEHP on reproductive endpoints have been tested in a variety of species including rats, mice, hamsters, ferrets and marmoset monkeys. The rat was the most sensitive followed by mice, hamsters and ferrets. Marmosets appear to be insensitive to DEHP-induced testicular toxicity. Key studies are described below.

3.8.2 Repeat Dose Toxicity Studies

Previous Evaluations

Oral

Poon et al (1997) exposed groups of 4-6 week old male and female Sprague-Dawley rats to 0, 5, 50, 500 and 5000 ppm DEHP in the diet for 13 weeks. Animals were reported to reach sexual maturity approximately 50 days into the study and thus were immature for only part of the study. These dietary concentrations corresponded to average DEHP doses of 0, 0.4, 3.7, 38, and 375 mg/kg bw/d, for the male rats. In the testes, Sertoli cell vacuolation, described as "mild," was seen in 7/10 males in the 500 ppm group, and 9/10 males in the 5,000 ppm group. The highest group also showed bilateral, multifocal, or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules. There was no measurement of reproductive function. The LOAEL, based on the testicular effects (Sertoli cell vacuolation) was 38 mg/kg bw/d and the NOAEL was 3.7 mg/kg bw/d.

David et al (2000a) fed 6-week old Fischer 344 rats (50-80 males/group) diets containing 0, 100, 500, 2,500, or 12,500 ppm DEHP (0, 5.8, 29, 147, and 789 mg/kg bw/d for males) for 104 weeks. Testes weight (absolute and relative) was reduced in rats of the high-dose group. Aspermatogenesis was observed in all rats in the highest dose group at study week 78 but not in rats treated with 2500 ppm or in the control group. At study week 105, the incidence of aspermatogenesis was significantly increased in rats exposed to 100 ppm and higher. The percentage of rats with aspermatogenesis from the control to high-dose group was 58, 64, 78, 74, and 97%, respectively. The authors identified the LOAEL for aspermatogenesis as 147 mg/kg bw/d and the NOAEL as 29 mg/kg bw. However, CERHR (2005) concluded that the findings indicate a NOAEL for testis effects of 5.8 mg/kg bw/d, because of the clear dose-response increase in the proportion of each group showing aspermatogenesis. However, this NOAEL may not be reliable because of the high frequency of aspermia in the controls. In fact the authors suggest that an increased incidence of aspermia may be a normal occurrence in the aging rat. CERHR (2005) also suggested that suboptimal testis fixation may have obscured any early vacuolar lesions produced by DEHP.

Akingbemi et al (2001, 2004) exposed rats to DEHP in two experimental series. In the first study to determine effects of gestational or lactational exposure, DEHP was administered daily by gavage in corn oil at 0 or 100 mg/kg bw/d to pregnant or nursing dams. In addition, to determine effects from exposure at different developmental ages, prepubertal male rats were dosed with 0, 1, 10, 100, or 200 mg/kg bw/d in two different 14 day periods: postnatal days (PND) 21–34 or 35-48 or for a longer 28 day period of PND 21-48.

In the second study (Akingbemi et al., 2004), young rats were gavaged daily with 0, 10 or 100 mg/kg bw/d DEHP on PND 21–48, 21–90 or 21-120. Within 24 hours of the final dose, measurements of LH and testosterone were made and the animals killed. Testicular histology was evaluated. Leydig cells were obtained from rats and incubated with LH to stimulate testosterone synthesis. Leydig cells were also incubated with testosterone biosynthesis substrates and enzyme activity measured.

There were no treatment-related effects on body weight gain or food consumption. There was no effect of DEHP treatment in young adults at any tested dose, on serum testosterone or LH or on *in vitro* Leydig cell steroidogenesis. There were no effects of any treatment on testicular histology. Treatment of prepubertal rats for 14 days (PND 21–34 or PND 35–48) did not produce alterations of serum LH or testosterone, but longer treatment (on PND 21–

48) produced increases in serum LH and testosterone and interstitial fluid testosterone that were statistically significant at 10 mg/kg bw/d. This study suggests that younger rats are more sensitive to the effects of DEHP and that there was a threshold duration of exposure.

Leydig cells isolated from prepubertal rats that had been treated on PND 21–34 or 35-48 showed a decrease in basal and LH-stimulated testosterone production at and above 100 and 10 mg/kg bw/d, respectively. There were no treatment effects on cultured Leydig cells derived from 35- and 90-day-old rats that had been prenatally exposed to 100 mg/kg bw DEHP. Paradoxically, exposure of prepubertal rats for 28 days (PND 21-48) was associated with an increased Leydig cell synthesis of testosterone and LH. Exposure on PND 35-48 affected all tested enzyme activities, with the most sensitive being 17β-hydroxysteroid dehydrogenase (reduced 74% at 10 mg/kg bw/d compared to control; other enzyme activities were significantly reduced at DEHP dose levels of 100 or 200 mg/kg bw/d). There were no effects of any treatment on testicular histology in any of the groups exposed during pregnancy, lactation, and post-weaning stages. The LOAEL was 10 mg/kg bw for increased serum LH and testosterone in rats exposed from PND 21-48 and the NOAEL was 1 mg/kg bw.

Dostal et al (1988) gave Sprague-Dawley rats oral doses of 0, 10, 100, 1000, or 2000 mg/kg bw of DEHP (>99% pure) by gavage in corn oil for 5 days (7-10 animals per group) at 1, 2, 3, 6, and 12 weeks of age. Absolute and relative testis weights were significantly reduced at doses of 1000 mg/kg bw/d in 1, 2, 3, and 6- week-old but not in 12-week-old rats compared to controls of the same age suggesting differential age sensitivity. Doses of 2000 mg/kg bw/d were fatal to suckling rats and caused decreased relative testis weight but no lethality in 6- and 12-week-old rats. The number of Sertoli cell nuclei per tubule was reduced by 35% at 1000 mg/kg bw in neonatal rats; two- and three-week old rats showed loss of spermatocytes but not of Sertoli cells. Loss of spermatids and spermatocytes in 6- and 12-week old rats at 1000 and 2000 mg/kg bw was shown. These results suggest that Sertoli cells are more sensitive during their proliferative stage.

Sjoberg et al (1986) studied the age-dependent testis toxicity of DEHP (1000 and 1700 mg/kg bw in the diet for 14 days) in rats at 25, 40, and 60 days of age. Body weight gain was retarded in all dosed groups and testicular weight was markedly reduced in 25- and 40-day-old rats given 1700 mg/kg bw. Severe testicular damage was shown for the 25-day- and 40-day-old rats at both dose levels. No changes were found in the 60-day-old rats.

A single bolus dose of DEHP (20, 100, 200, and 500 mg/kg bw) was given in corn oil to five neonatal rats (three-day old, CD Sprague-Dawley) pups per group (Li et al., 2000). MEHP (393 mg/kg bw), 2-EH (167 mg/kg), or vehicle was administered by gavage to 4 pups per group. All pups were killed 24 hours after dosing. The doses of MEHP and 2-EH were molar equivalent with 500 mg/kg DEHP. A time-course study was also conducted following a single dose of DEHP (200 mg/kg bw), where the pups were killed after 6, 9, 12, 24, or 48 h. Morphological examination revealed a dose-dependent presence of abnormally large, multi-nucleated germ cells (gonocytes) by 24 h post-treatment with DEHP (100-500 mg/kg bw). Sertoli cell proliferation was dose-dependently decreased from 100-500 mg/kg bw DEHP but not 20 mg/kg bw DEHP. There was a rebound in Sertoli cell proliferation at 48 hours following treatment with 200 mg/kg bw DEHP. MEHP (single dose group) induced similar effects as DEHP. A NOAEL for young pups of 20 mg/kg bw is derived for effects on altered gonocyte morphology and decreased Sertoli cell proliferation by a single oral dose of DEHP.

Two studies have administered oral doses of DEHP to pre- and post-pubertal marmosets for varying durations (Kurata et al., 1998*; Mitsubishi Chemical Safety Institute, 2003). Reproductive outcomes in particular were assessed.

Kurata et al (1998*) administered groups of 4 male and 4 female 12–15 months old (post-pubertal) marmosets, doses of 0, 100, 500, or 2500 mg/kg bw/d DEHP in corn oil by gavage for 13 weeks. There were no treatment-related decreases in testis weight, testosterone and estradiol levels. There were no testicular histopathological changes even at the highest dose. The NOAEL was 2500 mg/kg bw/d.

The authors of the Mitsubishi-Chemical-Safety-Institute (2003), in an unpublished report, administered DEHP by gavage in corn oil to juvenile marmosets (9 males and 6 females) beginning at 90–115 days of age until 18 months of age (young adulthood) at dose levels of 0, 100, 500, and 2500 mg/kg bw/d. Both males and females were assessed with in-life hormonal assays and with histopathology at necropsy. The results suggest little effect on testicular structure or function. Mean serum testosterone levels were highly variable, but the data suggested the possibility of a delay in the onset of puberty in male marmosets with increasing DEHP dose. Body weights and male organ weights were not affected. The NOAEL was 2500 mg/kg bw/d.

Inhalation

In a 4-week inhalation study conducted according to OECD guideline 412, male Wistar rats (10 rats per group) were exposed 5 days per week, 6 hours per day to 0, 0.01, 0.05, or 1 mg DEHP/L (0, 10, 50, or 1000 mg DEHP/m^3) (99.7% pure) as liquid aerosol (Klimisch et al., 1992*). The males were mated to untreated females. No effects on male fertility were observed 2 and 6 weeks after the end of exposure and no testicular toxicity was detected histologically. However, the results were not presented, and peroxisome proliferation also was not observed.

Parenteral

Sjoberg et al (1985b) exposed 25 or 40 day old rats to 0, 5, 50 or 500 mg/kg bw intravenously every other day for 10 days (time-weighted average 0, 2.5, 25 or 250 mg/kg bw/d). There was no change in testes weight but vacuolization of the Sertoli cells and spermatocyte degeneration was observed at 250 mg/kg bw/d. The NOAEL was 25 mg/kg bw/d.

In the second study, neonatal male rats or rabbits were injected with either 62 mg/kg bw/d DEHP or 4% bovine serum albumin during PND 3-21 (rats) or 14-42 (rabbits) (Baxter Healthcare Corporation, 2000*). Histopathological examination of the testes and other organs of DEHP-exposed animals revealed no alterations at this dose.

Similarly, Cammack et al (2003) conducted a 21-day repeat dose study of DEHP in neonatal (3- to 5-day old) rats. Rats were injected with 0, 60, 300 or 600 mg/kg bw/d or gavaged with 300 or 600 mg/kg bw/d. A second group of animals was dosed for 21 days then held for a recovery period until 90 days of age. At the end of the 21-day dosing period, testicular atrophy, decreased seminiferous tubules diameter and mild depletion of germinal epithelial cells were observed at 300 mg/kg bw/d. Although testicular atrophy persisted at the end of the recovery period, histopathological changes were not seen in the recovery group

previously exposed to a DEHP dose of 300 mg/kg bw/d for 21 days. At equivalent doses, oral exposure induced more significant changes in testicular weight and pathology. The NOAEL for intravenous exposure in the study was 60 mg/kg bw/d and the LOAEL was 300 mg/kg bw/d.

Data not Reported in Previous Evaluations

Tomonari et al (2006) gave 90-115 day old marmosets (5-6/sex/group) 0, 100, 500 or 2500 mg/kg bw/d by gavage for 65 weeks. Blood samples were taken throughout the study and analysed for DEHP, MEHP, zinc and testicular enzyme activity. At the end of the study, the liver, primary and secondary sex organs were weighed and examined histologically. There were no treatment-related changes in male organ weights, no microscopic changes in male gonads, secondary organs, Leydig, Sertoli or spermatogenic cells. No increases in hepatic peroxisomal enzyme activities were noted. The NOAEL was 2500 mg/kg bw/d.

3.8.3 Continuous Breeding Reproductive Toxicity Studies

Previous Evaluations

Lamb et al (1987) gave DEHP to male and female CD-1 mice (20 pairs per breeding group) at dietary levels of 0, 0.01, 0.1, or 0.3% (0, 14, 140, and 425 mg/kg bw/ day) from a 7-day premating period to 21 days after delivering litters (14 weeks in total). Decreased litters and viable pups were observed at 0.1% and above. No pairs were fertile at 0.3%. Also at 0.3% (the only dose examined), increased liver weights and decreased weights of the reproductive organs in parental animals (testes, epididymes, prostate, and seminal vesicles) were evident. All but one of the high-dose males showed some degree of bilateral atrophy of the seminiferous tubules. This dose also caused decreased sperm motility and sperm concentrations and increased incidences of abnormal sperm. The LOAEL was 0.1 % (140 mg/kg bw) based on decreased fertility and the NOAEL was 0.01% (14 mg/kg bw/d).

3.8.4 Two- and Three-Generation Reproductive Toxicity Studies

Previous Evaluations

Schilling et al (2001) fed Wistar rats (25 males and females in each group) DEHP in the diet at doses of 0, 1000, 3000, or 9000 ppm (0, 113, 340, or 1088 mg/kg bw/d) for two successive generations, from at least 70 days premating of the first parental generation. Increased focal tubular atrophy in the testis was observed in all treated groups (F0, F1 and F2). Decreased food consumption, body weight gain, testis weights and fertility index were seen in F0 and F1 adults at 9000 ppm. Decreased body weight gains, total number of pups, delayed vaginal opening and preputial separation, and increased numbers of stillborn pups were observed in F1 and/or F2 pups at 9000 ppm. Decreased AGD was observed from 1000 ppm and was statistically significantly different from 3000 ppm. Severe effects on testicular histology, sperm morphology, fertility, and sexual development of the offspring occurred in both generations at 9000 ppm. Reduced testis weights in F2 and focal tubular atrophy were observed in male offspring in F1 and F2 pups at 3000 ppm. Focal tubular atrophy also occurred at 1000 ppm. Vacuolisation of Sertoli cells was only observed in atrophic tubuli, which were present in all exposed groups. There was no indication that Sertoli cell vacuolation preceded focal or diffuse tubular atrophy and subsequent loss of sperm production. A NOAEL for fertility was not established as Sertoli cell vacuolation was recorded in the F1 offspring generation from the lowest dose level, 1000 ppm (113 mg/kg

bw). A developmental NOAEL was not established as Sertoli cell vacuolation was recorded in the F1 offspring generation at the lowest dose level, 1000 ppm (113 mg/kg bw).

Wolfe & Layton (2003) fed Sprague-Dawley rats (17 males and females in each group) DEHP in the diet at concentrations of 1.5, 10, 30, 100, 300, 1000, 7500, and 10000 ppm (0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, and 543-775 mg/kg bw/d) for two successive generations. The F_0 generation began exposure as adults. Clinical signs were generally comparable among all groups in all generations and were not treatment-related in incidence or severity. In the F_0 adults, a decreased number of live pups per litter were noted at 7500 ppm (592 mg/kg bw) and above. The only reproductive effects in the F_0 rats occurred at 10,000 ppm and included decreases in sperm counts and velocity, reductions in testis and epididymis weights, and increased numbers of rats with small testes in association with minimal-to-marked atrophy of seminiferous tubules characterized by loss of germ cells. The lowest dose level producing effects in F_1 offspring was 7500 ppm (391 mg/kg bw) and included decreases in number of live pups/litter, reduced male AGD, delayed testes descent, vaginal opening and preputial separation.

Fertility was compromised in the F_1 rats in the 10,000 ppm group which did not produce any viable litters. Other reproductive effects observed in F_1 parents were similar to those observed in F_0 parents but usually occurred at lower dose levels. For example, minimal to marked seminiferous tubule atrophy was noted at 10000 ppm in the F_0 and F_1 males, and at 7500 ppm in the F_1 and F_2 males. Minimal atrophy was noted 1 of 10 males in the 100 and 300 ppm F_1 groups. In the non-mating F_1 adult males of the 300 ppm group there was a small increase in the number of animals (3 of 45) with small testes and/or epididymides (none were observed in the F_0 males). The effects were not observed at the next higher dose (1000 ppm), but small testes were observed in 10 of 30 males of the 7500 ppm non-mating group. In F_2 non-mating males, small testes were also observed in 1 of 21 animals at 300 ppm and 1-3 animals at 1000 ppm. Small testes and epididymides were also observed in 7500 ppm F_3 males. While Sertoli cell vaculoation was observed in seminiferous tubules of the 1000 and 7500 ppm F_1 males (not 10000 ppm males), the vacuolation was similar to that in the controls. It was concluded that the vacuolation observed resulted from distortion during fixation and processing of the tissues (ECB, 2006). This distortion could also have obscured any minimal toxic effects that may have been present.

Increased liver weights were observed at 1000 ppm and above with accompanying histopathological changes. Decreased terminal body weights were observed at 7500 ppm and above. There was no general toxicity observed at doses below 1000 ppm. For fertility effects, the NOAEL was 1000 ppm (46 mg/kg bw/d) and the LOAEL was 7500 ppm (592 mg/kg bw/d) based on impaired litter parameters (F_1 pups). For developmental effects, the NOAEL was 100 ppm (4.8 mg/kg bw/d) and the LOAEL was 300 ppm (14 mg/kg bw) based on small testes size and minimal seminiferous tubule atrophy in the F_1 and F_2 generation. The case of a single male showing atrophy of seminiferous tubules in testis at 100 ppm was not considered significant, as there were no other accompanying findings.

3.8.5 Developmental/Postnatal Toxicity Studies

Previous Evaluations

The effect of DEHP on Leydig cell function in male Long-Evans rats exposed *in utero* (GD 12-21), during nursing, or during post-weaning stages has been evaluated (Akingbemi et al.,

2001). DEHP was administered to dams by gavage in corn oil at 0 or 100 mg/kg bw/d. Males were obtained for evaluation on PND 21, 35, or 90 (7 dams/group/stage). There were no effects of treatment during gestation on dam weight or weight gain or on offspring weight. Offspring testis and seminal vesicle weights also were not affected by treatment during gestation. Serum testosterone was reduced 31–33% and serum LH was reduced 50–64% in 21- and 35-day-old males exposed to DEHP during gestation. There were no effects on serum testosterone or LH in 90-day-old males. Prenatal exposure to DEHP resulted in decreased testosterone production by cultured progenitor Leydig cells obtained from 21-day-old males. Basal testosterone production was reduced 47%, and LH-stimulated testosterone production was reduced 56%.

The effects of DEHP were studied on male reproductive parameters in Sprague-Dawley rats exposed to 750 mg/kg/d DEHP by gavage commencing on GD 14 and ending at postnatal day (PND) 3 (Parks et al, 2000*). Exposed dams were sacrificed at GD 17, 18, 20, and at PND 2. Ex vivo testicular production of testosterone, testicular content of testosterone, and whole-body testosterone concentration were significantly reduced at all time points, with maximal effects at GD 20 where a 90% reduction in ex vivo testicular production of testosterone was noted. Anogenital distance was reduced at PND 2 and testicular weight was reduced at GD 20 and PND 2 respectively. Histopathological examination of the PND 2-testes showed increased numbers of Leydig cell hyperplasias and of multinucleated germ cells. DEHP exposure resulted in decreased testosterone, Leydig cell hyperplasia and formation of multinucleated germ cells in male fetuses and offspring.

Female rats received DEHP in the drinking water at 3.0-3.5 and 30-35 mg/kg bw/d from GD 1 to PND 21 (Arcadi et al., 1998*). Decreased pup kidney weights were observed at both doses, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between weeks 0 and 4 of age. Lower testicular weights were observed, associated with severe histopathological changes which included only a few elongated spermatids in tubules showing a pervious lumen at low dose level and a generalized disorganization of the tubular epithelium with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa, and with the tubular lumen filled with cellular deposits at high dose level. No NOAEL was established. There is doubt regarding the delivered dose in this experiment as DEHP is not soluble in water so was delivered as a suspension.

Data not Reported in Previous Evaluations

Female Wistar rats were given oral (gavage) doses of DEHP at 15, 45, 135, 405, 1215 µg/kg/bw/d or 5, 15, 45, 135 and 405 mg/kg bw/d on GD6 to PND21 (Grande et al., 2006). Exposure continued through lactation. In dams, liver and kidney weights were significantly increased at the highest dose level (405 mg/kg bw/d) No other signs of maternal toxicity were evident. Litter sizes, sex ratios, postimplantation losses and numbers of viable pups were also unaffected. In offspring, a significant increase in liver weight was observed on PND1 (but not PND22) at 135 and 405 mg/kg/d. A significant delay in the age of vaginal opening was observed at 15 mg/kg bw/d and above. Anogenital distance and nipple development were unaffected.

In a parallel study, effects of DEHP were studied on male offspring rats from the above study (Andrade et al., 2006). Nipple retention and reduced AGD were seen in males exposed to the highest dose (405 mg/kg/d). Delayed preputial separation was observed in

animals exposed to 15 mg/kg bw/d and above. Testes weights were significantly increased at 5, 15, 45 and 135 mg/kg bw/d (but not 405 mg/kg bw/d) on PND 22. Histopathological examinations of testes on PND 1 and 22 showed changes at the two highest doses (135 and 405 mg/kg/d). On PND 1, bi- and multinucleated gonocytes were evident. On PND 22, signs of reduced germ cell differentiation in seminiferous tubules were observed. The study concluded that DEHP acts as an anti-androgen in males at the highest dose level (405 mg/kg bw/d) but also induced subtle developmental effects at lower doses.

3.8.6 Prenatal Developmental Toxicity Studies

Previous Evaluations

Dietary levels of 0, 0.025, 0.05, 0.10, or 0.15% of DEHP (0, 44, 91, 190.6, or 292.5 mg/kg bw/d) were administered to mice throughout gestation (GD 0-17) (NTIS, 1984*; Tyl et al., 1988). Reduced maternal body weight gain was noted at 0.1% and above, mainly due to reduced gravid uterine weight. Increased resorptions, late foetal deaths and malformed foetuses, and decreased foetal weight and viable foetuses were observed at 0.1% and above. Increased malformed foetuses were seen at 0.05% and above. The external malformations included unilateral and bilateral open eyes, exophthalmia, exencephaly, and short, constricted or no tail. Visceral malformations were localised predominantly in the major arteries. Skeletal defects included fused and branched ribs and misalignment and fused thoracic vertebral centra. The NOAEL for maternal toxicity was 0.05% (91 mg/kg bw/d) and for developmental toxicity was 0.025% (44 mg/kg bw/d).

Pregnant rats received DEHP by gavage at doses of 0, 40, 200, or 1000 mg/kg bw/d from GD 6 to 15 (BASF, 1995*; Hellwig et al., 1997). Reduced uterine weights and increased relative kidney and liver weights were observed in dams at 1000 mg/kg bw/d. Also at this dose, decreased viable foetuses and foetal body weights, and increased implantation loss, external and skeletal malformed foetuses (predominantly of the tail, brain, urinary tract, gonads, vertebral column, and sternum) and foetuses with soft tissue, skeletal variations and retardations were seen. The NOAEL for maternal and developmental toxicity was 200 mg/kg bw/d.

DEHP at doses of 0, 40, 200, or 1000 mg /kg bw/d was administered by gavage to pregnant mice (15/group) from GD 6 to 15 (Huntingdon, 1997*). At GD 17, decreased viable pups and increased resorptions and post-implantation loss were observed at 1000 mg/kg bw/d. Cardiovascular abnormalities, tri-lobed left lungs, fused ribs, fused thoracic vertebral centres and arches, immature livers, and kidney anomalies were also observed at this dose. At 200 mg/kg bw/d, there was a slight increase in foetuses with intra-muscular or nasal haemorrhage or dilated orbital sinuses. There also were a small number of foetuses with anomalous innominate or azygous blood vessels at this dose level. A NOAEL of 200 mg/kg bw/d was established for maternal toxicity and 40 mg/kg bw/d for developmental toxicity.

Data not Reported in Previous Evaluations

Oral

Pregnant Sprague–Dawley rats were administered 0, 11, 33, 100, or 300 mg DEHP/kg/d by oral gavage starting on GD 8-18 (Calafat et al., 2006). Amniotic fluid samples were collected from each pup in the litters at necropsy on GD 18. Concentrations of MEHP in amniotic fluid were strongly correlated with corresponding maternal DEHP doses.

Pregnant Wistar rats were gavaged from GD 7 to 21 with vehicle or 10, 30, 100 or 300 mg/kg bw/d of DEHP. Male foetuses were examined on GD 21 (Borch et al., 2006). No maternal effects were reported. Testicular testosterone production *ex vivo* and testicular testosterone levels were reduced significantly at the highest dose. Histopathological effects on gonocytes were observed at 100 and 300 mg/kg bw/d. At the highest dose level Leydig cell effects and vacuolisation of Sertoli cell were observed. There was reduced testicular mRNE expression of the steroidogenesis related factors and reduced mRNE expression of a nuclear receptor involved in regulation steroid synthesis at the two highest doses. Even at the highest dose, there was no change in PPARα mRNA expression. The NOAEL for developmental effects was 30 mg/kg bw/d and the LOAEL was 100 mg/kg bw/d based on testicular pathology effects.

In a study of several phthalates, DEHP was administered orally to Sprague-Dawley rat dams at 750 mg/kg bw/d from GD 14 to PND 3 (Gray et al., 2000). There was no overt maternal toxicity or reduced litter sizes. DEHP treatment reduced maternal weight gain, pregnancy weight gain and pup weights. Male, but not female pups in both DEHP and BBP groups displayed shortened AGD and reduced testes weights. As infants, males had female-like areolas/nipples and increased incidence of reproductive malformations.

Inhalation

In rats, there was no consistent evidence of any treatment-related prenatal or postnatal developmental effects in the offspring of females (25/group) exposed to up to 300 mg/m^3 DEHP (the highest dose tested), 6 hours/d during the period of organogenesis (GD 6–15) (Merkle et al., 1988*). The number of live foetuses/dam was statistically significantly decreased and number of resorptions increased in the 50 mg/m^3 group but not at the next highest dose level.

Parenteral

There are insufficient data on the developmental toxicity of DEHP administered parenterally or intraperitoneally to identify LOAELs and NOAELs for these exposure routes. In the only published intravenous exposure study, no fetal toxicity was observed following intravenous administration of DEHP to pregnant rats (Lewandowski et al., 1980*). However the doses, 1 - 5 mg/kg bw/d were lower than those used in oral exposure studies. The lowest dose reported to produce fetal toxicity following intraperitoneal (IP) administration was 1,970 mg/kg bw/d (Peters & Cook, 1973*). Of 10 dams dosed on GD 3, 6, and 9 only one survived to deliver. Singh et al (1972*) administered 5 or 10 ml/kg bw (4,930 and 9,860 mg/kg bw) to groups of five Sprague-Dawley rats by IP injections on GD 5, 10, and 15. Maternal toxicity was not evaluated in this study. There was an increased frequency of resorptions at both doses and a decrease in fetal weights. Gross anomalies were only observed at the 9,860 mg/kg bw dose. The IP studies are limited as only high doses were tested and group size was small.

DEHP and MEHP were orally administered to pregnant ICR mice (9-11/group) at 0, 50, 100, 200, 400 mg/kg bw/d (MEHP) or 0, 250, 500, 1000 or 2000 mg/kg bw/d DEHP on GD 7-9 (Shiota and Mima, 1985). A second group received 0, 500, 1000, 2000, 4000, 8000 mg/kg bw/d (DEHP) or 0, 50, 100, 200 mg/kg bw/d (MEHP) by IP injections on GD 7-9. In groups given DEHP orally, resorptions and malformed foetuses (anencephaly and exencephaly) increased significantly above 500 mg/kg. No teratogenic effects were revealed

following IP doses of DEHP, or oral or IP doses of MEHP, although high doses were abortifacient and lethal to pregnant females. Thus, DEHP is embryotoxic and teratogenic in mice when given orally but not by IP administration. This difference may be a result of differences in metabolism, disposition, or excretion due to the route of administration. Although MEHP is a principal metabolite of DEHP and is more toxic than DEHP to adult mice, it seems that MEHP is not teratogenic in ICR mice.

3.8.7 Mode of Action

DEHP was not a competitive agonist at the oestrogen receptor in an *in vitro* competitive ligand-binding assay and did not induce oestrogen receptor-mediated gene expression in MCF-7 cells up to 10^{-5} M (Zacharewski et al., 1998). DEHP (up to 10^{-5}M) had no binding affinity for the oestrogen receptor α or β *in vitro* (Toda et al., 2004). DEHP, but not MEHP, demonstrated weak estrogenic activities in a human estogen receptor α (ERα) (but not ERβ) reporter gene assay in CHO-K1 cells transfected with expression vectors for human estrogen receptor ERα, ERβ and androgen receptor (AR) (Takeuchi et al., 2005). DEHP, but not MEHP, also demonstrated anti-estrogenic activity via ERα in the presence of 17β-estradiol and antiandrogenic activity in the hAR-transactivation assay. Neither DEHP nor its metabolite MEHP displayed affinity for the human androgen receptor at concentrations up to 10 μM in a reporter gene assay using COS monkey cells transfected with expression vectors for human androgen receptor (Parks et al., 2000).

DEHP (but not MEHP) increased proliferation of human breast cancer MCF-C7 cells (Okubo et al., 2003; Hong et al., 2005). However, the effects were not replicated *in vivo* as oral treatment with 600 mg/kg bw/d DEHP for 3 days did not increase expression of CaBP-9k mRNE in 7 day old female SD rats, a gene highly regulated by 17β-estradiol (Hong et al., 2005). DEHP did not induce estrogenic responses *in vivo* in a uterotrophic and vaginal cornification assays using immature and mature ovariectomised rats (Zacharewski et al., 1998).

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

Conclusion

Effects On Fertility

In vitro assays showed that neither DEHP nor its metabolite MEHP displayed affinity for the estrogen or androgen receptor suggesting that DEHP is not an androgen receptor antagonist, but acts as an antiandrogen.

There are very limited human data examining the reproductive effects of DEHP. These studies largely examine the relationship between urine levels of the DEHP metabolite, MEHP, and differing measurements of male and female reproductive health. The studies are small and largely negative. However, the most recent study suggests that at very high exposure levels to DBP and DEHP (mean serum MBP 644.3 μg/g creatinine; MEHP 565.7 μg/g creatinine), free testosterone levels are reduced (Pan et al., 2006).

There are many experimental animal studies, largely oral, using rats or mice. The most sensitive effects, perturbations in testicular structure and function, have been consistently

observed in several reproductive toxicity studies in rats and mice by both oral and parenteral routes of exposure (NTP, 1982*; Sjoberg et al., 1985b; Lamb et al., 1987; Poon et al., 1997; David et al., 2000a, b; Akingbemi et al., 2001; Schilling et al., 2001; Cammack et al., 2003; Wolfe & Layton, 2003; Akingbemi et al., 2004). *In vivo* and *in vitro* assays have demonstrated that the Sertoli cell is the most sensitive target of toxicity, causing subsequent germ cell depletion. Rats appear to be the more sensitive species than mice for testicular effects.

Increased LH and testosterone levels in Leydig cells were observed at 10 mg/kg bw/d with no effects at 1 mg/kg bw/d in 3 week old rats exposed for 28 days (Akingbemi et al., 2001). For testicular histopathology, the NOAEL and LOAEL were 3.7 and 38 mg/kg bw/d, respectively, in 4-6 weeks old rats exposed for 90 days (Poon et al., 1997). Decreases in testicular weight were reported at higher doses. For the endpoint of fertility, a NOAEL of 14 mg/kg bw/d is derived from a continuous breeding study in adult mice (Lamb et al., 1987). The LOAEL was 140 mg/kg bw/d.

The consistent finding of testicular effects in rats and mice is in contrast to studies in marmosets (Kurata et al., 1998*; Mitsubishi-Chemical-Safety-Institute, 2003*; Tomonari et al., 2006). No treatment-related changes in testicular histology or more gross parameters were observed at the highest dose used, 2500 mg/kg bw/d.

Developmental Effects

A number of human studies have attempted to link maternal MEHP levels with gestation length, onset of puberty and AGD. However, these studies, which were largely negative, are considered inadequate as they generally lacked an adequate control group and were of small sample size. Developmental studies in experimental animals comprise single and multiple-generation exposure largely by the oral route and predominantly in rodents.

There are no dermal studies, only a single inhalation study and few studies using parenteral routes of exposure. There are no developmental studies in primates.

Numerous studies have shown that DEHP is embryotoxic in rats at doses close to maternally toxic levels. In mice, several studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those producing observable evidence of toxicity to the dams.

In studies where exposure was limited to gestation, mice appear to be more sensitive to the developmental toxic effects of DEHP than rats. In mice, several studies have shown that DEHP is embryotoxic and teratogenic at dose levels below those producing observable evidence of toxicity to the dams. In rats, developmental studies have shown that DEHP is embryotoxic at doses close to maternally toxic dose levels when dosing encompassed early gestation exposure. DEHP induced overt structural malformations in rats exposed to 1000 mg/kg bw/d during the critical period of development (BASF, 1995*; Hellwig et al., 1997). More subtle endpoints were not recorded in all studies. Reduced AGD was reported in a number of studies. The LOAEL was at the non-maternotoxic dose of 113 mg/kg bw/d in rats (Schilling et al., 2001). Testes were reported to be small in male offspring of dams exposed to 250 mg/kg bw/d ie. non-maternotoxic doses during late gestation. Testicular pathology including Leydig cell hyperplasia was also noted at this dose. One other study reported decreased testicular weight and pathology at an estimated dose of 30-35 mg/kg bw/d but these results are questionable as doses administered were doubtful (Arcadi et al., 1998*). In

addition, data showed that DEHP disrupted male rat sexual differentiation by reducing testosterone levels (Akingbemi et al., 2001).

Developmentally induced effects are seen at lower doses in multigenerational studies. A well conducted oral 3-generational study in rats derived a NOAEL for developmental toxicity of 4.8 mg/kg bw/d based on the finding of small male reproductive organs at 14 mg/kg bw/d (Wolfe & Layton, 2003). At higher levels of exposure, effects on *in utero* survival, reduced AGD, undescended testes, retained nipples/areolae, incomplete preputial separation and disruption of spermatogenesis in offspring were evident. In this study, testicular abnormalities in the F_1 and F_2 generations were much more severe than in F_0, indicating the developmental phases were more sensitive to the testicular toxicity of DEHP.

The critical study for developmental toxicity is considered to be Wolfe & Layton (2003). For developmental effects, the NOAEL is 100 ppm (4.8 mg/kg bw/d) and the LOAEL is 1000 ppm (14 mg/kg bw/d), based on effects on the male reproductive organs.

4. HAZARD CHARACTERISATION

Toxicity data for DEHP are available for all health endpoints. For endpoints with 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 DEHP.

DEHP is rapidly absorbed from the GIT following oral administration. Up to approximately 50% of orally administered DEHP is absorbed by the GIT system in several species including humans. In contrast, the absorption of DEHP via the skin is low. The liver, kidney, testes and blood are the main sites of distribution following orally administered DEHP. DEHP is oxidised into a large number of metabolites with MEHP and 2-EH being the main constituents. There is no evidence of accumulation in animal tissues. DEHP and metabolites are excreted in the urine and faeces. A recent human study noted that 75% of orally administered DEHP was eliminated as metabolites via urine within 2 days.

In experimental animals, DEHP exhibits low acute oral, dermal and inhalation toxicity. Intravenous and intraperitoneal administration of DEHP results in higher acute toxicity than oral or dermal administration. DEHP induced minimal skin and eye irritation in animals and did not induce skin irritation in human volunteers. Data are insufficient to determine the respiratory irritant potential of DEHP.

Repeat dose effects of DEHP have been evaluated in a number of animal species by several routes of exposure. The most pronounced findings were effects on the liver (hepatomegaly, peroxisome proliferation), testes (tubular atrophy) and kidneys (increased kidney weights, mineralisation of renal papilla, tubule cell pigments and chronic progressive nephropathy). For liver and kidney toxicity (increases in serum albumin, absolute and/or relative liver and kidney weights and hepatic peroxisome proliferation) a LOAEL was established from a well-conducted 104-week rat dietary study at 146.6 mg/kg bw/d. The NOAEL was 28.9 mg/kg bw/d. For testicular effects, a LOAEL was established at 37.6 mg/kg bw/d based on an increased incidence of Sertoli cell vacuolation in a 13-week rat dietary study. The

NOAEL was 3.7 mg/kg bw/d. Studies of DEHP in monkeys failed to elicit the liver, kidney or testicular effects seen in rodents.

Peroxisome proliferation is observed in several animal species following administration of DEHP. Liver enlargement (hepatomegaly), hepatocyte hypertrophy and hyperplasia and proliferation of peroxisomes are recognised effects in rodents from exposure to peroxisome proliferators. In rodents, peroxisome proliferation has been shown to be mediated through the peroxisome proliferator-activated nuclear receptor alpha (PPARα) (Ward et al., 1998; Klaunig et al., 2003). Using PPARα-null mice, Lapinskas et al. (2005) recently showed that expression of PPARα is necessary for both DEHP and dibutyl phthalate (DBP) induced liver enlargement and induction of fatty acid metabolising enzymes confirming earlier knockout mouse studies of this receptor (Ward et al., 1998).

Studies conducted in patients treated with several hypolipidemic agents have provided no evidence for peroxisome proliferation or increased hepatocyte division in humans (Bentley et al., 1993*; Ashby et al., 1994*; Cattley et al., 1998[*]). The low levels of PPAR-α found in human liver could explain the low sensitivity of the human liver to the hepatotoxic effects of peroxisome proliferators (Tugwood et al., 1996*; Palmer et al., 1998*; Woodyatt et al., 1999*). Given also the minimal effects with DEHP in non-human primates, a similar low sensitivity of humans to the peroxisome proliferative effects of DEHP is therefore expected.

On a weight-of-evidence basis, DEHP is considered to be non-genotoxic. In carcinogenicity studies, DEHP showed positive transforming potential in some but not all mammalian cell transformation assays *in vitro*. *In vivo*, DEHP caused an increase in the incidence of liver tumour with a dose-response relationship in rats and mice of both sexes, and an increase in the incidence of Leydig cell tumours and MCL in male rats. DEHP-induced hepatocellular carcinomas are unlikely to be of relevance to humans since the hepatotoxic effects of DEHP, including hepatocellular tumour induction, are associated with peroxisome proliferation to which humans appear less sensitive.

Leydig-cell tumours were only reported in one animal study. Cook et al. (1999) noted similarities in the hypothalamo-pituitary-testis axis between rats and humans and concluded that substances that induce Leydig cell tumours via disruption of this axis in rats may have similar effects in humans albeit with a likely reduced Leydig cell proliferative response. An increased incidence of mononuclear cell leukaemia (MCL) was only seen in one of two rat studies and in neither of two mouse studies. This tumour type is well known to occur spontaneously, with a high incidence in the rat strain used in the study. It also has no comparable tumour type in humans (Caldwell, 1999) and so is unlikely to be relevant to humans.

The critical effects for DEHP are considered to be reproductive and developmental effects in males. Testicular toxicity appears to be the most sensitive toxicity endpoint but is significantly influenced by the age at exposure. Developing and prepubertal rats have been found to be much more sensitive to exposure to DEHP than adults. The younger animals responded to a much lower dose or produced a more serious lesion with a comparable dose on a mg/kg bw/d basis.

For effects on fertility, the NOAEL was 14 mg/kg bw/d based on a study in a continuous breeding study in mice (Lamb et al., 1987). The LOAEL in this study was 140 mg/kg bw/d but may not necessarily be due to male infertility as both sexes were exposed to DEHP in

the diet. However, a crossover study at the highest dose indicated both males and females had reduced fertility.

A number of key studies exposed animals during gestation and/or early postnatal life (Poon et al., 1987; Akingbemi et al., 2001; Wolfe & Layton, 2003; Andrade et al., 2006). The critical study for developmental toxicity was Wolfe & Layton (2003) for testicular effects during prenatal and neonatal development with a NOAEL of 4.8 mg/kg bw/d and a LOAEL of 14 mg/kg bw/d.

5. HUMAN HEALTH HAZARD SUMMARY TABLE

Phthalate	***Acute Toxicity***	***Irritation & Sensitisation***	***Repeated Dose Toxicity***	***Genetic Toxicity***	***Carcinogenicity***	***Fertility***	***Developmental Toxicity***
Diethylhexyl phthalate (DEHP)	Oral Rat: LD50 = 30600 to >40000 mg/kg bw Dermal Rabbit: LD50 = 24750 mg/kg bw Inhalation Rat: LC50 = >10.62 mg/L	Skin Irritation: ME Eye Irritation: ME Respiratory Irritation: Insufficient data Skin Sensitisation: negative Respiratory Sensitisation: Insufficient data	<u>Liver</u> Oral Rat: NOAEL = 28.9 mg/kg bw/d LOAEL = 146.6 mg/kg bw/d: ↑ serum albumin, ↑ liver wt and peroxisome proliferation <u>Kidney</u> Oral Rat: NOAEL = 28.9 mg/kg bw/d LOAEL = 146.6 mg/kg bw/d:↑ kidney weight <u>Testes</u> Oral Rat: NOAEL= 3.7 mg/kg bw/d LOAEL = 37.6 mg/kg bw/d: Sertoli cell vacuolation High doses: Hepatomegaly, hepatic peroxisome proliferation, kidney	*In vitro* Bacterial and fungul mutation assays: negative Primary DNE damage, sister chromatid exchange and chromosomal aberrations assays: negative Mammalian cell mutation assays: negative *In vivo* Drosophila melanogaster mutation assays: equivocal Chromosome aberration and DNE damage assays: negative Chromosome aberrations in sampled human lymphocytes: negative	*In vivo* Rat: NOAEL = 28.9 mg/kg bw/d LOAEL = 146.6 mg/kg bw/d: ↑ hepatocellular tumours and mononuclear cell leukaemia.	Continuous breeding study Mouse (adult): NOAEL = 14 mg/kg bw/d LOAEL = 140 mg/kg bw/d: ↓ fertility (adult)	Multigenerational study Rat: NOAEL = 4.8 mg/kg bw/d LOAEL = 14 mg/kg bw/d: ↓ testes wt; seminiferous tubule atrophy (F_1)

			proliferation, kidney and testicular degeneration				

6. ROBUST STUDY SUMMARIES

6.1 Repeated Dose Toxicity

Test Substance	:	DEHP
Type of Test	:	Chronic study
Species	:	Marmoset monkey, *Callithrix jacchus*, 90-115 days old.
Route of admin.	:	Oral (gavage)
Study Duration	:	65 weeks
Frequency of treatm.	:	Daily
Post exposure period	:	None
Doses	:	100, 500 or 2500 mg/kg bw/d in corn oil
Control group	:	Corn oil vehicle alone
NOAEL / NOEL	:	2500 mg/kg bw/d
LOAEL / LOEL	:	2500 mg/kg bw/d
GLP& QA	:	Not stated
Guidelines	:	Not stated
Method	:	Not a standard study
Result	:	DEHP at concentrations up to 2500 mg/kg bw/d did not have any treatment-related effects on male organ weights and no microscopic changes were found in male gonads or secondary sex organs. Sperm head counts, zinc levels, glutathione levels and testicular enzyme activites were comparable between groups. Electon microscope examination revealed no treatment-related abnormalities in Leydig, Sertoli or spermatogenic cells. Histochemical examination of the testis after 3 ß-hydroxysteroid dehydrogenase staining did not reveal any alterations in steroid synthesis in Leydig cells. In females, increased ovarian and uterine weights and elevated blood oestradiol levels were observed at 500 or 2500 mg/kg bw/d. These weight increases were associated with the presence of large corpus luteum, a common finding in older female marmosets. No abnormal histological changes were observed in ovaries or uteri in comparison to controls. No increases in hepatic peroxisomeal enzyme activites were noted in treated groups. Isolated hepatic enzyme activites (P-450 contents, testosterone 6 ß-hydroxylase and lauric acid ω-1 ω-hydroxylase activities) were increased in males and/or females at 500 or 2500 mg/kg bw/d but no consistent dose-related trends were observed.
Conclusion	:	The results demonstrated that chronic oral exposure to DEHP did not affect male reproductive tract development in the marmoset monkey.
Reference	:	Tomonari Y, Kurata Y, David RM, Gans G, Kawasuso T, & Katoh M (2006) Effect of di(2-ethylyhexyl) phthalate (DEHP) on genital organs from juvenile common marmosets: I. Morphological and biochemical investigation in 65-week toxicity study. J. Toxicol. Env. Health 69: 1651-1672.

6.2 Developmental Toxicity/ Teratogenicity

Test substance	:	DEHP, BBP, DINP, DEP, DMP, DOTP
Species	:	Sprague-Dawley female rats
Route of admin.	:	Oral (gavage)
Exposure period	:	GD 14 to PND 3
Study Duration	:	Not specified
Frequency of treatm.	:	Daily
Doses	:	750 mg/kg bw/d
Control group	:	Corn oil vehicle only
NOAEL maternal tox.	:	405 mg/kg bw/d
NOAEL teratogen.	:	5 mg/kg bw/d based on delayed vaginal opening
Guidelines	:	No information
GLP	:	No information
Method	:	No a standard test
Result	:	None of the phthalates induced overt maternal toxicity or reduced litter sizes. However, DEHP treatment reduced maternal weight gain and DEHP and DINP caused decreases in pregnancy weight gain. Decreased pup weights were observed in DEHP and BBP groups. Male, but not female pups in both DEHP and BBP groups displayed shortened anogenital distance and reduced testes weights. As infants, males in the DEHP, BBP and DINP groups had female-like areolas/nipples and increased incidence of reproductive malformations.
Conclusion	:	At this dose, DEHP, BBP and DINP altered sexual differentiation, whereas DOTP, DEP, and DMP were ineffective.
Reference		Gray LEJ, Ostby J, Furr J, Price M, Veeramachaneni D, & Parks L (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicological Sciences, 58: 350-365.

6.2 Developmental Toxicity/ Teratogenicity

Test substance	:	DEHP
Species	:	Female Wistar rats
Route of admin.	:	Oral (gavage)
Exposure period	:	GD 6 to PND 21
Study Duration	:	No information
Frequency of treatment.	:	Daily
Doses	:	15, 45, 135, 405, 1215 μg/kg/bw/d; 5, 15, 45, 135 and 405 mg/kg bw/d
Control group	:	Vehicle only (peanut oil)
NOAEL maternal tox.	:	135 mg/kg bw/d
NOAEL teratogen.	:	5 mg/kg bw/d
Guidelines	:	No information
GLP	:	No information
Method	:	Not a standard study
Result	:	In dams, liver and kidney weights were significantly increased at the highest dose level (405 mg/kg bw/d) No other signs of maternal toxicity were evident. Litter size, sex ratios, postimplantation losses and numbers of viable pups were also unaffected. In female offspring, a significant increase in liver weight was observed on PND1 (but not PND22) at 135 and 405 mg/kg/d. A significant delay in the age of vaginal opening was observed at 15 mg/kg bw/d and above. Anogenital distance and nipple development were unaffected.
Conclusion	:	The data showed that DEHP induced delayed vaginal opening in females exposed to 15 mg/kg/d and above.
Reference	:	Grande SW, Andrade AJ, Talsness CE, Grote K, & Chahoud I (2006) A Dose Response Study Following In Utero and Lactational Exposure to Di-(2-ethylhexyl) Phthalate (DEHP): Effects on Female Rat Reproductive Development. Toxicol. Sci. 91: 247-254

6.2 Developmental Toxicity/ Teratogenicity

Test substance	:	DEHP
Species	:	Female Wistar rats
Route of admin.	:	Oral (gavage)
Exposure period	:	GD 6 to PND 21
Study Duration	:	No information
Frequency of treatment.	:	Daily
Doses	:	15, 45, 135, 405, 1215 μg/kg/bw/d; 5, 15, 45, 135 and 405 mg/kg bw/d
Control group	:	Vehicle only (peanut oil)
NOAEL maternal tox.	:	135 mg/kg bw/d
NOAEL teratogen.	:	1215 μg/kg/bw/d
Guidelines	:	No information
GLP	:	No information
Method	:	Not a standard study
Result	:	In dams, liver and kidney weights were significantly increased at the highest dose level (405 mg/kg bw/d) No other signs of maternal toxicity were evident. Litter sizes, sex ratios, postimplantation losses and numbers of viable pups were also unaffected. In offspring, a significant increase in liver weight was observed on PND 1 (but not PND22) at 135 and 405 mg/kg/d. Testes weights were significantly increased at 5, 15, 45 and 135 mg/kg bw/d (but not 405 mg/kg bw/d) on PND 22. Nipple retention and reduced anogenital distance were seen with exposure to the highest dose (405 mg/kg/d). Delayed preputial separation was observed in animals exposed to 15 mg/kg bw/d and above. Histopathological examinations of testes on PND 1 and 22 showed changes at the two highest doses (135 and 405 mg/kg/d). On PND 1, bi- and multinucleated gonocytes were evident. On PND 22, signs of reduced germ cell differentiation in seminiferous tubules were observed.
Conclusion	:	The data showed that DEHP acts as an anti-androgen in males at the highest dose level (405 mg/kg bw/d) but also induced subtle developmental effects at lower doses.
Reference	:	Andrade AJM, Grande SW, Talsness CE, Grote K, Golombiewski A, Sterner-Kock A, & Chahoud I (2006) A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Toxicology 225:64-74.

7. APPENDIX A

Effects of DEHP following repeated oral, inhalation and parenteral administration (adapted from FDA, 2002; ECB, 2006)

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
Short-term studies (up to 28-days exposure)					
Rat, Sprague-Dawley Males 5 days (from day 6, 14-16, 21, 42, 86 of age) gavage, corn oil	0, 10, 100, 1000, 2000	10	100	liver: ↑ absw and relw from 100 mg/kg bw/d, ↑ pp, ↑ p.enz.act. ↓ TG, ↓ CHO	(Dostal et al., 1987a*)
Rat, Sprague-Dawley Females 5 days (2-6, 6-10, or 14-18 of lactation) gavage, corn oil	0, 2000	NE	2000	↓ bw liver: ↑ relw all groups, ↑ p.enz.act. ↓ TG, ↓ CHO	(Dostal et al., 1987b*)
Rat, F344 5 males/group 1 week, diet	0, 1.2% (0, 670)	NE	670	liver: ↑ absw and relw	(Takagi et al., 1992*)
Rat, F344 8 males 1 week, diet	0, 2% (0, 1600)	NE	1600	↓ CHO, ↓ TG ↑ absw and relw for liver and kidney no histological findings in liver, kidney, or testes	(Exxon, 1982*)
Rat, Wistar 4 males/ dose group 6 male controls 3, 10, or 21 days, diet	0 or 2% (1650-1830)	NE	1650-1830	↓ bw after 10-21 days liver: ↑ relw in all dosed males, ↑pp, ↑pSER, ↑p.enz.act., mitoch changed	(Mann et al., 1984*)
Rat, Alderley Park 20 rats/sex/group 30 rats/sex in control group 3, 7, 14, 28 days, or 9 months, diet	0, 50, 200, 1000	NE	50	liver: ↑ wt from 50 mg/kg bw/d, ↑ pp, ↑ pSER, ↑ p.enz.act., mitochondrial changes (males)	(CEFIC, 1982*) (Mitchell et al., 1985*)

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
Rat, Wistar 6 rats/sex 7 or 21 days, gavage	0, 2500	NE	2500	↓ bwg (males) liver: ↑relw, no histological findings, ↑nb peroxisomes, ↑pSER	(Mangham et al., 1981*)
Rat, F344 4-5 rats/sex/group 1 or 3 weeks, diet	0, 0.1, 0.6, 1.2% (0, 80, 480, 960)	NE	80	liver: ↑w, ↑ p.enz.act., ↓ TG	(CMA, 1982*)
Rat, F344 5 rats/sex/group 14 days, diet	0, 6300, 12500, 25000, 50000, 100000 ppm (0, 630, 1250, 2500, 5000, 10000)	630	1250	testes: atrophy from 12500 ppm	(NTP, 1982*)
Rat, Alderley Park 10 rats/sex/group 14 days, gavage	0, 2000	NE	2000	↓bwg (males) liver: ↑absw, ↑relw, ↑pp, ↑pSER, mitoch changes kidney: ↑weight (females), ↑pp testes: ↓weight, atrophy ↓CHO (males), ↓TG (males)	(ICI, 1982*) (Rhodes et al., 1986*)
Rat, Sprague-Dawley 6 males per group 14 day gavage	0, 1000	NE	1000	liver: ↑relw, ↑p.enz.act.	(Lake et al., 1984b*)
Rat, Sprague-Dawley 5 males per group 14 days, gavage	0, 25, 100, 250, 1000	NE	25	liver: ↑p.enz.act. from 25 mg/kg bw/d	(Lake et al., 1984a*)
Rat, Wistar 6 males per group 2 or 4 weeks, diet	0, 5, 18, 52, 182, 549	5	18	liver: ↑nb peroxisomes from 18 mg/kg bw/d	(RIVM, 1992*)
Rat, Wistar 5 males per group 14 days, gavage	0, 250, 500, 1000, 2000	250	500	liver: ↑relw from 500 mg/kg bw/d	(Khaliq & Srivastava, 1993*)
Rat, Wistar 5-6 males/group 16 days, diet	0, 0.01, 0.025, 0.05, 0.1, 0.5, 1.0% (0, 8, 22, 42, 88, 500, 900	42	88	liver: ↑relw at 88 mg/kg bw/d	(Fukuhara & Takabatake, 1977*)
Rat, F344 4-5 males per group	0, 2%	NE	NE	liver: ↑relw, ↑p.enz.act. hypolipidemia	(Moody & Reddy, 1978*)

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
3 weeks, diet					
Rat, Sprague-Dawley 4 males 3 weeks, diet	0, 2% (0, 900)	NE	900	↓bw and bwg liver: ↑absw and relw, ↑nb peroxisomes, ↑pSER, ↑p.enz.act. kidney: ↑absw and relw ↑CHO and TG (trend only)	(General Motors, 1982a,b*)
Rat, F344 5 rats/sex/group 21 days, diet	0, 0.01, 0.1, 0.6, 1.2, 2.5% (0, 11, 105, 667, 1224, 2101 [M]; 0, 12, 109, 643, 1197, or 1892 [F])	NE	11	↑TG at 0.01% (males)	(CMA, 1984*) (Barber et al., 1987)
Rat, F344, 5 males per group 21 days, diet	0, 100, 1000, 6000, 12000, 25000 ppm (0, 11, 105, 667, 1223, 2100)	11	105	liver: ↑p.enz.act. from 1000 ppm	(Short et al., 1987*)
Rat, Wistar 3 males/group 2-4 weeks, diet	0, 2% (4 w) or 2 w, 2% +2 w control diet	NE	NE	liver: ↑ wt during treatment period, ↓ during withdrawal period; ↑ p.enz.act. during treatment, ↓ during withdrawal	(Miyazawa et al., 1980*)
Rat, F344 5 rats/sex/group 28 days, diet	0, 0.2, 0.67, 2.0% (0, 150, 504, 1563 [M]; 0, 147, 490, 1416 [F])	NE	150	liver: ↑absw and relw from 0.2%, ↑p.enz.act., ↓ total lipids	(Nuodex, 1981c*)
Rat, F344 5 males 28 days, gavage	0, 1000	NE	1000	liver: ↑absw and relw	(Tenneco, 1981*)
Rat, F344 5 rats/sex/group 28 days, diet	0, 0.67% (0, 350)	NE	350	liver: ↑relw, ↑nb peroxisomes, and ↑p.enz.act. at 0.67%	(Hodgson, 1987*)
Rat, F344 5 rats/sex/group 21 days, gavage	0 700	NE	700	liver: ↑relw, ↑nb peroxisomes, and ↑p.enz.act. at 700 mg/kg bw/d	(Hodgson, 1987*)
Rat, F344 5 males per dose group	0, 0.02, 0.05, 0.1, 0.5, 1.0, 2.5% (0,	NE	24	liver: ↑relw from 0.02%	(BIBRA, 1990*)

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
10 male controls 28 days, diet	24, 52, 115, 559, 1093, 2496)				
Mouse, B6C3F1 10 males per group 5 days, gavage	0, 1879, 2844, 4304, 6514, 9860	NE	1879	liver: enlarged with a slight dose-response trend from 1879 mg/kg bw/d	(Nuodex, 1981b*)
Mouse, CD-1 10 females per group 8 days, gavage, corn oil	0, 6000, 7690, 9860	NE	6000	clinical signs of toxicity from 6000 mg/kg bw/d	(Hazleton, 1983*)
Mouse, B6C3F1 5 mice/sex/group 14 days, diet	0, 6300, 12500, 25000, 50000, 100000 ppm (0, 630, 1250, 2500, 5000, 10000)	1250	2500	↓bw, ↓bwg from 25000 ppm (males)	(NTP, 1982*)
Mouse, B6C3F1 10 mice/sex/group 4 weeks, diet	0, 1000, 5000, 10000, 25000 ppm (0, 250, 1210, 2580, or 6990 [M]) 0, 270, 1430, 2890, 7900 [F])	250	1210	↓bw and bwg from 5000 ppm (males) liver: ↑absw and relw from 5000 ppm kidney: ↓absw from 5000 ppm (males), inflammation from 5000 ppm	(Eastman Kodak, 1992a*)
F344 rats, 5 males/gp. and B6C3F1 mice, 5 females/gp 14 days, gavage	0, 2000 mg/kg bw	NE	2000	palmitoyl CoA oxidase): 9-fold and 21-fold ↑ in rats and mice, respectively. catalase: 2-fold and 3-fold ↑ in rats and mice, respectively. glutathione peroxidase: ↓ to 50% and 35% of the control in rats and mice respectively.	(Tomaszewski et al., 1986*)
Subchronic Studies (>28-days exposure <chronic exposure)					
Rat, Sprague-Dawley 10 rats/sex/group 13 weeks, diet	0, 5, 50, 500, 5000 ppm (0, 0.4, 3.7, 37.6, 375.2 [M]) 0, 0.4, 4.2, 42.2, 419.3 [F])	3.7	37.6	testes: mild to moderate Sertoli cell vacuolation from 500 ppm	(Poon et al., 1997)
Rat, F344	0, 1600, 3100,	320	630	↓bwg at 25000 ppm	(NTP, 1982*)

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
10 rats/sex/group 13 weeks, diet	6300, 12500, 25000 ppm (0, 80, 160, 320, 630, 1250)			testes: atrophy from 12500 ppm	
Rat, F344 10 rats/sex/group 13 weeks, diet	0, 1000, 4000, 12500, 25000 ppm (0, 63, 261, 859, 1724 [M]; 0, 37, 302, 918, 1858 [F])	NE	37 (females) 63 (males)	liver: ↑absw and relw from 1000 ppm	(Eastman Kodak, 1992b*)
Mouse, B6C3F1 10 mice/sex/group 13 weeks, diet	0, 800, 1600, 3100, 6300, 12500 ppm (0, 100, 200, 400, 800, 1600)	NE	100	↓bwg from 800 ppm (females)	(NTP, 1982*)
Chronic Toxicity studies (>10% of the test animals lifespan					
Rat, Sprague-Dawley 15 rats/sex/group 17 weeks, diet;	0, 0.2, 1.0, or 2.0% (0, 143, 737, 1440 [M]; 0, 154, 797, 1414 [F])	NE	143	liver: ↑absw and relw from 0.2%, no histological findings	(Gray et al., 1977*)
Rat, F344 5-10 males per group 1, 2, 4, 8, 18, 39, 77, 151, or 365 days, diet	0 or 1.2% (0, 600)	NE	600	↑p.enz.act.	(Conway et al., 1989*)
Rat, Sprague-Dawley 520 males in total1 102 weeks, diet	0, 0.02, 0.2, 2.0% (0, 7, 70, or 700)	NE	7	↓bw from 0.2% liver: ↑p.enz.act. from 0.02%, no tumours testes: atrophy and inhibition of spermatogenesis from 0.02%	(Ganning et al., 1987*; Ganning et al., 1990*)
Rat, F344 50 rats/sex/group 103 weeks, diet	0, 6000, 12000 ppm (0, 322, 674 [M]; 0, 394, 774 [F])	NE	322	liver: neoplastic lesions from 6000 ppm; testes: seminiferous tubular degeneration at 6000 ppm	(NTP, 1982*)
Rat, F344 70-85/sex/group	0, 100, 500, 2500, 12500 ppm (0, 5.8,	28.9 [M] 36.1 [F]	146.6-181.7	liver: ↑ wt (males) and peroxisome proliferation at 2,500 ppm;	(Moore, 1996*)

Species, Study Duration, Route	***Doses (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d)***	***Effects at LOAEL***	***References***
recovery group: 55/sex 104 weeks, diet	28.9, 146.6, 789.0 [M]; 0, 7.3, 36.1, 181.7, 938.5 [F]) or 12500 ppm for 78 weeks, followed by a recovery period of 26 weeks			kidney: ↑ weight from 2500 ppm testes: ↓ wt, ↑ incidence and severity of bilateral aspermatogenesis; ↓ incidence of interstitial cell neoplasms	
Rat, Sprague-Dawley 5 dosed males, 8 control males 2 years, diet	0, 2% (0, 1000)	NE	1000	liver: ↑relw, ↑ nb mitoch ↑nb peroxisome ↑p.enz.act. and lipid peroxidation	(Lake et al., 1987*)
Mouse, B6C3F1 50 mice/sex/group 103 weeks, diet	0, 3000, 6000 ppm (0, 672, 1325 [M] 0, 799, 1821 [F])	672-799	1325-1821	↓bw at 6000 ppm (females) liver: hepatocellular neoplastic lesions kidney: inflammation at 6000 ppm (males) testes: seminiferous tubular degeneration and testicular atrophy at 6000 ppm	(NTP, 1982*)
Mouse, B6C3F1 70-85/sex/group; recovery group: 55/sex 104 weeks, diet	0, 100, 500, 1500, 6000 ppm (0, 19.2, 98.5, 292.2, 1266.1 [M]; (0, 23.8, 116.8, 354.2, 1458.2 [F]) or 6000 ppm followed by a recovery period of 26 weeks	19.2 [M] 23.8 [F]	98.5-116.8	liver: peroxisome proliferation and ↑ weight (males) from 500 ppm	(Moore, 1997*)
Male rats, One year, by gavage, three times weekly	0, 0.9, 0.9 leachate from toluene extraction	NE	0.9	kidneys: statistically significant ↑ incidence of focal cystic changes; ↓ creatinine clearance	(Crocker et al., 1988*)
Marmoset monkeys (4/sex) 13 weeks, gavage	0, 100, 500 2500	2500	NE	No effects	(Kurata et al., 1998*)
Marmoset monkeys	0, 100, 500 2500	2500	NE	No effects on liver and testes wt, accessory male	(Mitsubishi-Chemical-

Species, Study Duration, Route	*Doses (mg/kg bw/d)*	*NOAEL (mg/kg bw/d)*	*LOAEL (mg/kg bw/d)*	*Effects at LOAEL*	*References*
(9M, 6 F) 65 weeks, daily gavage				reproductive organs; no testicular lesions, no differences in sperm counts.	Safety-Institute, 2003*) (McKee et al., 2004)
Marmoset monkeys (5-6/sex/group) 65 weeks, gavage	0, 100, 500 2500	2500	NE	No effects on body weight or liver weight. No changes in hepatic peroxisomal enzyme activities.	(Tomonari et al., 2006)
INHALATION					
Rats, Wistar (10/group) 4 weeks	0, 0, 10, 50, 1000 mg/m^3	50	1000	↑ lung wt	(BASF, 1990*; Klimisch* et al., 1992)
Rats, Wistar (4/group) 4-8 weeks	0, 5, 25 mg/m^3	5	25	↓ seminal vesicle wt	(Kurahashi et al., 2005)
Hamster, Syrian golden 23 months	15 ug/m^3	15	NE	No effects	(Schmezer et al., 1988*)
PARENTERAL					
Rats, Sprague-Dawley (5-6/gp) 12 days, IV	0, 2.5, 25, 250	25	250	Testes: Sertoli cell change at 250	(Sjoberg et al., 1985b*)
Rat, strain not specified (12/group) 18 days, IV	0, 30.8, 91.7, 164.8	92	165	↓ body wt, ↑ liver wt at 165	(Greener et al., 1987*)
Rat, strain not specified (7/group) 18 days, IV	0, 62	62	NE	No effect	(Baxter Healthcare Corporation, 2000*)
Rabbit, strain not specified (5/group) 28 days, IV	0, 62	62	NE	No effect	(Baxter Healthcare Corporation*, 2000)
Rat, Sprague-Dawley (7/group) 21 days, IV	0, 60, 300, 600	60	300	Testicular atrophy, ↓ testes wt,↑ liver wt.	(Cammack et al., 2003)
Mice, Swiss Webster (6 male/group) 5 days, IP or every other day for 20 days	0, 50, 100	100	NE	No effects	(Curto & Thomas, 1982*)
Rat, Sprague-Dawley	0, 50, 100	50	100	↓ gonadal zinc at 100	(Curto & Thomas,

Species, Study Duration, Route	***Doses (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d)***	***Effects at LOAEL***	***References***
Mice, Swiss Webster (6 male/group) every other day for 20 days, IP					1982*)
Monkey, rhesus (3 male/group) 6-12 month, IV	0, 8 (plasma stored at 4°C), 27 (plasma stored at 20°C), 32 (platelet poor plasma stored at 22°C)	32	NE	Probable non-treatment related effects due to disease in colony	(Jacobson et al., 1977*)
Rat (uremic), 14 days, IP	2000	2000	NE	No adverse effects	(Leber & Uviss, 1979*)
Rat 56 doses over 19 weeks, IP	500		210.5	Hepatomegaly, ↑ MFO activity, ↓ GST activity	(Leber & Uviss, 1979*)
Rat 7 days, IP	3906	NE	3906	Hepatomegaly	(Pollack et al., 1989*)
Rat Every other day for 12 days, IP	Up to 7.5	3.8	NE	No adverse effects, ↓ liver vitamin A at 0.75 and above	(Nair et al., 1998*)
Dog. 6 doses/week for 6 weeks, IV	NA	1	NE	No adverse effects	(Rutter, 1975*)

↓ / ↑ = decreased/increased; absw: absolute weight; bw: body weight; bwg: body weight gain; CHO / TG: cholesterol/triglyceride; mitoch: mitochondria; [M]: male; [F]: female; NA:not available; nb:number; NE: not established; p.enz.act.: peroxisomal enzyme activity or activities; pp: peroxisome proliferation; pSER: proliferation of smooth endoplasmic reticulum; relw: relative weight

8. APPENDIX B

Key reproductive studies with DEHP in laboratory animals (adapted from FDA, 2002; ECB, 2006)

Study design/ Species	***Route***	***Doses (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d) & endpoint***	***References***
Two-generation studies					
Rat, Sprague-Dawley 17males/group	Diet	1.5, 10, 30, 100, 300, 1000, 7500, 10000 ppm ($F_0/F_1/F_2$: 0.1, 0.5-0.8, 1.4-2.4, 4.8-7.9, 14-23, 46-77, 359-592, 543-775)	Sys: 14-23 Fert: 46 Devp: 4.8	Sys: 46, ↑ liver, kidney wt Fert: 592, ↓ nb live pups (F_1); Devp: 14, testes, epididymis atrophy ($F_{1,2}$)	(Wolfe & Layton, 2003)
Rat, Wistar; 25/gp	Diet	0, 1000, 3000, 9000 ppm (0, 113, 340, 1088)	Mat: 340 Fert: NE Devp: NE	Mat: 1088, ↓ body wt & food consumption Fert: 113, minimal testes atrophy (F_2); Devp: 113, testes atrophy (described above)	(Schilling et al., 2001)
Repeat dose studies					
Study design/Species	***Route***	***Doses (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d) & endpoint***	***Reference***
11-week-old CD-1 mice. 20 pairs of mice 98 days	Diet	0.01, 0.1, 0.3 % (0, 14, 141, 425)	Sys: 141 Fert: 14	Sys: 425 Fert: 141, reduced fertility; ↓ Live pups per litter	(Lamb et al., 1987)
Rat, Long-Evans; 10/group 70 or 100 days	Gavage	0, 10, 100	Sys: NE Fert: NE	Sys: NE Fert: 10, ↑ serum LH and testosterone, ↑ nb Leydig cells	(Akingbemi et al., 2004)
Rat, F344 24 males/group 60 days	Diet	0, 320, 1250, 5000, 20000 ppm (0, 18, 69, 284, or 1156 mg/kg bw/d)	Sys: 69 Fert: 69	Sys: 284, ↓ body wt Fert: 284, ↓ testis, epididymis, and prostate wt	(Agarwal et al., 1986a*; Agarwal et al., 1986b*)
Fischer-344 rats 50-80/group 104 weeks	Diet	0, 100, 500, 2500, 12500 ppm (0, 5.8, 29, 147, 789)	Sys: 29 Fert: 5.8	Sys: 147, ↑ liver & kidney wt (M) Fert: 29, ↑ aspermatogenesis (age related ?)	(David et al., 2000a)
Marmosets - 4 male and 4 female 12–15 months	Gavage	0, 100, 500, 2500	Sys: 2500 Fert: 2500	Sys: no effect Fert: no effect	(Kurata et al., 1998*)

(post pubertal) 13 weeks					
Rat, Sprague-Dawley (4–6-week-old 10 rats/sex/group 90 days	Diet	0, 5, 50, 500, 5000 ppm (0, 0.4, 3.7, 37.6, 375.2 [M])	Sys: 37.6 Fert: 3.7	Sys: 375.2, ↑ liver & kidney wt Fert: 37.6, Mild Sertoli cell vacuolation	(Poon et al., 1997)
Rat, Long-Evans PND 21–34, 35–48, or 21–48, PND 62–89. 10/group 14 or 28 days,	Gavage	0, 1, 10, 100, 200	Sys: 200 Fert: 1	Sys: no effect on body wt Fert: 10, ↓ 17α-hydroxylase in testis, altered ex vivo Leydig cell testosterone synthesis	(Akingbemi et al., 2001)
Rat, Sprague Dawley 1, 2, 3, 6, and 12 weeks of age (7-10/group) 5 days	Gavage	0, 100, 200, 500, 1000	Sys: NE Fert: 200	Sys: 100: ↑ abs & rel liver wt Fert: 500, ↓ Sertoli cell nuclei in 1 wk old rats, loss of spermatocytes in 2- & 3-week old rats	(Dostal et al., 1988)
Rat, Sprague Dawley 25, 40, 60 day old (6/group) 14 days	Diet	0, 1000, 1700	Sys: 1000 Fert: 1000 (25, 40 day old)	Sys: 1700, ↓ bwg Fert: 1700, ↓ testicular wt; testicular damage in 25- & 40-day-old rats given. No changes in 60-day-old rats	(Sjoberg et al., 1986)
marmosets 90–115 days of age 65 weeks	Gavage	0, 100, 500, 2500	Sys: 2500 Fert: 2500	Sys: NE Fert: NE	(Mitsubishi-Chemical-Safety-Institute, 2003*)
Prenatal Developmental toxicity studies					
Study design/ Species	***Route***	***Dose (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d) & endpoint***	***Reference***
Mouse, 1-CR 30-31 females/group GD 0-20	Diet	0, 0.025, 0.05, 0.10, or 0.15% (0, 44, 91, 190.6, 292.5)	Mat: 91 Devp: 44	Mat: 190.6, ↓ maternal bwg, ↓ uterine wt, Devp: 91, ↓ foetal bw and nb live foetuses/litter); ↑ nb & % of resorptions, late foetal deaths, dead & malformed foetuses, & % malformed foetuses/litter,	(NTIS, 1984*; Tyl et al., 1988)

				visceral malformations & skeletal defects	
Mouse, CD-1 15 females/ group GD 6-15,	Gavage	0, 40, 200, 1000	Mat: 200 Devp: 40	Mat: 1000, ↓ bwg Devp: 200, foetotoxic effects, ↓ nb viable foetuses	(Huntingdon, 1997*)
Mice, CD-1 28–29 per group GD 0 – 17	Diet	0, 0.01, 0.025, 0.05% (0, 19, 48, 95)	Mat: 95 Devp: 48	Mat: no effect Devp: 95, ↑ prenatal mortality, ↓ litter size	(Price et al., 1988*)
Rat, F344/CrlBr 34-25 females/group GD 0-20	Diet	0, 0.5, 1.0, 1.5, or 2% (0, 357, 666, 856, 1055)	Mat: 357 Devp: 357	Mat: 666, ↓ maternal food intake, ↓ maternal bwg Devp: 666, ↓ foetal wt	(NTIS, 1984*; Tyl et al., 1988)
Rats, Fischer 344 GD 0 - 20	Diet	0, 0.25, 0.5, 1.0% (0, 164, 313, 573)	Mat: 313 Devp: 164	Mat: 573, ↓ maternal bwg Devp: 313, ↓ litter size, ↓ pup bw (pup wt in high-dose group recovered by PND4).	(Price et al., 1988*)
Rat, Wistar 9-10 females/group GD 6-15	Gavage	0, 40, 200, 1000	Mat: 200 Devp: 200	Mat: 1000, ↓ maternal bw, ↑ maternal relative kidney and liver wt Devp: 1000, ↓ nb live foetuses/dam, ↓ foetal body weights, ↑ nb malformed foetuses/dam	(BASF, 1995*; Hellwig et al., 1997)
Rat, Sprague-Dawley 3-9/group GD 7–18 Examined: GD 20, 5 or 10 weeks (Exp 2)	Gavage	0, 125, 250, 500	Mat: 500 Devp: 250	Mat: NE Devp: 500, Leydig cell hyperplasia	(Shirota et al., 2005)
Rat, Wistar 8/gp GD7-21	Gavage	0, 10, 30, 100, 300	Mat: NR Devp: 30	Mat: NR Devp: 100, ↑ testicular path	(Borch et al., 2006)
Rat, Sprague-Dawley 6 dams/group GD 7–18 Evaluated: GD 12, 14, 16, 18, 20, 7 weeks	Gavage	0, 500, 1000	Mat: 500 Devp: 250	Mat: 1000, ↓ dam bw Devp: 500, small fetal testes, hyperplasia of interstitial cells	(Shirota et al., 2005)
Rat, Long-	Gavage	0, 100	Mat: 100	Mat: NE, no effects on dam bw or bwg	(Akingbemi et al.,

Evans 7/group GD 12–21 Evaluated: PND 21, 35, or 90			Devp: NE	Devp: 100, ↓ Serum testosterone and LH at PND 21- and 35 but not at 90 days	2001)
Rat, Wistar 18/dose group GD 7-21	Gavage	0, 300, 750	Mat: 750 Devp: NE	Mat: NE Devp: 750, testicular histological effects	(Borch et al., 2005)
Developmental/postnatal toxicity studies					
Study design/ Species	***Route***	***Dose (mg/kg bw/d)***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d) & endpoint***	***Reference***
Rat, Sprague-Dawley 11 rats/dose GD 14-PND2	Gavage	0, 750	Mat: NE Fert: NE Devp: NE	Mat: 750, ↓ mat wt gain Fert: NE Devp: 750, ↓ testes wt at GD 20, PND2, ↓ body weight at PND2 not GD20, ↓ testosterone and AGD (abs and rel) from GD17, ↑ Leydig cell hyperplasia and multinucleated gonocytes observed in testes.	(Parks et al., 2000)
Rat, Sprague-Dawley 5-19/dose GD 14-PND 3	Gavage	0, 750	Mat: 750 Fert: 750 Devp: NE	Mat: NE Fert: NE. Devp: 750, ↓ maternal wt gain, ↓ pup wt in male pups, ↓ AGD and ↓ testis wt, retention of female-like areolas/nipples, ↑ incidence of reproductive malformations	(Gray et al., 2000*)
Rat, Wistar 20/group GD 7-PND17	Gavage	0, 300, 750	Mat: NE Fert: NE Devp: NE	Mat: NE Fert: NE Devp: 300, ↓ AGD; abnormal testis histology	(Jarfelt et al., 2005*)
Rat, Sprague-Dawley 5-8 /group GD3-PND21	Gavage	0, 375, 750, 1500	Mat: 375 Fert: 750 Devp: 375	Mat: 750, ↓ wt gain Fert: 1500, ↓ litter size Devp: 750, ↓ pup survival, ↓ AGD	(Moore et al., 2001*)
Rat, Wistar 10/group GD 1 – PND21	Gavage	0, 20, 100, 500	Mat: 500 Fert: 100 Devp: 100	Mat: NE Fert: 500, ↓ litter size, Devp: 500, ↓ prostate wt, ↓ sperm nb & production	(Dalsenter et al., 2006)

Rat, Long Evans 12 rats/group GD 1-PND21	Oral, drinking water	0, 3.0-3.5, 30-35	Mat: 30-35 Fert: NE Devp: NE	Mat: NE Fert: 3.0-3.5, ↓ testicular wt with only a few elongated spermatids in tubules at the low dose level Devp: 3.0-3.5, ↓ pup kidney wt	(Arcadi et al., 1998*)
Inhalation					
Study design/species	***Route***	***Doses***	***NOAEL (mg/kg bw/d)***	***LOAEL (mg/kg bw/d) & endpoint***	***Reference***
Rat, Wistar 25 females/ group GD 6-15	Head-nose	0, 0.01, 0.05, or 0.3 mg/litre (0, 10, 50, or 300 mg/m^3) 6h/d	Mat: 300 Fert: 300 Devp: 300	Mat: NE Fert: NE Devp: NE (↓ nb live foetuses at 50 mg/m^3 but not 300 mg/m^3; ↑ % of resorptions at 50 mg/m^3 but not 300 mg/m^3	(Merkle et al., 1988*)
Parenteral					
Rat GD 6–15.	IV	0, 1.3-1.4, 4.7-5.3 mg/kg/d	Mat: 4.7-5.3 Fert: 4.7-5.3 Devp: 4.7-5.3	Mat: NE Fert: NE Devp: NE	(Lewandowski et al., 1980*)
Rat, Sprague-Dawley 5/group GD 5, 10, and 15	IP	0, 4930 and 9860 mg/kg bw	Mat: NE Fert: NE Devp: NE	Mat: Fert: 4930, ↓ implantations Devp: 4930, ↓ foetal wt	(Singh et al., 1972*)
Rat, Sprague-Dawley 5/group GD3, 6, and 9 Examined at weaning	IP	0, 1972, 3944 mg/kg/d	Mat: NE Fert: NE Devp: NE	Mat: 1972, ↑ dam deaths Fert: 1972, ↓ implantations Devp: 1972, ↓ nb of weaned pups	(Peters & Cook, 1973*)

AGD: anogenital distance; bw: body weight; bwg: body weight gain; nb: number NE: not established; NR: not reported; Mat: maternal; Fert: fertility; Devp: development; wt: weight

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