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This document is an **External Peer Review draft**. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage by construed to represent the Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

IRIS SUMMARY FOR DIBUTYL PHTHALATE

0038
Dibutyl Phthalate; CASRN 84-74-2; 00/00/0000

Human health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several program offices, regional offices, and the Office of Research and Development. Sections I (Chronic Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the positions that were reached during the review process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website at http://www.epa.gov/iriswebp/iris/backgr-d.htm.

STATUS OF DATA FOR Dibutyl Phthalate

File First On-Line 01/31/1987

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	00/00/0000
Acute RfD		
Short term RfD		
Subchronic RfD		
Chronic RfD		
Inhalation RfC Assessment (I.B.)	discussion	00/00/0000
Carcinogenicity Assessment (II.)	on-line	00/00/0000

_I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

__I.A. REFERENCE DOSES (RfD) FOR ORAL EXPOSURE

Dibutyl Phthalate
CASRN –84-74-2
Section I.A. Last Revised -- 00/00/0000

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iriswebp/iris/backgr-d.htm for an elaboration of these concepts. Because RfDs can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

This summary replaces the summary dated 08/01/1990. The previous RfD (verified 08/26/1987) of 1E-1 mg/kg-day was based on the 1 year study of Smith (1953) with a NOAEL of 125 mg/kg-day, a LOAEL for increased mortality of 600 mg/kg-day, and a total uncertainty factor of 1000 (10 each for interspecies and intraspecies extrapolation and 10 for duration of exposure and deficiencies in the study).

___I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	RfD
Developmental (decrease in fetal testosterone)	NOAEL: 30 mg/kg-day	100	Acute 0.3 mg/kg-day Short term 0.3 mg/kg-day Subchronic 0.3 mg/kg-day
	LOAEL: 50 mg/kg-day		Chronic 0.3 mg/kg-day

Rat developmental oral gavage study
Lehmann et al., 2004

The same study and uncertainty factor are used to derive the reference values for all durations of exposure. See Section I.A.2 of this summary and Section 5.1 of the Toxicological Review of Dibutyl Phthalate.

*Conversion Factors and Assumptions – Animals were exposed by gavage in corn oil on gestational days 12 to 19.

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES

Lehmann et al. (2004) investigated the exposure-response relationships for the effect of dibutyl phthalate on steroidogenesis in fetal rat testes. Pregnant Sprague-Dawley rats (n = 7 in control and 5 in each exposed group) were treated with dibutyl phthalate by gavage in corn oil at 0, 0.1, 1.0, 10, 50, 100, or 500 mg/kg-day from gestational day 12 to 19. This phase of the study measured gene expression and protein synthesis. Separate groups of animals (n = 7) were treated with dibutyl phthalate by gavage in corn oil at 0, 0.1, 1.0, 10, 30, 50, 100, or 500 mg/kg-day on gestational days (GDs) 12–19 exposure to determine fetal testosterone concentration. Testes were isolated on GD 19 and changes in gene and protein expression were quantified by reverse

transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis. For determination of mRNA gene expression, total RNA was isolated from the testes of five individual fetuses representing four to five litters per treatment group. Rat specific primers and probes were used for the genes of interest. For Western blot analysis, whole testes from four individual fetuses per treatment group were solubilized. The samples were heated at 95 °C and equal concentrations of protein were added to each lane of a sodium dodecyl sulfate-polyacrylamide minigel. After electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes and quantified using specific primary antibodies. Fetal testicular testosterone concentration was determined from three to four individual fetuses from one to four litters per exposure group. Testes were homogenized and extracted with ethyl acetate and chloroform (4:1). After drying, the extract was dissolved in methanol and testosterone was quantified with a radioimmunoassay.

Exposure to dibutyl phthalate at 50 mg/kg-day and above resulted in significant reductions in mRNA and protein concentration for steps involved in cholesterol transport and synthesis of testosterone including scavenger receptor-1 (SR-B1), steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage ($P450_{SCC}$), and 3β-hydroxysteroid dehydrogenase (3β-HSD). mRNA expression and protein synthesis of insulin-like growth factor 3 (Insl3) was reduced at 500 mg/kg-day. Reductions in Insl3 result in cryptorchidism in rats. There was a statistically significant decrease ($p<0.05$) in mean testosterone concentration per testis at 50 mg/kg-day and above, but not at 30 mg/kg-day. These data are reported in Appendix C of the Toxicological Review of Dibutyl Phthalate. In this study, the NOAEL for the decrease in testosterone concentration is 30 mg/kg-day and the LOAEL is 50 mg/kg-day.

Thompson et al. (2004a) investigated the time course of onset and reversibility of the effects of dibutyl phthalate on the fetal testis and on cholesterol transport and steroidogenesis. Pregnant Sprague-Dawley rats (n = 4–5 per group) received dibutyl phthalate by gavage in corn oil at 0 or 500 mg/kg-day on GDs 12–17 with sacrifice on GDs 17–19; on GDs 12–18 with sacrifice on GD 18–19; or on GDs 12–19 with sacrifice on GD 19. Testes were removed for testosterone, mRNA, and protein analysis. Significant decreases in testosterone production and mRNA expression of SR-B1, $P450_{SCC}$, StAR, and P450c17 were observed as early as GD 17. Testosterone, mRNA, and protein levels remained low 24 hours after exposure but increased 48 hours after exposure. The concentration of testosterone relative to age-matched control was 46.6% on GD 17 and 17.8% on GD 18. The mean expression of the four genes relative to age-matched control was 46.4% on GD 17 and 15.4% on GD 18.

In another experiment Thompson et al. (2004a) treated pregnant dams with dibutyl phthalate by gavage in corn oil at 0 or 500 mg/kg-day beginning at GD 12 with sacrifice on GD 19. The start of exposure was shifted from GD 12 to one day later in gestation for each treatment group. The final group was treated only on GD 19. Testicular testosterone was measured in four fetuses, each from a separate litter. Significant decreases in fetal testicular testosterone, mRNA expression, and protein expression were evident in each exposure group. In groups treated on GD12 - 19, GD 13 - 19, GD 14 - 19, GD 15 - 19, GD 16 - 19, GD 17 - 19, or GD 18 - 19, the concentration of fetal testicular testosterone was decreased an average of 87%. In groups treated only on GD 19 and sacrificed 3 hours after exposure, the concentration of fetal testicular testosterone was decreased 56%. In a testis explant system, dibutyl phthalate caused diminished transport of cholesterol across the mitochondrial membrane and diminished function

at each point in the testosterone biosynthetic pathway, except for the step catalyzed by 17β-HSD. The transcriptional repression caused by dibutyl phthalate was not mediated by interference with SF-1.

Thompson et al. (2005) studied the precise timing of dibutyl phthalate-associated changes in testosterone concentration and gene expression in the fetal testis and corticosterone and gene expression in the fetal adrenal gland. For the study in the fetal testis, pregnant Sprague-Dawley rats were treated with 0 or 500 mg/kg dibutyl phthalate by gavage in corn oil at 0.5, 1, 2, 3, 6, 12, 18, or 24 hrs before sacrifice on GD 19. Testosterone concentration was measured by radioimmunoassay in fetal testes (4 litters, 3 fetuses per litter). Gene and protein expression was also measured (4-5 fetuses per group with each fetus taken from a separate litter). Testicular testosterone was decreased within one hour of exposure to dibutyl phthalate and preceded the repressed transciption of StAR (steroid acute regulatory protein), Scarb1 (scavenger receptor class B, member 1; also known as Sr-b1), Cyp11a1 (cytochrome P450 family 11, subfamily a, polypeptide 1; also known as $P450_{SCC}$), and Cyp17a1 (cytochrome P450 family 17, subfamily a, polypeptide 1; also known as CYP17). StAR mRNA was signficantly diminished 2 hours after exposure to DBP, but Cyp11a1, Cyp17a1, and Scarb1 did not show a significant decrease in expression until 6 hours after exposure to dibutyl phthalate. The decrease in testicular testosterone averaged 43% in animals sacrificed 1 to 6 hours after exposure. The decrease in testicular testosterone averaged 77% in animals sacrificed 12, 18, or 24 after exposure. For the study in the fetal adrenal gland, pregnant Sprague-Dawley rats were treated with 0 or 500 mg/kg dibutyl phthalate by gavage in corn oil daily from GD 12 to 19 and sacrificed on GD 19. Total corticosterone was measured by radioimmunoassay in fetal adrenal glands (4 litters, 2 fetuses per litter). Protein expression for StAR, Scarb1, and Cyp11a1 was also measured in the fetal adrenal gland (4-5 fetuses per group with each fetus taken from a separate litter). The decrease in corticosterone production in the fetal adrenal (approximately 45% decrease) was not statistically significant. In addition the expression of Star, Scarb1, and Cyp11a1 in the adrenal was unaffected by dibutyl phthalate. Together these studies demonstrate that dibutyl phthalate initiates a rapid and dynamic change in gene expression in the fetal testis that likely plays a role in the reduction in steroidogenesis that is unique to the fetal testis relative to the steroidogenically active fetal adrenal.

Mylchreest et al. (2000) conducted a study to establish a NOAEL for developmental toxicity. Pregnant Sprague-Dawley CD rats were given dibutyl phthalate by gavage in corn oil at 0, 0.5, 5, 50, or 100 mg/kg-day (n = 19–20 per group) or 500 mg/kg-day (n = 11) on GDs 12–21. In male offspring anogenital distance (AGD) was decreased at 500 mg/kg-day. A statistically significant increase ($p<0.05$, using a nested analysis) in retained areolas or nipples on post natal day (PND) 14 was present in 31 and 90% of male pups at 100 and 500 mg/kg-day, respectively (80 and 100% of litters affected, respectively). The individual litter data are presented in Appendix C of the Toxicological Review of Dibutyl Phthalate. At 500 mg/kg-day, male pups had nipple buds similar to those of females. The areolas and nipples in the thoracic area were the most prominent. Nipple development was more rudimentary at 100 mg/kg-day but was still clearly different from control males. The nipple bud was rarely visible, but a dark spot on the skin was apparent at the position of the nipple, also mainly in the thoracic area. In the control group, only one male pup had nipple buds; others had a discoloration of the skin in the nipple region, but it was faint and did not show a predilection for the thoracic area. In contrast to

Mylchreest et al. (1999), preputial separation was not delayed at any exposure in males with normal external genitalia. Hypospadias was observed in 5/58 rats (4/11 litters) at 500 mg/kg-day. Absent or partially developed epididymis (23/58 rats in 9/11 litters), vas deferens (16/58 animals in 9/11 litters), seminal vesicles (4/58 rats in 4/11 litters), and ventral prostate (1/58 animals) occurred at 500 mg/kg-day. In 110-day-old males at 500 mg/kg-day, the weights of the testes, epididymis, dorsolateral and ventral prostates, seminal vesicles, and levator ani-bulbocavernosus muscle were decreased. At 500 mg/kg-day, widespread seminiferous tubule degeneration was seen in 25/58 rats (in 9/11 litters), focal Leydig cell hyperplasia in 14/58 rats (in 5/11 litters), and Leydig cell adenoma in 1/58 rats (in 1/11 litters). The NOAEL is 50 mg/kg-day, and the LOAEL is 100 mg/kg-day.

NTP (1995) conducted a continuous breeding study in Sprague-Dawley rats. These data are also reported in Wine et al. (1997) and NTP (1991). Animals (n = 40 in the control group and 20 in each exposure group) received 0, 1000, 5000, or 10,000 ppm dibutyl phthalate in feed (equivalent to 0, 52, 256, or 509 mg/kg-day in males and 0, 80, 385, or 794 mg/kg-day in females) during a 16-week mating period. Selected data are presented in Appendix D of the Toxicological Review of Dibutyl Phthalate. Mean body weights of exposed dams at delivery and during lactation generally decreased with increasing exposure concentration. Mating, pregnancy, and fertility indices of F_1 rats were lower in the 10,000 ppm group than in the controls. Germinal epithelial degeneration of the testes and absence or under development of the epididymides were noted in F_1 males in the 10,000-ppm group. Interstitial cell hyperplasia was noted in 7 of 10 males in the 10,000 ppm-group. There was a decrease in mean live pups per litter in F_1 and a decrease in adjusted mean live pup weight in F_2 at the lowest exposure (see Appendix D). Based on the results of the series of studies by Ema et al. (2000b, 1998, 1994, 1993) showing postimplantation loss and a decrease in fetal body weight and the crossover trial in NTP (1995) showing a decrease in fetal body weight when only pregnant females were exposed to dibutyl phthalate, these effects are most likely due to effects in the female, rather the male. Therefore, the LOAEL is 80 mg/kg-day (1000 ppm) in female rats.

Duty et al. (2003a) recruited 168 men who were part of subfertile couples and who presented to the Massachusetts General Hospital andrology laboratory for semen analysis between January 2000 and April 2001. This was a cross sectional study in which semen and urine samples were collected from each subject on the same day as part of an infertility work-up. Semen parameters were categorized based on 1999 World Health Organization reference values for sperm concentration (<20 million/ml) and motility (<50% motile), as well as Tygerberg strict criteria for morphology (<4% normal). The comparison group was men for whom these semen parameters were all above the reference values. The concentration of eight phthalate monoesters was measured in a single spot urine sample collected on the same day as the semen sample with high-performance liquid chromatography and tandem mass spectrometry. Exposure to chemicals other than phthalate esters was not evaluated. Specific gravity-adjusted phthalate monoester levels were subdivided into tertiles (0–11.64, 12.24–20.13, and 20.16–433.93 ng/ml). There was a statistically significant relationship between tertiles of monobutyl phthalate and decreased sperm motility (odds ratio per tertile = 1.0, 1.8 [95% CI 0.7–4.6], 3.0 [95% CI 1.2–7.6]; *p*-value for trend = 0.02; n = 15, 21, or 27 in each tertile, respectively) but not for decreased sperm concentration (1.0, 1.4 [95% CI 0.3–6.0], 3.3 [95% CI 0.9–12.6]; *p*-value for trend = 0.07; n = 6, 6, or 10 for each tertile, respectively). There was also a statistically significant relationship for

monobenzyl phthalate with sperm concentration. The authors pointed out that a strength of the study included a reliable biomarker of exposure (phthalate monoesters in urine) rather than self-reported exposures, but a weakness in that the phthalate levels were based on a single spot urine sample from a limited number of subjects. The authors further cautioned that the results were also based on a single sperm analysis and that until the results are replicated in larger and more diverse populations, the wider applicability and consistency of the results remains unclear.

Duty et al. (2003b) further analyzed the integrity of the DNA in the sperm samples using the neutral single-cell microgel electrophoresis assay (comet assay). The concentration of monobutyl phthalate in the semen sample was not significantly associated with DNA fragmentation patterns as observed in the comet assay.

Duty et al. (2004) explored whether phthalates were associated with altered sperm movement characteristics. Two-hundred twenty subjects (male partners of a subfertile couple between 20 and 54 years of age and who presented to an andrology laboratory between January 2000 and October 2001) provided a semen sample for computer-aided sperm analysis (CASA) and a urine sample for measurement of phthalate monoesters (monoethyl phthalate, monobenzyl phthalate, monobutyl phthalate, mono-2-ethylhexyl phthalate, and monomethyl phthalate). Only data for monobutyl phthalate are reported here. Three CASA parameters, straight-line velocity (VSL), curvilinear velocity (VCL), and linearity (LIN), were used as measures of sperm progression, sperm vigor, and swimming pattern, respectively. As stated by the authors, there was a "suggestive negative" dose-response relationship (shown as the predicted change in mean sperm motion parameter for the second and third tertiles compared with the first tertile; p value for trend) for MBP and VSL (-3.07 µm/s, -2.87 µm/s; p = 0.08) and VCL (-3.25 µm/s, -3.46 µm/s; p = 0.2).

Swan et al. (2005) investigated AGD and other genital measurements in male infants and prenatal exposure to phthalates. A standardized measure of AGD was obtained from boys 2-30 months of age (n = 134). AGD was significantly correlated with penile volume ($r^2 = 0.24$, p=0.005) and the proportion of boys with incomplete testicular descent ($r^2 = 0.23$, p=0.007). The researchers defined the anogenital index (AGI) as the AGD divided by body weight (kg) at examination and calculated the age-adjusted AGI by regression analysis. Nine phthalate monoester metabolites were measured in a single sample of the mother's prenatal urine (n = 85) and were examined as predictors of age-adjusted AGI in regression and categorical analyses. An individual mother's urine sample was matched with the age-adjusted AGI for the individual boy. Exposure to other chemicals was not investigated. The urinary phthalate monoesters that were inversely related to age-adjusted AGI included monoethyl phthalate (p=0.012), monoisobutyl phthalate (p=0.014), monobutyl phthalate (p=0.023), and monobenzyl phthalate (p=0.05). Odds ratios were calculated for shorter than expected age-adjusted AGI with the concentration of phthalate monoester in the mother's urine. The exposure groups were low (<25th percentile), medium (≥25th to <75th percentile), and high (≥75th percentile). The reported odds ratios for monoethyl phthalate were 1.0, 2.6 [95% CI, 0.9–7.8], and 4.7 [95% CI, 1.2–7.4]; for monoisobutyl phthalate were 1.0, 2.5 [95% CI, 0.8–7.4], and 7.3 [95% CI, 1.9–27.9]; for monobutyl phthalate were 1.0, 3.8 [95% CI, 1.2–12.3], and 10.2 [95% CI, 2.5–42.2]; and for monobenzyl phthalate were 1.0, 3.1 [95% CI, 1.001–9.8], and 3.8 [95% CI, 1.03–13.9]. The associations between male genital development and phthalate exposure are consistent with the

effects in prenatal rodents following oral exposure to monobutyl phthalate. The concentration of monobutyl phthalate in prenatal urine associated with short AGI in this study (50th percentile = 22.3 μg/L and 75th percentile = 47.3 μg/L) are comparable to the concentration of monobutyl phthalate reported for women in the National Health and Nutrition Examination Survey (50th percentile = 30.0 μg/L and 75th percentile = 59.5 μg/L) (Silva et al., 2004; DHHS, 2003). These data support the hypothesis that prenatal exposure to phthalates at environmental levels can adversely affect male reproductive development in humans.

The associations between male genital development and phthalate exposure reported by Swan et al. (2005) are consistent with the effects found by others in prenatal rodents following oral exposure to dibutyl phthalate and monobutyl phthalate (see data reported in Section 4.3.1 and Section 4.3.2 following) and support the hypothesis that prenatal exposure to phthalates at environmental levels can affect male reproductive development in humans. However, as pointed out by the authors, Swan et al. (2005) has a number of limitations. The analysis is based on a single measurement of AGD in boys and a single measurement of phthalate esters in maternal urine. Although the AGD is a standard measure in the rodent developmental bioassay, the reliability of this measurement in humans has not been established. Use of the measurement of AGD in larger studies in a range of diverse human populations will be needed to obtain normative data. The measurement of AGD in this study was not conducted in infants of the same age as is done in the standard rodent developmental bioassay. The optimal timing for measurement of AGD in boys has not been established. In an attempt to provide a standard measure for comparison in boys of different ages, the measured AGD was normalized to body weight to give the AGI. The reliability of the AGI has not been established for humans. The maternal urine samples were collected late in pregnancy (mean = 28.3 weeks) and the measured phthalate metabolite levels may not reflect exposure during the most sensitive developmental window in the male fetus. Finally, the mothers were exposed to multiple phthalates at detectable levels.

Additional limitations on Swan et al. (2005) were discussed in a commentary by Sharpe (2005). Sharpe points out that Swan et al. (2005) show an association between maternal exposure to phthalates and AGI in boys, but they do not show that one caused the other or that exposure to phthalates caused reduced production of testosterone. They also do not show that exposure to phthalates caused abnormalities as all the boys were “normal” at the time of examination. Sharp (2005) also points out that the findings need to be confirmed independently as the association with AGI with maternal exposure to phthalates could be fortuitous. There could be other lifestyle factors that cause a woman to be exposed to phthalates that might themselves cause the reduction in AGI or reduced testosterone in the fetus.

Main et al. (2006) investigated whether phthalate monoester contamination of human breast milk had any influence on the postnatal surge of reproductive hormones in newborn boys as a sign of testicular dysgenesis. Main et al. obtained biologic samples from a prospective Danish-Finnish cohort study on cryptorchidism from 1997 to 2001. They analyzed individual breast milk samples collected as additive aliquots postnatally (n = 130; 62 cryptorchid and 68 healthy boys) for phthalate monoesters [mono-methyl phthalate, mono-ethyl phthalate, mono-n-butyl phthalate, mono-benzyl phthalate, mono-2-ethylhexyl phthalate, and mono-isononyl phthalate]. They analyzed serum samples (obtained in 74% of all boys) for gonadotropins, sex-

hormone binding globulin, testosterone, and inhibin B. All phthalate monoesters were found in breast milk with large variations. The median, minimum, and maximum values for monobutyl phthalate were 9.6, 0.6, and 10,900 μg/L, respectively. Values for the other phthalate monoesters are not reported here. No association was found between phthalate monoester levels and cryptorchidism. However, mono-ethyl phthalate and monobutyl phthalate showed positive correlations with sex-hormone binding globulin ($r = 0.323$, $p = 0.002$ and $r = 0.272$, $p = 0.01$, respectively); mono-methyl phthalate, mono-ethyl phthalate, and monobutyl phthalate with the luteining hormone:free testosterone ratio ($r = 0.21$-0.323, $p = 0.002$-0.044); and mono-isononyl phthalate with luteinizing hormone ($r = 0.243$, $p = 0.019$). Monobutyl phthalate was negatively correlated with free testosterone ($r = -0.22$, $p = 0.033$). Other phthalate monoesters showed similar but non-significant tendencies. These data show concordance with rodent data and suggest human Leydig cell development and function may also be vulnerable to perinatal exposure to some phthalates.

The effects on testosterone concentration in the fetal testes (Lehmann et al., 2004) and the increase in retained areolas or nipples in male pups (Mylchreest et al., 2000) occur at the lowest exposure. The increase in retained areolas or nipples is considered an adverse effect and is irreversible. The NOAEL and LOAEL in this study are 50 and 100 mg/kg-day, respectively. In Lehmann et al. (2004) there was a statistically significant decrease in fetal testosterone concentration at 50 mg/kg-day, but not at 30 mg/kg-day. Although the decrease in testosterone in the fetal testis is reversible and returns to normal levels after the metabolites of dibutyl phthalate are cleared from the circulation, this biochemical change during the critical developmental window may initiate the cascade of irreversible malformations in the male reproductive tract as described in Section 4.5.2. Therefore, EPA considers the decrease in testosterone concentration an adverse effect in this study. Accordingly, the critical effect for chronic exposure to dibutyl phthalate is developmental toxicity (decreased testosterone in the fetal testes) and the principal study is Lehmann et al. (2004) with a NOAEL of 30 mg/kg-day and a LOAEL of 50 mg/kg-day. In this study exposure was on GDs 12 –19 and there was a 61% decrease in testosterone in the fetal testis at the LOAEL. Other studies (Thompson et al., 2005, 2004a) showed a comparable decrease in fetal testosterone (43 - 77%) following a single exposure to dibutyl phthalate at 500 mg/kg-day on GD 19. It is not known whether the effects seen following repeated (8 days) administration of 50 mg/kg-day dibutyl phthalate would be evident after a single exposure. However, it is a plausible assumption for developmental toxic effects that "a single exposure at a critical time in development may produce an adverse developmental effect, i.e., repeated exposure is not a necessary prerequisite for developmental toxicity to be manifested" as discussed in U.S. EPA's 1991 Guidelines for Development Toxicity Risk Assessment. Therefore, EPA concluded that a single exposure to 50 mg/kg-day dibutyl phthalate during the critical developmental window may initiate the cascade of malformations in the male reproductive tract. Therefore, the Lehmann et al. (2004) study is applicable to the derivation of both the acute and short-term reference values. The effects on testosterone levels observed by Lehmann et al. (2004) also occur at doses that are lower than those observed in the available subchronic studies. Thus, Lehmann et al. (2004) was selected for the derivation of the subchronic and chronic reference values. Using the decrease in testosterone concentration in the fetal testes (Lehmann et al., 2004) as the critical effect to derive the reference value for all durations of exposure will likely protect children and adults from the other effects of dibutyl phthalate that require a higher exposure.

___I.A.3. UNCERTAINTY FACTORS

UF = 100 for reference values for all durations of exposure

Interspecies. A ten-fold uncertainty factor is used for interspecies extrapolation as the current data are not sufficiently robust to depart from the default value.

Intraspecies. A ten-fold uncertainty factor is used for intraspecies extrapolation as there are no data to justify a departure from the default value.

LOAEL to NOAEL. A factor for extrapolation from a LOAEL to NOAEL was not used as the principal study established a NOAEL for the critical effect.

Subchronic to Chronic. A factor for extrapolation for duration of exposure was not used. Consistent with EPA practice (U.S EPA, 1991), an uncertainty factor was not used to account for the extrapolation from less than chronic exposure because developmental toxicity resulting from a narrow period of exposure was used as the critical effect. The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure. In addition, the developmental effect occurs at an exposure lower than the exposure required for effects in other target organ (liver, testes, nervous system) following subchronic exposure.

Data Base. A factor for data base uncertainty was not used. Although the data base lacks focused studies on developmental neurotoxicity, immunotoxicity studies, chronic studies, and cancer bioassays, an uncertainty factor for data base deficiencies is not considered necessary. Neither developmental neurotoxicity nor immunotoxicity are likely to be critical effects for dibutyl phthalate. There is considerable evidence that chemicals that cause peroxisome proliferation in rodent are likely to cause liver tumors in rodents at an exposure similar to that causing peroxisome proliferation (Klaunig et al., 2003). Dibutyl phthalate causes effects in the rodent liver when exposure is 279 mg/day. These liver effects are correlated with markers for peroxisome proliferation and they occur at an exposure much greater than the exposure causing testicular effects in the male fetus. Accordingly, the lack of chronic studies and cancer bioassays for dibutyl phthalate is not considered a data base deficiency.

___I.A.4. ADDITIONAL STUDIES/COMMENTS

As documented in the Toxicological Review of Dibutyl Phthalate, there is a large number of supporting studies. The following studies are included in the IRIS Summary as they document exposure-response levels for toxicity to the liver, testis, and nervous system in laboratory animals.

Srivastava et al. (1990) administered dibutyl phthalate to young male Wistar rats (5 weeks old, n = 6 in each group) by gavage in peanut oil at 0, 250, 500, or 1000 mg/kg-day for 15 days. A significant decrease in testis weight was observed at 500 and 1000 mg/kg-day (64 and 48%, respectively). Histopathological examination revealed degeneration of seminiferous tubules at all exposures (50, 20, and 70% at the low, mid, and high exposure, respectively). The

activities of testicular enzymes associated with postmeiotic spermatogenic cells, such as sorbitol dehydrogenase and acid phosphatase, were decreased significantly at 500 and 1000 mg/kg-day (17 and 26%, respectively, for sorbitol dehydrogenase and 25 and 36%, respectively, for acid phosphatase), while that of lactate dehydrogenase was significantly increased at all exposures (16, 25, and 48%, respectively). The activities of enzymes associated with premeiotic spermatogenic cells, Sertoli cells or interstitial cells, β-glucuronidase, γ-glutamyl transpeptidase, and glucose-6-phosphate dehydrogenase were significantly increased at all exposures (β-glucuronidase 22, 39, and 74%, respectively; γ-glutamyl transpeptidase 26, 40, and 67%, respectively; glucose-6-phosphate dehydrogenase 32, 39, and 52%, respectively). The LOAEL is 250 mg/kg-day.

NTP (1995) conducted a 13-week evaluation of the toxicity of dibutyl phthalate in male and female F344 rats. Rats (n = 10 of each sex in each group) received dibutyl phthalate in the diet at 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm (equivalent to 0, 176, 359, 720, 1540, or 2964 mg/kg-day in males and 0, 177, 356, 712, 1413, or 2943 in females). No deaths occurred. Markedly reduced final mean body weights were observed in males and females in the 40,000 ppm groups (a decrease of 45 and 73%, respectively). An increase in relative liver weight was observed in males that received 5000 ppm or greater (an increase of 18, 32, 54, and 70%, respectively) and in females that received 10,000 ppm or greater (an increase of 11, 25, and 78%, respectively). Testis and epididymal weights of males in the 20,000- and 40,000-ppm groups were lower than those of the controls. Hypocholesterolemia was observed in male and female rats receiving 20,000 or 40,000 ppm, and hypotriglyceridemia was detected in males in all exposed groups and in females receiving 10,000 ppm or greater. Elevations in alkaline phosphatase activity and bile acid concentration in male and female rats were considered indicative of cholestasis.

Morphologic evaluation confirmed the toxicity of dibutyl phthalate to the liver and testis of rats. Microscopic examination of the liver revealed hepatocellular cytoplasmic alterations consistent with glycogen depletion in male and female rats receiving 10,000 ppm or greater. In the liver of rats in the 40,000-ppm groups, small, fine eosinophilic, granules were also observed in the cytoplasm of hepatocytes. Ultrastructural examination suggested the presence of increased numbers of peroxisomes, and peroxisomal enzyme activity (palmitoyl-CoA oxidase activity) was elevated in the livers of rats administered 5000 ppm or greater. In males increases of 1.9-, 5.7-, 9.7-, and 13.5-fold, respectively were observed; in females increases of 1.7-, 2.6-, 11-, and 32.5-fold, respectively, were observed. Lipofuscin accumulation was detected in rats receiving 10,000 ppm or greater.

Histopathologic examination of the testes revealed degeneration of the germinal epithelium. There was a mild to marked focal lesion in the 10,000- and 20,000-ppm groups and a marked diffuse lesion in all males in the 40,000 ppm group resulting in almost complete loss of the germinal epithelium at 40,000 ppm. Testicular zinc concentrations were lower in the 20,000- and 40,000-ppm groups than in the controls. Serum testosterone values were also lower at these concentrations than in the controls. Spermatogenesis was evaluated in males in the 0-, 2500-, 10,000-, and 20,000-ppm groups. At 20,000 ppm, spermatid heads per testis and per gram testis, epididymal spermatozoal motility, and the number of epididymal spermatozoa per gram epididymis were lower than in the controls. All of these findings are consistent with the marked

loss of germinal epithelium at this exposure. The NOAEL for effects in the testis is 359 mg/kg-day (5000 ppm), and the LOAEL is 720 mg/kg-day (10,000 ppm). The NOAEL for effects in the liver is 176 mg/kg-day (2500 ppm), and the LOAEL is 359 mg/kg-day (5000 ppm).

BASF (1992, as summarized in NTP-CERHR, 2000) conducted a 3 month study in male and female Wistar rats. Rats (10 of each sex per dose, 6 weeks old) received dibutyl phthalate in the diet at 0, 400, 2000, or 10,000 ppm (equivalent to 0, 27, 142, or 688 mg/kg-day in males and 0, 33, 162, or 816 mg/kg-day in females). A battery of hematological, clinical chemical, and urinalysis tests were conducted after approximately 45 days of exposure and at the end of the study. Cyanide insensitive palmitoyl-CoA oxidation was also determined as a measure of peroxisome proliferation. Neurological function, using the EPA functional observation battery, was assessed prior to exposure and on days 34, 59, and 90. Testes were examined for histological changes after fixing in Bouin's solution. No effects were observed on body weight, neurological function, or the testis at any exposure. There was a statistically significant increase in relative liver and kidney weights in females and an increase in palmitoyl-CoA oxidation in both males and females at the highest exposure. However, quantitative data for these effects are not reported in NTP-CERHR (2000). The NOAEL for neurological and testicular effects is 688 mg/kg-day, the highest exposure tested. The NOAEL for effects in the liver is 142 mg/kg-day and the LOAEL is 688 mg/kg-day.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study -- high
Data Base -- medium
RfD -- high

Dibutyl phthalate has been reviewed by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2003, 2000; Kavlock et al., 2002). Limited epidemiological studies in humans have shown an association between some sperm parameters (Duty et al., 2003a), a decreased AGI (Swan et al., 2005), and decrease in testosterone in infants (Main et al., 2006) and environmental exposure to phthalate monoesters. There is high confidence in the conclusion that dibutyl phthalate is a reproductive and developmental toxicant in a number of laboratory animal species and would present a reproductive and developmental hazard to humans. The exposure-response relationships for these effects are adequately documented in laboratory animals and were used to calculate benchmark doses for the effects. The principal study for the derivation for all duration RfDs is Lehmann et al. (2004). The critical effect is the decreased testosterone concentration in the fetus (developmental toxicity) with a NOAEL of 30 mg/kg-day and a LOAEL of 50 mg/kg-day. This biochemical effect is the key step that leads to the cascade of malformations in the developing male reproductive tract and is considered an adverse effect in this study. Using this effect as the basis for the RfDs will protect from the other effects of dibutyl phthalate in the testes and liver that require a higher exposure. The data base merits a medium to high confidence rating. The data base is missing chronic bioassays and studies focusing on immunotoxicity and developmental neurotoxicity. However it is unlikely that either developmental neurotoxicity or immunotoxicity would be a critical effect. It is not likely that a chronic study in adults would result in effects at an exposure lower than that which disrupts the testosterone concentration in

the fetal testes. Dibutyl phthalate is a peroxisomal proliferator in rodents. If chronic bioassays were conducted, any liver tumors would likely occur at an exposure greatly in excess of the exposure known to cause the critical effect for developmental toxicity. Accordingly, the RfDs merit a high confidence rating.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- Toxicological Review of Dibutyl Phthalate.

This assessment was peer reviewed by a group of external scientists. Comments from the peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. A record of these comments is included in Appendix A of the Toxicological Review of Di-n-Butyl Phthalate (U.S. EPA, 2006).

Agency Completion Date -- __/__/__ *[note: leave this BLANK until completion is reached*]

___I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

__I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Dibutyl Phthalate
CASRN – 84-74-2
Section I.B. Last Revised -- 00/00/0000

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a

summary of that evaluation will be contained in Section II of this file.

__I.B.1. INHALATION RfC SUMMARY

No data are available to derive a Reference Concentration.

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Dibutyl Phthalate
CASRN – 84-74-2
Section II Last Revised -- 00/00/0000

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is an upper bound on the estimate of risk per unit of concentration, either per μg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

No data on carcinogenicity are available. Consequently, under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is inadequate information to assess carcinogenic potential.

_III. [reserved]
_IV. [reserved]
_V. [reserved]

_VI. BIBLIOGRAPHY

Dibutyl Phthalate
CASRN -- 84-74-2
Section VI. Last Revised -- 00/00/0000

__VI.A. ORAL RfD REFERENCES

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__VI.B. INHALATION RfC REFERENCES

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__VI.C. CARCINOGENICITY ASSESSMENT REFERENCES

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_VII. REVISION HISTORY

Dibutyl Phthalate
CASRN – 84-74-2
File First On-Line 01/31/1987

Date	Section	Description
09/07/1988	II.	Carcinogen summary on-line
08/01/1989	VI.	Bibliography on -line
03/01/1990	I.A.4.	Text corrected
05/01/1990	II.A.4.	First sentence revised
08/01/1990	I.A.	Oral RfD summary noted as pending change
09/01/1990	I..B.	Not verified; data inadequate
09/01/1990	IV.F.1.	EPA contact changed
10/01/1990	I..B.	Inhalation RfC message on-line
10/01/1990	VI..B.	Inhalation RfC references added
08/01/1991	II.D.3.	Primary and secondary contacts changed
01/01/1992	I.A.7.	Secondary contact changed
01/01/1992	IV.	Regulatory actions updated
02/01/1993	II.D.3.	Primary contact changed
08/01/1995	I.A.	EPA's RfD/RfC and CRAVE workgroups were discontinued in May, 1995. Chemical substance reviews that were not completed by September 1995 were taken out of IRIS review. The IRIS Pilot Program replaced the workgroup functions beginning in September, 1995.
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA Regulatory Actions, and Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
01/09/2002	I., II.	This chemical is being reassessed under the IRIS Program.
12/03/2002	I.A.6., I.B., II.D.2.	Screening-Level Literature Review Findings message has been added.

_VIII. SYNONYMS

Dibutyl Phthalate
CASRN – 84-74-2
Section VIII Last Revised -- 00/00/0000

84-74-2
1,2-Benzenedicarboxylic Acid Dibutyl Ester
o-Benzenedicarboxylic Acid, Dibutyl Ester
Benzene-o-Dicarboxylic Acid Di-n-Butyl Ester
Butylphthalate

Celluflex DPB
Dibutyl 1,2-Benzene dicarboxylate
Dibutyl phthalate
Di-n-Butylphthalate
Dibutyl-o-Phthalate
DPB
Elaol
Ergoplast FDB
Genoplast B
Hexaplast M/B
N-Butylphthalate
Palatinol C
Phthalic Acid Dibutyl Ester
Polycizer DBP
PX 104
RC Plasticizer DBP