**CHEMICALS UNDER CONSIDERATION FOR POSSIBLE LISTING VIA THE AUTHORITATIVE BODIES MECHANISM**

**PACKAGE 21**
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Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

The chemicals listed in the Table below meet the criteria for listing under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code section 25249.5 et seq.), more commonly known as Proposition 65, via the authoritative bodies listing mechanism as known to the State to cause reproductive toxicity. The regulatory requirements for listing by this mechanism are set forth in Title 22, California Code of Regulations §12306[1]. For example, the regulations include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body the Office of Environmental Health Hazard Assessment (OEHHA) uses to determine whether listing under Proposition 65 is required.

The National Toxicology Program solely as to final reports of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) is one of five institutions which have been identified as authoritative bodies for identification of chemicals as causing reproductive toxicity for the purposes of Proposition 65 (§12306(1)(3)). This body has identified the chemicals in the Table below as causing reproductive toxicity. OEHHA has found that these chemicals appear to be "formally identified" as causing reproductive toxicity as required by §12306(d). The chemicals below are the subjects of reports published by the authoritative body which conclude that the chemicals cause reproductive toxicity. Also, the documents specifically and accurately identify the chemicals and the documents meet one or more of the criteria required by §12306(d)(2).

OEHHA also finds that the criteria in regulation for "as causing reproductive toxicity" (§12306(g)) have been satisfied for the chemicals in the Table below. In making this evaluation, OEHHA relied upon the discussion of data by the authoritative body in making its finding that the specified chemicals cause reproductive toxicity. A brief discussion of the relevant reproductive and developmental toxicity studies providing evidence for the findings is presented below. Much of the discussion is taken verbatim from the NTP-CERHR (2003a, b, c, d) reports. The statements in bold reflect data and conclusions that satisfy the criteria for the sufficiency of evidence for reproductive toxicity (§12306(g)). The full citations for the authoritative body documents are given in this report.

[1] All further references are to Title 22 of the California Code of Regulations unless otherwise indicated.

## Chemicals Under Consideration for Listing as Known to the State to Cause Reproductive Toxicity

| Chemical | CAS No. | Toxicological Endpoints | Chemical Use | Reference |
|---|---|---|---|---|
| Butyl benzyl phthalate (BBP) | 85-68-7 | developmental toxicity | Plasticizer in plastics used primarily in vinyl tiles, also in food conveyer belts, artificial leather, automotive trim and traffic cones. | NTP-CERHR (2003a) |
| Di-*n*-butyl phthalate (DBP) | 84-74-2 | developmental toxicity<br>female reproductive toxicity<br>male reproductive toxicity | Coalescing aid in latex adhesives, plasticizer in cellulose plastics and solvent for dyes. | NTP-CERHR (2003b) |
| Di-*n*-hexyl phthalate (DnHP) | 84-75-3 | female reproductive toxicity<br>male reproductive toxicity | Used in the making of plastisols that are subsequently used in the manufacture of automobile parts (air filters, battery covers) and dip-molded products (tool handles, dishwasher baskets). Commercial phthalate substances containing DnHP may be added to the polyvinyl chloride (PVC) utilized in the manufacture of flooring, canvas tarps, and notebook covers. Substances containing DnHP may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations. | NTP-CERHR (2003c) |
| Di-isodecyl phthalate (DIDP) | 68515-49-1[1]<br>26761-40-0 | developmental toxicity | Plasticizer in a wide range of PVC plastic products. These include coverings on wires and cables, artificial leather, carpet backing and pool liners. It has only limited use in food packaging or handling. | NTP-CERHR (2003d) |

[1]DIDP is a complex substance, assigned two different CAS Numbers. See NTP-CEHR (2003d) for details.

Butyl Benzyl Phthalate (BBP) (CAS No. 85-68-7).

**Studies in rats and mice have shown that prenatal exposure to high levels of BBP can result in a range of developmental effects that include prenatal mortality, reduced growth, and skeletal, visceral, and external malformations.**

The NTP-CERHR has concluded that there is clear evidence of adverse effects for developmental toxicity in laboratory animals (NTP-CERHR, 2003a). Further, NTP-CERHR states that "Although there is no direct evidence that exposure of people to BBP adversely affects reproduction or development, studies reviewed by the expert panel and subsequently published studies with laboratory rodents show that exposure to BBP can adversely affect development, including development of the male reproductive tract. The NTP believes it is reasonable and prudent to conclude that the results reported in laboratory animals indicate a potential for similar or other adverse effects in human populations if exposures are sufficiently high." (NTP-CERHR, 2003a, p. 2).

The *NTP-CERHR Expert Panel Report on Butyl Benzyl Phthalate* by the NTP-CERHR Phthalates Expert Panel is incorporated into NTP-CERHR (2003a) as Appendix II. The Expert Panel reviewed numerous studies of BBP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003a, Appendix II), is summarized below.

Field *et al*. (1989) conducted a dietary study in CD (Sprague-Dawley) rats involving exposure of 30 pregnant rats per group to 0, 0.5, 1.25, and 2.0% BBP (0, 420, 1,100, and 1,640 mg/kg bw/day) on gestation day (gd) 6-15. The dams were killed on gd 20, necropsied, and pups examined and evaluated.

At 1,640 mg/kg bw/day, there were increased resorptions and concomitant reduced numbers of live fetuses per litter, reduced fetal body weight, and increased fetal malformations. Urogenital malformations, analyzed separately, were increased; they included distended ureters and distended or absent kidneys. Other fetal malformations at the high dose were anophthalmia (missing eyes), fused or malaligned vertebrae, and fused ribs. There were increased incidences of fetal variations per litter at both the 1,100 and 1,640 mg/kg bw/day doses. Significant developmental toxicity occurred at the 1,100 and 1,640 mg/kg bw/day doses; teratogenicity was observed at 1,640 mg/kg bw/day. Maternal toxicity was observed at doses that caused developmental toxicity.

Ema *et al*. (1992) treated 10 Wistar rats/group with BBP by gavage with 0, 500, 750, or 1,000 mg/kg bw/day on gd 7–15. Dams and fetuses were evaluated following sacrifice on gd 20. Maternal body weight gains were reduced at doses of 750 and 1,000 mg/kg bw/day, but the corrected weight gain (maternal body weight excluding the gravid uterus) was decreased only at the high dose. Food intake was reduced at all dose levels. Four dams in the high-dose group died and entire litters were resorbed in the six surviving dams. Complete litter resorptions were observed in 3/10 dams in the 750 mg/kg bw/day group. Other effects at that dose included increased fetal death due to postimplantation loss, reduced fetal weight, and increased external, skeletal, and internal malformations. The malformations

consisted primarily of cleft palate, fused sternebrae, and dilated renal pelves. An earlier dietary study by Ema *et al*. (1990) was without effect.

A developmental toxicity dietary study in CD-1 mice by Price *et al*. (1990) involved exposure of 30 pregnant mice per group to 0, 0.1, 0.5, and 1.25% BBP (0, 182, 910, and 2,330 mg/kg bw/day), on gd 6-15. Embryofetal effects included increased incidences of resorptions and late fetal deaths, with concomitant reductions in live fetuses per litter, and increased malformations (external and skeletal) at 910 and 2,330 mg/kg bw/day. Malformations included exencephaly, short tail, cardiovascular defects, fused ribs, and abnormal or fused sternebrae and vertebrae. Fetal body weight per litter was decreased and fetal variations were increased at the 2,330 mg/kg bw/day dose. Maternal and developmental toxicity was present at the two highest doses.

An oral developmental toxicity study by the Monsanto Company (1978) performed in New Zealand white rabbits was without effect.

Ema *et al.* (1995) compared effects of BBP and DBP administered by gavage to pregnant Wistar rats at 0, 750, 1,000 or 1,250 mg/kg bw/day on gd 7–9, gd 10–12, or gd 13–15. Increased postimplantation loss was observed for both compounds at all doses from all exposure periods. Malformations were observed in groups treated with both phthalate esters at ≥750 mg/kg bw/day on gd 7–9 (vertebral column and ribs) and on gd 13–15 (cleft palate and fused sternebrae). No malformations were observed with either compound at any dose when they were administered on gd 10–12.

Imajima *et al.* (1997) gavaged pregnant Wistar-King A (WKA) rats with monobutyl phthalate (MBuP; a metabolite of BBP) in sesame oil at 0 or 300 mg/day on gd 15–18 (equivalent to approximately 1,000 mg/kg bw/day). Male offspring were evaluated on gd 20 and on postnatal days (pnd) 30–40 to determine the position of the testes. In control males, all testes were located in the lower abdomen on gd 20 (19 pups, 3 litters) and had descended into the scrotum on pnd 30–40 (15 pups, 3 litters). In stark contrast, in males exposed *in utero* to MBuP, on gd 20 all testes were located high in the abdominal cavity (15 pups, 3 litters) with significantly higher testes ascent. On pnd 30–40, MBuP exposed males exhibited cryptorchidism (22/26 pups, 5 litters with uni-or bi-lateral undescended testes); 87% of the undescended testes were in the abdominal cavity, the remaining 13% were located at the external inguinal ring.

Since BBP and DBP share MBuP as a metabolite, a study by Mylchreest *et al*. (2000), in which pregnant rats were orally dosed on gd 12–21 with DBP at 0, 0.5, 5, 50, 100, or 500 mg/kg bw/day, is germane and was discussed in NTP-CERHR (2003a). The male offspring were evaluated until puberty. The maternal NOAEL was 500 mg/kg bw/day. The developmental NOAEL was 50 mg/kg bw/day, based on the presence of retained nipples and areolae in pre-weanling males at 100 mg/kg bw/day and malformations of the male reproductive tract, testicular lesions (Leydig cell hyperplasia and one Leydig cell adenoma),

increased incidence of undescended testes, reduced anogenital distance, and retained nipples and areolae in males at 500 mg/kg bw/day.

Piersma *et al*. (1995) reported the results of a standard general and reproductive toxicity screen, conducted according to the OECD 421 protocol, for BBP. Male and female WU rats (10/sex/group), 10–11 weeks old at the start of exposure, were gavaged for 14 days with BBP in corn oil at dose levels of 0, 250, 500, or 1,000 mg/kg bw/day, and then paired (1:1) and allowed to mate for a maximum of 14 days while dosing continued. Once evidence of mating was observed, the animals were separated. Males continued to be dosed daily, and were then killed and necropsied after a total dosage period of 29 days. Reproductive organs were removed and placed in Bouins fixative. Dosing of females continued until pnd 6, after which the females were killed and necropsied and ovaries and uteri examined. Pups were counted, sexed, weighed, and examined for external malformations on pnd 1 and 6 and then killed. Parental toxicity was observed at the high dose. The numbers of animals achieving a pregnancy were 9, 8, 7, and 4 (of 10) in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively. Postnatal pup mortality did not differ across dose groups, but average litter sizes at birth were 9.4, 11.4, 8.4, and 1.5 in the 0, 250, 500, and 1,000 mg/kg bw/day groups, respectively, with statistical significance achieved at the highest dose. Absolute pup weight was significantly reduced at birth in the high- (29%) and mid- dose (7%) groups. Testicular degeneration accompanied by interstitial cell hyperplasia was significantly increased in the high-dose $F_0$ males. The high-dose group had lower fertility (decreased numbers of litters and decreased numbers of pups per litter) in the F0 generation with marked histopathology in the testes. F1 pup weight was reduced at birth in the mid-and high-dose groups and a developmental NOAEL of 250 mg/kg bw/day was identified. The reproductive NOAEL was identified as 500 mg/kg bw/day.

Di-*n*-butyl Phthalate (DBP) (CAS No. 84-74-2).

**DBP is a testicular toxicant in three species of young adult laboratory animals, elicits malformations of the male rat reproductive tract via a disturbance of the androgen status, is developmentally toxic to both rats and mice by the oral route and causes adult female functional reproductive toxicity (decreases in fertility) in rats.**

The NTP-CERHR concluded that there is clear evidence that DBP causes adverse developmental and reproductive effects in laboratory animals (NTP-CERHR, 2003b). NTP-CERHR (2003b) further stated that, although there is no direct evidence that exposure of people to DBP adversely affects reproduction or development, it is reasonable and prudent to conclude that the results reported in laboratory animals indicate a potential for similar or other adverse effects in humans.

The *NTP-CERHR Expert Panel Report on Di-n-Butyl Phthalate* by the NTP-CERHR Phthalates Expert Panel is incorporated into NTP-CERHR (2003b) as Appendix II. The

Expert Panel reviewed numerous studies of DBP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003b, Appendix II), is summarized below.

Shiota *et al*. (1980) and Shiota and Nishimura (1982) evaluated the effects of oral exposure to DBP in concentrations of 0, 0.05, 0.1, 0.2, 0.4, and 1.0% in the diet in mice. Female ICR-JCL mice ate the DBP diet from the day a cervical plug was observed (gd 0) until they were killed on gd 18. Using food consumption data, the authors calculated mean daily intake of DBP to be 0, 80, 180, 350, 660, and 2,100 mg/kg bw/day. Six-to-nine litters were examined per dose group, except that 15 litters were examined from the highest dose group. Food intake levels were not affected in pregnant dams. Maternal weight gain was significantly reduced at the high dose (2,100 mg/kg bw/day), but the effect might have been secondary to increased fetal loss. Resorptions (prenatal mortality) were significantly increased (98.4%) in the high-dose group. Delayed ossification was observed at all dose levels as indicated by a reduction in the number of ossified coccygia in treated fetuses (n=9.4, 5.1, 4.5, 6.0, and 2.6 in the control to 660 mg/kg bw/day groups). Reduced fetal body weight was observed at the two highest doses.

Ema *et al.* (1993, 1994, 1998) evaluated the developmental toxicity of DBP in Wistar rats by exposure through gavage and feed. In all studies, dams were sacrificed on gd 20–21 and examined for implantation sites. Fetuses were weighed and examined for external, skeletal, and visceral malformations. In one study (Ema *et al*., 1993), 12 dams/group were gavaged with 0, 500, 630, 750, or 1,000 mg/kg bw/day (0, 1.80, 2.27, 2.70, or 3.60 mmol/kg bw/day) on gd 7–15 (Table 7-6). Gestational weight gain was reduced in dams of the 630 mg/kg bw/day group and adjusted weight gain (dam weight not including gravid uterus) was reduced in dams exposed to 750 mg/kg bw/day and higher. Complete resorptions occurred in 2/12, 10/12, and 9/9 litters of the 630, 750, and 1,000 mg/kg bw/day dose groups, respectively, thus resulting in decreased live fetuses/litter. Fetal weight was reduced in groups exposed to 630 mg/kg bw/day and higher. External malformations, consisting entirely of cleft palate, were increased in the 750 mg/kg bw/day group. In Ema *et al*. (1998), additional endpoints including anogenital distance and testicular descent were studied. Eleven dams/group were fed diets containing 0, 0.5, 1.0, or 2.0% DBP on gd 11–21. Authors estimated daily intake rates of 0, 331, 555, and 661 mg/kg bw/day for the control to high-dose groups, respectively. Maternal gestational and corrected weight gain were reduced in dams exposed to 555 mg/kg bw/day and higher and were accompanied by a reduction in food intake. Fetal weight was reduced and the incidence of external malformations (cleft palate) and skeletal malformations (fused sternebrae) were increased in the 661 mg/kg bw/day dose group. Reduced anogenital distance and increased incidence of undescended testes were observed in male fetuses exposed to 555 and 661 mg/kg bw/day.

Ema *et al*. (1994, 1995) focused on the time- and dose-dependency of DBP developmental toxicity. In the studies, groups of 10–13 pregnant rats were gavaged with 0, 750, 1,000,

1,250, or 1,500 mg/kg bw/day on gd 7–9, 10–12, or 13–15. Resorptions were increased in all dose groups at all time points. All dams treated with 1,500 mg/kg bw/day experienced complete litter resorptions. However, the types and frequencies of malformations varied according to the exposure time course. Treatment on gd 10–12 did not result in an increased malformation rate. Treatment with doses of 750 mg/kg bw/day and higher on gd 7–9 resulted in increased skeletal malformations (fusion or absence of vertebral arches and ribs). Administration of 750 mg/kg bw/day and higher on gd 13–15 resulted in the greatest incidence of teratogenicity, including increased external malformations (cleft palate) and skeletal malformations (fusion of sternebrae).

Saillenfait *et al*. (1998) exposed Sprague-Dawley rats (27 per group) to a single administration of DBP by gavage on gd 14 at 0, 500, 1,000, 1,500, or 2,000 mg/kg body weight. Increased resorptions at 1,500 and 2,000 mg/kg and reduced fetal body weights at 2,000 mg/kg were observed. Skeletal variations were also increased at these doses.

In a study by Wine *et al*. (1997), CD Sprague Dawley rats, 10 weeks old at the start of exposure, were used for continuous-breeding phase and cross-over mating studies. There were 20 breeding pairs in each treated dose group, and 40 pairs in the control group. DBP was mixed with feed to levels of 0, 0.1, 0.5 and 1.0% (w/w); this yielded calculated doses of 0, 52, 256, and 509 mg/kg bw/day for males and 0, 80, 385, and 794 mg/kg bw/day for females. Following a 7-day premating period, the rats were housed as breeding pairs for 14 weeks. Litters were removed immediately after birth. Endpoints in-life included clinical signs, parental body weight and food consumption, fertility (numbers of pairs producing a litter/total number of breeding pairs), number of litters/pair, number of live pups/litter, proportion of pups born alive, sex ratio, and pup body weights within 24 hours of birth. In the F0 generation there was no effect the ability to produce litters with at least one live pup; all produced approximately five litters. There was clear indication that DBP, when administered in the diet, affected total number of live pups per litter in all treated groups (reduced by ~ 8–17%) and live pup weights in the 256-385 and 509-794 mg/kg bw/day groups by 6–12 %.

A cross-over mating study was conducted between the high-dose treatment group and the controls. The percent of pairs mating, becoming pregnant, and delivering a litter was unaffected, as was litter size, although adjusted live pup weight was reduced in litters from treated females. At F0 necropsy, there were no gross or histopathologic effects in the reproductive tracts of treated animals. Epididymal sperm count, testicular spermatid number, and estrous cycle length were not affected by DBP treatment in the F0 animals. Systemic effects in the F0 rats included decreased body weight in females and increased liver and kidney to body weight ratios in both sexes of the high-dose group. The final F1 litters following the continuous F0 breeding phase were weaned and raised to sexual maturity (pnd 88) and received the same dose in feed as their parents. Upon reaching sexual maturity,

20 non-sibling F1 males and females within the same treatment group were housed in pairs for 1 week and then housed individually until delivery of an F2 litter.

F1 pup weight was significantly reduced in the high-dose group on pnd 0, 14, and 21. During rearing, three high-dose males were found to have small and malformed prepuces and/or penises and were without palpable testes. Mating, pregnancy, and fertility were significantly lower in the high-dose F1 group with only 1 of 20 pairings resulting in a litter. While litter size was unaffected, F2 pup weight was reduced in all treatment groups. All dose groups were killed and necropsied, at which point the body weights of the high-dose animals were 8-14 % lower than controls, but unchanged at other dose levels. For males only, kidney to body weight ratio increased at the 256-509 mg/kg bw/day levels and liver to body weight ratio was increased at the highest level. The relative weights of the ventral prostate and seminal vesicles and the absolute weight of the right testis were decreased in the F1 males from the high-dose group. There were no effects on the ovary of F1 females. Epididymal sperm count and testicular spermatid count was significantly reduced in the high-dose F1 males. Histologic analysis was only performed on selected males (n=10) from the control, mid-, and high- dose groups (the solution used to preserve testes is not clear). Widespread seminiferous tubular degeneration was noted in 1/10 controls, 3/10 in the mid-dose group, and 8/10 in the high-dose group. The high-dose group also exhibited interstitial cell hyperplasia. Five of ten high-dose males also had underdeveloped or defective epididymides. No ovarian or uterine lesions were noted in F1 females and there was no effect on ante-mortem estrous cyclicity. The F1 high-dose group had a high rate of infertility, the middle dose had fewer (F0 mating) and lighter pups (F0 and F1 matings), while the low-dose animals had fewer pups (F0 mating) and lighter pups (F1 mating).

Mylchreest *et al*. (1998) administered DBP by gavage to pregnant CD rats (10 per group) at 0, 250, 500, or 750 mg/kg bw/day from gd 3 until pnd 20. At birth, pups were counted, sexed, weighed, and examined for signs of toxicity. Sexual maturity was assessed by observing age of vaginal opening and preputial separation in females and males, respectively. Estrous cycles were assessed in females for 2 weeks. The F1 rats were sacrificed at 100–105 days of age. Necropsies were conducted on all males and up to three females per litter. A histological examination of sex organs was conducted on all rats with lesions and up to two unaffected rats per litter. Testes were preserved in Bouin's fixative.

Maternal body weight gain was comparable to controls throughout the dosing period. At 750 mg/kg bw/day, the number of live pups per litter at birth was decreased and maternal effects on pregnancy and postimplantation loss are likely to have occurred. Anogenital distance was decreased at birth in the male offspring at 500 and 750 mg/kg bw/day. The epididymis was absent or underdeveloped in 0, 9, 50, and 71% of adult offspring (100 days old) at 0, 250, 500, and 750 mg/kg bw/day, respectively, and was associated with testicular atrophy and widespread testicular germ cell loss. Hypospadias occurred in 0, 3, 21, and 43% of males, and ectopic or absent testes in 0, 3, 6, and 29% of males at 0, 250, 500, and 750

mg/kg bw/day, respectively. Absence of prostate gland and seminal vesicles as well as small testes and seminal vesicles were noted at low incidence in the 500 and 750 mg/kg bw/day dose groups. Dilated renal pelves, frequently involving the right kidney, were observed in all DBP dose groups. Vaginal opening and estrous cyclicity were not affected in the female offspring, although low incidences of reproductive tract malformations, mainly involving development of the uterus, were observed in 2 rats and 1 rat at the 500 and 750 mg/kg bw/day doses, respectively. In this study, all exposed groups showed adverse effects on male reproductive tract structure and indices of puberty.

In a subsequent study by Mylchreest *et al*. (1999) DBP exposure was reduced to just late gestation (gd 12–21) and compared the effects of DBP to the pharmacological androgen receptor antagonist, flutamide. Pregnant CD rats received DBP at 0, 100, 250, or 500 mg/kg bw/day by gavage with corn oil (n =10) or flutamide at 100 mg/kg bw/day (n =5) on gd 12–21. Males were killed at approximately 100 days of age and females at 25–30 days of age. In F1 males, DBP (500 mg/kg bw/day) and flutamide caused hypospadias, cryptorchidism, agenesis of the prostate, epididymis, and vas deferens, degeneration of the seminiferous epithelium, and interstitial cell hyperplasia of the testis. Agenesis of the epididymis was also observed at 250 mg/kg bw/day. Flutamide and DBP (250 and 500 mg/kg bw/day) also caused retained thoracic nipples and decreased anogenital distance. Interstitial cell adenoma occurred at 500 mg/kg bw/day in two males from the same litter. The only effect seen at 100 mg/kg bw/day was delayed preputial separation. The low incidence of DBP-induced intra-abdominal testes contrasted with the high incidence of inguinal testes seen with flutamide. Thus, the prenatal period is sensitive for the reproductive toxicity of DBP. There were no signs of maternal toxicity with the exception of a 16% body weight loss at the time of birth and complete fetal mortality in one dam of the 500 mg/kg bw/day group. In addition, testicular focal interstitial cell hyperplasia and an adenoma (in one male) were observed in males at 500 mg/kg bw/day at 3 months of age.

Mylchreest *et al*. (2000) gavaged 19–20 Sprague-Dawley CD rats/group with 0, 0.5, 5, 50, or 100 mg/kg bw/day and 11 Sprague-Dawley CD rats with 500 mg/kg bw/day in corn oil on gd 12–21 (Table 7-12). Dams and pups were weighed and examined at birth. After the pups were weaned, dams were killed and implantation sites and organ weights were evaluated. Pups were weighed weekly and examined for sexual maturation. When pups reached puberty they were killed and organ weights were determined. The testes and epididymides were preserved in Bouin's solution and examined histologically. There was no evidence of maternal toxicity at any dose. In male pups, the incidence of retained aereolas or nipples was increased at the 100 and 500 mg/kg doses (31% of rats in 16/20 litters and 90% of rats in 11/11 litters, respectively). Malformations observed in the highest dose group included: hypospadias (9% of rats in 4/11 litters); and agenesis of the epididymis (36% of rats in 9/11 litters), vas deferens (28% of rats in 9/11 litters), and prostate (1/58 rats). Reduced testis, epididymis, prostate, and levator muscle weight and reduced anogenital distance in males were also observed at the high dose. Histological effects in high-dose males included

interstitial cell hyperplasia (35% of rats in 3/5 litters), adenoma (1/23 rats), and seminiferous tubule degeneration (56% of rats in 3/5 litters). The single case of seminiferous tubule degeneration in the 100 mg/kg bw/day group was considered equivocal because the lesion does occur spontaneously in a small number of Sprague-Dawley rats. In female offspring, the age of vaginal opening and reproductive organ weight and histology were unaffected.

Gray *et al*. (1999) gavaged 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3 with corn oil vehicle or DBP at 500 mg/kg bw/day, and groups of 4-6 Long Evans Hooded rats with 0 or 500 mg/kg bw/day on gd 16–19. This study also compared the effects of DBP at 500 mg/kg bw/day and an equimolar concentration of 750 mg/kg bw/day DEHP administered by gavage to 8–10 Sprague-Dawley rats/group from gd 14 to lactation day 3. The male F1 pups were evaluated for sexual maturation and were then killed and necropsied at 5 months of age. Organ weights were measured and a histological examination of reproductive organs (preserved in Bouin's) was conducted. The presence or absence of maternal toxicity was not described. Effects in F1 males included reduced anogenital distance, and increases in percent areolas and nipples at birth, numbers of areolas and nipples at birth and adulthood, hypospadias, and testicular and epididymal atrophy or agenesis. A decrease in weight for prostates, epididymides, testes, penis, and the levator ani muscle was also observed in the treated rats. None of the control pups were found to have nipple development, malformations, or testicular degeneration. DEHP and DBP exposure resulted in effects that were qualitatively similar, but quantitatively DEHP was considerably more toxic to the male reproductive system.

The study by Gray *et al*. (1999) also included a multigeneration reproductive component conducted to assess effects of DBP exposure in Long Evans Hooded rats. Weanling male and female rats of the parental (F0) generation (10–12/sex/group) were gavaged daily with DBP in corn oil through puberty, adulthood, mating, gestation, and lactation. Females received 0, 250, or 500 mg/kg bw/day; male rats received 0, 250, 500, or 1,000 mg/kg bw/day. Sexual maturation and estrous cycles of the F0 were evaluated. Treated rats were mated with untreated controls. When the F1 litters were weaned, the parental rats were killed and necropsied. Implantation sites, serum hormone levels, organ weights, and testicular histology were evaluated. A delay in puberty was observed in all treated F0 males based on the age of preputial separation (42.6, 43.4, and 44.4 days from low to high-dose group vs 39.6 days in control group). Fertility was reduced in F0 males and females in the 500 mg/kg bw/day group. Infertility in F0 males was apparently due to testicular atrophy and reduced sperm counts. F0 females in the 500 mg/kg bw/day group cycled and mated successfully, but experienced an increased incidence of mid-term abortion. Malformations were significantly increased in F1 pups from the 250 and 500 mg/kg bw/day groups. Types of malformations included low numbers of hypospadias, abdominal testes, anophthalmia, uterus unicornous, and renal agenesis. The F1 pups were not treated with DBP after weaning. Four to eighteen pairs of F1 pups from treated dams were selected for continuous mating within dose groups for 11 cycles and the remaining F1 pups were necropsied. The F2

pups born during the continuous breeding phase were counted and discarded. Fecundity was reduced in F1 rats from treated dams and the number of F2 pups born was reduced in breeding pairs from the 250 and 500 mg/kg bw/day groups. At necropsy, a non-significant reduction in caudal sperm counts (19%) and a significant reduction in caudal sperm levels (34%) were noted in F1 males from the 250 and 500 mg/kg bw/day groups, respectively. This study by Gray *et al.* (1999) was considered somewhat limited because many endpoints and details of the experimental methods were not reported.

In Lamb *et al*. (1987) and Reel *et al*. (1984), DBP was one of four phthalate esters compared using the Continuous Breeding protocol in CD-1 mice; the same basic protocol as reported in Wine *et al*. (1997). Male and female CD-1 mice, 20 pairs/treatment group and 40 pairs in control, were fed a diet with DBP at 0 300, 3,000, or 10,000 ppm (doses of 53, 525, and 1,750 mg/kg bw/day as reported by Reel *et al*. (1984)) for 7 days prior to and during a 98-day cohabitation period. Litters were removed immediately after birth. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair and of live pups per litter, pup weight, and offspring survival. Testes were fixed in Bouin's solution for histological evaluation. DBP exposure reduced litter size, numbers of litters per pair, number of fertile pairs, live pups per litter, and proportion of pups born alive in the high-dose group. These effect were not seen at lower dose levels. A crossover mating trial demonstrated that female, but not male, mice were affected by DBP, as shown by significant decreases in the percentage of fertile pairs, the number of live pups per litter, the proportion of pups born alive, and live pup weight. Only the control and high-dose F0 DBP groups were necropsied. There were no effects on sperm parameters in the males, although body weight was significantly decreased (8%) and liver to body weight ratio significantly increased (11%). For females, liver to body weight ratio was significantly increased (19%) and relative uterine weight significantly decreased (28%), but there was no effect on estrous cycles. No treatment-related gross or histological lesions were noted. A second generation was not evaluated.

In Lamb *et al*. (1987), the high-dose group was subfertile and the middle-dose and the low-dose groups were functionally unaffected. The mid- and low-dose groups were not necropsied or evaluated for reproductive development or performance.

Di-*n*-hexyl Phthalate (DnHP) (CAS No. 84-75-3).

**DnHP is a reproductive toxicant causing fertility effects in both sexes of two rodent species.**

The NTP-CERHR has concluded that there is clear evidence of adverse reproductive effects (male and female) in laboratory animals (NTP-CERHR, 2003c).

The *NTP-CERHR Expert Panel Report on Di-n-Hexyl Phthalate* by the NTP-CERHR Phthalates Expert Panel is incorporated into the NTP-CERHR (2003c) as Appendix II. The Expert Panel reviewed several studies of DnHP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003c, Appendix II), is summarized below.

DnHP was evaluated in the Chernoff-Kavlock screening assay (Hardin *et al*., 1987). CD-1 mice (48-50 dams/group) were gavaged on gd 6-13 with 9,900 mg/kg bw/day (undiluted chemical, 10 mL/kg/day) or corn oil. According to the standard protocol, dams are allowed to litter and a postnatal evaluation is conducted. However, there were no live litters (0/34). One exposed dam died. Body weight changes in dams could not be evaluated due to complete litter loss.

The key study for the assessment of the reproductive toxicity of DnHP is reported by Lamb *et al*. (1987) and Reel *et al*. (1985). In Lamb *et al*., twenty pairs of male and female CD-1 mice (40 pairs in control group) were dosed with DnHP for 7 days prior to and during a 98-day cohabitation period. The doses were 0, 0.3, 0.6, or 1.2% w/w in the diet. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair, number of live pups per litter, pup weight, and offspring survival. Organs were collected for histological evaluation and testes were preserved in Bouin's solution. DnHP exposure resulted in a dose-related reduction in the proportion of pairs able to produce even a single litter during the continuous breeding phase. No litters were produced at the high dose (1,670 mg/kg bw/day), one litter in the mid-dose group (800 mg/kg bw/day), 14 of 17 pairs had litters in the low-dose group (380 mg/kg bw/day), compared to all pairs having litters in the control group. The numbers of litters per pair, the number of live pups per litter, and the proportion of pups born alive were also significantly affected by DnHP exposure. Significant effects occurred at the lowest dose level with clear adverse effects seen in the absence of any body weight effects. A crossover mating trial was performed between the high-dose males and control females. There was a significant decrease in detected matings (56%) compared to controls (90%), and only 1 of 18 treated males sired a litter. When the high-dose females were mated with control males, there was no decrease in copulatory plugs, but none of the females became pregnant. Only the control and high-dose DnHP groups were necropsied. Sperm assessment showed a significant decrease in sperm number (7% of control) and motility (22% of control) parameters. Only 3 of 18 males had sufficient numbers of sperm to allow assessment of abnormal forms; incidence in these 3 was diminished in number compared to control. There were significant decreases in the relative weights of the epididymis, testis, and seminal vesicle. There was extensive atrophy of the seminiferous epithelium with mature sperm markedly diminished in the epididymis. No treatment-related microscopic lesions were detected in the ovaries, uterus, or vagina of the female mice. For females, liver to body weight ratio was significantly increased (31%) and uterine weight significantly decreased (31%). Body and relative kidney/adrenal weights were significantly decreased and liver to body weight ratio was significantly increased in

both males and females of the high-dose group, but histological changes were not noted. A second generation was not evaluated.

In a short-term study (Foster *et al*., 1980) which employed a single dose level of DnHP (2.4 g/kg bw/day) given by gavage in corn oil to a group of 12 pubertal male Sprague Dawley rats (4-weeks-old) for 4 days, marked effects on testis weight (65% of control value) were noted in the absence of body weight effects. Histologic examination of formalin-preserved testes revealed a marked seminiferous tubular atrophy with the majority of tubules showing few spermatogonia and Sertoli cells, but normal Leydig cell morphology.

Di-isodecyl Phthalate (DIDP) (CAS Nos. 68515-49-1, 26761-40-0).

**Prenatal oral exposure to DIDP results in developmental toxicity: Exposure of pregnant dams to relatively high doses of DIDP causes abnormal development of the fetal skeleton, and reduced weight gain and survival of pups; DIDP exposure was also associated with abnormalities of the urinary tract.**

The NTP-CERHR has concluded that there is clear evidence for adverse developmental effects in laboratory animals for DIDP (NTP-CERHR, 2003d). Further NTP-CERHR (2003e) states "Although there is no direct evidence that exposure of people to DIDP adversely affects reproduction or development, studies with rats have shown that exposure to DIDP can cause adverse developmental effects, but it does not affect reproduction."

The *NTP-CERHR Expert Panel Report Di-Isodecyl Phthalate* by the NTP-CERHR Phthalates Expert Panel is incorporated into the NTP-CERHR (2003d) as Appendix II. The Expert Panel reviewed several studies of DIDP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003d, Appendix II), is summarized below.

Effects were not seen in the Chernoff-Kavlock assay, a developmental toxicity screening test in CD-1 mice (Hardin *et al*., 1987).

Waterman *et al*. (1999) administered DIDP (CAS No. 68515-49-1) to 25 Sprague-Dawley rats/group on gd 6–15 by gavage at 0, 100, 500, and 1,000 mg/kg bw/day. The dams were sacrificed on gd 21 and implantation sites were evaluated. Fetuses were weighed and examined for external, visceral, and skeletal malformations. At 1,000 mg/kg bw/day, maternal toxicity was indicated by decreased weight gain and food consumption. Effects on fetal mortality or weight were not observed at any dose. Signs of developmental toxicity were seen in fetuses from dams that received 500 and 1,000 mg/kg bw/day. There was a statistically significant increase in the percent litters with 7th cervical ribs at the 1,000 mg/kg bw/day dose; a numerical increase in litter incidence with increasing dose (8.0, 18.2, 25, 41.7%) was also observed. A dose-related increase in the percent fetuses with a 7th cervical

rib was observed, with the incidence at the two highest doses attaining statistical significance (1.0, 2.3, 6.2, 9.2%). A second skeletal variant, rudimentary lumbar (14th) rib(s), showed increased incidence at the two highest doses that was significant on a percent litter basis at the highest dose and on a percent fetus basis at the two highest doses. Litter incidence values were 40.0, 36.4, 62.5, and 95.8%, while fetal incidence was 8.2, 9.0, 21.2, and 52%. Waterman *et al*. (1999) interpreted their results as indicating a LOAEL for maternal and developmental toxicity at 1,000 mg/kg bw/day and a NOAEL of 500 mg/kg bw/day. The Expert Panel concurred with the maternal NOAEL but selected a developmental NOAEL of 100 mg/kg bw/day based on the significant incidence of cervical and accessory 14th ribs. The Expert Panel informed the sponsor of the Waterman *et al*. study that the Panel believed that there were more recent and superior methods for the analysis of pup incidence. The sponsor statistically reanalyzed findings of toxicological interest using the generalized estimating equation (GEE) approach to the linearized model and shared its reanalysis results with the Panel. This is a pup level analysis within a model that uses the GEE approach to account for the litter effect, i.e., the correlation between outcomes measured on pups within the same litter. The dose groups were tested pair-wise versus controls; this gave similar results to a trend test based on a dose-response model fit with all dose levels up to that of interest included. The results are consistent with the interpretation of the Expert Panel.

Hellwig *et al*. (1997) investigated the comparative developmental toxicity of a number of phthalates. They administered DIDP (CAS No. 26761-40-0) by gavage in olive oil at 0, 40, 200, and 1,000 mg/kg bw/day to Wistar rats on gd 6–15 in 7–10 pregnant rats per group. The dams were sacrificed on gd 20 and implantation sites were evaluated. Fetuses were weighed and examined for external, visceral, and skeletal malformations. At 1,000 mg/kg bw/day, there was maternal toxicity expressed as reduced feed consumption, vaginal hemorrhage in 3 dams, and increased absolute and relative liver weights. Kidney weight was unaffected. Developmental effects included increased incidences of percent fetal variations per litter (24.3, 37.2, 38.4, and 44.2% at 0, 40, 200, and 1,000 mg/kg bw/day, respectively) with the values at 200 and 1,000 identified as statistically significant. In the high-dose group, there were clear increases in rudimentary cervical ribs and accessory 14th ribs. An increased incidence of dilated renal pelves and hydroureter was observed at all treatment levels which apparently contributed to a statistically significant increase in the mean percent of fetuses affected per litter with variations at the 200 and 1,000 mg/kg bw/day doses. The data at 200 mg/kg bw/day are at odds with the authors' statement that "no substance-related effects were observed on dams, gestational parameters or fetuses among the two lower dose groups." Since there were increased incidences of total fetal variations at both 200 and 1,000 mg/kg bw/day, the Expert Panel concluded that 40 mg/kg bw/day was the developmental NOAEL and 200 mg/kg bw/day the maternal NOAEL.

Developmental effects were also observed in one- and two-generation reproductive toxicity studies in rats (Exxon Biomedical Sciences Incorporated, 1997, 2000). In both studies, dams were exposed to DIDP through diet from 10 weeks prior to mating through gestation and

lactation. Dietary dose levels were 0, 0.25, 0.2, 0.4, and 0.8% for the two-generation study. In the one-generation pilot study, fetal body weights were lower in groups exposed to 0.5% DIDP and higher. There was no effect on offspring survival.

For the two-generation study in 30 Crl:CDBR VAF Plus rats/sex/group, developmental effects in F1 offspring included a decrease in live pups at birth and on pnd 4 and a decrease in pup birth weight and weight gain in the high-dose group on pnd 0, 7, 14, and 21 for both sexes and also on pnd 4 for males. In F1 pups, relative liver weights were significantly increased in females in the mid- and high-dose groups and males in the high-dose group. Liver cell hypertrophy and eosinophilia were also observed in the mid- and high-dose groups. F1 females in the mid- and high-dose groups experienced a delay in vaginal opening (33.5 and 34.2 days, respectively, vs 32.2 days in control). The age of preputial separation was not affected in males, but the frequency of evaluation was not sufficient to rule out an effect. Developmental effects in F2 pups were similar to those observed in F1 pups. F2 pup survival was reduced on pnd 1 and 4 in all treated groups, and also on pnd 7 and at weaning in the high-dose group. An unusually high incidence of pup deaths in 4, 2, and 4 litters of the low-, mid-, and high dose groups respectively was noted; it was opined that reduced survival is usually observed in small numbers of pups distributed over many litters. F2 pup birth weight was reduced in males of the high-dose group and postnatal weight gain was reduced in all pups of the high dose-group on pnd 1, 4, 7, 14, and 21. Four high-dose male pups had undescended testes, an effect that was probably related to delayed development. Although F2 pup liver weight was not increased, liver cell hypertrophy and eosinophilia were observed in mid- and high-dose males and females.

The two generation study was repeated by ExxonMobil Biomedical using lower doses of 0, 0.02,0.06, 0.2, and 0.4% in feed. In addition to lower doses, this study incorporated measurement of anogenital distance on day of birth and assessment of nipple retention on pnd 13 or 14, on all offspring of both generations. Age at which vaginal patency and preputial separation occurred was noted for 2 rats/sex/dose for both F1 and F2 offspring. Dams were exposed for 10 weeks prior to mating throughout pregnancy and gestation. In the F1 offspring there were no effects on pup survival, body weight, or organ weights. However, an increased incidence of dilated renal pelves (8/29 vs 0/30) were noted in adult F1 males of the high-dose group (0.4%). The authors did not consider the effect to be biologically significant. Developmental results in F2 offspring were consistent with findings of the previous 2-generation study. Effects at the 0.2% dose level included significant reductions in F2 pup survival on pnd 1 and 4 and significant decreases in body weights of female F2 pups on pnd 14 and male pups on pnd 35. At the 0.4% dose level, F2 pup survival was significantly decreased on pnd 1 and 4 and body weights were significantly lower for female F2 pups on pnd 14 and 21 and for males F2 pups on pnd 14, 28, and 35. At the high dose, the liver to body weight ratio was increased in F2 female pups sacrificed on pnd 21, but authors stated that the result was not biologically significant due to a lack of absolute organ weight change. A histological examination was not conducted. No treated F1 and F2 pups

experienced differences from controls in either anogenital distance or abnormal nipple retention.

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