Proposition 65 Maximum Allowable Dose Level (MADL) for Reproductive Toxicity for Di-*n*-Hexyl Phthalate (DnHP)

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Summary

The maximum allowable dose level (MADL) for di-*n*-hexyl phthalate (DnHP) is **2,200 micrograms/day** (µg/d) for the oral route of exposure. This MADL is based on the male and female reproductive toxicity of DnHP as observed in mice in a study conducted by the National Toxicology Program (NTP). Studies have shown that phthalates are rapidly and nearly completely absorbed following oral administration in rodents. Therefore, exposure by the dermal or the inhalation routes or via multiple routes that leads to an absorbed dose equivalent to **2,200 micrograms/day** (µg/d) should also be considered the maximum allowable dose level.

Background

This report describes the derivation of a MADL for DnHP (CAS No. 84-75-3). DnHP is produced as a component (up to 25%) in di-*iso*-hexyl phthalate (DiHP); it can also be present in levels of less than 1% in C6-C10 phthalates. Both DiHP and C6-C10 phthalates are used as plasticizers in the manufacture of polyvinyl chloride (PVC) and other plastic products. Consumer products that may contain DnHP include automobile parts (e.g., air filers, battery covers), tool handles, dishwasher baskets, vinyl floorings, canvas tarps, and notebook covers (NTP-CERHR, 2003). This chemical was listed under the Safe Drinking Water and Toxic Enforcement Act of 1986 (commonly known as Proposition 65, codified at Health and Safety Code Section 25249.5 et seq.) as known to the State to cause reproductive toxicity (male and female endpoints), effective December 2, 2005. The Proposition 65 listing of DnHP was based on the formal identification of DnHP as causing male and female reproductive toxicity by the NTP in its final report titled "NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Hexyl Phthalate (DnHP)" (NTP-CERHR, 2003). The NTP, solely as to final reports of the NTP's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR), is a body recognized as authoritative under Proposition 65 (Title 22, California Code of Regulations, section 12306(1))[1].

[1] All further references to regulations are to Title 22, California Code of Regulations unless otherwise noted.

Procedures for the development of Proposition 65 MADLs are provided in regulations (Sections 12801 and 12803). Exposure at a level 1,000 times greater than the MADL is expected to have no observable effect. As defined in regulations, a MADL is derived from a No Observable Effect Level (NOEL) based on the most sensitive study deemed to be of sufficient quality (Section 12803).

Study Selection

Relevant studies and reports on the male and female reproductive toxicity of DnHP have been identified through a comprehensive literature search. There are no data on the reproductive toxicity of DNHP in humans. There are three animals studies including a one-generation reproductive study in mice (Lamb, 1987), a four-day exposure study in rats (Foster et al., 1980), and a 21-day exposure study in rats (Mann et al., 1985). All three studies were included in the "NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Hexyl Phthalate (DnHP)" (NTP-CERHR, 2003).

The study reported by Lamb (1987) is a NTP-sponsored one-generation reproductive study in mice following the continuous breeding reproduction study design by the NTP (Reel et al., 1985). The studies in rats by Foster et al. (1980) and Mann et al. (1985) only used one high dose level of DnHP (2,400 and 1,824 mg/kg-day, respectively) and found effects at the high doses used. The NTP study (final report by Reel et al., 1985 and published by Lamb, 1987) covers a broader dose range and found effects at lower doses. Thus it represents a more sensitive study and is used as the basis for establishing MADLs for DnHP. Major findings from this study are summarized below.

Four groups of CD-1 mice (40 pairs of males and females in the control group and 20 pairs in each of the three DNHP-treated groups) were treated with DnHP in the diet at concentrations of 0, 0.3% (low), 0.6% (middle), or 1.2% (high). The author estimated that the doses are equivalent to approximately 0, 380, 800, or 1,670 mg/kg-day, respectively. The animals were treated for seven days before mating, and were then randomly grouped as mating pairs, cohabited, and treated for 13 weeks (total of 14 weeks or 98 days; continuous breeding phase). Pups from the final litters in each dose group were observed until postnatal day 21, when all the pups were sacrificed for final examination. Following the continuous breeding phase, a cross-over mating trial was performed by mating animals in the high-dose group (18 males and 17 females) to animals in the control group of opposite sex for seven days to determine which sex was adversely affected. Animals in the high dose group were not treated during the seven-day cross-over mating trials.

For the general toxicity endpoints, DnHP treatment did not affect food consumption in any group. The body weights of male mice in the DnHP-treated groups in Week 13 (41.5 g ± 0.9, 39.5 g ± 0.5, and 38.4 g ± 0.6, mean ± SE, in the 0.3%, 0.6%, and 1.2% groups, respectively) were lower than that of the control in the same week (42.2 g ± 0.6), indicating a dose-related decrease in body weight gain by DnHP in the diet. Body weights of postpartum dams after the cross-over trials were similar to those of the

controls. Necropsy was only performed in animals that went through the cross-over trials, including rats from the control groups and the high dose group. None of the deaths (three males in the control, three females in the low dose, one female in the middle dose, two males and two females in the high dose group) appeared to be treatment related. Although the weights of the liver (increased) and kidney (decreased) in the high dose group were significantly different from the weights of liver and kidney of the controls, no apparent histopathological changes in the liver or kidney of DnHP-treated rats were found. These findings indicate that treatment with DnHP at doses up to 1,670 mg/kg-day does not cause apparent general toxicity in rats of either sex.

Obvious reproductive toxicity was observed in all DnHP-treated groups. The control group had a fertility index (percentage of fertile pairs divided by the total number of pairs cohabitated) of 100%, delivering an average of 4.89±0.05 litters per pair during the continuous breeding period (mean±SE). There were no litters produced in the high dose group (16 pairs) and only one litter produced by 19 mating pairs in the middle dose group during the 13-week continuous breeding phase. In the low dose group, 14 of 17 pairs (82%) of the treated rats (three females died during the treatment) produced 3.43±0.34 litters per pair ($p<0.01$, compared to the controls). The number of live pups per litter in the low dose group (3.43±0.48) was significantly lower than that of the controls (12.29±0.40, $p<0.01$). Among the 33 final litters from the control group, pups from one litter died before postnatal day four. In contrast, all the live pups from two final litters in the low dose group died by postnatal day 14. All five pups in the single final litter in the middle dose group survived until postnatal day 21.

Mating of 18 males from the high dose group with untreated females from the control group only produced one litter, while no litter of pups was produced from mating of 17 females from the high dose group with untreated male controls. Necropsy of male rats in the high dose group after cross-over mating trials found decreased weights of the testis, epididymis, and seminal vesicles. The percentage of mobile sperm and the sperm concentration in the cauda epididymis were significantly lower in the high dose group than that of the controls. Histopathological evaluation of the testis revealed severe degenerative changes in the seminiferous epithelium. However, no treatment-related histopathological changes were observed in the ovary, uterus, or vagina of the females in the high dose group.

Thus, treatment with DnHP in the diet at concentrations of 0.3% and above resulted in severe reproductive effects in both male and female CD-1 mice in this study. NOELs for male and female end-points were not observed in this study. The lowest dose used in the study, 0.3% in the diet or 380 mg/kg-day, is therefore considered as the lowest observable effect level (LOEL) for both male and female reproductive toxicity and is used as the basis for the MADL calculation. At the LOEL, DnHP caused significant reduction in fertility (fertility index, number of litters per pair, number of live pups per litter) and in offspring viability during the postnatal development.

MADL Calculation

The NOEL is the highest dose level which results in no observable reproductive effect, expressed in milligrams of chemical per kilogram of bodyweight per day (Section 12803(a)(1)). When a NOEL is not provided from the relevant studies, the LOEL is converted to a NOEL for purposes of assessment by dividing by 10 (Section 12803(a)(7)). The NOEL is converted to a milligram per day dose level by multiplying the assumed human body weight by the NOEL (Section 12803(b)). When the applicable reproductive effect is upon the male, a human body weight of 70 kilograms shall be assumed. When the applicable reproductive effect is upon the female, a human body weight of 58 kilograms shall be assumed.

For the oral route of exposure, the following calculations were performed to derive the $MADL_{oral}$ for DnHP, based on the NTP study in mice (Reel et al., 1985; Lamb, 1987) that provided a LOEL of 380 mg/kg-day for both male and female reproductive toxicity.

Conversion from a LOEL to NOEL:
380 mg/kg-day ÷ 10 = 38 mg/kg-day

Calculation of the NOEL for a 70 kg man:
38 mg/kg-day × 70 kg = 2,660 mg/day

Calculation of the NOEL for a 58 kg woman:
38 mg/kg-day × 58 kg = 2,204 mg/day

Section 12803(a)(1) requires that "the reproductive effect for which studies produce the lowest NOEL shall be utilized for the determination of the NOEL," when multiple reproductive effects provide the basis for listing. Thus, the NOEL for a 58 kg woman (2,204 mg/day) is used to calculate a MADL.

The MADL is derived by dividing the NOEL by one thousand (Section 12801(b)(1)). Thus, the adjusted NOEL was divided by 1,000 to obtain the MADL.

$MADL_{oral}$ = 2204 mg/day ÷ 1000 = 2204 µg/day or **2200 µg/day** after rounding.

This MADL applies to exposure to DnHP by the oral route.

There is no empirical data available to establish MADLs for DnHP by other routes of exposure. There is no specific data on absorption of DnHP via the oral route of exposure. However, numerous data have shown that phthalates are rapidly and nearly completely absorbed following oral administration in rodents (e.g., NTP-CERHR, 2005). The MADL for the oral route of exposure should be thus considered as an absorbed dose. For the purpose of Proposition 65, exposure by the dermal or the inhalation routes or via multiple routes that leads to **2,200 µg/day** should be considered the maximum allowable dose level.

References

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