Proposition 65 Maximum Allowable Dose Level (MADL) for Reproductive Toxicity for Linuron

August 2002

Office of Environmental Health Hazard Assessment (OEHHA)
Reproductive and Cancer Hazard Assessment Section

Summary

The maximum allowable dose level (MADL) for linuron exposure is **460 micrograms/day (μg/d)** for the oral and inhalation routes of exposure. These values were derived based on a two-generation reproductive toxicity study (Haskell Laboratory,1990).

Background

This report describes the derivation of a MADL for linuron (CAS No. 330-55-2). Linuron, or 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, is a substituted urea herbicide with a reported average use in California over the five years between 1994 and 1998 of 83,203 lbs/year. (Pesticide Use Report, California Department of Pesticide Regulation, 2001). It is listed under Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986) as known to the State to cause reproductive toxicity (developmental toxicity), effective March 19, 1999. The Proposition 65 listing of linuron was based on a formal identification by the U.S. Environmental Protection Agency (U.S. EPA) of linuron as causing developmental toxicity (U.S. EPA 1994a, 1994b). U.S. EPA is an authoritative body under Proposition 65 for identification of chemicals as causing reproductive toxicity (Title 22, California Code of Regulations, Section 12306(l)) (22 CCR 12306(l)).

Procedures for the development of Proposition 65 MADLs are provided in regulation (22 CCR 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 (22 CCR 12803).

Study Selection

Relevant studies on the toxicity of linuron have been identified through literature searches and have been reviewed. No human data specifically relevant to the developmental toxicity of linuron were identified from literature searches. Subsequent to the identification by U.S. EPA of linuron as causing developmental toxicity, several studies examining the developmental toxicity of linuron have been reported (Gray et al., 1999; McIntyre et al., 2000, 2002; Lambright et al., 2000). Major findings of these studies and those relevant to MADL development conducted to meet with federal regulations and guidelines are summarized in Table 1.

Linuron is an androgen receptor (AR) antagonist and competes with androgens for AR binding. It has been documented to inhibit androgen-induced gene expression *in vitro* and short-term exposure to linuron reduces the size of androgen-dependent tissues *in vivo*. *In utero* linuron exposure appears to affect androgen-dependent development of the male reproductive system (McIntyre et al., 2000, 2002). Testing regimes that include dosing during the androgen-dependent period of sexual differentiation and examination of neonates~~and young adult animals~~ would therefore allow detection of reproductive tract abnormalities that would not be evident in the previously-standard teratology study (dosing from gestation day 6-15, with examination of only fetal animals required). These findings suggest that the appropriate way to determine the developmental toxicity of linuron would be to include assessment of effects on the development of the male reproductive system.

Linuron clearly demonstrates effects on the male reproductive system of the offspring after maternal exposure during mid and late gestation (McIntyre et al., 2000). These findings indicate that the male reproductive system is particularly sensitive to linuron exposure during this period of development. The values noted at the low dose level (12.5 mg/kg/day) were not statistically significant by pairwise comparison with controls. However, the effects at the low dose level were interpreted by the authors to be biologically significant and OEHHA determined that there was a significant dose-related trend for the incidence of hypoplastic testes. Hence a NOEL could not be obtained from this study.

The study that is used to obtain the MADL is the two-generation reproduction study (Haskell Laboratory, 1990) conducted to satisfy the data requirements for a multigeneration reproductive toxicity study in rats under Federal Insecticide Fungicide and Rodenticide Act (FIFRA). This study includes exposure during late gestation and yields a NOEL of 100 ppm. In this study Sprague-Dawley rats (20 rats/sex/group) were administered linuron (purity 96.2%) in the diet at 12.5, 100, or 625 ppm (males: 0.84, 6.8, or 44.75 mg/kg/day; females: 1.0, 8.3, or 54.1 mg/kg/day). At the 625 ppm level, a statistically significant decrease in pup weights at birth, (F1 and F2 generations) was observed. A significant decrease in pup weight at birth was also noted in the 100 ppm group for the F1 generation; however, the lower weight in this group appeared to be related to the large litter size. Also the mean pup weights of the 100 ppm F1 and F2 litters were comparable to each other as well as the mean weight of the F2 control litters.

This statistically significant decrease in pup weights, litter size and pup viability (day 0-4) is an effect on overall development detected in a two-generation reproduction study yielding a pup NOEL of 100 ppm in diet. In addition, increased estradiol and luteinizing hormone (LH) levels in the serum at 625 ppm suggest a potential for antiandrogenic activity of linuron and correlate with decreased fertility for F1 parents at 625 ppm. Exposure in this study for the F1 generation includes gestational as well as postnatal exposure (via lactation until weaning) and subsequent exposure via diet. Despite this additional exposure, F1 animals (males) at the 100 ppm dose level did not demonstrate testicular lesions in the young adults examined (as was observed by McIntyre, et al., 2000), nor did they demonstrate a decrease in fertility. Also the NOEL is not above the lowest dose from the study by McIntyre et al., 2000. This further supports using the two-generation reproduction study (Haskell Laboratory, 1990) to obtain the MADL.

MADL Calculation

The NOEL is the highest dose level that results in no observable developmental effect, expressed in milligrams of chemical per kilogram of bodyweight per day (22 CCR 12803(a)(1)). The results obtained for the most sensitive and relevant study have been used. The mean daily intake of linuron in the diet for the female animals at the 100 ppm dose level was estimated to be 8.3 mg/kg/day (U.S. EPA RED, 1995).

NOEL = 8.3 mg/kg/day
Adjusting for purity of the test article (96.2 %):
NOEL = 7.98 mg/kg/day

The NOEL is converted to a milligram per day dose level by multiplying the assumed human body weight by the NOEL (22 CCR 12803(b)). When the applicable reproductive effect is upon the female or conceptus, human body weight of 58 kilograms is assumed.

7.98 mg/kg/day x 58 kg = 463.1 mg/day

The MADL is derived by dividing the NOEL by one thousand (1,000) to arrive at the maximum allowable dose level (22 CCR 12801(b)(1)). Thus, the adjusted NOEL is divided by 1,000 to obtain the MADL:

MADL = 463.1 mg/day ÷ 1000 = 0.463 mg/day = 463 µg/day or **460 µg/day** after rounding.

This value is applicable to oral and inhalation routes of exposure, in the absence of sufficient data for developing a separate MADL for inhalation exposure.

Table 1. Evidence on Developmental Toxicity of Linuron

STUDY (SPECIES)	EXPOSURE	FINDINGS	NOEL
Developmental Study (Rat) Haskell Laboratory, 1979	0, 50, 125 or 625 ppm (oral) days 6-15	Reduced maternal weight gain at 625 ppm; minor skeletal anomalies at 625 ppm	Developmental NOEL = 125 ppm (12.1 mg/kg) Maternal NOEL = 125 ppm
Developmental Study (Rabbit NZW) Argus Laboratories, 1985	0,5, 25, 100 mg/kg; (oral) days 7-19	Decreased maternal weight gain and increased abortions at high dose of 100 mg/kg	Maternal NOEL = 5 mg/kg/day; Developmental NOEL = 25 mg/kg/day
Reproduction Study (Rat) Haskell Laboratory, 1990	0, 12.5, 100, or 625 ppm (20 rats/sex/group) M: 0, 0.84, 6.8, or 44.75 mg/kg/day; F: 0, 1.0, **8.3**, or 54.1 mg/kg/day Animals examined at 147-161 days of feeding	Decreased pup weights, decreased litter size (F2) and pup viability	**Developmental NOEL = 100 ppm in diet of dams (8.3 mg/kg/day)**
Reproduction Study (Rat) Haskell Laboratory, 1986	0, 25, 125, 625 ppm (1.25, 6.25, and 31.25 mg/kg/day)	At 2 years: Interstitial cell adenomas, ISC hyperplasia at ≥125 ppm males, cystic endometrial hyperplasia in females	NOEL = 25 ppm in diet (males) NOEL = 25 ppm in diet (females) = 1.25 mg/kg/day
Multigeneration (Rat) Reproduction Study Haskell Laboratory, 1984	0, 25, 125, 625 ppm	Weight gain decrements at 125 ppm and 625 ppm in females Smaller litter, reduced 24 hour survival of pups, reduced pup weights at 625 ppm	Parental NOEL = 25 ppm in diet Repro/lactation NOEL = 125 ppm in diet
McIntyre et al., 2000 (Rat)	0, 12.5, 25 or 50 mg/kg from gestation day 12-21 Pups examined at PND1, PND21 and PND 100-105 Crl:CD(SD)BR rats (11 rats per group)	Dose-related retention of areolae/nipples, epididymidal and testicular lesions (seminiferous tubular degeneration) at 12.5 mg/kg/day (statistical significance only at 50 mg/kg/day)	**LOEL = 12.5 mg/kg/day**
Gray et al., 1999 (Rat)	40 mg/kg/day from weaning through puberty, mating gestation, and lactation. Altered Reproductive test Protocol (Long-Evans hooded Rats) (continuous breeding over 12 breeding cycles) 100 mg/kg/day GD 14-gd 18 (Sprague-Dawley rats)	Reduced testicular and epididymal weight, fewer pups, decreased spermatid counts. Increased incidence of hypospadias, testicular and epididymal atrophy or agenesis at 9 months.	LOEL = 40 mg/kg/day LOEL = 100 mg/kg/day

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