Proposition 65 Maximum Allowable Dose Level (MADL) for Reproductive Toxicity for Thiophanate-methyl for the Oral Route of Exposure

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Reproductive and Cancer Hazard Assessment Section

Summary

The maximum allowable dose level (MADL) for thiophanate-methyl exposure by the oral route is **600 micrograms/day (µg/day)**. This value was derived as described below, based upon a chronic study in rats (TINS 1993).

Background

This report describes the derivation of a MADL for thiophanate-methyl (CAS No. 23564-05-8).

Thiophanate-methyl is a fungicide registered and used in California. It was listed under Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986) as known to the State to cause reproductive toxicity (male and female reproductive toxicity), effective May 18, 1999. The Proposition 65 listing of thiophanate-methyl was based on formal identification by the U.S. Environmental Protection Agency (U.S. EPA) of thiophanate-methyl as causing reproductive toxicity (U.S. EPA 1994a, 1994b). The U.S. EPA is an authoritative body under Proposition 65 for identification of chemicals as causing reproductive toxicity (Title 22, California Code of Regulations, § 12306 (*l*)).

Procedures for the development of Proposition 65 MADLs are provided in regulations (Title 22, Cal. Code of Regs. §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 (Title 22, Cal. Code of Regs. §12803(a)(4)).

Study Selection

Relevant studies on the reproductive toxicity of thiophanate-methyl have been identified from the California Department of Pesticide Regulation's Pesticide Registration Database, a recent U.S. EPA Toxicology Chapter for the Reregistration Eligibility Decision (draft) (U.S. EPA 2001), and searches of the peer-reviewed literature. All of the studies identified are referenced in the Bibliography at the end of this document. No human data specifically relevant to the reproductive toxicity of thiophanate-methyl were identified.

The listing of thiophanate-methyl was based upon three studies (U.S. EPA 1994a). For male reproductive toxicity, the supporting study was a chronic rat study (Nisso 1972a). For female reproductive toxicity, the two supporting studies were a three-generation rat reproductive study (Huntingdon 1972) and a mouse developmental study (Nisso 1970c). In addition to these studies, a rat chronic study (TINS 1993) and a rat two-generation reproductive study (Hazleton 1993, 1995) also provide dose-response data for reproductive endpoints. Overall, for male and female reproductive toxicity, the rat appears to be the more sensitive species. The reproductive effect observed in the mouse developmental study (Nisso 1970c) was at a much higher dose than the effects observed in the rat reproductive studies (Huntingdon 1972, Hazleton 1993, 1995). Additional information on the key studies (Nisso 1970c, 1972a; Huntingdon 1972; Hazleton 1993, 1995; TINS 1993), including the NOELs and Lowest Observable Effect Levels (LOELs) is given in Table 1, below.

Table 1. Summary of key thiophanate-methyl studies of reproductive toxicity.

Study Reference	Animals	Treatment	Systemic Toxicity and LOEL	Reproductive Toxicity and LOEL	Reproductive NOEL
Hazleton (1993, 1995)	Sprague-Dawley rats, male and female. 25 animals/ sex/group	**Feed** at 0, 200, 630, or 2,000 ppm. Reproductive study, two generations with one litter first generation, two litters second generation.	Males: reduced body weight gain at 630 ppm. Increased liver, thyroid weight at 2,000 ppm. Females: reduced body weight gain, increased liver, thyroid weights at 2,000 ppm.	Reduced litter size, post-natal day (pnd) 1 pup weight, adjusted (for litter size) pnd 1 pup weight at 630 ppm	Female: 200 ppm (15.7 mg/kg-day)
Huntingdon (1972)	Sprague-Dawley rats, male and female. 10 males/ group, 21-22 females/ group.	**Feed** at 0, 40, 160, or 640 ppm. Reproductive study, three-generations with two litters per generation	Males: reduced body weight at 640 ppm. Females: None	Reduced litter size, pup birth weight, litter weight at 640 ppm.	Female: 160 ppm
Nisso (1970c)	ICR mice, mated females. 20 mice/group	**Gavage** at 0, 40, 200, 500, or 1,000 mg/kg-day. Developmental study, treatment gestation day (gd) 1-15, sacrifice gd 19.	None	Reduced live litter size, mostly due to reduced implantations at 1,000 mg/kg-day	Female: 500 mg/kg-day
Nisso (1972a)	Sprague-Dawley rats, male and female. 50 animals/ sex/control group, 35 animals/ sex/other groups	**Feed** at 0, 10, 40, 160, or 640 ppm. Chronic study, up to two years.	Males and females: reduced body weight and weight gain at 640 ppm.	Male: degeneration of seminiferous tubules and reduced spermatogenesis by histopathology at 640 ppm. Female: none (no effect ovary weight, gross or histopathology)	Male: 160 ppm Female: 640 ppm
TINS (1993)	Fischer 344 rats, male and female. 60 animals/ sex/group	**Feed** at 0, 75, 200, 1,200 or 6,000 ppm. Chronic study, up to 2 years.	Males and females: reduced body weight, increased liver, kidney and thyroid weights and histopathological effects at 1,200 ppm	Males: Increased incidence and/or severity of testicular atrophy among animals which died on study at 1,200 ppm. Female: none (no effect ovary weight, gross or histopathology)	Males: 200 ppm (8.8 mg/kg-day) Female: 6,000 ppm (335 mg/kg-day)

Indications of male reproductive toxicity were found in both of the rat chronic studies. In the earlier study (Nisso 1972a), male and female Sprague-Dawley rats were treated in feed at 0, 10, 40, 160, or 640 ppm for up to 2 years. Incidence of testicular histopathology, described as "…the seminiferous tubuli underwent… degeneration and atrophy, and sometimes… complete aspermatogenesis," was increased at 640 ppm over control animals at the terminal sacrifice. The frequencies of this observation were 1/16, 1/8, 1/9, 1/10, and 5/8 for the 0, 10, 40, 160, and 640 ppm groups, respectively. This effect was statistically significant at 640 ppm ($p < 0.01$, Fischer Exact Test, calculation by OEHHA staff); consequently, the NOEL for this endpoint was 160 ppm. In the later rat chronic study (TINS 1993), male and female Fischer 344 rats were treated in feed at 0, 75, 200, 1,200, or 6,000 ppm for up to 2 years. Histopathological observations revealed increased incidence and/or severity of testicular atrophy at 1,200 and 6,000 ppm over controls in the animals which died on study. The increases were statistically significant using the Mann-Whitney U-test. The NOEL for this endpoint was 200 ppm. It appears that the endpoints reported in these two studies reflect the same underlying pathology. In terms of ppm, the NOEL from the TINS 1993 study (200 ppm) was higher than the NOEL from the Nisso 1972a study (160 ppm), but did not exceed the LOEL from that study (640 ppm). The TINS 1993 study was reported in considerably greater detail than was the Nisso 1972a study. The Nisso 1972a study report did not provide doses in terms of mg/kg-day, nor was there sufficient information in the report to calculate doses directly. The TINS 1993 study report provided the doses in terms of mg/kg-day. The NOEL of 200 ppm was equivalent in males to 8.8 mg/kg-day. The NOEL is the maximum dose level that has no observable reproductive effect (Title 22, Cal. Code of Regs. § 12801(c)). Within the studies which identified male reproductive endpoints, i.e. the Nisso 1972a and TINS 1993 studies, the TINS 1993 study had the higher NOEL in terms of ppm, but this NOEL did not exceed the LOEL of the Nisso 1972a study, so the TINS 1993 study is the most sensitive study deemed to be of sufficient quality (Title 22, Cal. Code of Regs. §12803(a)(4)). Consequently, the MADL for male reproductive toxicity was based on the NOEL from this study.

Indications of female reproductive toxicity were found in both of the rat reproductive studies. In the earlier study (Huntingdon 1972), male and female Sprague-Dawley rats were treated in feed at 0, 40, 160, or 640 ppm for three generations with two litters per generation (six litters total). Pup birth weight in the 640 ppm group was lower than control in all litters except the F2a litter. Litter size and litter weight in the 640 ppm group were lower than control in all litters except the F3a litter. The NOEL for these endpoints was 160 ppm. In the later study (Hazleton 1993, 1995), male and female Sprague-Dawley rats were treated in feed at 0, 200, 630, or 2,000 ppm for two generations with one litter in the first generation and two litters in the second generation. Lower litter sizes compared to controls were observed in all litters at 2,000 ppm and two of three litters at 630 ppm. None of these effects was statistically significant. Lower postnatal day 1 (pnd 1) average pup weight (adjusted for litter size) compared to controls was observed in all litters at 630 ppm and 2,000 ppm. The only statistically significant effect was in the F2b litter at 630 ppm. The NOEL for these effects was 200 ppm. It appears that the endpoints reported in these two studies are consistent. In terms of ppm, the NOEL from the Hazleton 1993 study (200 ppm) was higher than the NOEL from the Huntingdon 1972 study (160 ppm), but did not exceed the LOEL from that study (640 ppm). The Hazleton 1993 study was reported in considerably greater detail than was the Huntingdon 1972 study. The Huntingdon (1972) study report did not

provide doses in terms of mg/kg/d, nor was there sufficient information in the report to calculate doses directly. The Hazleton 1993 study report provided the doses on a weekly basis, but not for the total exposure periods. The range of doses for females treated at 200 ppm was reported as 12.8-24.5 mg/kg-day for the pre-mating and gestation periods. OEHHA staff has calculated the average doses for the pre-mating and gestation periods. The NOEL of 200 ppm was equivalent in females to an average dose of 15.7 mg/kg-day. The NOEL is the maximum dose level that has no observable reproductive effect (Title 22, Cal. Code of Regs. § 12801(c)). Within the studies which identified female reproductive endpoints, i.e. the Huntingdon 1972 and Hazleton 1993 studies, the Hazleton 1993 study had the higher NOEL in terms of ppm, but this NOEL did not exceed the LOEL of the Huntingdon 1972 study, so the Hazleton 1993 study is the most sensitive study deemed to be of sufficient quality (Title 22, Cal. Code of Regs. §12803(a)(4)). Consequently, the MADL for female reproductive toxicity was based on the NOEL from the Hazleton 1993 study.

In cases where multiple reproductive effects (e.g. male and female) provide the basis for the determination that a chemical is known to the state to cause reproductive toxicity, the reproductive effect for which studies provide the lowest NOEL shall be utilized for the determination of the NOEL , expressed in milligrams of chemical per kilogram of bodyweight per day (Title 22, Cal. Code of Regs. § 12803(a)(1)). The determination of the appropriate NOELs for male and female reproductive effects have been described above. Comparing the male and female reproductive endpoints, the NOEL concentration in food was the same: 200 ppm. However, the male NOEL corresponds to a lower dose than the female NOEL when the doses are expressed as mg/kg-day (8.8 mg/kg-day vs. 15.7 mg/kg-day). Thus, expressed in mg/kg-day, the NOEL for the male reproductive endpoint is lower than the NOEL for the female reproductive endpoint in these studies. Therefore, the male reproductive endpoint from the later chronic rat study (TINS 1993) was used to calculate the MADL.

MADL Calculation

The NOEL is the highest dose level which results in no observable reproductive effect, expressed in milligrams of chemical per kilogram of body weight per day (Title 22, Cal. Code of Regs. § 12803(a)(1)). The NOEL is converted to a milligram per day dose level by multiplying the NOEL by the assumed human body weight (Title 22, Cal. Code of Regs. §12803(b)). For male reproductive toxicity, the assumed male body weight is 70 kg. The NOEL from the TINS 1993 study of 8.8 mg/kg-day was adjusted for the reported purity of 96.55% to 8.5 mg/kg-day.

8.5 mg/kg-day x 70 kg = 595 mg/day

The MADL is derived by dividing the NOEL by 1,000 (Title 22, Cal. Code of Regs. §12801(b)(1)).

MADL = 595 mg/day ÷ 1,000 = 595 µg/day.

The MADL for the oral route is **600 µg/day** after rounding.

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Note: This section contains all of the studies reviewed by OEHHA staff for the preparation of the thiophanate-methyl MADL, including those referenced within the MADL document itself.

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