

Proposition 65 Maximum Allowable Dose Level (MADL) for Reproductive Toxicity for *m*-Dinitrobenzene

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

Summary

The maximum allowable dose level (MADL) for *m*-dinitrobenzene is **38 micrograms/day ($\mu\text{g}/\text{d}$) for the oral route of exposure**, derived from an oral reproductive toxicity study in rats conducted by Linder et al. (1986). Dermal or inhalation exposure leading to an absorbed dose of 36 $\mu\text{g}/\text{d}$ should be considered the maximum allowable for Proposition 65 purposes.

Background

This report describes the derivation of maximum allowable dose levels (MADLs) for *m*-dinitrobenzene (*meta*-dinitrobenzene, or *m*-DNB, CAS No. 99-65-0). *m*-DNB is used as an intermediate in organic synthesis and in the production of dyes, explosives, industrial solvents, and pesticides (HSDB, 2002). This chemical 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 reproductive toxicity), effective July 1, 1990. The Proposition 65 listing of *m*-DNB was based on a formal identification by the U.S. Environmental Protection Agency (U.S. EPA) of *m*-DNB as causing reproductive toxicity (male reproductive toxicity) (U.S. EPA 1989a, 1989b). 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 [22 CCR 12306]).

Procedures for the development of Proposition 65 MADLs are provided in regulations (22 CCR 12801 and 12803). Exposure at a level 1,000 times greater than the MADL is expected to have no observable effect. As specified in regulations, a MADL is derived from 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 male reproductive toxicity of *m*-DNB have been identified through literature searches. These studies, listed in the Bibliography for this document, have been reviewed and considered for the establishment of the MADL.

The male reproductive toxicity of *m*-DNB has been clearly shown in numerous toxicological studies in laboratory animals including rats and mice. Decreased testicular weights, morphological degeneration of germ cells, decreased testicular sperm head counts or epididymal sperm counts, or reduced fertility have been observed in rats or mice following oral treatment or intraperitoneal injection with *m*-DNB. Major findings from several relevant animal studies with long-term oral treatment with *m*-DNB are briefly summarized in Table 1. No male reproductive toxicity studies using inhalation or dermal exposure were found in the literature. No human data relevant to the male reproductive toxicity of *m*-DNB were found in the literature.

Table 1. Summary of the Male Reproductive Toxicity of *m*-DNB

Study Reference	Animals	Treatment	General Toxicity	Reproductive Effects & LOEL	NOEL (adjusted)
Cody et al., 1981	Rats (species not reported), 20 rats per group	Drinking water , 0, 3, 8, 20 mg/L for 16 weeks	No effect on body weights; no clinical sign of toxicity; increased spleen weights at ≥ 8 mg/L	Decreased testicular weights and “slight to moderate decrease in spermatogenesis.” LOEL = 20 mg/L (3.1 mg/kg/d)	8 mg/L (1.13 mg/kg/d as reported by the authors)
Linder et al., 1986	Male Sprague-Dawley rats, 12 animals per group	Gavage , 0, 0.75, 1.5, 3.0, 6.0 mg/kg, five d/wk for 12 weeks	No effect on body weights; no clinical sign of toxicity at ≤ 3.0 mg/kg; increased spleen weights at ≥ 1.5 mg/kg.	Decreased testicular & epididymal weights; testicular atrophy; decreased testicular sperm head counts and epididymal sperm counts; decreased fertility. LOEL= 1.5 mg/kg (1.07 mg/kg/d)	0.75 mg/kg (0.54 mg/kg/d)
Irimura et al., 2000	Male Sprague-Dawley rats, 4-5 animals per group	Gavage , 0, 25, 50 mg/kg/d for four weeks or 0, 25, 50, 75 for two weeks	No sign of general toxicity.	Decreased testicular weights; testicular atrophy and decreased number of sperms in the ducts of the epididymides. LOEL= 50 mg/kg/d	25 mg/kg/d

Note: Adjusted NOELs: NOELs reported in the original reports were adjusted to mg/kg/d following the methods described in “MADL Calculation” section of this document.

The pertinent regulation specifies that the NOEL is to be based on the most sensitive study deemed to be of sufficient quality (22 CCR 12803(a)(4)). The study that meets this criterion is that by Linder et al. (1986). In this study, groups (12 animals/group) of 29-day-old male Sprague-Dawley rats were treated by gavage with *m*-DNB at doses of 0, 0.75, 1.5, 3.0, or 6.0 mg/kg, five days per week for up to twelve weeks. After ten weeks of treatment, each male was mated with two virgin females for a maximum of one week for evaluation of the effect of *m*-DNB on male fertility. The authors stated that no clinical signs of toxicity or effects on growth rate were observed in rats treated with ≤ 3.0 mg/kg *m*-DNB. The spleen weights were increased in rats treated with 1.5 or 3.0 mg/kg *m*-DNB. Due to severe toxicity including mortality observed in animals treated with 6.0

mg/kg, treatment in this group was terminated in Week 10 (after four days of breeding). Main quantitative data on the male reproductive effects of *m*-DNB as reported by the authors are summarized in Table 2. In addition, the authors observed atrophy and incomplete spermatogenesis in seminiferous tubules in animals treated with 3.0-mg/kg of *m*-DNB. One of 12 animals in the 1.5-mg/kg group exhibited decreased spermatogenesis. The authors stated that spermatogenesis in all animals dosed with 0.75-mg/kg of *m*-DNB appeared to be normal. Since no obvious effects on sperm parameters or testicular morphology were observed in rats treated with 0.75 mg/kg *m*-DNB, 0.75 mg/kg was determined to be the NOEL. This dose is equivalent to 0.54 mg/kg/d after adjusting from the dosing regimen in the study of five days per week to seven days per week.

Table 2. Major findings on the male reproductive toxicity of m-DNB from the study in rats by Linder et al. (1986)

Endpoints		Control	0.75 mg/kg	1.5 mg/kg	3.0 mg/kg
Implantation sites (No./litter)		15.6±0.4	14.5±0.7	14.1±0.6	0
Resorptions (%)		5.7±1.9	11.6±2.3	9.8±2.4	No data
No. of live pups per litter	Male	7.2±0.5	5.5±0.4*	6.6±0.6	No data
	Female	7.5±0.5	7.1±0.6	6.1±0.3	No data
	Total	14.7±0.5	12.7±0.7*	12.8±0.5*	No data
Testicular weights (g)		1.68±0.05	1.61±0.03	1.61±0.06	0.68±0.03*
Epididymal weights (g)		0.54±0.01	0.52±0.01	0.52±0.02	0.30±0.01*
Sperm parameters	No. (10 ⁶) per mg epididymal fluid	2.44±0.06	2.44±0.08	2.47±0.09	No data
	Sperm head (10 ⁶) per gram testis	147±3	141±6	115±9*	7±1*
	Motility (%)	63±2	60±3	61±4	No data
	Normal morphology (%)	95.4±2.2	97.6±0.7	96.7±0.9	15.1±13.0*

Note: *: Compared to control, p<0.05.

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 (22 CCR 12803(a)(1)). 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)). For male reproductive toxicity, the assumed body weight of man is 70 kg (22 CCR 12803(b)).

For the oral route of exposure, the following calculations were performed to derive the MADL for *m*-DNB, based on a NOEL of 0.75 mg/kg found in the rat study by Linder et al. (1986):

$$\begin{aligned} &\text{Adjusting of NOEL (0.75 mg/kg) from five days/week to seven days/week:} \\ &0.75 \text{ mg/kg} \times (5 \text{ days} \div 7 \text{ days}) = 0.54 \text{ mg/kg/d} \end{aligned}$$

Calculation of NOEL for a 70 kg man:
 $0.54 \text{ mg/kg/d} \times 70 \text{ kg/man} = 37.8 \text{ mg/d}$

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_{oral} = $37.8 \text{ mg/d} \div 1000 = 37.8 \text{ } \mu\text{g/d}$ or **38 $\mu\text{g/d}$** after rounding.

Approximately 80-96% of *m*-DNB was absorbed when administered orally in rats (Nystrom & Rickert, 1987; McEuen et al., 1995). Using the highest reported absorption value for oral exposure, 38 $\mu\text{g/d}$ is approximately equivalent to an absorbed dose of 36 $\mu\text{g/d}$ ($38 \text{ } \mu\text{g/d} \times 96\% = 36 \text{ } \mu\text{g/d}$). No MADLs can currently be provided for dermal or inhalation exposures. However, dermal or inhalation exposure leading to an absorbed dose of 36 $\mu\text{g/d}$ should be the maximum allowable dose level for purposes of Proposition 65.

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