Proposition 65 Maximum Allowable Dose Level (MADL) for Reproductive Toxicity for 2,4-D Butyric Acid

March 2003

Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Section

Summary

The maximum allowable dose level (MADL) for 2,4-D butyric acid (2,4-DB; 2,4-dichlorophenoxybutyric acid) is **910 micrograms/day (µg/d)**. This value was derived as described below, based on a one-year oral toxicity study in dogs conducted by Hazleton Laboratories (1990).

Background

This report describes the derivation of a maximum allowable dose level (MADL) for 2,4-D butyric acid (CAS No. 94-82-6). 2,4-D butyric acid is an herbicide used for weed control in agriculture and in consumer lawn-care products. 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 (male reproductive toxicity), effective June 18, 1999. The Proposition 65 listing of 2,4-D butyric acid was based on a formal identification by the U.S. Environmental Protection Agency (U.S. EPA) of 2,4-D butyric acid as causing male reproductive 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 (22 CCR 12306)).

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 specified in regulation, 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

No human data specifically relevant to the male reproductive toxicity of 2,4-D butyric acid were identified from literature searches. Relevant data on the male reproductive toxicity of 2,4-D butyric acid were obtained from the California Department of Pesticide Regulation pesticide regulation database, including a chronic toxicity study in rats (MacKenzie 1987), a 13-week toxicity study in dogs (Hazleton Laboratories 1969), a one-year toxicity study in dogs (Hazleton Laboratories 1990) and a multigeneration study in rats (Bottomley 1986). No additional relevant animal data concerning male

reproductive toxicity of 2,4-D butyric acid were identified through searches of the peer-reviewed literature.

U.S. EPA (1994b) based its identification of male reproductive toxicity on a subchronic toxicity study (Hazleton Laboratories 1969). In this 13-week oral toxicity study, four male and four female dogs were administered 0, 2.5, 8, 25 or 80 mg/kg/d 2,4-D butyric acid. The agent was originally administered to these groups in diet. After two weeks, the dosing was conducted by administration in gelatin capsules seven days per week to these groups. The authors reported many histopathological changes in the testes and epididymides of all four beagle dogs administered 25 mg 2,4-D butyric acid/kg/d orally for 5-13 weeks (Hazleton Laboratories, 1969), including, lack of seminiferous epithelia and aspermatogenesis in the testes, and decreased number of spermatozoa in the epididymides. Two of the four male dogs in this group were sacrificed prior to the end of the 13 week period due to morbidity. All of the dogs in the 80 mg/kg/d group died or were sacrificed moribund by the third week of the study. The major general toxicity finding at pathology in the 25 and 80 mg/kg/d groups was "compound-related changes consisting primarily of hemorrhage and edema throughout the body tissues and organs." No deaths, moribund sacrifice, or effects on spermatogenesis were reported at a lower dose of 8 mg 2,4-D butyric acid/kg/d.

In addition to the Hazleton (1969) study discussed by U.S. EPA, additional relevant studies were identified by OEHHA in connection with MADL development (Table 1). No other studies found effects on endpoints of male reproductive toxicity. This includes the later Hazleton (1990) study in dogs (6 animals per group) treated with 2,4-D butyric acid via dietary administration for one year at 4 concentrations corresponding to doses of 0, 2.4, 6, and 13 mg/kg/day. This study was conducted in a lower dose range than Hazleton Laboratories (1969), which used capsule administration to reduce gastric irritation. Elevations in clinical chemistry parameters, and increased incidence of kidney tubule pigment and distended gallbladder, were identified at a lowest observable effect level (LOEL) of 2.4 mg/kg/d. No effects were reported on testes pathology.

The NOEL is required to be based on the most sensitive study deemed to be of sufficient quality (22 CCR Section 12803(a)(4). The relevant studies are outlined in Table 1. Two available studies in rats (Bottomley 1986, McKenzie 1987) were not considered appropriate for NOEL identification since no male reproductive effects were reported in rats. The Hazleton (1990) dog study was considered most sensitive because it used a longer exposure period and had a larger group size than the Hazleton (1969) study. Thus, the NOEL for male reproductive toxicity was identified at 13 mg/kg body weight per day from the Hazleton (1990) study. This NOEL is the highest NOEL lower than the 25 mg/kg/d LOEL identified by Hazleton Laboratories (1969).

Table 1. LOELs/NOELs for endpoints relevant to 2,4-D butyric acid male reproductive toxicity

			LOEL/NOEL
Hazleton Laboratories 1969	0, 2.5, 8, 25, 80 mg/kg/day capsules/diet; 13 weeks	dog	LOEL 25 mg/kg/d NOEL 8 mg/kg/d
Hazleton Laboratories 1990	0, 75, 225, 675/450 ppm diet (0, 2.4, 6, 13 mg/kg/day) one year	dog	LOEL none NOEL 13 mg/kg/d (675/450 ppm diet)
Bottomley 1986	0, 60, 300 ppm diet multigeneration	rat	LOEL none NOEL 75 mg/kg/d
McKenzie 1987	0, 60, 600, 1800 ppm diet two years	rat	LOEL none NOEL 450 mg/kg/d

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 Section 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 Section 12803(b)). For male reproductive toxicity, the assumed body weight of a man is 70 kg (22 CCR 12803(b)). The MADL is derived by dividing the NOEL by one thousand (1,000) to arrive at the maximum allowable dose level (22 CCR Section 12801(b)(1)). Thus, the adjusted NOEL was divided by 1,000 to obtain the MADL.

NOEL = 13 mg/kg-d × 70 kg = 910 mg/d

MADL = 910 mg/d ÷ 1000 = **910 µg /d**

References

Bottomley AM (1986). 2,4-DB effect on two generations of the rat. Project # UNC/138-R, Huntingdon Research Centre Ltd.

Hazleton Laboratories (1990). One-year oral toxicity study in beagle dogs with 2,4-DB technical. Final Report. Project # 400-724. Hazleton Laboratories America, Inc., Wisconsin.

Hazleton Laboratories (1969). 13-Week Oral Administration – Dogs: 2,4 DB Acid; Final Report. Project #656-110, Hazleton Laboratories, Inc., Virginia.

MacKenzie KM (1987). Lifetime dietary combined chronic toxicity and oncogenicity study in albino rats with 2,4-DB. Project # HLS 6158-103, Hazleton Laboratories America Inc., Wisconsin.

US Environmental Protection Agency (US EPA, 1994a). Proposed Rule: Addition of Certain Chemicals; Toxic Chemical Release Reporting; Community Right to Know. Federal Register (59 FR 1788).

US Environmental Protection Agency (US EPA, 1994b). Final Rule: Addition of Certain Chemicals; Toxic Chemical Release Reporting; Community Right to Know. Federal Register (59(229) FR 61432).