INITIAL STATEMENT OF REASONS
TITLE 27, CALIFORNIA CODE OF REGULATIONS

PROPOSED AMENDMENTS TO SECTION 25805(b), SPECIFIC REGULATORY LEVELS: CHEMICALS CAUSING REPRODUCTIVE TOXICITY

MAXIMUM ALLOWABLE DOSE LEVELS FOR *n*-HEXANE, ORAL AND INHALATION ROUTES

PURPOSE AND BACKGROUND OF PROPOSED AMENDMENTS

PURPOSE

The proposed regulatory amendments would adopt two Maximum Allowable Dose Levels (MADLs) for *n*-hexane under Proposition 65[1] in Title 27, California Code of Regulations section 25805(b)[2]. The proposed MADLs were derived using scientific methods outlined in Section 25803. The proposed oral MADL for *n*-hexane is 28,000 micrograms per day and the proposed inhalation MADL for *n*-hexane is 20,000 micrograms per day.

PROPOSITION 65 AND LISTING OF *n*-HEXANE

Proposition 65 was enacted as a ballot initiative on November 4, 1986. The Office of Environmental Health Hazard Assessment (OEHHA) within the California Environmental Protection Agency is the lead state entity responsible for the implementation of Proposition 65[3]. OEHHA has the authority to adopt and amend regulations to further the purposes of the Act[4].

The Act requires businesses to provide a warning when they cause an exposure to a chemical listed as known to the state to cause cancer or reproductive toxicity. The Act also prohibits the discharge of listed chemicals to sources of drinking water. Warnings are not required and the discharge prohibition does not apply when exposures are sufficiently small, as specified in the Act[5].

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[1] The Safe Drinking Water and Toxic Enforcement Act of 1986, codified at Health and Safety Code section 25249.5 *et. seq*., hereafter referred to as “Proposition 65” or “The Act”.
[2] All subsequent citations are to Title 27, California Code of Regulations, unless otherwise noted.
[3] Health and Safety Code, section 25249.12 and Cal. Code of Regs., Title 27, section 25102(o).
[4] Health and Safety Code, section 25249.12(a).
[5] Health and Safety Code, section 25249.9(b) and 25249.10(c).

On December 15, 2017, *n*-hexane was added to the Proposition 65 list as known to the state to cause reproductive toxicity (male reproductive endpoint), based on the findings of the state's qualified experts, the Developmental and Reproductive Toxicant Identification Committee (DARTIC)[6]. The DARTIC determined that *n*-hexane had been clearly shown, through scientifically valid testing according to generally accepted principles, to cause male reproductive toxicity.

STUDY SELECTION

Relevant studies that provide information on the male reproductive toxicity of *n*-hexane were identified in the materials that formed the basis for listing *n*-hexane as causing reproductive toxicity with the male reproductive endpoint. A comprehensive literature search did not find additional relevant studies since the Proposition 65 listing of *n*-hexane. All of the relevant studies were reviewed as the possible basis for establishing a MADL for *n*-hexane. The most sensitive studies deemed to be of sufficient quality were selected to provide a basis for the MADLs[7].

Human Studies

No human data were identified in the materials that formed the basis for listing *n*-hexane as causing reproductive toxicity, or in a subsequent literature search by OEHHA.

Studies in Laboratory Animals

Six inhalation and two oral study reports provided relevant data on the male reproductive toxicity of *n*-hexane. Three inhalation studies were conducted in mice, none of which found clear evidence for male reproductive toxicity of *n*-hexane[8]. Three inhalation studies in rats reported various adverse effects of *n*-hexane on the male

[6] Health and Safety Code section 25249.8(b) and Section 25302 *et seq*.
[7] Section 25803(a)(5)
[8] Litton Bionetics Inc (1980). Mutagenicity evaluation of n-hexane in the mouse dominant lethal assay. Final report. Study performed under contract PS-39 by: Litton Bionetics, Inc. LBI project no. 21141-01. Washington DC.
Mast TJ, Hackett PL, Decker JR, Westerberg RB, Sasser LB, McClanahan BJ, Rommereim RL and Evanoff JJ (1988). Inhalation Reproductive Toxicology Studies: Sperm morphology study of n-hexane in B6C3Fl mice. Washington DC. National Institute of Environmental Health Sciences, National Toxicology Program Contract DE-AC06-76RLO 1830.
Mast TJ, Rommereim RL, Evanoff JJ, Sasser LB, Decker JR, Stoney KH, Weigel RJ and Westerberg RB (1988). Inhalation Reproductive Toxicology Studies: Male Dominant Lethal Study of n-Hexane in Swiss (CD-1) Mice. Washington DC. National Institute of Environmental Health Sciences, National Toxicology Program Contract DE-AC06-76RLO 1830.

reproductive system following inhalation exposure for different periods of time[9,] Oral treatment of adult rats with *n*-hexane by gavage at 46.2 millimoles per kilogram of bodyweight per day (mmol/kg-day) (approximately 4000 mg/kg-day) for 120 days was stated to cause atrophy of the seminiferous epithelium[10]. In another oral study, a single dose of 20,000 milligrams per kilogram of bodyweight (mg/kg) or five daily doses of 10,000 mg/kg by gavage of *n*-hexane to adult rats resulted in abnormal changes in several parameters of male reproductive toxicity, although the effects of *n*-hexane on the male reproductive system were not as significant as those observed with several other chemicals tested in the same study[11]. No studies using other routes of exposure were identified.

Brief summaries of major findings on male reproductive toxicity in rats from the three inhalation male reproductive toxicity studies are presented in Table 1. Each of these studies was reviewed and considered by OEHHA for the establishment of the inhalation MADL.

[9]De Martino C, Malorni W, Amantini MC, Scorza Barcellona P, Frontali N (1987). Effects of respiratory treatment with N-hexane on rat testis morphology. I. A light microscopic study. Exp Mol Pathol. 46(2):199-216. PubMed PMID: 3556533.
Imai T and Omoto M (1999). A preliminary report on the tumorigenic effect of long-term exposure to n-hexane in the rat testis. J Occup Health 41(4): 261-262.
Nylen P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson AC, Kronevi T, Kvist U, Sjöstrand NO, Höglund G (1989). Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch Toxicol 63(4):296-307.
[10] Krasavage WJ, O'Donoghue JL, DiVincenzo GD and Terhaar CJ (1980). The relative neurotoxicity of methyl-n-butyl ketone, n-hexane and their metabolites. *Toxicol Appl Pharmacol* **52**(3): 433-441.
[11] Linder RE, Strader LF, Slott VL, Suarez JD (1992). Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. Reprod Toxicol 6(6):491-505. PubMed PMID: 1288759

Table 1. Male Reproductive Effects of *n*-Hexane in Rats in Inhalation Studies

STUDY (SPECIES)	EXPOSURE	FINDINGS	NOEL/LOEL
De Martino et al. 1987 (Rat)[12]	INHALATION (a) Single 24 hour exposure to solvent vapor at 0 or 5000 ppm. (b) Repeated 16 hours/day exposures daily up to 8 days or 6 days/week up to 6 weeks at 0 or 5000 ppm.	(a) (Testes): Focal degeneration of spermatocytes; increased severity of spermatocyte degeneration, severe depletion of spermatids and spermatocytes; total aplasia of seminiferous tubules with drastic decreases of the spermatogonia; epididymal tubules devoid of exfoliated germ cells; necrotic germ cells in epididymis (b) After 2-8 days, there were testicular lesions; massive exfoliation of normal and degenerated spermatids and spermatocytes; retracted apical cytoplasm of Sertoli cells.	LOEL = 5000 ppm
Imai and Omoto 1999 (Rat)[13]	INHALATION Measured concentration range 1100-900 ppm (mean 983 ± 32 ppm) for 4h/d 6 days/wk for 415 days, control received fresh air.	Leydig cell hyperplasia and Leydig cell tumors.	LOEL = 900-1100 ppm (mean 983 ± 32 ppm)
Nylen et al. 1989 (Rat)[14]	INHALATION 986 ppm ± 55 ppm 21 hrs/day 7 days/wk for 28 days; or, 999 ppm ± 29 ppm 18 h/day 7 days/wk for 61 days.	Severe testicular atrophy 2 wks, and 10, 12 and 14 mos post-exposure. Bilateral testicular damage 1 year after exposure; decreased testicular weight 10 months after exposure.	LOEL=~1000 ppm

[12] De Martino C, Malorni W, Amantini MC, Scorza Barcellona P, Frontali N (1987). Effects of respiratory treatment with N-hexane on rat testis morphology. I. A light microscopic study. Exp Mol Pathol. 46(2):199-216. PubMed PMID: 3556533.

[13] Imai T and Omoto M (1999). A preliminary report on the tumorigenic effect of long-term exposure to n-hexane in the rat testis. J Occup Health 41(4): 261-262.

[14] Nylen P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson AC, Kronevi T, Kvist U, Sjöstrand NO, Höglund G (1989). Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch Toxicol 63(4):296-307.

A brief summary of major findings on the dose-response relationship from the two oral male reproductive toxicity studies in rats is presented in Table 2. The studies identified were reviewed and considered by OEHHA for the establishment of the oral MADL.

Table 2. *n*-Hexane Male Reproductive Toxicity Findings from Oral Exposure Studies

STUDY (SPECIES)	EXPOSURE	FINDINGS	NOEL/LOEL
Linder et al., 1992 (Rat)	GAVAGE Dosed on day 0 (single dose or equal portion at 9 am and then at 4 pm)—20,000 mg/kg, and sacrificed on day 2 or 14. If results were negative or questionably positive, additional groups of animals were given 5 (10,000 mg/kg) daily doses (days 0-4) and sacrificed on day 8 or 17 (3 or 13 days after the last dose).	Single day exposure: increase in # of seminal vesicles in the group sacrificed on day 14 of study; decrease in testicular sperm head count in the animals sacrificed on day 2 of the experiment. 5-day exposure groups: decrease in prostate weight; increase in cauda sperm counts.	LOEL: 10,000 mg/kg
Krasavage et al., 1980 (Rat)	Oral Daily dose of 6.6 or 13.2 mmol/kg for 90 days, 46.2 mmol/kg for 120 days.	Reported atrophy of seminiferous epithelium at the end of 120-day treatment with 46.2 mmol/kg. No information on the testicular effects at lower doses.	LOEL = 46.2 mmol/kg (approximately 4000 mg/kg)

Among the three studies relevant to establishment of the inhalation MADL (Table 1), the study by De Martino et al. (1987) had a Lowest Observable Effect Level (LOEL) of 5000 parts per million (ppm), which is higher than the LOELs of about 1000 ppm in the studies by Imai and Omoto (1999) and Nylen et al. (1989). The effects seen in Imai and Omoto (1999) included Leydig cell hyperplasia and Leydig cell tumors. The study by Nylen et al. (1989) reported male reproductive toxicity endpoints of severe testicular atrophy, bilateral testicular damage, and decreased testicular weight. These effects were seen at approximately 1000 ppm both in rats exposed to the chemical for 21 hours/day for 28 days and in rats exposed to the chemical for 18 hours/day for 61 days. There were no No Observable Effect Levels (NOELs) in these studies.

Among the two studies relevant to establishment of the oral MADL (Table 2), the study by Krasavage et al., 1980 identifies a LOEL of 4,000 milligrams per kilogram of bodyweight per day (mg/kg-day) in rats based on reported atrophy of the seminiferous epithelium. This LOEL is lower than the LOEL of 10,000 mg/kg-day that resulted in a decrease in prostate weight and testicular sperm head count in the study by Linder et al. (1992). There were no NOELs in these studies.

STUDY BASIS FOR MADL CALCULATIONS

Among the three inhalation studies in rats, the study by Nylen et al. (1989) was identified as the most sensitive study deemed to be of sufficient quality, and thus was selected as the basis for the inhalation MADL.

The LOEL in the oral study by Krasavage et al. (1980) is lower than that observed in the study by Linder et al. (1992). Thus, for the purpose of calculating an oral MADL for *n*-hexane, the dose of 4000 mg/kg in the study of Krasavage et al. (1980) is identified as a LOEL.

MADL CALCULATIONS

The following calculations were performed in accordance with Section 25803 to derive the MADLs for *n*-hexane:

Inhalation exposure:

Nylen et al. exposed rats to *n*-hexane at a target concentration of 1000 ppm (actual reported values 986 ppm ± 55 ppm (mean ± standard error) for 21 hours per day, 7 days per week for 28 days or alternatively to 999 ppm ± 29 ppm for 18 hours per day, 7 days per week for 61 days). A concentration of 1000 ppm was used as the basis for the MADL.

Because the Nylen et al. (1989) study provided a LOEL rather than a NOEL, a NOEL was calculated by dividing the LOEL of 1000 ppm by 10, resulting in a NOEL of 100 ppm[15]. The dose group exposed for the fewest hours per day (18 hours) serves as the basis for the NOEL. The average bodyweight of the rats in this study was 0.275 kg.

[15] Section 25803(a)(8)

Conversion of the *n*-hexane air concentration in ppm to milligrams per cubic meter of air (mg/m^3) was performed by using a molecular weight for *n*-hexane of 86.18 grams per mole (g/mol) and a partial molar volume (i.e., the volume occupied by one mole of an ideal gas) of 24.45 at 25°C:

$$(100 \text{ ppm} \times 86.18 \text{ g/mol}) \div 24.45 = 352.47 \text{ mg/m}^3$$

The inhalation rate (IR) for male rats, in cubic meters per day (m^3/day), was calculated using the OEHHA (2018) inhalation rate equation for rats[16], which was derived using experimental data on animal breathing rates (m^3/day) and corresponding body weights (kg), and which is included here as an Attachment:

$$IR_{rats} = 0.702 \times bw_{rats}^{2/3}$$

The constant 0.702 in the rat inhalation rate equation is in m^3/day. This equation results in a rat inhalation rate of 0.30 m^3/day ($=0.702 \times 0.275^{2/3}$).

Calculation of the NOEL dose corresponding to a 0.275 kg rat breathing *n*-hexane at an air concentration of 352.47 mg/m^3, 18 hours per day, at an inhalation rate of 0.30 m^3/day is as follows:

$$(352.47 \text{ mg/m}^3 \times 0.3 \text{ m}^3/\text{day} \times 18 \text{ hours}/24 \text{ hours}) \div 0.275 \text{ kg (average rat weight)} = 288.4 \text{ mg/kg/day}$$

Calculation of the NOEL dose for a 70 kg man:

$$288.4 \text{ mg/kg/day} \times 70 \text{ kg} = 20{,}188 \text{ mg/day}$$

The inhalation MADL is derived by dividing the inhalation NOEL by one thousand (Section 12801(b)(1)).

$$20{,}188 \text{ mg/day} \div 1000 = 20{,}188 \text{ µg/day or } 20{,}000 \text{ µg/day after rounding}$$

$MADL_{inhalation}$ = 20,000 µg/day

[16] OEHHA (2018). Calculation of Rat Breathing Rate Based on Bodyweight. OEHHA, California Environmental Protection Agency, May 2018. Included here as an Attachment.

Oral exposure:

Because the Krasavage et al. (1980) study provided a LOEL rather than a NOEL, a NOEL for purposes of assessment was calculated by dividing the LOEL of 4,000 mg/kg-day by 10, resulting in a NOEL of 400 mg/kg-day[17].

Calculation of NOEL in mg/day for a 70 kilogram (kg) man (Section 25803(b)):

400 mg/kg-day x 70 kg = 28,000 mg/day

The oral MADL is derived by dividing the oral NOEL expressed in mg/day by one thousand (Section 25801(b)(1)):

28,000 mg/day ÷ 1000 = 28,000 µg/day

$MADL_{oral}$ = 28,000 µg/day

PROPOSED REGULATORY AMENDMENTS

The proposed changes to Section 25805(b) are provided below in underline:

Chemical name	*Level (micrograms per day)*
n-Hexane (oral)	28,000
n-Hexane (inhalation)	20,000

PROBLEM BEING ADDRESSED BY THIS PROPOSED RULEMAKING

Proposition 65 does not provide guidance regarding how to determine whether a warning is required or a discharge is prohibited. OEHHA is the implementing agency for Proposition 65 and has the authority and expertise to examine the scientific literature and calculate a level of exposure, in this case a MADL, that does not require a warning or at which a discharge is not prohibited.

[17] Section 25803(a)(8)

NECESSITY

These proposed regulatory amendments would adopt oral and inhalation MADLs that conform to the Proposition 65 implementing regulations and reflect the currently available scientific knowledge about *n*-hexane. A MADL provides assurance to the regulated community that exposures or discharges at or below it are considered not to pose a significant risk of developmental or reproductive harm. Exposures at or below the MADL are exempt from the warning and discharge requirements of Proposition 65[18].

BENEFITS OF THE PROPOSED REGULATION

See "Benefits of the Proposed Regulation" under ECONOMIC IMPACT ANALYSIS below.

TECHNICAL, THEORETICAL, AND/OR EMPIRICAL STUDIES, REPORTS, OR DOCUMENTS

In determining the evidence and standards that formed the basis for listing *n*-hexane under Proposition 65, OEHHA reviewed the scientific literature. These documents included numerous studies of the effects of *n*-hexane, including *in vivo* studies in experimental animals that provide evidence of male reproductive toxicity.

OEHHA relied on the following studies:

- Krasavage WJ, O'Donoghue JL, DiVincenzo GD and Terhaar CJ (1980). The relative neurotoxicity of methyl-n-butyl ketone, *n*-hexane and their metabolites. *Toxicol Appl Pharmacol* **52**(3): 433-441.
- Nylen P, Ebendal T, Eriksdotter-Nilsson M, Hansson T, Henschen A, Johnson AC, Kronevi T, Kvist U, Sjöstrand NO and Höglund G (1989). Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to *n*-hexane and a protective effect of simultaneous exposure to toluene or xylene. *Arch Toxicol* 63 (4): 296-307.

OEHHA also relied on the 2018 OEHHA document entitled "Calculation of Rat Breathing Rate Based on Bodyweight"[19] included here as an Attachment, and on the attached Economic Impact Analysis in developing this proposed regulation.

[18] Health and Safety Code sections 25249.9(b) and 25249.10(c).
[19] OEHHA (2018), full citation provided in footnote 18.

REASONABLE ALTERNATIVES TO THE REGULATION AND THE AGENCY'S REASONS FOR REJECTING THOSE ALTERNATIVES

MADLs provide "safe harbor" values that aid businesses in determining if they are required to provide a warning for a given exposure or prohibited from discharging a listed chemical. The alternative to the proposed amendments to Section 25805(b) would be to not promulgate MADLs for the chemical. Failure to promulgate these MADLs would leave the business community without a safe harbor level to assist businesses in complying with Proposition 65. No alternative that is less burdensome yet equally as effective in achieving the purposes of the regulation in a manner that achieves the purposes of the statute has been proposed.

REASONABLE ALTERNATIVES TO THE PROPOSED REGULATORY ACTION THAT WOULD LESSEN ANY ADVERSE IMPACT ON SMALL BUSINESSES

OEHHA is not aware of significant cost impacts that small businesses would incur in reasonable compliance with the proposed action. Use of the proposed MADLs by businesses is voluntary and therefore does not impose any costs on small businesses. In addition, Proposition 65 is limited by its terms to businesses with 10 or more employees (Health and Safety Code, section 25249.11(b)), so it has no effect on very small businesses.

EVIDENCE SUPPORTING FINDING OF NO SIGNIFICANT ADVERSE ECONOMIC IMPACT ON BUSINESS

Because the proposed MADLs provide "safe harbor" levels for businesses to use to comply with Proposition 65, OEHHA does not anticipate that the proposed regulation will have a significant statewide adverse economic impact directly affecting businesses, including the ability of California businesses to compete with businesses in other states.

EFFORTS TO AVOID UNNECESSARY DUPLICATION OR CONFLICTS WITH FEDERAL REGULATIONS CONTAINED IN THE CODE OF FEDERAL REGULATIONS

Proposition 65 is a California law that has no federal counterpart. There are no federal regulations addressing the same issues and, thus, there is no duplication or conflict with federal regulations.

ECONOMIC IMPACT ANALYSIS
Gov. Code section 11346.3(b)

It is not possible to quantify any monetary values for this proposed regulation because its use is voluntary and it only provides compliance assistance for businesses subject to the Act.

Creation, Elimination, or Expansion of Jobs/Businesses in California

This regulatory proposal will not affect the creation or elimination of jobs within the State of California. Proposition 65 requires businesses with ten or more employees to provide warnings when they expose people to chemicals that are known to cause cancer or developmental or reproductive harm. The law also prohibits the discharge of listed chemicals into sources of drinking water. n-Hexane is listed under Proposition 65; this regulatory proposal identifies a level of exposure to *n*-hexane that exempts r businesses from the warning requirement and discharge prohibition.

Creation of New Businesses or Elimination of Existing Businesses within the State of California

This regulatory action will not impact the creation of new businesses or the elimination of existing businesses within the State of California. The regulatory proposal does not create additional compliance requirements, but instead provides “safe harbor” values that aid businesses in determining if they are complying with the law with respect to n-hexane.

Expansion of Businesses within the State of California

This regulatory action will not impact the expansion of businesses within the State of California. The regulatory proposal does not create additional compliance requirements, but instead provides “safe harbor” values that aid businesses in determining if they are complying with the law.

Benefits of the Proposed Regulation

The MADL provides a “safe harbor” value that aids businesses in determining if they are complying with the law. Some businesses may not be able to afford the expense of establishing a MADL and therefore may be exposed to litigation for a failure to warn or for a prohibited discharge of the listed chemical. Adopting this regulation will save these businesses those expenses and may reduce litigation costs. By providing a safe harbor level, this regulatory proposal does not require, but may encourage, businesses to lower the amount of the listed chemical in their product to a level that does not cause a significant exposure, thereby providing a public health benefit to Californians.