EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

CYCLOHEXANOL

DRAFT

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Preface

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that one of the mechanisms by which "a chemical is known to the state to cause cancer or reproductive toxicity [is] if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant (DART) Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (Title 22, California Code of Regulations, Section 12301 (22 CCR 12301)). The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency.

Another of the mechanisms by which a chemical may be put on the Proposition 65 list is if the chemical has been formally identified as causing cancer or reproductive toxicity by an organization that has been designated by the State's qualified experts as "authoritative" for purposes of Proposition 65. One such "authoritative body" is the U.S. Environmental Protection Agency (22 CCR 12306).

Cyclohexanol was added to the Proposition 65 list of chemicals known to the state to cause reproductive toxicity under the authoritative bodies provision of Proposition 65, based on formal identification as causing male reproductive toxicity by the U.S. Environmental Protection Agency. Subsequent to that listing, a Court of Appeal decision restricted the evidence that could be reviewed by OEHHA for potential authoritative bodies listings [Third District Court of Appeal, Western Crop Protection et al. vs. Gray Davis et al. (Case No. CO29727, May 9, 2000 as modified on denial of rehearing, June 8, 2000)]. In conducting the more restrictive review of the evidence, per that decision, OEHHA has found that there is no substantial evidence that the scientific criteria for listing cyclohexanol were met (22 CCR 12306(g)). As required by regulation (22 CCR 12306(j)), cyclohexanol is being referred to the DART Identification Committee for a recommendation as to whether it should remain on the Proposition 65 list.

This draft document provides the DART Identification Committee with information relevant to the reproductive toxicity of this chemical. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held on December 17, 2001, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether cyclohexanol "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

A. ABSTRACT

Cyclohexanol (CAS No. 108-93-0) is used in the production of nylon, lacquers, paints, varnishes, degreasers, plastics and plasticizers, soaps and detergents, textiles, and insecticides. Cyclohexanol can be absorbed via the oral, inhalation or dermal routes of exposure.

There were no human data available pertaining to the potential developmental or reproductive toxicity of cyclohexanol. With respect to developmental toxicity, data from a study conducted in mice indicated an adverse effect of cyclohexanol on pup postnatal growth when treatment was continuous from prior to conception, throughout gestation and lactation, and into the postweaning period. No multigeneration reproductive toxicity studies were available, nor were there any data pertaining to endpoints of female reproductive toxicity. A seven-day gavage study of male rats showed no effect of cyclohexanol on testicular weights. Two longer-term studies focusing on the male reproductive system in gerbils, rats, and rabbits found cyclohexanol exposure to be associated with changes in testes and epididymal weights, histopathological changes in these organs, and altered biochemistry.

B. INTRODUCTION

B.1 Chemical Structure and Main Physical Characteristics

Cyclohexanol (CAS No. 108-93-0) (Hexahydophenol, Hexalin, Hydroxycyclohexane) is a colorless to light-yellow, viscous liquid, or a sticky solid, with a molecular weight of 100.16, and a formula of $C_6H_{12}O$ (HSDB, 2001; ACGIH, 1996; Merck, 1989). It is described as having a camphor- or menthol-like odor. It is soluble in water (3.6 g/100 g at 20 degrees C); and miscible with aromatic hydrocarbons, ethanol, ethyl acetate, linseed oil and petroleum solvents.

B.2 Regulatory History

The American Conference of Governmental Industrial Hygienists lists a TWA of 50 ppm for cyclohexanol (ACGIH, 1999), with a skin notation, based on irritation and CNS effects. The OSHA PEL and NIOSH REL-TWA are in agreement with the ACGIH recommendations (OSHA, 2000). The NIOSH IDLH (Immediately Dangerous to Life or Health) level has been set at 3,500 ppm (NIOSH/CDC, 1996).

Cyclohexanol was added to the Proposition 65 list of chemicals known to the state to cause reproductive toxicity effective November 6, 1998. This listing took place under the authoritative bodies provision of Proposition 65, based on a formal identification of cyclohexanol as causing male reproductive toxicity by the U.S. Environmental Protection Agency, an identified authoritative body (22 CCR 12306). Cylcohexanol is not listed as a carcinogen under Proposition 65, or as a Toxic Air Contaminant under California AB 1807, and there is no Public Health Goal for cyclohexanol in California drinking water.

B.3 Exposure Information

Cyclohexanol is used in the production of nylon, lacquers, paints, varnishes, degreasers, plastics and plasticizers, soaps and detergents, textiles, and insecticides (ACGIH, 1999). The 1983 National Occupational Exposure Survey estimated 68,715 U.S. workers are potentially exposed to cyclohexanol (HSDB, 2001). Occupational exposures may occur though inhalation, or via dermal contact with cyclohexanol-containing solutions (HSDB, 2001).

Reported environmental releases of cyclohexanol for the U.S. in 1999 totaled 3,892,943 pounds (U.S. EPA, 2001). Toxic Release Inventory (TRI) data specific to California were not reported. Exposure of the general population to cyclohexanol is most likely to occur via ingestion of contaminated drinking water or food, inhalation of contaminated air, or dermal contact with contaminated water (HSDB, 2001).

B.4 Pharmacokinetics

There are no toxicokinetic data in humans, and no quantitative data on absorption and distribution of cyclohexanol in animals (Dutch Expert Committee, 1990).

B.4.1 Absorption

Cyclohexanol can be absorbed via the oral, inhalation, or dermal routes of exposure (HSDB, 2001; ACGIH, 1999). Rabbits displayed similar pathologic profiles when cyclohexanol was administered by either the oral or dermal routes, thus providing evidence of toxicologically significant dermal absorption (Rowe and McCollister, 1982). Cyclohexanol has even been reported to be one of a number of alcohols that can increase the skin penetration of externally applied drugs (Opdyke, 1975).

B.4.2 Metabolism and excretion

Hepatic NAD-dependent alcohol dehydrogenase (ADH) is primarily responsible for the oxidation of cyclohexanol (HSDB, 2001). At the same time, the potential for cyclohexanol to serve as a substrate for ADH in tissues such as placenta, ovary, testis, and adrenal appears to be low (Dutch Expert Committee, 1990). Compared to ethanol, cyclohexanol as a substrate for ADH has a relative velocity or maximal turnover rate of 67%, but a stronger affinity for the enzyme (Rowe and McCollister, 1982). One study demonstrated an initial rate of oxidation for cyclohexanol of 135 mol/L/min per mole of ADH (the same as for ethanol and *n*-octanol) (Rowe and McCollister, 1982).

Following oral or inhalation exposure of rabbits to cyclohexanol, most of the compound is excreted in the urine following sulfation or glucuronidation (Rowe and McCollister, 1982; U.S. EPA, 1985). The majority is excreted as cyclohexyl glucuronide (Dutch Expert Committee, 1990).

With inhalation exposure to cyclohexanol at concentrations ranging from 145 to 1229 ppm, some conjugation with sulfates occurred during exposure to all but the lowest concentration (Treon et al., 1943b). Pre-exposure urine samples demonstrated a daily average glucuronic-acid content of 53 mg per rabbit. Following exposure to 145 ppm cyclohexanol, these same animals showed an increase of glucuronic acid output to 289 mg per rabbit. When treatment ceased, normal excretion levels (58 mg/rabbit) returned.

Since the ratio of inorganic sulfates to total sulfates, and glucuronic-acid excretion in the urine were altered during exposure, and returned to normal with the cessation of exposure, Treon et al. (1943b) concluded that the metabolic disposition of cyclohexanol was rapid and without prolonged retention in the organism. The Dutch Expert Committee (1990) calculated a $t_{1/2}$ for metabolite excretion of about 12 hours, and opined that accumulation may occur over the short term.

B.5 Non-DART Toxicity

B.5.1 Acute

Human subjects exposed to 100 ppm cyclohexanol for three to five minutes experienced irritation of the eyes, nose, and throat (U.S. EPA, 1985).

According to the Registry of Toxic Effects of Chemical Substances (RTECS, 2000), the lowest published toxic dose/concentration (TDLo/TCLo) for acute inhalation exposure in humans was 75 ppm. The reported effects included conjunctival irritation.

The acute oral LD_{50} for cyclohexanol in the rat has been reported as 2.06 g/kg (ACGIH, 1999; U.S. EPA, 1985; Dutch Expert Committee, 1990). RTECS (2000) lists an oral LD_{50} for the rat of 1400 mg/kg, and an inhalation lethal concentration (LC) for that species of 6500 mg/m^3.

For the mouse, the Dutch Expert Committee (1990) lists an intravenous LD_{50} of 270 mg/kg, subcutaneous LD_{50}s of 2.48 and 1.42 g/kg, and an intraperitoneal LD_{50} of 760 mg/kg.

The minimum lethal dose by the oral route in rabbits was between 2.2 and 2.6 g/kg bw (Treon et al., 1943a). All four rabbits given oral doses of cyclohexanol of between 0.8 and 2.2 g/kg survived. The sequence of symptoms following oral administration of cyclohexanol to rabbits was: ataxia, lethargy, collapse, narcosis, loss of reflexes, and death. Animals were described as being on their sides in 10 to 45 minutes following dosing. Surviving animals recovered from narcosis in 5.5 to 16 hours.

RTECS (2000) records a TDLo of 794 mg/kg for dermal exposure in the rabbit. Twenty-four hour skin irritation tests in the rabbit showed a mild or moderate reaction with an application of 500 µl (RTECS, 2000). Twenty-four hour eye irritation tests in the rabbit showed a mild or moderate reaction at 100 µl (RTECS, 2000). Treon et al. (1943a) reported a minimum lethal dose of 12.4-22.7 g/kg for cyclohexanol applied dermally to rabbits. Prior to death, the clinical symptoms observed for these exposures included marked hypothermia, convulsive movements, and narcosis.

B.5.2. Subchronic

A number of intermediate-duration studies have been conducted on cyclohexanol by the dermal, oral, or inhalation routes of exposure. Little data are available from human studies, most of the available information comes from studies conducted in experimental animals.

B.5.2.1. Human studies

Human subjects given a 48-hour closed patch test using 4% cyclohexanol in petrolatum, showed no evidence of irritation (Rowe and McCollister, 1982).

B.5.2.2. Animal studies

B.5.2.2.1. Oral route

Cyclohexanol was evaluated in a study of dicyclohexyl phthalate (DCHP), along with another DCHP metabolite, monocyclohexyl phthalate (MCHP) (Lake et al., 1982). Cyclohexanol was given to twelve 30-day old rats of the Sprague-Dawley strain (initial body weights not specified), by gavage, at a dose of 455 mg/kg-day for seven days. Control animals were given 5 ml corn oil/kg bw by gavage.

All three compounds were associated with liver enlargement, and the induction of some parameters of hepatic xenobiotic metabolism. In the case of cyclohexanol, relative liver weights were significantly increased ($p < 0.001$), as were the activities of hepatic biphenyl 4-hydroxylase, 7-ethoxycoumarin o-deethylase, and aniline 4-hydroxylase ($p < 0.001$); and cytochrome P-450 content ($p < 0.05$). There was no effect of treatment on relative kidney or testes weights. While histological evaluation of the testes was performed for animals treated with the other two compounds, there was no mention of a histological evaluation of testicular tissue from cyclohexanol-treated animals.

B.5.2.2.2. Dermal route

As a model for cyclohexanol-containing soap products, five grams of an ointment consisting of potassium oleate with 0, 5, 10, or 15% cyclohexanol was applied to rabbit skin (clipped free of fur) for 15 consecutive days, Sundays excluded (Treon et al., 1943a). It is not noted whether the treated areas were covered, or if the animals were restrained during the treatment period. One hour following each day's application, the treated area of skin was rinsed with water and dried. The skin of treated animals became reddened and streaked with dilated blood vessels. The effect was minimal at the lowest percentage of cyclohexanol, but graded into superficial sloughing of the skin and lacrimation (apparently resulting from absorbed cyclohexanol) at the higher concentrations. None of these animals died or exhibited hypothermia, but treated animals did lose weight over the dosing period--an effect not seen in control animals. Weight gain and skin appearance returned to normal in a short time after cessation of treatment. Reproductive endpoints were not evaluated.

In another experiment reported in the same paper (Treon et al., 1943a), a rabbit had a total of 94.4 g cyclohexanol/kg bw applied to the skin daily for 10 days (Sundays excepted); the material was washed off one hour following each application. Initial responses consisted of local injury, temporary narcosis, tremor, and athetoid movements. Hypothermia became increasingly marked with subsequent exposures. The treatment area eventually developed ulcers and localized thickening of the skin. The rabbit died on the day following the 10^{th} treatment.

B.5.2.2.3. Inhalation route

Treon et al. (1943b) conducted four experiments in which rabbits (four rabbits per concentration) were exposed to cyclohexanol by inhalation at concentrations ranging from 145 ppm for 300 hours, to 1229 ppm for 150 hours. Exposure periods were 6 hours/day, 5 days/week, for 5 to 11 weeks. Reproductive endpoints were not evaluated in these experiments. The lowest lethal concentration was 997 ppm. Two out of four rabbits exposed to cyclohexanol by inhalation at concentrations of 1229 ppm for 150 hours, or to 997 ppm for 300 hours, died after demonstrating convulsions and tremors. Exposure to the two highest concentrations was always accompanied by conjunctival irritation, lacrimation, salivation, lethargy, and distention of the ear veins. Narcosis was induced only during the first few exposures to these concentrations, and animals lost weight over the exposure period.

No rabbits died at the lower exposure level of 272 ppm (300 hours), although slight conjunctival irritation and ear-vein distention were observed (Treon et al., 1943b). No deaths or clinical symptoms were observed at the lowest concentration of 145 ppm for 300 hours. Animals exposed to these two lower concentrations gained weight during the exposure period. Minimal, but definite, degenerative changes were found in the liver and kidneys of rabbits exposed only to the lower concentration of 145 ppm cyclohexanol.

One monkey was exposed to cyclohexanol by inhalation at a concentration of 693 ppm, for a total of 300 hours (Treon et al., 1943b). The animal survived this exposure, demonstrating a weight loss of 193 grams, as well as lethargy, conjunctival irritation, and slight ear-vein distention. "Barely demonstrable" microscopic changes were observed in the heart muscle, lungs, liver, and kidneys of this animal.

U.S. EPA (1985) summarizes additional studies of cyclohexanol toxicity; in some cases these summaries are based on other reviews of the literature. The Dutch Expert Committee (1990) includes discussion of some of the same studies. One study, published in the Russian language, involved exposure of rats to 0.15 ppm cyclohexanol by inhalation, 24 hours/day, for 87 days—including a 6-day period on rations restricted to 20% of the normal quantity consumed. Findings included "changes in the interrelationship of the chonaxie of the extensor and flexor muscles," as well as a decrease in cholinesterase activity, and an increase in the ascorbic acid content of the liver. These effects were not reported at a lower concentration of 0.014 ppm, presumably under the same conditions of exposure.

Another Russian study found hypoxia in rats exposed to 10 ppm cyclohexanol, 5-6 hours/day, for 100 days (as summarized by: U.S. EPA, 1985; Dutch Expert Committee, 1990). Lung and blood levels of Cu were decreased, and Fe and Ni levels were increased.

B.5.3 Chronic

Twenty-five percent of 453 people exposed daily to "less than the 'permitted' concentration of cyclohexanol" showed "nonspecific disturbances of the autonomic nervous system" during a two-year period. Only eight percent of 100 non-exposed controls had similar disturbances (Rowe and McCollister, 1982; ACGIH, 1999).

B.5.4 Other

Cyclohexanol was not found to be mutagenic in any of several strains of *Salmonella typhimurium*, at concentrations of 500 µg/plate, with or without the presence of a rat liver homogenate activation system (ACGIH, 1999). U.S. EPA's (1985) *Health and Environmental Effects Profile for Cyclohexanol* summarizes the results of three mutagenicity studies on cyclohexanol, one conducted using cultured human leukocytes, and two using Drosophila melanogaster. None were positive.

C. DEVELOPMENTAL TOXICITY

No information was available on the potential developmental toxicity of cyclohexanol in humans, and few data were available from experimental animals.

C.1. Mammalian studies. Developmental toxicity in TB and NMRI mice: Gondry 1972

A 1.0% solution of cyclohexanol was given as a dietary additive to female mice of the TB or NMRI strains. The number of parental animals was not specified in the paper. Treatment was begun prior to conception, and continued throughout gestation and lactation. Weaned young of 21 days postnatal age were continued on the diet. The Dutch Expert Committee (1990) estimated that the dose of cyclohexanol used in the Gondry (1972) study was approximately 1200 mg/kg-day. Pup mortality was determined at the time of weaning (i.e. postnatal day 21), and growth studies were initiated at that time. Pre-weaning pups were not weighed.

When offspring mortality was evaluated on postnatal day 21, five out of 35 cyclohexanol-treated TB pups had died, as compared to 11 deaths out of 92 control pups (14.1 and 11.9%, respectively). For NMRI mice, 22 out of 51 cyclohexanol-exposed pups died, as compared to 14 out of 116 controls (43.1 and 12.2%, respectively). No statistical evaluation of the data was reported in this study.

Treatment of TB animals was continued for an additional generation, and the mortality of the second generation was increased to 52 deaths out of 97 animals (53.5%). There were no data for a second generation of control animals.

"Considerable" inhibition of postnatal growth between postnatal days 21 and 110 was also observed in first and second-generation females exposed to cyclohexanol during gestation. The data do not appear to have been subjected to statistical analysis. The

growth of male offspring was less affected, even showing increases over control values at some time points.

C.2. Non-mammalian studies. Toxicity studies in fertilized zebrafish eggs: Groth et al. 1993.

Fertilized zebrafish eggs were cultured individually on microplates. Test substances were added to the culture media when embryos reached the 8-cell stage. Cyclohexanol was tested at concentrations ranging from 1-30 mmol/L. Twelve eggs were used for each concentration and the control group. Eggs were observed microscopically for about 96 hours until hatching.

No deaths or morphological changes were observed in control fish embryos. For cyclohexanol-treated embryos, the NOEL was 3 mMol cyclohexanol/L culture medium. The LC_{50} (95% confidence interval) was 13.8 mMol/L (13.7-13.9 mMol/L); the LC_{100} was 16 mMol/L. The abnormalities observed included: edematous enlargement of the pericardial space in approximately 20% of treated embryos, deformation of the skeleton and muscle apparatus in approximately 33% of treated embryos, and retardation of body development in approximately 20% of treated embryos.

D. FEMALE REPRODUCTIVE TOXICITY

No data concerning the potential for cyclohexanol to cause female reproductive toxicity in either humans or animals were available in the literature. Gondry (1972) treated female mice during mating, gestation, and lactation, but no data were presented on parameters of relevance to female reproductive toxicity. The study is discussed above (Section C.1).

E. MALE REPRODUCTIVE TOXICITY

No data were available pertaining to the male reproductive toxicity of cyclohexanol in humans. Relevant animal studies were available, but there were no standard multigeneration reproductive toxicity studies.

E.1. Studies of the male reproductive system

E.1.1. Antispermatogenic activity of cyclohexanol in gerbils and rats: Tyagi et al. 1979.

Cyclohexanol, at a dose of 15 mg/kg-day, was given by subcutaneous (s.c.) injection to 20 adult male gerbils (*Meriones hurriane* Jerdon) and 20 adult male rats (*Rattus rattus* Rufescens). The ages of the animals used in these experiments were not reported. No mention is made of the purity of the cyclohexanol, or whether any injection vehicle was used, or of the injection volume, or the site of the injection. Control animals were given injections of distilled water. Treatment periods were 21 days for gerbils and 37 days for rats. There is no mention of in-life observations or interim body weights. The animals

were sacrificed for evaluation 24 hours following the final dose of cyclohexanol.

Whole body weights were taken, and the male reproductive organs and glands were removed, weighed, and processed for light microscopy. Tissues were fixed in Bouin's fluid and infiltrated with paraffin. Sections of six µm thickness were cut and stained with hematoxylin and eosin. Diameters of seminiferous tubules and Leydig cell nuclei were measured on camera lucida tracings of selected sections. There is no mention of any pathological evaluation of non-reproductive tissues.

Though actual body weights were not provided in the paper, cyclohexanol exposure was reported not to be associated with loss in body weight for either species. In both species, significant weight reductions were reported for testes, epididymides, and ventral prostate ($p<0.01$ or 0.001). In the rat, mean weight of the seminiferous vesicles was reported to be significantly decreased relative to controls ($p < 0.01$). For gerbils, the mean and standard error for seminiferous vesicle weight of cyclohexanol-exposed animals were reported as 255 ± 49 mg/100g body weight, as compared to 407 ± 83 mg/100g body weight for controls. This difference was not flagged as statistically significant in the tabulated results. Thyroid and adrenal gland weights were not observed to be affected. Specific data for body, thyroid, and adrenal weights were not presented in the paper.

Histopathology revealed marked degenerative changes in the seminiferous tubules of males of both species. The paper reports losses of type-A spermatozoa, spermatocytes, spermatids, and spermatozoa. Sertoli cells were described as demonstrating vacuolation of the cytoplasm to varying degrees. Seminiferous tubules and Leydig cell nuclei were described as shrunken. Leydig cell cytoplasm was considered to be weakly eosinophilic and highly vacuolated.

Luminal epithelia of the epididymides of both gerbils and rats were stated to be normal, and sterocilia present. The lumina were considered to be empty, though some showed the presence of degenerating cells. Lumina of the ductus deferens were also reported to be empty.

The protein, RNA, and sialic acid contents of testis, epididymis, and seminal vesicles were significantly ($p < 0.01$) decreased (on a µg/mg tissue basis) in cyclohexanol-treated males of both species. Testicular glycogen content was significantly decreased in treated males of both species ($p < 0.01$), while testicular chlolesterol and alkaline phosphatase contents of treated animals were significantly increased (on a mg/g tissue basis) over control values ($p < 0.01$).

Quantitatively, serum protein and cholesterol, blood sugar, urea, alkaline phosphatase, and serum transaminases for treated animals were all considered to be within the normal range. Alkaline phosphatase activity, however, was significantly increased ($p < 0.02$) in cyclohexanol-exposed gerbils. Hematological parameters (red cell count, hemoglobin, packed cell volume, and leucocytes) were also considered to be normal. These endpoints were taken to indicate normal functioning of the liver, kidney, and other organs in cyclohexanol-treated animals, while at the same time specific male reproductive toxicity

was indicated.

E.1.2. Effects on testes and epididymides of male rabbits: Dixit et al. 1980

Cyclohexanol diluted with olive oil was given orally to male rabbits (of unspecified strain and age) at a dose of 25 mg/kg-day for 40 days. At commencement of the experimental period, the animals weighed between 1.5 and 2.0 kg. The specific means of oral administration was not stated, but is presumed to have been gavage. Purity of the cyclohexanol used is not noted in the paper.

There is no mention of in-life observations or interim body weights. One group of five treated animals ("treated") was evaluated 24 hours following administration of the final dose of cyclohexanol. An additional group of five treated animals ("treated/recovery") was allowed a 70-day recovery period before evaluation. The third group of animals ("control") served as a vehicle control.

For each animal, the right testis and epididymis were weighed, and fixed using Bouin's fluid in preparation for paraffin sectioning. The left testis and epididymis were frozen for later determinations of total protein, RNA, sialic acid, glycogen, and acid phosphatase; adrenal ascorbic acid contents were also determined. General data were also collected on: blood hemoglobin content, packed cell volume, serum proteins, cholesterol, phospholipids, triglycerids, hepatic function, serum transaminase (SGPT), acid/alkaline phosphatase, blood sugar, and bilirubin.

Final mean body weights and adrenal weights (relative to body weights) did not differ among groups of rabbits. As neither mean starting weights, nor interim weights were provided, it is not clear whether animals within each group might have lost (or failed to gain) weight over the course of the study. As compared to controls, significant reductions ($p < 0.01$) in relative testes and epididymal weights were found in treated rabbits evaluated shortly after the cessation of treatment.

Histological examination of the livers did not reveal necrosis in any of the groups. Histopathological evaluation of the testes of treated animals noted loss of type A spermatogonia, spermatocytes, spermatids, and spermatozoa. In the epididymides of these animals, the luminal epithelium was reported to be reduced and the stereocilia scanty. The diameters of seminiferous tubules and Leydig cell nuclei were significantly reduced ($p < 0.01$).

Testicular and epididymal contents of protein, RNA, sialic acid, glycogen, and acid phosphatase were all significantly reduced ($p < 0.01$) in treated animals, as was adrenal ascorbic acid content. Serum analysis of treated animals demonstrated significant increases ($p < 0.01$) in cholesterol, SGPT, phospholipids, triglycerids, alkaline phosphatase activity, and bilirubin. Serum protein was significantly decreased ($p < 0.01$) in this same group of animals, while acid phosphatase activity, blood sugar, and urea levels were unchanged from control values.

Marked recovery was noted in treated/recovery animals. The relative testicular and epididymal weights of these male rabbits did not quite reach control values, but were significantly greater ($p < 0.01$) than those of treated animals that were not allowed to recover. In treated/recovery animals, following the 70-day recovery period, the histological evidence indicated normal spermatogenesis. Along with organ weights, the seminferous tubule and Leydig cell nuclear dimensions were restored to normal. Biochemical endpoints for reproductive tissues showed at least some recovery as compared to the treated group; most measures were restored to control values. Serum protein, SGPT, tryglycerid, acid phosphatase activity, blood sugar, and urea levels were not significantly different from controls. Significant departures ($p < 0.01$) from control values were seen in this group for cholesterol, phospholipids, alkaline phosphatase, and bilirubin.

E.1.3. Effects of orally administered dicyclohexyl phthalate and cyclohexanol in rats: Lake et al. 1982

Cyclohexanol was evaluated in a study of dicyclohexyl phthalate (DCHP), along with another DCHP metabolite, monocyclohexyl phthalate (MCHP) (Lake et al., 1982). Cyclohexanol was given to twelve 30-day old rats of the Sprague-Dawley strain (initial body weights not specified), by gavage, at a dose of 455 mg/kg-day for seven days. Control animals were given 5 ml corn oil/kg bw by gavage. While the cyclohexanol is stated to have been purchased from a major vendor, the exact purity is not noted. Animals were sacrificed for evaluation at 24 hours following the last dose.

All three compounds were associated with liver enlargement, and the induction of some parameters of hepatic xenobiotic metabolism. DCHP treatment did not affect kidney or testes weights, but histological evidence of testicular damage was reported for animals exposed to 2500 mg/kg-day DCHP. Histopathological evaluation of tissues from one animal of this group revealed "bilateral tubular atrophy of 30-40% of the germinal cells of the testes." MCHP was reported to produce "marked testicular atrophy," in terms of significantly reduced testes weights, as well as almost complete bilateral atrophy of the germinal epithelium of the seminiferous tubules by histological evaluation.

In the case of cyclohexanol, relative liver weights were significantly increased ($p < 0.001$), as were the activities of hepatic biphenyl 4-hydroxylase, 7-ethoxycoumarin o-deethylase, and aniline 4-hydroxylase ($p < 0.001$); and cytochrome P-450 content ($p < 0.05$). There was no effect of treatment on relative kidney or testes weights. There was no mention of a histological evaluation of testicular tissue from cyclohexanol-treated animals.

F. INTEGRATIVE EVALUATION

F.1. Developmental Toxicity

The available data on the potential developmental toxicity of cyclohexanol consists of one study conducted in mice (Gondry, 1972), and a supplementary study conducted in

zebra fish (Groth et al., 1993). In the case of the Gondry (1972) study, cyclohexanol was administered continuously from prior to conception, throughout gestation and lactation, and into the postweaning period. Offspring mortality and growth were not evaluated prior to postnatal day 21. As currently interpreted, Proposition 65 excludes consideration of developmental toxicity resulting from exposures during postnatal life.

The zebra fish embryo study reported an LC_{50} (95% confidence interval) of 13.8 mMol cyclohexanol/L culture medium for morphological abnormalities. The abnormalities among the treated embryos included edematous enlargement of the pericardial space, deformation of the skeleton and muscle apparatus and retardation of body development.

F.2. Female Reproductive Toxicity

None of the available data address the potential female reproductive toxicity of cyclohexanol. There are no relevant studies conducted in humans, and none of the subchronic toxicity studies conducted in animals appear to have collected data on the weights or histopathology of female reproductive organs. While the Gondry (1972) study involved treatment of dams prior to conception, as well as throughout pregnancy and lactation, the only data presented were taken on post-weaning-age offspring. While the mortality and growth deficits observed in these offspring might have been at least partially due to effects on the reproductive capacity of their dams (such as lactational insufficiency), the data do not directly address this possibility.

F.3. Male Reproductive Toxicity

Neither human data relevant to the potential male reproductive toxicity of cyclohexanol, nor multigeneration reproductive toxicity studies were available. Two studies reported adverse effects on the male reproductive system following cyclohexanol exposure (Tyagi et al., 1979; Dixit et al., 1980). The Tyagi et al. (1979) study used subcutaneous injection as the route of exposure for rats and gerbils, while Dixit et al. (1980) used the oral route for rats. A third study, an oral toxicity study conducted in male rats (Lake et al., 1982), found no evidence for a change in testes weights associated with cyclohexanol exposure.

The findings of the Dixit et al. (1982) and Tyagi et al. (1979) studies were substantially in agreement, despite the use of different species and routes of exposure. Dixit et al. (1982) gave oral cyclohexanol to male rabbits at a dose of 25 mg/kg-day for 40 days. Animals evaluated 24 hours following the end of treatment showed reductions in relative testes and epididymal weights, and adverse structural effects observable via histopathology. Alterations were also noted in certain testicular and epididymal biochemical parameters (such as protein and RNA content). Significant recovery was noted in animals returned to control conditions for 70 days following the treatment period. When cyclohexanol was given by the subcutaneous route at a dose of 15 mg/kg-day for 21 or 37 days (gerbils and rats, respectively), adverse effects were seen on the weights and histological appearance of male reproductive organs (Tyagi et al., 1979). Biochemical endpoints were altered in

male reproductive tissues, but serum biochemistry and hematological data were within control ranges.

Lake et al. (1982) evaluated cyclohexanol as part of a seven day study on dicyclohexyl phthalate (DCHP) and its metabolites. Cyclohexanol was given to 30-day old male rats, by gavage, at a dose of 455 mg/kg-day. Cyclohexanol had no effect on relative kidney or testes weights, though exposure was reported to be associated with liver enlargement and induction of some parameters of hepatic xenobiotic metabolism. There was no mention of a histological evaluation of testicular tissue from cyclohexanol-treated animals.

G. SUMMARY

Cyclohexanol (CAS No. 108-93-0) is a colorless to light-yellow, viscous liquid, or a sticky solid, with a molecular weight of 100.16, and a formula of $C_6H_{12}O$. Cyclohexanol is used in the production of nylon, lacquers, paints, varnishes, degreasers, plastics and plasticizers, soaps and detergents, textiles, and insecticides. Workplace exposures are most likely to occur through the inhalation or dermal routes, while the general population may be exposed via contaminated air, ingestion of contaminated food or drinking water, or dermal contact with cyclohexanol-containing water.

No California-specific data on production volumes or worker exposure are available. Reported environmental releases for the U.S. in 1999 totaled 3,892,943 pounds. A 1983 survey estimated that 68,715 U.S. workers are potentially exposed to cyclohexanol in the workplace.

Cyclohexanol can be absorbed via the oral, inhalation or dermal routes of exposure. Hepatic NAD-dependent-alcohol dehydrogenase (ADH) is primarily responsible for the oxidation of cyclohexanol. Most of the compound is excreted in the urine in conjugation with sulfuric or glucuronic acid. Metabolic disposition is thought to be relatively rapid, though accumulation may occur.

Cyclohexanol is irritating to the skin and mucous membranes. The minimum acute lethal dose in rabbits was 2.2 g/kg-bw by the oral route, and 12.4 g/kg-bw by the dermal route. Effects observed in repeated dose studies in rabbits, conducted by either the dermal or inhalation route, included: conjunctival irritation, lacrimation, salivation, lethargy, distention of the ear veins, narcosis, tremor, hypothermia, and death.

There were no human data available pertaining to the potential developmental or reproductive toxicity of cyclohexanol. Data from a study conducted in mice indicated an adverse effect of cyclohexanol on pup postnatal growth when treatment was continuous from prior to conception, throughout gestation and lactation, and into the postweaning period. As currently interpreted, however, Proposition 65 excludes consideration of developmental toxicity resulting from exposures during postnatal life. In the case of the mouse study on cyclohexanol, pup mortality and growth were not evaluated until postnatal day 21.

Neither human data relevant to the potential male reproductive toxicity of cyclohexanol, nor multigeneration reproductive toxicity studies were available. In a seven-day gavage study, male rats exposed to cyclohexanol showed no change in testicular weights. Two other, longer-term studies of the male reproductive system in gerbils, rats, and rabbits found cyclohexanol exposure to be associated with effects on the male reproductive organs, including: changes in testes and epididymal weights, histopathological changes in these organs, and altered biochemistry.

Table 1. Effects of Cyclohexanol Exposure on the Male Reproductive System in Laboratory Animals

Reference	Study Design	Reproductive Effects	Systemic Effects
Tyagi et al., 1979	Gerbils, s.c. injection; 15 mg/kg-day to 20 adult males for 21 days (20 untreated controls) Evaluation 24 hrs after last dose	Decreased weights of: testes, epididymides, seminal vesicles, and ventral prostate Degeneration of seminiferous tubules, loss of sperm precursors, abnormal Sertoli and Leydig cells Decreased protein, RNA, and sialic acid in testis, epididymis, and seminal vesicles Decreased testicular glycogen Increased testicular cholesterol and alk phosphatase	No effect on body, thyroid, or adrenal weight No effect on serum protein, cholesterol, blood sugar, urea, or serum transaminases Increased serum alk phosphatase No effect on haematological parameters
	Rats, s.c. injection; 15 mg/kg-day to 20 adult males for 37 days Evaluation 24 hrs after last dose	Decreased weights of: testes, epididymides, seminal vesicles, and ventral prostate Degeneration of seminiferous tubules, loss of sperm precursors, abnormal Sertoli and Leydig cells Decreased protein, RNA, and sialic acid in testis, epididymis, and seminal vesicles Decreased testicular glycogen Increased testicular cholesterol and alk phosphatase	No effect on body, thyroid, or adrenal weights No effect on serum protein, cholesterol, alk phosphatase, blood sugar, urea, or serum transaminases No effect on haematological parameters
Dixit et al., 1980	Rabbits, oral gavage; 25 mg/kg-day; 40 days 5 males/group: 1) untreated control, 2) treated, evaluated 70 days post treatment, 3) treated, evaluated 24 hrs post treatment	**Group 3:** Reduced relative testis and epididymis weights Loss of sperm precursors; abnormal epididymides, seminiferous tubules, and Leydig cells Reduced testicular and epididymal protein, RNA, sialic acid, glycogen, and acid phosphatase **Group 2:** Marked recovery of most endpoints to control, or near-control values	No differences in body or relative adrenal weights No histological evidence of liver necrosis **Group 3:** Decreased adrenal ascorbic acid Increased serum cholesterol, SGPT, phospholipids, triglycerides, alk phosphatase, and bilirubin Decreased serum protein No change in acid phosphatase, blood sugar, or urea **Group 2:** Most endpoints recovered to normal levels Elevated serum cholesterol, phospholipid, alk phosphatase, and bilirubin
Lake et al.; 1982	Rats; oral gavage; 455 mg/kg-day, 7 days 12 males/group	No effect on testis weight	No effect on kidney weight Increased relative liver weight; evidence for induction of certain parameters of xenobiotic metabolism

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