National Toxicology Program
Toxicity Report Series
Number 20

NTP Technical Report
on Toxicity Studies of

Diethanolamine

(CAS No. 111-42-2)

Administered Topically and in Drinking Water
to F344/N Rats and B6C3F$_1$ Mice

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NIH Publication No. 92-3343
October 1992

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

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The NTP report on the toxicity studies of diethanolamine is based primarily on 2- and 13-week studies that began in October, 1986, and ended in January, 1988, at Battelle Memorial Laboratories, Columbus, OH.

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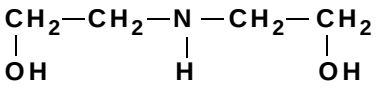
Diethanolamine



Molecular formula: $C_4H_{11}NO_2$
CAS Number: 111-42-2 **Molecular Weight**: 105.14
Synonyms: 2,2'-iminodiethanol; 2,2'-iminobisethanol; diethylolamine; bis(hydroxyethyl)amine; 2,2'dihydroxydiethylamine; 2,2'-aminodiethanol

ABSTRACT

Diethanolamine is a high-production chemical used in cosmetics, in cutting fluids, as a dispersing agent for agricultural chemicals, and as an absorbent for acidic gases. Toxicology studies of diethanolamine were conducted in F344/N rats and B6C3F$_1$ mice of both sexes for 2 weeks (5/sex/species/dose) and 13 weeks (10/sex/species/dose) to characterize and compare the effects of oral and dermal exposure. In addition to histopathology, evaluations included clinical pathology, urinalyses, and sperm morphology or vaginal cytology. *In vitro* genetic toxicity studies included assessments of mutagenicity in *Salmonella typhimurium* and mouse lymphoma L5178Y cells, analysis of chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells, and determination of micronuclei formed in mice during the 13-week dermal exposure study.

Groups of rats and mice received drinking water containing diethanolamine at concentrations of up to 10000 ppm during studies of 2 or 13 weeks duration. In the 2-week studies, rats and mice of both sexes received in the were 0, 630, 1250, 5000, and 10000 ppm diethanolamine in the drinking water. In the 13-week studies, rats received 0, 320, 630, 1250, 2500, and 5000 ppm (males) or 0, 160, 320, 630, 1250, and 2500 ppm (females) in drinking water; male and female mice received 0, 630, 1250, 2500, 5000, and 10000 ppm. All female rats in the 2 highest dose groups and 2 males in the 10000 ppm group in the 2-week study died before the end of the study. In the 13-week study, deaths of mice occurred in the 3 highest dose groups; 2 male rats in the top dose group also died. Surviving animals in the higher concentration groups in both studies exhibited depressed weight gains. Rats receiving diethanolamine developed a poorly regenerative, microcytic anemia in both studies. In the 2-week study, dosed male and female rats had increased kidney weights, renal tubular cell necrosis, and decreased renal function; rats in the 13-week study also showed increased incidences or severity of nephropathy, tubular necrosis, and mineralization. Degeneration of the seminiferous tubules of the testis was noted in dosed males in both the 2- and 13-week studies, and sperm motility and count were decreased in the 13-week study. Demyelination in the brain (medulla oblongata) and spinal cord was observed in male and female rats in the 13-week study. In mice, dose-dependent increases in liver weight were observed in males and females in the 2-week study; cytologic alteration and

necrosis of individual hepatocytes were observed in the highest dose group. In the 13-week drinking water study in mice, nephropathy and tubular necrosis were observed in males, and degeneration of cardiac myocytes, and hepatocellular necrosis were seen in males and females. Cytologic alteration in the submandibular salivary gland was noted in male and female mice. Hepatocyte cytologic alteration also was noted in all dosed groups of mice.

In the 2-week dermal studies, groups of rats and mice were administered daily doses of diethanolamine in 95% ethanol, ranging from 160 to 2500 mg/kg for mice, and from 125 to 2000 mg/kg for rats, 5 days per week. In 13-week studies, dermal doses ranged from 32 to 500 mg/kg for rats, and from 80 to 1250 mg/kg for mice. In the 2-week study, early deaths of male rats and male and female mice occurred in the highest dose groups and in female rats in the 2 highest dose groups (1000 and 2000 mg/kg). Body weight gains were reduced in rats and mice in the higher dose groups. Early deaths in the 13-week study were observed in the highest dose groups of rats (500 mg/kg) and mice (1250 mg/kg). Body weight gains were reduced in rats and mice given the higher doses. Rats in the dermal studies exhibited dose-dependent hematologic and renal function changes similar to those observed in rats in the drinking water study. In addition, in the 2-week study, rats exhibited ulcerative skin lesions at the site of application, accompanied by inflammatory cell infiltration, hyperkeratosis, and acanthosis (hyperplasia) of the epidermis. Hyperkeratosis, without ulceration, was observed in some animals. Ulceration at the site of application was observed in male and female mice. Acanthosis, without ulceration or inflammatory cell infiltration, was observed in mice in all lower dose groups. In the 13-week study, skin lesions at the site of application included ulceration and inflammation, hyperkeratosis, and acanthosis. Liver weights were increased in male and female rats, but there were no associated histopathological changes. Other treatment-related effects observed in rats included demyelination in the brain and spinal cord, and nephropathy, renal tubular necrosis, and/or tubular mineralization; mice exhibited cytological alterations in the liver and/or hepatocellular necrosis, renal tubular epithelial necrosis, and cardiac myocyte degeneration.

In *in vitro* genetic toxicity studies, diethanolamine was not mutagenic in *Salmonella typhimurium* or mouse L5178Y $TK^{+/-}$ cells. Diethanolamine did not induce sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, nor did it induce micronuclei in peripheral blood erythrocytes in mice exposed by topical application for 13 weeks. All *in vitro* studies were conducted with and without S9 activation.

Target organs of diethanolamine toxicity identified in these studies included bone marrow, kidney, brain, spinal cord, testis, and skin in rats, and liver, kidney, heart, salivary gland, and skin in mice. A no-observed-adverse-effect-level (NOAEL) was not achieved for hematological changes or nephropathy in rats (< 160 ppm), or for cytologic alteration of the liver in mice (< 630 ppm) in the drinking water studies. In the dermal studies, a NOAEL was not achieved for hematological changes, nephropathy, or hyperkeratosis of the skin in rats (< 32 mg/kg), or for cytologic alteration of the liver or acanthosis of the skin in mice (< 80 mg/kg).

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies on diethanolamine on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies are appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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Summary of Peer Review Comments

Dr. R.L. Melnick, NIEHS, introduced the short-term toxicity studies of diethanolamine by reviewing the uses, experimental design, and results.

Dr. Carlson, a principal reviewer, said this was a well-written report. He questioned the statement that a NOAEL was not achieved for female mice in the dermal studies, commenting that 80 mg/kg appeared to be a NOAEL based on lack of cytologic alteration of the liver. Dr. Melnick agreed but pointed out that 80 mg/kg was not a NOAEL for dermal lesions. Dr. Carlson thought that while perhaps statistically correct, it seemed to be stretching a point to say that a NOAEL was not observed in the rat based on hematologic studies when the change was 1 percent or less.

Dr. Garman, a second principal reviewer, agreed that this was a very well-written, thorough, and well-documented report. He noted that, although there were neurologic signs in rats on the 2-week water studies, neuropathologic changes were not noted until 13 weeks and wondered if additional stains might be warranted to be sure the clinical signs were not indicative of early neuropathologic changes. Dr. J.F. Mahler, NIEHS, responded that the brain sections from the 2-week studies were reviewed with particular attention to the same areas that were affected in the 13-week studies, and no lesions were observed. Dr. Garman asked that more precise neuroanatomic locations be stated in photomicrographs of brain lesions.

Dr. Bailey commented that there had been extensive, long-time use of diethanolamine and related amines in industry, primarily in formulations, and that corresponding subchronic testing has been done. He said he was unaware of findings of neurologic and testicular toxicity. He suggested that the NTP might want to solicit information on these studies, many of which would be unpublished.

Seeing no objections, Dr. Klaassen accepted the report with the suggested editorial and other changes on behalf of the panel.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

Diethanolamine (DEA) exists either as colorless crystals or as a colorless liquid at ambient temperatures. It has a melting point of 28°C, a boiling point of 268.8°C (Mullins, 1978; Windholz, 1983), and is miscible with water, forming alkaline solutions (e.g., the pH of a 0.1 N solution is 11).

Diethanolamine undergoes reactions characteristic of secondary amines and of alcohols. Along with monoethanolamine and triethanolamine, DEA is produced by ammonolysis of ethylene oxide, then separated by distillation (Mullins, 1978). In 1989, 202 million pounds (92 x 10^6 kg) of diethanolamine were produced in the United States (USITC, 1990). Its reactions with long-chain fatty acids form neutral ethanolamine soaps (Mullins, 1978) which are used extensively as emulsifiers, thickeners, wetting agents, and detergents in cosmetic formulations (including skin cleaners, creams, and lotions) (Beyer *et al.*, 1983). Diethanolamine has numerous other uses: as a dispersing agent in various agricultural chemicals, as an absorbent for acidic gases (hydrogen sulfide and carbon dioxide), as a humectant, as an intermediate in the synthesis of morpholine, as a surface active agent in cutting fluids, as a corrosion inhibitor, as a component in textile specialty agents, and as a secondary vulcanization accelerator in the rubber industry. It also is an ingredient in cleaners and pharmaceutical ointments, in polyurethane formulations, in herbicides, and in a variety of organic syntheses (Mullins, 1978; Windholz, 1983; Beyer *et al.*, 1983). Approximately 830,000 workers potentially are exposed to diethanolamine annually, as estimated from data compiled from the National Occupational Exposure Survey (NIOSH, 1990). This widespread industrial and consumer use of diethanolamine results in large, unaltered amounts of the chemical being discharged into water and sewage (Yordy and Alexander, 1981).

OSHA has not set any occupational standard for exposure to diethanolamine, but the American Conference of Governmental Industrial Hygienists (ACGIH) recommends a time-weighted average threshold limit value (TLV) of 3 ppm (ACGIH, 1980). The ACGIH-TLV was derived from results of a 90-day feed study, which showed no toxic effects at 20 mg/kg/day (Smyth *et al.*, 1951), and by applying a safety factor of 10 (ACGIH, 1980).

Absorption, Disposition, and Excretion

Dermal absorption of diethanolamine was suggested to occur in rats, since N-nitroso-diethanolamine (NDEA) was excreted in the urine of male Sprague-Dawley rats that had been exposed to diethanolamine by topical application and given nitrite in their drinking water (Preussman *et al.*, 1981). However, because of a lack of specific, published information on the absorption, metabolism and excretion of diethanolamine, the NTP is studying the fate of diethanolamine in male F344 rats and B6C3F_1 mice following oral gavage, topical application, and i.v. injection (RTI, 1991).

Preliminary results of these studies (RTI, 1991) indicated that, following i.v. administration of 7.5 mg/kg, ^{14}C-labeled diethanolamine to male F344 rats, the radiolabel was excreted almost exclusively in urine, with less than 1 percent of the radiolabel found in feces or exhaled breath. Excretion in urine, however, was less than anticipated for such a polar compound, and accounted for only 16% and 28% of the total dose at 24 and 48 hours after administration, respectively. Most of the label was retained in tissues, with the highest concentrations detected in liver and kidney 48 hours after dosing. Tissue/blood ratios for liver and kidney were approximately 150; for lung and spleen, 35 to 40; for the heart approximately 20; for other tissues, less than 10.

Studies of dermal absorption (RTI, 1991) using ^{14}C-labeled diethanolamine (at doses of 2.1, 7.6 or 27.5 mg/kg, in 95% ethanol, applied to 2 cm^2 of skin and using an occlusive cover) revealed that absorption from skin increased with dose from approximately 3% at 2.1 mg/kg to approximately 16% at 27.5 mg/kg. Distribution of dermally absorbed radiolabel to the tissues was similar to that after administration by i.v. injection; excretion was almost exclusively in urine, and more of the absorbed dose was retained in tissues than was excreted. Diethanolamine-derived radioactivity retained in tissues was concentrated in the liver and kidney; tissue/blood ratios were similar to those seen following i.v. administration.

Oral administration of ^{14}C-labeled diethanolamine resulted in nearly complete absorption from the gastrointestinal tract. As observed with i.v. and topical administration, excretion of radiolabel was almost exclusively in urine. Excretion in feces (~ 2.5%) was approximately twice that observed following i.v. administration; some of this material probably was unabsorbed diethanolamine. Tissue distribution following oral administration was similar to i.v. administration, with the greatest concentrations in liver and kidney and lesser concentrations in lung, spleen, and heart.

The large amounts of diethanolamine-derived radioactivity retained in tissues suggests that diethanolamine may bioaccumulate in tissues with repeat exposure. To test this hypothesis, rats were administered an oral dose, 5 days a week, for up to 8 weeks; their tissues were assayed after 1, 2, 4, or 8 weeks of exposure, and 4 weeks after a 4-week exposure. The results suggested that diethanolamine-derived radioactivity accumulated in tissues and reached steady-state levels at approximately 4 weeks. The highest concentrations of diethanolamine-derived radiolabel occurred in liver and kidney, but tissue/blood ratios were lower than observed with single exposures. Animals treated for 4 weeks and held in metabolism cages for an additional 4 weeks to permit collection of excreted material, eliminated diethanolamine-derived radioactivity with a half-life of approximately 1 week, a rate consistent with achievement of steady-state tissue levels at approximately 4 weeks.

Less extensive studies with $B6C3F_1$ mice produced results that were consistent with those in rats. Excretion was largely limited to urine; about 25% of the dose was excreted in urine in 48 hours. As observed in rats, the highest concentrations of diethanolamine-derived radioactivity were in liver and kidney, which had tissue/blood ratios of approximately 100. Lower concentrations were observed in heart, spleen, and lung, with tissue/blood ratios of approximately 20, 30, and 50, respectively. Absorption of diethanolamine from mouse skin was approximately 60%, significantly greater than observed for rats; but the data are not directly

comparable, as a larger dose was used in the mouse study (81 mg/kg), and absorption from rat skin was found to increase as the dose increased (RTI, 1991).

Toxic Effects

The acute, oral LD_{50} of diethanolamine in unspecified strains of rats was reported to be 1820 mg/kg (Smyth *et al.*, 1951) and 780 mg/kg (Smyth *et al.*, 1970). In an unspecified strain of mice, the intraperitoneal LD_{50} was 2300 mg/kg (Blum *et al.*, 1972), and the subcutaneous LD_{50} was 3553 mg/kg (NIOSH, 1979). The estimated lethal dose of diethanolamine in humans is 20 g (Dreisbach, 1980). Symptoms associated with diethanolamine intoxication include increased blood pressure, diuresis, salivation, and pupillary dilation (Beard and Noe, 1981). Diethanolamine causes mild skin irritation to the rabbit at concentrations above 5%, and severe ocular irritation at concentrations above 50% (Carpenter and Smyth, 1946; Beyer *et al.,* 1983).

The liver and kidneys have been identified as target organs of diethanolamine toxicity in rats and mice following systemic exposure. In rats receiving diethanolamine in their diets for 90 days, microscopic lesions (not characterized) and deaths occurred at daily doses of 170 mg/kg and higher, while alterations in liver and kidney weights occurred at doses of 90 mg/kg and above. No toxic effects were observed at 20 mg/kg (Smyth *et al.*, 1951).

Single i.p. injections of neutralized diethanolamine to male Sprague-Dawley rats, at doses of 100 or 500 mg/kg, produced cytoplasmic vacuolization, basophilia, and mitochondrial swelling in hepatocytes, and necrosis and cytoplasmic vacuolization of the renal tubular epithelium at 4 and 24 hr after dosing (Grice *et al.*, 1971). Repeated i.p. administration (250 mg/kg) caused an increase in liver weight (Hartung *et al.*, 1970). A single LD_{50} dose (2300 mg/kg), administered by i.p. injection to Swiss Webster mice, produced extensive fatty degeneration in the liver (Blum *et al.*, 1972); ultrastructural alterations included swollen mitochondria, and dilated and degranulated endoplasmic reticulum.

A dose- and time-dependent loss in respiratory control was observed in hepatic mitochondria isolated from male Sprague-Dawley rats treated with diethanolamine (42, 160, or 490 mg/kg/day) in their drinking water for 1, 2, or 3 weeks (Barbee and Hartung, 1979a). However**,** the loss in mitochondrial function was not produced when diethanolamine was administered in the drinking water for only 24 hours or when diethanolamine (5 mM) was added to isolated mitochondria.

Inhalation exposure of male rats to diethanolamine (25 ppm continuously for 216 hours) resulted in increased liver and kidney weights, and in elevations in serum glutamic-oxaloacetic transaminase (SGOT) activity, and blood urea nitrogen levels (Hartung *et al.*, 1970). Exposure of male rats to 6 ppm, 8 hours per day, for 13 weeks resulted in depressed growth rates and increases in lung and kidney weights.

Korsrud *et al.* (1973) reported that there were dose-related increases in relative liver and kidney weights in male Sprague Dawley rats, 18 hours after a single oral administration of neutralized diethanolamine, at doses ranging from 100 to 3200 mg/kg. There also was minimal parenchymal cell damage (less acidophilic) in livers of animals treated with doses ranging from 200 to 1600 mg/kg. Large lipid droplets and focal cytoplasmic degeneration were seen in hepatocytes of

animals in the 1600 mg/kg treatment group. A single oral dose of 800 mg/kg caused increases in serum concentrations of urea and in serum activities of sorbitol dehydrogenase, isocitrate dehydrogenase, and SGOT; it also resulted in a decrease in serum arginine concentrations. A single oral dose of 1600 mg/kg caused increases in the serum levels of ornithine carbamyl transferase, glutamate dehydrogenase, fructose-1,6-diphosphate aldolase, and lactate dehydrogenase activities. In addition, doses of 400 mg/kg and higher produced renal tubular cell necrosis.

Hruban *et al.* (1965) observed large vacuoles and fat droplets in hepatocytes and ultrastructural changes in the endoplasmic reticulum and mitochondria from livers of rats treated orally for 1 or 4 days with 1000 mg diethanolamine/kg/day. Ultrastructural changes in the endoplasmic reticulum (release of ribosomes) and in the mitochondria (swollen) were similar to those described by Blum *et al.* (1972). In addition, Hruban *et al.* (1965) noted depletion of zymogen granules and disruption of the rough endoplasmic reticulum into vacuoles in pancreatic acinar cells.

A normocytic anemia, without bone marrow depression or increases in reticulocyte counts, was observed in male rats treated with 4 mg/ml of neutralized diethanolamine in their drinking water for 7 weeks (Hartung *et al.*, 1970).

The mechanism of diethanolamine toxicity is unknown, but it may be related to an alteration of phospholipid metabolism. Treatment of Wistar or Sherman rats with diethanolamine caused increased formation of hepatic phospholipids (Artom *et al.*, 1949). Repeated oral administration in drinking water (1 to 3 weeks, at a dose of 320 mg/kg/day) reduced the level of incorporation of ethanolamine and choline into hepatic and renal phospholipids in Sprague-Dawley rats (Barbee and Hartung, 1979b), and led to incorporation of diethanolamine into phospholipids in these organs. The half-life for disappearance of the phospholipid derivatives of diethanolamine was about twice as long as those for choline or ethanolamine phospholipids. The accumulation of atypical, diethanolamine-containing phospholipids may disrupt normal membrane structure and lead to alterations in the functional properties of subcellular membranes. Similar mechanisms may be involved in the inhibitory effect of repeated exposures of rats to 100 to 750 mg/kg/day diethanolamine on liver microsomal hydroxylase and N-demethylase activities (Foster *et al.*, 1971).

Carcinogenicity

No carcinogenicity studies have been reported for diethanolamine. In the presence of nitrite or oxides of nitrogen, diethanolamine may be nitrosated to N-nitrosodiethanolamine (NDEA) (Loeppky *et al.*, 1983), a potent liver and nasal cavity carcinogen in rats (Lijinsky *et al.*, 1980; Preussmann *et al.*, 1982). The reaction of diethanolamine with N-nitrosating agents has resulted in the detection of NDEA in synthetic cutting and grinding fluids (Fan *et al.*, 1977b), cosmetics, lotions, and shampoos (Fan *et al.*, 1977a), and in processed tobacco (Brunnemann and Hoffmann, 1981; Schmeltz *et al.*, 1977). Preussmann *et al.* (1981) suggested that NDEA could also form *in vivo*, since this nitrosamine was excreted in the urine of rats given nitrite in drinking water following topical application of diethanolamine.

Genetic Toxicity

The genotoxic potential of diethanolamine has been examined in various short-term tests. The data indicate that the substance was not mutagenic in bacteria (Dean *et al.*, 1985; Haworth *et al.*, 1983) nor in the yeast, *S. cerevisiae* (Dean *et al.*, 1985). It did not induce sister-chromatid exchanges or chromosomal aberrations (Dean *et al.*, 1985; Sorsa *et al.*, 1988; Loveday *et al.*, 1989) or cell transformation (Inoue *et al.*, 1982) in hamster cells *in vitro.* Positive results were reported for an *in vitro* hepatocyte single strand-break assay, where hepatocytes freshly isolated from rats, hamsters, or pigs, were incubated with diethanolamine (Pool *et al.,* 1990). Positive responses were seen with all 3 species.

Short-term testing of structural analogs of diethanolamine indicates that these substances are not genotoxic. Triethanolamine was extensively studied and found to be negative in bacterial mutagenicity assays (Dean *et al.*, 1985; Mortelmans *et al.,* 1986), in the Drosophila sex-linked recessive lethal assay (Yoon *et al.,* 1985), and in Chinese hamster ovary cell cytogenetic tests (Galloway *et al.*, 1987).

Rationale for Conducting Studies

Diethanolamine was nominated for study by the National Cancer Institute because of its large annual production, known human exposure, potential for conversion to a known carcinogen in the presence of nitrite (NDEA), and because there was little adequate toxicity and carcinogenicity data on this chemical. Topical application and oral administration (drinking water) were chosen as routes of administration for 2- and 13-week toxicity studies in F344 rats and B6C3F$_1$ mice to permit comparison of the potential toxicities via these routes, both of which are typical for human exposure to this chemical. The studies performed included hematology and clinical chemistry analyses, urinalyses, and reproductive system and histopathologic evaluations. Diethanolamine also was evaluated for mutagenicity in *Salmonella typhimurium* and in mouse lymphoma L51178Y cells, for induction of sister-chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, and for induction of micronuclei in red blood cells in mice in the 13-week studies.

MATERIALS AND METHODS

Procurement and Characterization of Diethanolamine

Diethanolamine (CAS 111-42-2) was obtained from Kodak Laboratory and Specialty Chemicals (Rochester, NY). Cumulative analytical data indicated that the purity was greater than 99%. Infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of diethanolamine and with available literature references. Results of elemental analysis for carbon, hydrogen, and nitrogen agreed with theoretical values. Karl Fischer analysis indicated 0.1% water. No impurities greater than 0.1% relative to the diethanolamine peak were observed by gas chromatography. The bulk chemical was stored at room temperature and protected from light. Reanalysis of diethanolamine by gas chromatography and nonaqueous amine titration revealed no degradation of the bulk chemical during storage over the course of these studies.

Dose Formulations

For the drinking water studies, diethanolamine doses were prepared with deionized water as the delivery vehicle; the pH was adjusted to 7.4 ± 0.2 with 1 N hydrochloric acid. Dose solutions were stored no longer than 20 days at room temperature in polypropylene carboys. The solutions were analyzed by gas chromatography before and after administration to animals and found to be within 15% of the theoretical concentrations.

For the dermal studies, solutions of diethanolamine were prepared in 95% ethanol (USP grade). Dose solutions were stored no longer than 20 days at room temperature, protected from light. Results of analyses of dose formulations by gas chromatography before and after administration to animals were within 10% of theoretical values.

Toxicity Study Designs

Male and female F344/N rats and B6C3F$_1$ mice used in this study were produced under strict barrier conditions at Taconic Farms, Germantown, NY, (13-week drinking water and dermal studies and 2-week dermal studies) or Simonsen Labs, Inc., Gilroy, CA (2-week drinking water studies). Animals were progeny of defined microflora-associated parents, transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at approximately 4 weeks of age, quarantined for 11-13 days, and placed on study at about 6 weeks of age. Blood samples were collected from 5 animals per sex and species at the start and termination of the 13-week drinking water and dermal studies. The sera were analyzed for viral titers; data from 5 viral screenings performed in rats and 12 screenings performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989; 1989a) showed no positive antibody titers. Additional details concerning study design and performance are listed in Table 1.

Groups of 5 rats and 5 mice of each sex received drinking water solutions containing diethanolamine at concentrations of 0, 630, 1250, 2500, 5000, or 10000 ppm (0, 0.63, 1.25, 2.5, 5.0, or 10 mg/ml) *ad libitum* daily for 14 days. In 13-week drinking water studies, 10 animals per

sex per species received diethanolamine in the drinking water at concentrations of 0, 320, 630, 1250, 2500, and 5000 ppm for male rats; 0, 160, 320, 630, 1250, and 2500 ppm for female rats; and 0, 630, 1250, 2500, 5000, and 10000 ppm for male and female mice. NIH-07 Open Formula diet in pellet form (Zeigler Brothers, Inc., Gardners, PA) was available *ad libitum*.

In the 2-week dermal studies, diethanolamine in 95% ethanol was administered at doses of 0, 63, 125, 250, 500, and 1000 mg diethanolamine/ml to groups of 5 rats and 5 mice of each sex, once daily for twelve days (excluding weekends) over a 16-day interval. The volume of the dosing solution was adjusted weekly based on the most recent mean body weight of each dose group, with target doses of 0, 125, 250, 500, 1000, and 2000 mg diethanolamine/kg body weight for rats, and 0, 160, 320, 630, 1250, and 2500 mg diethanolamine/kg body weight for mice. The dosing solution was applied to the shaved back of each animal from the mid-back to the interscapular region using a calibrated micropipette. In the 13-week dermal studies, 10 animals per sex per species were administered diethanolamine in 95% ethanol once per day, except for weekends and holidays, for 13 weeks, at concentrations of 0, 37.5, 75, 150, 300, and 600 mg/ml for both species (0, 32, 63, 125, 250, or 500 mg/kg for rats and 0, 80, 160, 320, 630, or 1250 mg/kg for mice). City water and NIH-07 Open Formula diet in pellet form (Zeigler Brothers, Inc., Gardners, PA) were available *ad libitum*.

Rats were housed individually for dermal studies and five per cage for drinking water studies. Mice were housed individually in both the dermal and drinking water studies. Animal rooms were maintained at 72 ± 3°F and 50 ± 15% relative humidity with 12 fresh air changes per hour and 12 hours of subdued fluorescent light daily.

Urine samples were collected from rats housed individually in polycarbonate metabolism cages for approximately 16 hours during the second week of the 2-week studies (study day 12) and the twelfth week of the 13-week studies. Collection tubes were immersed in ice/water baths and food was removed from the cages. Volume, appearance, specific gravity, and pH were measured for each urine sample. Concentrations of glucose, protein, urea nitrogen, and creatinine, and activities of alkaline phosphatase and lactate dehydrogenase also were measured using a Hitachi 704® Chemistry Analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN) and reagents obtained from the manufacturer.

Clinical pathology studies were performed on all rats and mice that survived until the end of the 2-week and 13-week drinking water and dermal studies. Animals were anesthetized with carbon dioxide, and blood samples were collected from the retroorbital sinus using heparinized microcapillary tubes. Biochemical analyses were performed on blood samples collected in Microtainers® (Becton Dickinson, Rutherford, NJ) with no preservative or anticoagulant. Clinical chemistry parameters, measured using a Hitachi 704® Automatic Chemistry Analyzer (Indianapolis, IN), included sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), total protein (TP), albumin, urea nitrogen (UN), creatinine, glucose, and total bile acids. Reagents for these assays were obtained from the manufacturer, except for SDH and total bile acids, which were obtained from Sigma Chemical Co. (St. Louis, MO). Additional blood samples, from rats only, were collected in Microtainers® containing dipotassium EDTA and were analyzed with an Ortho ELT-8 Laser Hematology Counter (Ortho Instruments, Westwood, MA). Hematologic analyses included erythrocyte count (RBC), leukocyte count (WBC), mean corpuscular volume

(MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HGB), hematocrit (HCT), leukocyte differential count, erythrocyte morphologic assessment, reticulocyte count, platelet count and platelet morphologic assessment. Differential leukocyte counts were determined by microscopic evaluation of blood smears stained with Wright Giemsa. Reticulocytes were stained by mixing equal volumes of whole blood with new methylene blue. Preparations were incubated for 20 minutes and smears were made. The number of reticulocytes per 1000 RBC was determined microscopically and used to calculate absolute counts.

Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed; organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically, as required by protocols, were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all control animals, all early death animals, and all animals in the highest dose groups with at least 60% survivors. Target tissues were examined in animals from lower dose groups until a no-effect level was determined. All lesions observed at necropsy were examined microscopically. Target tissues examined, and those required by protocol to be examined for the diethanolamine studies, are listed in Table 1.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.*, (1985).

Vaginal cytology and sperm morphology evaluations were performed on rats and mice exposed to 0, 630, 1250, and 2500 ppm diethanolamine in drinking water. In dermal studies, rats exposed to 0, 63, 125, and 250 mg/kg, and mice exposed to 0, 160, 320, and 630 mg/kg were evaluated. Methods were those described by Morrissey *et al.* (1988). Briefly, for the 7 days prior to sacrifice, females were subjected to vaginal lavage with saline. The aspirated cells were scored for the relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: Sperm that were extruded from a small cut made in the epididymis were dispersed in a warm, buffered solution, and the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field were counted. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline and incised with a razor blade, the solution mixed gently, then heat-fixed at 65°C. Sperm density was subsequently determined using a hemocytometer.

To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer.

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Diethanolamine

EXPERIMENTAL DESIGN	
Study Dates	2-Week Dermal Studies: October--November, 1986 2-Week Drinking Water Studies: March--April, 1987 13-Week Dermal Studies: September--December, 1987. 13-Week Drinking Water Studies: October, 1987--January, 1988
Size of Study Groups	2-Week Studies: 5 males and 5 females of each species per dose group 13-Week Studies: 10 males and 10 females of each species per dose group
Doses/Duration of Dosing	Drinking Water Studies: 2-Weeks: Rats--0, 630, 1250, 2500, 5000, or 10000 ppm in water *ad libitum.* Mice--0, 630, 1250, 2500, 5000, or 10000 ppm in water *ad libitum*. 13-Weeks: Rats: male-- 0, 320, 630, 1250, 2500, or 5000 ppm in water *ad libitum.* Rats: female--0, 160, 320, 630, 1250, or 2500 ppm in water *ad libitum*.. Mice--0, 630, 1250, 2500, 5000, or 10000 ppm in water *ad libitum*.. Dermal Studies: 2-Weeks: Rat--0, 125, 250, 500, 1000, or 2000 mg/kg, 5 days per week for 2 weeks. Mice--0, 160, 320, 630, 1250, 2500 mg/kg, 5 days per week for 2 weeks. 13-Weeks: Rats--0, 32, 63, 125, 250, 500 mg/kg, once daily, except for holidays and weekends, for 13 weeks. Mice--0, 80, 160, 320, 630, or 1250 mg/kg, once daily, except for holidays and weekends, for 13 weeks.
Type and Frequency of Observation	2-Week Studies: observed 2x/day for mortality/moribundity; 1 x week for clinical signs of toxicity; weighed initially, on day 8, 1 x week, and at necropsy. Water consumption was measured weekly for drinking water studies. 13-Week Studies: observed 2x/day for mortality/moribundity; body weight and clinical observations recorded weekly and at necropsy. Water consumption was measured twice weekly for drinking water studies.
Necropsy and Histologic Examinations	Complete necropsy performed on all animals. Protocol-required tissues examined microscopically in all control animals, all early death animals, and all animals in the highest dose group with 60% survivors. These tissues included: adrenal glands, brain (3 sections), clitoral glands, esophagus, eyes (if grossly abnormal), bone (femur, sternebrae, or vertebrae) with marrow, gallbladder (mouse), gross lesions, heart/aorta, intestine-large (cecum, colon, rectum), intestine-small (duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary glands, seminal vesicles, skin (in dermal studies: skin sections of gross lesions at the site of application, skin at the site of application without gross lesions, and undosed inguinal control skin), spinal cord and sciatic nerve (13-week rat studies only), spleen, stomach (forestomach and glandular stomach), testes with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The following target organs were identified in the indicated studies and examined at lower dose levels until a no-effect level was determined: kidney, testis: 2-week drinking water study, rats; kidney, testis, and application site skin: 2-week dermal study, rats; kidney, brain and spinal cord, testis, adrenal gland, lymphoid tissues: 13-week drinking water study, rats; kidney, brain, and application site skin: 13-week dermal study, rats; liver: 2-week drinking water study, mice; liver, application site skin: 2-week dermal study, mice; liver, kidney, heart, salivary gland: 13-week drinking water study, mice; liver, kidney, heart, salivary gland, application site skin: 13-week dermal study, mice.

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Studies of Diethanolamine (continued)

ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N rats $B6C3F_1$ mice
Animal Source	Simonsen Laboratories, Gilroy, CA (2-Week Drinking Water Studies), Taconic Farms, Germantown, NY.
Study Laboratory	Battelle Memorial Laboratories, Columbus, Ohio
Time Held Before Study	2-Week Drinking Water Studies: rats--12 days; mice--11 days 2-Week Dermal Studies: rats--12 days; mice-- 13 days 13-Week Drinking Water Studies: rats--12 days; mice--13 days 13-Week Dermal Studies: rats--13 days; mice--12-13 days
Age When Placed on Study	2-Week Drinking Water Studies: rats--41 days; mice--40 days 2-Week Dermal Studies: rats--42 days; mice--43 days 13-Week Drinking Water Studies: rats--6 weeks; mice 5-6 weeks 13-Week Dermal Studies: rats--7 weeks; mice 5-6 weeks
Age When Killed	2-Week Drinking Water Studies: rats--55 days; mice--54 days 2-Week Dermal Studies: rats--58 days; mice--60 days 13-Week Drinking Water Studies: rats--19 weeks; mice 18-19 weeks 13-Week Dermal Studies: rats--20 weeks; mice 18-19 weeks
Method of Animal Distribution	Animals were weighed and randomized using a Xybion® computer program (partitioning algorithm).
Diet	NIH-07 Open Formula Pellets, (Zeigler Bros., Inc., Gardners, PA) available *ad libitum*,
Animal Room Environment	Rats housed 5/cage for drinking water studies, individually for dermal studies. Mice housed individually for all studies; 72 ± 3°F; 50 ± 15% humidity; 12 hours fluorescent light/day; 10-12 air changes/hour.

Mutagenicity Studies

Mutagenicity studies of diethanolamine in *Salmonella typhimurium* were conducted as described in Haworth *et al.* (1983). Briefly, diethanolamine was tested in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, using a preincubation assay in both the absence or presence of Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. Diethanolamine was tested at doses up to 3333 μg/plate; higher concentrations were toxic. A positive response is defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any single strain/activation combination. An equivocal response is defined as an increase in revertants which is not dose-related, not reproducible, or is of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

Induction of Trifluorothymidine (TFT) Resistance in Mouse Lymphoma L5178Y Cells

The experimental protocols and statistical methods are presented by McGregor *et al.* (1988). Mouse lymphoma L5178Y/$TK^{+/-}$ cells were maintained at 37°C as suspension cultures in supplemented Fischer's medium. All treatment levels and controls within an experiment were replicated. Cells were incubated with the study chemical for 4 hours, after which the medium plus chemical was removed and the cells resuspended in fresh medium and incubated for an

additional 2 days to express the mutant phenotype. Log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of TFT-resistant cells ($TK^{-/-}$), and in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37°C in 5% CO_2 for 10 - 12 days.

Chinese Hamster Ovary Cytogenetics Assays

Testing was performed as reported by Loveday *et al.* (1989). Briefly, Chinese hamster ovary cells (CHO) were incubated with diethanolamine or solvent (dimethylsulfoxide) for induction of sister-chromatid exchanges (SCE) and chromosomal aberrations (ABS) both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were incubated for sufficient time to reach second metaphase division. Additional procedural details are provided in Appendix D.

Mouse Peripheral Blood Micronucleus Assay

At the termination of the 13-week study, blood smears were prepared from peripheral blood obtained by cardiac puncture of all dosed and control mice. The slides were stained with Hoechst 33258/pyronin Y (McGregor *et al.*, 1983). Ten thousand normochromatic erythrocytes from each animal were scored for micronuclei.

Statistical Methods

All numerical data from the 2-week studies were reported as group means and standard deviations. Body weights, organ weights, and clinical pathology data were tested for homogeneity of variance by Bartlett's test. If the data were non-homogeneous, a separate variance t-test was performed. If the data were homogeneous, a 1-way analysis of variance (ANOVA) was performed, followed by Dunnett's test (pairwise comparisons with control).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971; 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose-response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Analysis of Vaginal Cytology data

Since the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

Analysis of Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells

All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P < 0.05$) for a chemical to be considered capable of inducing TFT-resistance; a single significant response led to a "questionable" conclusion; and the absence of both a trend and a peak response resulted in a "negative" call. Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are in Caspary *et al.* (1988).

Analysis of CHO Cytogenetics Assays

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at a single dose point is less than 0.01; the probability for such a chance occurrence at 2 dose points is less than 0.001. For a single trial, an increase at a dose of 20% or greater is considered weak evidence of a positive response (w+); increases at 2 or more doses is evidence of a positive (+) response.

Chromosomal aberration data are presented as percentage of cells with aberrations. Both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P<.05$) difference for 1 dose point and a significant trend ($P<0.015$) were considered weak evidence for a positive response (W+); significant differences for 2 or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

Analysis of Micronucleus Data

Statistical analyses for micronuclei were completed using linear trend tests on polychromatic erythrocyte data, log-transformed data for normochromatic erythrocytes, and analysis of variance on ranks (ANOVA) for percentage polychromatic cells among total erythrocytes. The frequencies of micronuclei in the dosed groups were compared with the frequencies determined for the concurrent untreated control animals using the Student t-test.

Quality Assurance

The animal studies of diethanolamine were performed in compliance with FDA Good Laboratory Practices regulations (Code of Federal Regulations, 21 CFR Part 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the animal studies.

RESULTS

2-Week Drinking Water Studies in F344/N Rats

Two males in the 10000 ppm group and all females in the 5000 and 10000 ppm groups died or were killed while in a moribund condition before the end of the study (Table 2). All female rats in the top dose group died by study day 6. Chemically-related clinical signs included abnormal posture, tremors, and hypoactivity. Body weight gains were reduced in male rats that received 5000 or 10000 ppm and in female rats that received 1250 or 2500 ppm. Water consumption was lower in all treatment groups compared to controls, especially in the 5000 and 10000 ppm groups; reduced palatability of the 5000 and 10000 ppm drinking water solutions may have contributed to reduced body weight gains at these levels. Based on water consumption and body weight data, daily doses of diethanolamine were estimated to range from about 80 to 1040 mg/kg.

TABLE 2 Survival, Weight Gain, and Water Consumption of F344/N Rats in the 2-Week Drinking Water Studies of Diethanolamine

Dose (ppm)	Survival[a]	Mean Body Weight (grams) Initial[b]	Final	Change[c]	Final Weight Relative to Controls (%)[d]	Average Water Consumption[e]	Estimated DEA Consumed[f]
MALE							
0	5/5	72	149	77	100	16.2	0
630	5/5	73	152	79	102	14.2	77
1250	5/5	73	149	76	100	15.0	162
2500	5/5	73	144	71	97	14.6	319
5000	5/5	74	131	57	88	13.5	622
10000	3/5[g]	73	98	25	66	9.2	1016
FEMALE							
0	5/5	80	136	56	100	16.3	0
630	5/5	81	134	53	99	13.9	79
1250	5/5	80	126	46	93	13.5	158
2500	5/5	80	107	27	79	13.8	371
5000	0/5[h]	79	j	j	j	10.6[k]	670
10000	0/5[i]	80	j	j	j	8.3[k]	1041

a Number surviving at 15 days/number of animals per dose group.
b Body weights were measured 4 days before the first dose.
c Mean weight change of the animals in each dose group.
d (Dosed group mean/Control group mean) x 100.
e Average water consumption in ml/animal/day.
f Chemical consumption in mg/kg/day.
g Day of Death: 14, 14.
h Day of Death: 5, 5, 5, 8, 8.
i Day of Death: 4, 4, 4, 6, 6.
j All animals in group died before scheduled termination.
k Average for first week only.

Exposure to diethanolamine produced a moderate, poorly regenerative, normochromic, microcytic anemia in male and female rats, as indicated by dose-dependent decreases in erythrocyte and reticulocyte counts, MCV, hemoglobin concentration, and hematocrit (Table 3). In male and female rats, serum concentrations of creatinine, total protein, UN, albumin, and bile acids (male rats) were increased by treatment with diethanolamine. Activities of ALT (SGPT) were increased in female rats at the highest dose with surviving animals (2500 ppm); however, no corresponding histopathologic lesions were seen in the liver.

TABLE 3 Hematological Changes in Peripheral Blood of F344/N Rats in the 2-Week Drinking Water Studies of Diethanolamine[a]

Dose (ppm)	0	630	1250	2500	5000	10000
MALE						
RBC (10^6/μL)	7.65 ± 0.28	7.45 ± 0.29	7.25 ± 0.18	6.64 ± 0.20**	6.56 ± 0.32**	5.70 ± 0.31**[b]
HGB (g/dL)	14.4 ± 0.4	13.8 ± 0.5	13.2 ± 0.2**	12.3 ± 0.3**	12.5 ± 0.3**	10.7 ± 0.7**[b]
HCT (%)	44 ± 1	42 ± 1	40 ± 1**	37 ± 1**	36 ± 2**	32 ± 2**[b]
MCV (fL)	58 ± 1	56 ± 1*	55 ± 1**	56 ± 1**	55 ± 0**	56 ± 6**[b]
MCH (pg)	18.8 ± 0.3	18.5 ± 0.4	18.3 ± 0.3	18.5 ± 0.2	19.0 ± 0.9	18.8 ± 0.4
Retic. (10^6/μL)	0.18 ± 0.02	0.18 ±0.03	0.19 ± 0.02	0.11 ± 0.01*	0.07 ± 0.01**	0.05 ± 0.01**
FEMALE						
RBC (10^6/μL)	7.79 ± 0.40	7.54 ± 0.07	7.23 ± 0.08*	6.97 ± 0.64	c	c
HGB (g/dL)	14.7 ± 0.2	14.0 ± 0.3*	13.1 ± 0.1**	12.8 ±1.0*	c	c
HCT (%)	45 ± 1	43 ± 1*	40 ± 0**	38 ± 3**	c	c
MCV (fL)	58 ± 2	57 ± 1*	56 ±1*	55 ± 1**	c	c
MCH (pg)	19.0 ± 1.0	18.6 ± 0.5	18.2 ± 0.2	18.5 ± 0.4	c	c
Retic. (10^6/μL)	0.16 ± 0.01	0.08 ± 0.01**	0.08 ± 0.01**	0.05 ± 0.01**	c	c

a Values are means ± S.D.
b N = 3.
c All animals in group died before scheduled termination.
* Significantly different from control, P 0.05 (ANOVA, Dunnett).
** Significantly different from control, P 0.01 (ANOVA, Dunnett).

The kidneys in male and female rats were affected by diethanolamine. Increases in absolute and relative kidney weight, incidence of renal tubular epithelial necrosis, urine concentrations of urea nitrogen, glucose, protein, and lactate dehydrogenase activity were observed in exposed male and female rats (Table 4). Urine parameters were normalized per mg of urinary creatinine, because of differences in the 16-hour urine volume between the various dose groups. Renal tubular necrosis was characterized microscopically by large areas of the renal parenchyma in which tubules were denuded of epithelium; lumens of affected tubules were filled with eosinophilic debris of sloughed epithelial cells, and the nuclei exhibited karyolysis or karyorrhexis. Many tubules were lined by attenuated basophilic epithelial cells with a high nuclear/cytoplasmic ratio, indicative of regenerative changes. There was a tendency for tubular necrosis to be localized to the outer stripe of the outer medulla and the medullary rays, especially in females (Plate 1). Tubular necrosis was seen in males that received 10000 ppm diethanolamine and in females that received 2500, 5000, or 10000 ppm; the lesion in early death animals generally was more severe than in animals that survived until study termination. Minimal mineralization of necrotic renal tubules also was observed. Tubular lesions were associated with vascular congestion, but no inflammatory cell reaction was present.

Several other microscopic findings in some high-dose animals were considered chemically-related. Mild to marked seminiferous tubule degeneration, characterized by a reduction in tubule size and in the number of spermatogenic cells, was observed in all high dose males; accompanying this lesion was the appearance of large numbers of degenerate cells in the lumen of epididymal tubules. A minimal to mild depletion of femoral bone marrow cells was seen in high dose male rats surviving to study termination, but this was not a consistent finding in lower dose groups, and it is uncertain if this was a direct chemical effect or secondary to other

factors. Other microscopic findings were considered secondary to stress, inanition, or renal failure, including minimal to mild gastric ulceration, hemorrhage or inflammation, and lymphoid depletion of the spleen and thymus.

TABLE 4 Renal Toxicity in F344/N Rats in the 2-Week Drinking Water Studies of Diethanolamine[a]

Dose (ppm)	0	630	1250	2500	5000	10000
MALE						
Final body weight	149	152	149	144	131*	98**[b]
Right kidney weight	0.733	0.768	0.848*	0.821	0.855*	0.918**[b]
Relative kidney weight	4.92	5.05	5.69**	5.70*	6.53**	9.37**[b]
Tubular epithelial necrosis[c]	0/5	0/5	0/5	0/5	0/5	3/5*[b](2.2)
Urinalysis						
Urea nitrogen (mg/mg creatinine)	28	27	28	29	32**	62**
Glucose (mg/mg creatinine)	0.3	0.3	0.2	0.2	0.4	4.8**
Protein (mg/mg creatinine)	0.7	0.4	0.3	0.3	0.5	3.1**
Lactate dehydrogenase, (IU/mg creatinine)	0.08	0.08	0.08	0.11**	0.15**	0.48**
FEMALE						
Final body weight	136	134	126*	106**	d	d
Right kidney weight	0.628	0.740*	0.696	0.821**	d	d
Relative kidney weight	4.62	5.52*	5.52*	7.75**	d	d
Tubular epithelial necrosis	0/5	0/5	0/5	5/5 (1.6)	5/5 (3.4)	5/5 (3.2)
Urinalysis						
Urea nitrogen (mg/mg creatinine)	33	35	33	46**	e	e
Glucose (mg/mg creatinine)	0.2	0.2	0.3	0.9**	e	e
Protein (mg/mg creatinine)	0.4	0.5	0.7	1.8**	e	e
Lactate dehydrogenase, (IU/mg creatinine)	0.06	0.07	0.11*	0.21**	e	e

a Organ and body weights given in grams; organ-weight-to-body-weight ratios given as mg organ weight/g body weight.
b N = 3.
c Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 5 unless otherwise noted.
d All animals in group died before scheduled termination.
e All animals in group died before urine collection.
* Significantly different from control (P 0.05).
** Significantly different from control (P 0.01).

13-Week Drinking Water Studies in F344/N Rats

Two males in the high dose (5000 ppm) group died before the end of the study (Table 5). One female death in the lowest dose group (160 ppm) was not considered treatment-related. Body weight gains were depressed in a dose-related fashion in both sexes (Figure 1). Decreased water consumption among the higher dose groups may have contributed in part to the decreased body weight gain. Based on water consumption and body weight data, average daily doses of diethanolamine were estimated to range from about 25 to 440 mg/kg in males and about 15 to 240 mg/kg in females. Clinical signs of toxicity included tremors, emaciation, abnormal posture, and rough hair coat in the 2 highest dose groups of each sex.

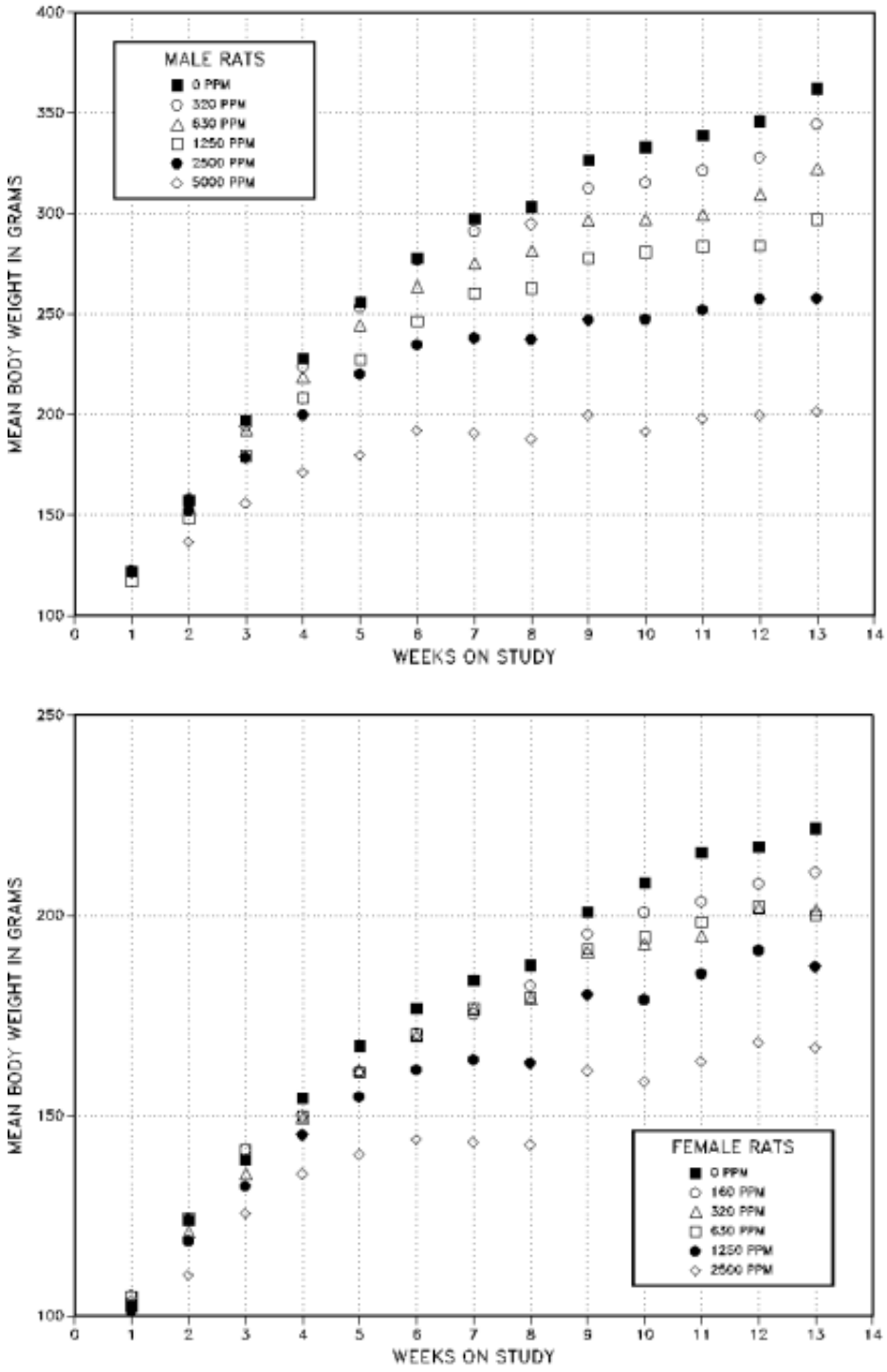

Figure 1 Body Weights of F344/N Rats Exposed to Diethanolamine by Drinking Water for 13 Weeks

TABLE 5 Survival, Weight Gain, and Water Consumption of F344/N Rats in the 13-Week Drinking Water Studies of Diethanolamine

Dose (ppm)	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Water Consumption[d]	Estimated DEA Consumed[e]
MALE							
0	10/10	122	362	240		20.9	0
320	10/10	123	344	221	95	20.2	25
630	10/10	122	322	200	89	19.2	48
1250	10/10	117	297	180	82	18.3	97
2500	10/10	123	258	135	71	17.7	202
5000	8/10	121	202	81	56	15.6	436
FEMALE							
0	10/10	102	222	120		15.5	0
160	9/10	105	211	106	95	14.9	14
320	10/10	103	201	98	91	16.9	32
630	10/10	105	200	95	90	15.2	57
1250	10/10	102	187	85	84	15.8	124
2500	10/10	104	167	63	75	13.9	242

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.
d Average water consumption in ml/animal/day.
e Chemical consumption in mg/kg body weight/day.

Diethanolamine administration produced a moderate, poorly regenerative, microcytic, normochromic anemia in male and female rats (Table 6; Appendix B). Hematologic effects were dose-dependent and included decreases in erythrocyte and reticulocyte counts, hemoglobin concentration, hematocrit, MCV, and MCH. The magnitude of the responses was greater in the 13-week study than in the 2-week study; MCV was reduced in rats at all dose levels. Hematologic effects were not associated with microscopic changes in the femoral bone marrow.

No significant gross lesions attributable to diethanolamine were found at necropsy. Dose-related increases in relative kidney weights were observed in males and females (Table 7; Appendix A). Kidney weight changes were accompanied by increases in the incidence and/or severity of nephropathy, renal tubular cell necrosis, or tubular mineralization (Table 8). Nephropathy consisted of tubules lined by epithelial cells with more basophilic staining of the cytoplasm and a higher nuclear/cytoplasmic ratio; occasionally, thickened basement membranes were seen around these tubules. This lesion was present to a minimal degree in controls, particularly in male rats, but was increased in incidence and severity in high dose males and in most female treatment groups. Increased nephropathy was considered a regenerative change and was supported by the observation of tubular necrosis at the higher doses. Tubular necrosis was minimal in severity and was characterized by eosinophilic tubular epithelial cells with pyknotic nuclei, frequently seen desquamated into the lumen of renal tubules. Mineralization was observed as basophilic concretions within necrotic tubules which were present primarily along the outer stripe of the outer medulla. Mineralization was present in all female control rats; however, there was a dose-related increase in severity and/or incidence in both females and males.

TABLE 6 Hematological Changes in Peripheral Blood of F344/N Rats in the 13-Week Drinking Water Studies of Diethanolamine

Dose (ppm)	0	160[a]	320	630	1250	2500	5000[b]
MALE							
RBC ($10^6/\mu L$)	8.79	--	8.75	8.20**	7.33**	6.40**	5.71**
HGB (g/dL)	14.8	--	14.3*	13.3**	11.6**	9.8**	8.9**
HCT (%)	47.8	--	46.1	42.5**	36.9**	31.4**	27.8**
MCV (fL)	54	--	53**	52**	50**	49**	49**
MCH (pg)	16.9	--	16.4**	16.2**	15.9**	15.3**	15.5**
Reticulocytes ($10^6/\mu L$)	0.23	--	0.23	0.23	0.24	0.14**	0.16**
FEMALE							
RBC ($10^6/\mu L$)	8.40	8.51	7.84**	7.56**	6.78**	6.43**	--
HGB (g/dL)	15.1	15.2	13.8**	13.0**	11.3**	10.5**	--
HCT (%)	47.3	47.0	42.3**	39.7**	34.4**	31.2**	--
MCV (fL)	56	55**	54**	53**	51**	49**	--
MCH (pg)	17.9	17.8*	17.7**	17.2**	16.7**	16.3**	--
Reticulocytes ($10^6/\mu L$)	0.17	0.16	0.13**	0.12*	0.09**	0.08**	--

a Females only, N = 9.
b Males only, N = 8.
* Significantly different from control (p 0.05) by Dunn's or Shirley's test.
** Significantly different from control (p 0.01) by Dunn's or Shirley's test.

TABLE 7 Kidney, Liver, Testis, and Epididymis Weights of Rats Administered Diethanolamine in Drinking Water for 13 Weeks[a]

Dose (ppm)	0	160	320	630	1250	2500	5000
MALE							
Necropsy body weight	366	--	339	326	302	265	205
Kidney weight	1.29	--	1.34	1.30	1.21	1.18	1.26
Relative kidney weight	3.54	--	3.94**	3.99**	3.98**	4.44**	6.14**
Liver weight	15.09	--	13.87	14.92	14.82**	14.18	11.59**
Relative liver weight	41.28	--	40.79	45.61**	48.90	53.27**	56.71**
Right testis weight	1.49	--	1.46	1.47	1.27**	0.97**	0.54**
Relative testis weight	4.08	--	4.31	4.50	4.22	3.64**	2.63**
Epididymis weight	0.426	--	0.453	0.392	0.309**	0.184**	0.134**
Relative epididymis weight	1.17	--	1.34**	1.20	1.02**	0.68.**	0.65**
FEMALE							
Necropsy body weight	218	208	201	202	188	162	--
Kidney weight	0.66	0.86**	0.84**	0.83*	0.87**	0.92**	--
Relative kidney weight	3.03	4.12	4.21**	4.12**	4.63**	5.67**	--
Liver weight	6.08	6.36	7.04**	6.99**	7.78**	7.32**	--
Relative liver weight	27.86	30.54	35.09**	34.52**	41.41**	45.26**	--

a Organ weights and body weights given in grams; organ-weight-to-body-weight ratios given as mg organ weight/g body weight.
* Significantly different from the control group by Williams' or Dunnett's test (P 0.05).
** Significantly different from the control group by Williams' or Dunnett's test (P 0.01).

The brain and spinal cord also were identified as targets of diethanolamine toxicity. In the brain, microscopic change was observed in coronal sections of the medulla oblongata and consisted of bilaterally symmetrical areas of vacuolization of the neuropil (Table 8). Vacuoles were most consistently seen as sharply delimited, round-to-oval, clear spaces arranged symmetrically around the midline of the medulla in areas of transversely sectioned white matter identified as the tectospinal tract. In more severe cases, there was involvement of more peripheral white matter tracts at the same level of the medulla (Plate 2). Generally, vacuoles were empty and not associated with a glial response, although some contained debris, and a minimal cellular reaction was present. Special stains for myelin demonstrated only a focal loss of myelin sheaths in these vacuolated areas. In transverse sections of the spinal cord, vacuoles were randomly scattered in the dorsal, ventral, and lateral columns of the white matter and in spinal nerves. No lesions were observed in sections of the sciatic nerve. Minimal to mild demyelination of the brain and spinal cord was observed in all male and female rats in the 2500 and 5000 ppm dose groups (Table 8). There were no neurologic clinical signs that could be clearly attributed to these lesions.

TABLE 8 Incidence and Severity of Kidney, Brain, and Spinal Cord Lesions in F344/N Rats Administered Diethanolamine in Drinking Water for 13 Weeks[a]

Dose (ppm)	0	160	320	630	1250	2500	5000
MALE							
Kidney							
Nephropathy	6/10 (1.0)	–	2/10 (1.0)	2/10 (1.0)	3/10 (1.0)	6/10 (1.0)	10/10 (2.4)
Tubular epithelial necrosis	0/10	–	0/10	0/10	0/10	0/10	10/10 (1.0)
Tubular mineralization	0/10	–	0/10	0/10	1/10 (1.0)	10/10 (1.8)	10/10 (1.7)
		–					
Brain, medulla							
Demyelination	0/10	–	0/10	0/10	0/10	10/10 (1.7)	10/10 (2.0)
Spinal cord							
Demyelination	0/10	–	0/10	0/10	0/10	10/10 (1.9)	10/10 (2.0)
FEMALE							
Kidney							
Nephropathy	2/10 (1.0)	9/10 (1.0)	10/10 (1.5)	10/10 (1.4)	9/10 (1.0)	2/10 (1.0)	–
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	1/10 (1.0)	3/10 (1.0)	–
Tubular mineralization	10/10 (1.3)	10/10 (2.0)	10/10 (2.5)	10/10 (3.0)	10/10 (2.4)	10/10 (1.7)	–
Brain, medulla							
Demyelination	0/10	0/10	0/10	0/10	10/10 (1.5)	10/10 (1.9)	–
Spinal cord							
Demyelination	0/10	0/10	0/10	0/10	10/10(1.0)	10/10(1.9)	–

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

Decreases in testis and epididymis weights (Table 7) were associated microscopically with degeneration of seminiferous epithelium and with hypospermia. The testicular lesion was morphologically similar to that seen in the 2-week drinking water study and consisted of decreased numbers of spermatogenic cells, reduced size of seminiferous tubules, and scant intraluminal sperm. Testicular degeneration was diagnosed in all high dose (5000 ppm) males and in 3 of 10 males at the 2500 ppm dose level. Intraluminal cellular debris and reduced numbers of sperm cells were present in the epididymis. These findings correlated with

decreases in sperm motility and sperm count per gram caudal tissue (Appendix C). Atrophy of the seminal vesicles and prostate glands in male rats from the higher dose groups were additional treatment-related lesions. There were no noteworthy changes among female rats in estrous cycle length (Appendix C).

Cytoplasmic vacuolization of the zona glomerulosa of the adrenal cortex was a treatment-related effect in high dose male rats (9 of 10) and in females in the 2500 (2 of 10) and 5000 ppm (10 of 10) dose groups. This was a minimal change consisting of small clear vacuoles in the cytoplasm of these cells and may have been related to increased mineralocorticoid production secondary to renal damage and/or dehydration.

Dose-related increases in relative liver weights occurred in male and female rats (Table 7). Although the changes in liver weights were not associated with microscopic lesions in the liver, there were mild to moderate increases in serum concentrations of total bile acids in female rats in all dose groups, and in male rats in all dose groups except the lowest (320 ppm)(Appendix B). Other relevant biochemical changes in male and female rats included increases in concentrations of albumin, total protein, and UN in serum (Appendix B).

Treatment-related microscopic lesions in the 2 high-dose group male rats that died before study termination were similar to those of rats that survived to the end of the study.

2-Week Dermal Studies in F344/N Rats

Three males and all females in the highest dose groups died before the end of the study (Table 9). In addition, 1 female in the 1000 mg/kg dose group died early. Animals exhibited emaciation, dyspnea, hypoactivity, and crusting at the site of application, and all but 1 of the rats that died were killed in a moribund condition. Body weight gains of the 1000 mg/kg group of females and the 1000 and 2000 mg/kg groups of males were reduced compared to controls.

Dose-dependent, poorly regenerative, microcytic, normochromic anemia occurred in male and female rats exposed dermally to diethanolamine (Table 10). Corresponding bone marrow changes were not seen histologically. Increases in white blood cell counts at the higher dose levels may have been related to the severity of ulceration and inflammation caused by diethanolamine at the site of application.

Kidney changes similar to those observed in the drinking water study were observed in male and female rats in the dermal study (Table 11). These included increases in absolute and relative kidney weight, in the incidence of tubular epithelial necrosis, and in urine levels of urea nitrogen, glucose, protein (females), and lactate dehydrogenase activity. Renal tubular necrosis was found only in early death animals, and was similar morphologically to that seen in the 2-week drinking water studies, consisting primarily of denuded tubules filled with cellular debris. The incidence of this lesion was greater in females than males. Mineralization of necrotic tubules was present in many animals.

Irritation and crusting of the skin at the site of application were observed in males and females in the 3 highest dose groups (500, 1000, and 2000 mg/kg). In these groups, microscopic

TABLE 9 Survival and Weight Gain of F344/N Rats in the 2-Week Dermal Studies of Diethanolamine

Dose (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
0	5/5	111	168	57	100
125	5/5	110	163	53	97
250	5/5	112	169	57	101
500	5/5	115	168	53	100
1000	5/5	111	146	35	87
2000	2/5[d]	114	132	18	79
FEMALE					
0	5/5	91	120	29	100
125	5/5	91	117	26	98
250	5/5	94	120	26	100
500	5/5	93	115	22	96
1000	4/5[e]	92	101	09	84
2000	0/5[f]	93	g	g	g

a Number surviving at 17 days/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.
d Day of Death: 6,8,14.
e Day of Death: 5.
f Day of Death: 5, 5, 5, 5, 15.
g All animals in group died before scheduled termination.

examination of the treatment-related lesions of the application site showed ulceration with associated acanthosis and inflammation at the ulcer margins (Table 12). Ulceration was characterized by necrosis of the epidermis and, to a variable degree, the dermis; severity was dose-related and ranged from focal, superficial epidermal lesions to more diffuse involvement which extended deeper into the dermis. An accompanying inflammatory reaction consisted predominantly of neutrophils, many of which were degenerate and created a zone of cell debris at the borders of necrotic areas.

Animals surviving to the end of the study sometimes evidenced more chronic, inflammatory changes of beginning fibrovascular proliferation at the ulcer margins. Acanthosis (epidermal hyperplasia) was characterized by a proliferation of squamous epithelial cells, resulting in increased thickness of the epithelium above the normal 2-3 cell layers seen in controls. Acanthosis was focal and most pronounced at the margins of ulcerated areas, where it was associated with vacuolar and ballooning degeneration of the epithelium. Decreased severity scores at the highest dose may have been due to more extensive ulceration of the application site in these animals or due to early death, resulting in less time for a reparative response. Ulcers, inflammation, and acanthosis were observed in the 500, 1000, and 2000 mg/kg dose groups of each sex (Table 12). Acanthosis of minimal severity also was seen in female rats in the 250 mg/kg group. Hyperkeratosis was observed in all groups of animals exposed to diethanolamine.

Mild to moderate seminiferous tubule degeneration in the testis, morphologically similar to that described in drinking water studies, was observed in 4 of 5 high dose males.

TABLE 10 Hematological Changes in Peripheral Blood of F344/N Rats in the 2-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	125	250	500	1000	2000
MALE						
RBC (10^6/μL)	8.06 ± 0.31	8.02 ± 0.44	7.56 ± 0.23*	6.99 ± 1.43	6.75 ± 0.13**	5.26 ± 0.57[b]
HGB (g/dL)	14.8 ± 0.5	14.5 ± 0.6	13.6 ± 0.3**	13.1 ± 1.6	11.8 ± 0.3**	9.1 ± 1.1[b]
HCT (%)	48 ± 2	47 ± 2	44 ± 2	40 ± 8	38 ± 1**	28 ± 2*[b]
MCV (fL)	60 ± 1	59 ± 1	58 ± 1	58 ± 1**	57 ± 1**	54 ± 1**[b]
MCHC (pg)	18.4 ± 0.4	18.1 ± 0.6	18.0 ± 0.3	19.0 ± 2.2	17.5 ± 0.6	17.3 ± 0.3[b]
WBC (10^3/μL)	7.4 ± 1.5	7.7 ± 0.5	6.9 ± 0.8	8.4 ± 0.8	10.3 ± 2.0**	9.1 ± 1.5[b]
Retic. (10^6/μL)	0.16 ± 0.02	0.15 ± 0.02	0.12 ± 0.02	0.08 ± 0.02*	0.10 ± 0.00	0.11 ± 0.01[b]
FEMALE						
RBC (10^6/μL)	8.23 ± 0.33	7.85 ± 0.50	7.81 ± 0.25	7.52 ± 0.32*	6.74 ± 0.37*[c]	d
HGB (g/dL)	15.2 ± 0.6	14.6 ± 0.8	14.2 ± 0.4*	13.6 ± 0.3**	12.3 ± 0.5**[c]	d
HCT (%)	50 ± 2	48 ± 2	46 ± 1*	44 ± 1**	39 ± 2**[c]	d
MCV (fL)	61 ± 1	61 ± 1	60 ± 1	59 ± 1*	57 ± 1**[c]	d
MCHC (pg)	18.4 ± 0.5	18.6 ± 0.5	18.2 ± 0.4	18.2 ± 0.4	18.3 ± 0.5[c]	d
WBC (10^3/μL)	8.4 ± 1.5	5.9 ± 1.7*	7.6 ± 0.9	7.4 ± 1.6	10.0 ± 0.3 [c]	d
Retic. (10^6/μL)	0.11 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.07 ± 0.01[c]	d

a Values are means ± SD.
b N = 2
c N = 4
d All animals in group died before scheduled termination.
* Significantly different from control, p 0.05 (ANOVA, Dunnett).
** Significantly different from control, p 0.01 (ANOVA, Dunnett).

TABLE 11 Renal Toxicity in F344/N Rats in the 2-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	125	250	500	1000	2000
MALE						
Necropsy weight	168	163	169	168	146	132[b]
Right kidney weight	0.87	0.94	0.98	1.01	1.03	1.18*[b]
Relative kidney weight	5.18	5.77	5.80	6.01**	7.08**	8.97**[b]
Tubular epithelial necrosis	0/5	0/5	0/5	0/5	0/5	3/5 (2.0)[c]
Urinalysis						
Urea nitrogen (mg/mg creatinine)	35	34	36	38	44*	79**[d]
Glucose (mg/mg creatinine)	0.2	0.1	0.1	0	0.3	2.5*[d]
Protein (mg/mg creatinine)	1.7	0.7*	0.6**	0.3**	0.5**	1.3*[d]
Lactate dehydrogenase (IU/mg creatinine)	0.06	0.06	0.07*	0.11**	0.17**	0.39**[d]
FEMALE						
Necropsy weight	120	117	120	115	101**	f
Right kidney weight	0.63	0.75*	0.74*	0.79**	0.79**[e]	f
Relative kidney weight	5.21	6.35**	6.15**	6.82**	7.87**[e]	f
Tubular epithelial necrosis (b)	0/5	0/5	0/5	0/5	1/5 (2.0)[c]	5/5 (2.0)[c]
Urinalysis						
Urea nitrogen (mg/mg creatinine)	42	45	48	47	57**[e]	78[g]
Glucose (mg/mg creatinine)	0.4	0.4	0.3	0.3	0.3[e]	1.0[g]
Protein (mg/mg creatinine)	0.7	0.2	0.4	0.4	0.3[e]	1.3[g]
Lactate dehydrogenase (IU/mg creatinine)	0.08	0.07	0.07	0.11	0.19*[e]	0.29[g])

a Organ weights and body weights given in grams; organ-weight-to-body-weight ratios given as mg organ weight/g body weight.
b N = 2.
c Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 5 unless otherwise noted.
d N = 3.
e N = 4.
f All animals in group died before scheduled termination.
g N = 1.
* Significantly different from the control group (p 0.05).
** Significantly different from the control group (p 0.01).

TABLE 12 Incidence and Severity of Skin Lesions in F344/N Rats in the 2-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	125	250	500	1000	2000
MALE						
Ulcer	0/5	0/5	0/5	4/5 (1.3)	5/5 (3.2)	5/5 (3.6)
Inflammation	0/5	0/5	0/5	3/5 (1.7)	5/5 (3.0)	5/5 (3.0)
Acanthosis	0/5	0/5	0/5	5/5 (1.8)	5/5 (3.0)	4/5 (2.0)
Hyperkeratosis	0/5	4/5 (1.0)	4/5 (1.0)	5/5 (1.8)	5/5 (2.8)	5/5 (2.0)
FEMALE						
Ulcer	0/5	0/5	0/5	5/5 (2.4)	5/5 (3.2)	5/5 (3.2)
Inflammation	0/5	0/5	0/5	5/5 (1.8)	5/5 (2.8)	5/5 (3.2)
Acanthosis	0/5	0/5	2/5 (1.0)	5/5 (2.4)	5/5 (3.2)	4/5 (1.8)
Hyperkeratosis	0/5	5/5 (1.0)	5/5 (1.0)	5/5 (2.2)	5/5 (2.6)	5/5 (1.8)

a Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 5.

13-Week Dermal Studies in F344/N Rats

One 500 mg/kg male died during week 9, and 2 females administered 500 mg/kg diethanolamine were killed in a moribund condition during week 10 (Table 13). Final mean body weights of males receiving doses of 250 or 500 mg/kg, and of females receiving doses of 125 mg/kg or higher, were lower than those of controls (Figure 2). The primary clinical signs of toxicity in the 3 highest dose groups were irritation and crusting of the skin at the site of diethanolamine application.

TABLE 13 Survival and Weight Gain of F344/N Rats in the 13-Week Dermal Studies of Diethanolamine

Dose (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
0	10/10	124	342	218	
32	10/10	122	337	215	99
63	10/10	123	323	200	94
125	10/10	120	336	216	98
250	10/10	124	294	170	86
500	9/10	120	237	117	69
FEMALE					
0	10/10	107	192	85	
32	10/10	113	193	80	100
63	10/10	114	191	77	99
125	10/10	106	178	72	93
250	10/10	109	172	63	90
500	8/10	109	151	42	79

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.

A moderate, poorly regenerative, microcytic, normochromic anemia developed in male and female rats exposed dermally to diethanolamine for 13 weeks (Table 14). Decreases in red blood cell variables were observed even at the lowest dose, 32 mg/kg; thus, a no-observable-adverse-effect level (NOAEL) for diethanolamine-induced anemia was not achieved. No histologic changes in femoral bone marrow were observed. Serum biochemical changes in male rats included mild increases in concentrations of UN and albumin at the 4th and 2nd highest dose groups, respectively, and mild increases in activities of ALT in animals in the 3 highest dose groups (Appendix B). In female rats, UN, albumin, and total protein increased in all dose groups (except at the lowest dose for total protein), and total bile acids increased in the 2 highest dose groups. A mild increase in activity of ALT occurred in female rats in the highest dose group (Appendix B).

TABLE 14 Hematological Changes in Peripheral Blood of F344/N Rats in the 13-Week Dermal Studies of Diethanolamine

Dose (mg/kg)	0	32	63	125	250	500
MALE						
RBC (10^6/μL)	8.87	8.81	8.79	8.57*	7.90**	6.80**
HGB (g/dL)	15.5	15.3	15.1*	14.3**	12.9**	11.0**
HCT (%)	47.6	46.4	45.6*	43.1**	38.8**	32.6**
MCV (fL)	54	53**	52**	50**	49**	48**
MCH (pg)	17.5	17.3**	17.1**	16.7**	16.3**	16.1**
Reticulocytes (10^6/μL)	0.20	0.21	0.20	0.21	0.18	0.18
FEMALE						
RBC (10^6/μL)	8.45	8.14**	7.83**	7.38**	6.91**	6.23**
HGB (g/dL)	15.5	14.8**	14.1**	13.2**	12.0**	10.5**
HCT (%)	48.9	46.7**	44.2**	40.6**	36.9**	31.9**
MCV (fL)	58	57	56**	55**	53**	51**
MCH (pg)	18.4	18.2	18.1*	17.8**	17.4**	16.8**
Reticulocytes (10^6/μL)	0.16	0.13*	0.12**	0.10**	0.14*	0.12**

* Significantly different from the control group (p 0.05) by Dunn's or Shirley's test.
** Significantly different from the control group (p 0.01) by Dunn's or Shirley's test.

As in the drinking water studies and the 2-week dermal study, the kidney was identified as a target organ in the 13-week dermal study. Absolute and relative kidney weights were increased in male and female rats (Table 15). These weight changes were associated with increased severity or increased incidences of nephropathy, renal tubular cell necrosis, or tubular mineralization (Table 16). A dose-dependent increase in incidence and severity of nephropathy was evident at the lower dose levels in females, but there was no clear treatment effect on this lesion in male rats as there was in the 13-week drinking water study. Tubular necrosis was observed in females in the 2 highest dose groups, but no active necrosis was found in the corresponding male groups. Tubular mineralization, consistent with previous necrosis, was present in high-dose males, as well as being increased in incidence and severity in most treated female groups.

There was a dose-dependent increase in absolute and relative liver weights in both male and female rats (Table 15). Although mild serum biochemical changes occurred (Appendix B), no corresponding microscopic lesion was observed. Dermal exposure to diethanolamine was not associated with testicular or epididymal changes as had been observed in the drinking water study. Sperm morphology and vaginal cytology evaluations (Appendix C) did not show adverse effects.

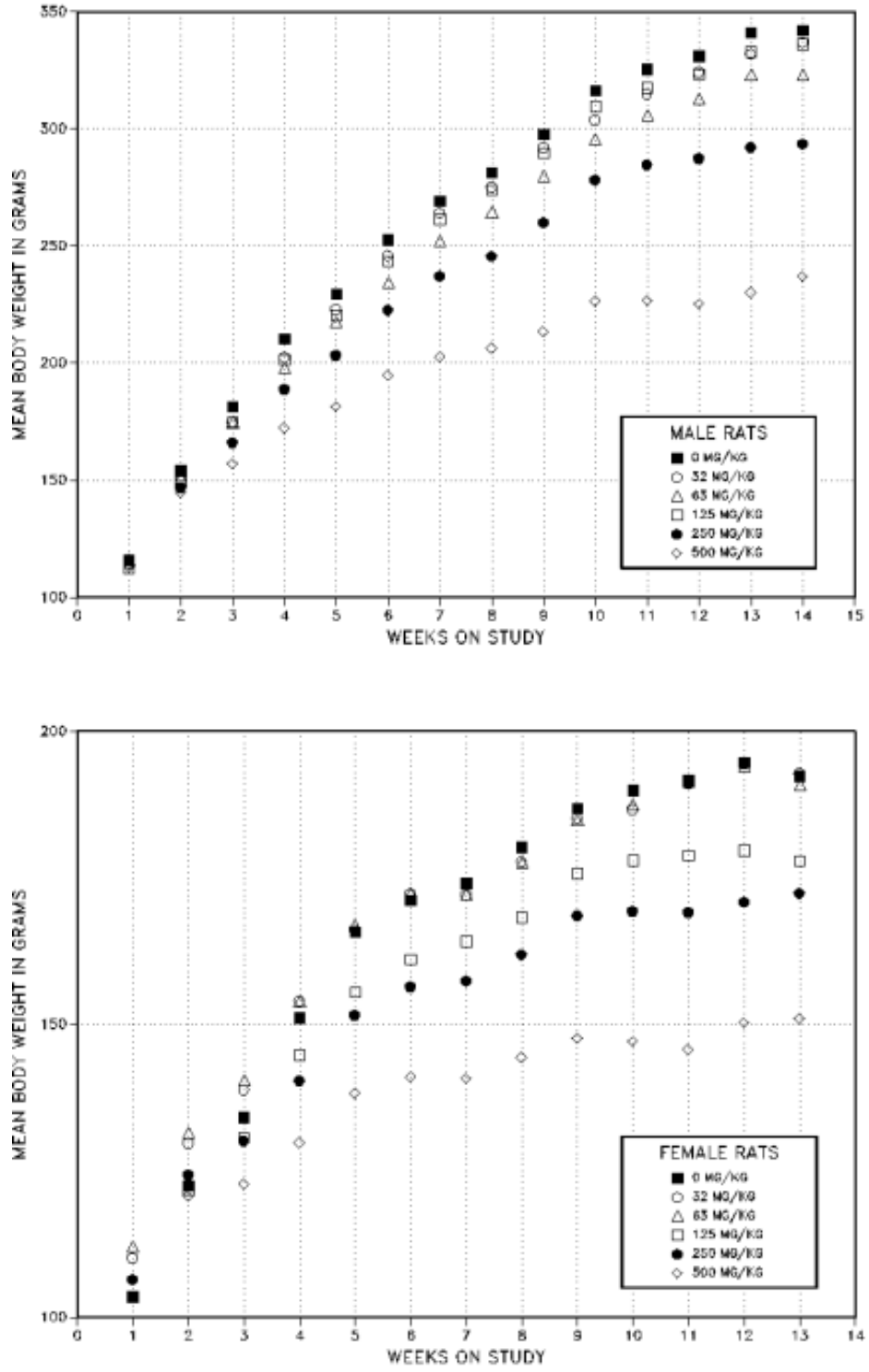

Figure 2 Body Weights of F344/N Rats Exposed Dermally to Diethanolamine for 13 Weeks

TABLE 15 Kidney and Liver Weights of F344/N Rats Administered Diethanolamine Dermally for 13 Weeks[a]

Dose (mg/kg)	0	32	63	125	250	500
MALE						
Necropsy weight	347	342	328	342	300	241**
Kidney weight	1.17	1.41**	1.21	1.32*	1.20	1.31
Relative kidney weight	3.39	4.12**	3.68**	3.87**	4.04**	5.38**
Liver weight	13.25	14.10	13.29	16.00 *	15.12*	14.05*
Relative liver weight	38.2	41.2*	40.5**	46.6**	50.3**	58.3**
FEMALE						
Necropsy weight	193	194	191	180**	174**	154**
Kidney weight	0.69	0.97**	0.90**	0.92**	0.91**	1.05**
Relative kidney weight	3.59	5.00**	4.69**	5.12**	5.25**	6.83**
Liver weight	6.48	7.56**	7.59**	7.79**	8.17**	9.00**
Relative liver weight	33.5	38.9**	39.7**	43.4**	47.1**	58.4**

[a] Body weights and organ weights are given in grams; relative organ-weight-to-body-weight ratios are given in mg organ weight/gm body weight.
* Significantly different from the control group (P 0.05) by Williams' or Dunnett's test.
** Significantly different from the control group (P 0.01) by Williams' or Dunnett's test.

Lesions of the treated skin were similar to those present in the 2-week study, and were dose-related in incidence and severity (Table 16). The lesion was diagnosed as ulceration and ranged from small, superficial foci of epidermal loss to extensive areas of coagulation necrosis of the epidermis and dermis. The ulcers were accompanied by inflammatory cell infiltration that was prominent at the borders between necrotic and viable tissue. Inflammation was primarily neutrophilic, but was designated "chronic-active" due to the frequent appearance of fibrovascular tissue proliferation in the vicinity of ulcers. Minimal to moderate acanthosis (epidermal hyperplasia) invariably was present at ulcer margins in the higher dose groups; at lower dose levels, only minimal acanthosis and hyperkeratosis were present.

Demyelination in the medulla oblongata was observed in all males and females in the 500 mg/kg dose group, and in 7 females in the 250 mg/kg dose group (Table 16). The lesion was morphologically and topographically similar to that diagnosed in the 13-week drinking water study; it was characterized by intramyelinic vacuoles arranged symmetrically around the medial medulla oblongata in the region of the tectospinal tract. Unlike the drinking water study, however, all lesions were minimal in severity and there was no spinal cord involvement.

All early-death rats in this study had lesions of the kidney, skin, and brain as described above. The severity of these lesions, however, was no greater than was seen in animals that survived to the end of the study.

TABLE 16 Incidence and Severity of Kidney, Brain, and Skin Lesions in F344/N Rats Administered Diethanolamine Dermally for 13 Weeks[a]

Dose (mg/kg)	0	32	63	125	250	500
MALE						
Kidney						
Nephropathy	9/10 (1.0)	6/10 (1.0)	5/10 (1.0)	6/10 (1.0)	4/10 (1.0)	5/10 (1.0)
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	0/10	0/10
Tubular mineralization	0/10	0/10	0/10	0/10	0/10	9/10 (1.9)
Brain, medulla						
Demyelination	0/10	0/10	0/10	0/10	0/10	10/10 (1.0)
Skin						
Ulcer	0/10	0/10	0/10	0/10	3/10 (1.3)	10/10 (2.6)
Chronic active inflammation	0/10	0/10	0/10	0/10	3/10 (1.3)	10/10 (1.7)
Acanthosis	0/10	0/10	3/10 (1.0)	6/10 (1.0)	6/10 (1.5)	10/10 (2.2)
Hyperkeratosis	0/10	0/10	5/10 (1.0)	10/10 (1.1)	10/10 (1.4)	10/10(1.9)
FEMALE						
Kidney						
Nephropathy	3/10 (1.0)	9/10 (1.3)	10/10 (1.4)	10/10 (1.7)	7/10 (1.1)	4/10 (1.0)
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	2/10 (1.0)	10/10 (1.0)
Tubular mineralization	4/10 (1.0)	9/10 (1.0)	10/10 (1.6)	10/10 (1.9)	10/10 (1.1)	10/10 (1.0)
Brain, medulla						
Demyelination	0/10	0/10	0/10	0/10	7/10 (1.0)	9/10 (1.0)
Skin						
Ulcer	0/10	0/10	0/10	1/10 (1.0)	7/10 (1.9)	10/10 (3.4)
Chronic active inflammation	0/10	0/10	0/10	3 /10 (1.0)	7/10 (1.6)	10/10 (2.5)
Acanthosis	0/10	0/10	1/10 (1.0)	6 /10 (1.2)	7/10 (2.0)	10/10 (2.6)
Hyperkeratosis	0/10	5/10 (1.0)	6/10 (1.0)	9 /10 (1.2)	10/10 (1.7)	10/10 (2.1)

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions.

2-Week Drinking Water Studies in B6C3F$_1$ Mice

There were no deaths in the control or treatment groups of male and female mice (Table 17). Body weights were reduced in male and female mice that received 10000 ppm diethanolamine and in female mice that received 5000 ppm. Chemically-related clinical signs were limited to animals in the top dose groups and included rough haircoat, emaciation, and abnormal posture. Water consumption was somewhat depressed in the high dose group; reduced palatability of the 10000 ppm drinking water solution may have contributed to the body weight effect at this dose level. Based on water consumption and body weight data, daily doses of diethanolamine were estimated to range from about 110 to 1360 mg/kg in males and about 200 to 2170 mg/kg in females.

No gross lesions related to treatment were observed at necropsy. Dose-dependent increases in absolute and relative liver weight were seen in male and female mice, and correlated with microscopic findings of hepatocellular cytologic alteration (Table 18). Cytologic alteration refers to a spectrum of hepatocytic changes which included cellular enlargement, increased cytoplasmic eosinophilia, and increased occurrences of binucleated hepatocytes (Plate 3). These changes resulted in disruption of the hepatic cords. Cytologic alteration was generally diffuse, but with a periportal distribution in some minimal cases. Necrosis of single, random hepatocytes was

frequently associated with cytologic alteration, and increased mitosis was present in a few affected animals. Serum sorbitol dehydrogenase activity was higher in female mice that received 10000 ppm diethanolamine than in controls (Table 18). Mild myocardial degeneration, consisting of small foci of myocyte fragmentation with inflammation and/or mineralization, was observed in 1 male and 1 female at the highest dose level.

TABLE 17 Survival, Weight Gain, and Water Consumption of B6C3F$_1$ Mice in the 2-Week Drinking Water Studies of Diethanolamine

Dose Concentration (ppm)	Survival[a]	Mean Body Weight (grams) Initial[b]	Final	Change[c]	Final Weight Relative to Controls (%)[d]	Average Water Consumption[e]	Estimated DEA Consumed[f]
MALE							
0	5/5	20.8	25.2	4.4		3.8	0
630	5/5	20.9	24.7	3.8	98	4.0	110
1250	5/5	20.8	25.1	4.3	100	3.8	205
2500	5/5	21.0	25.3	4.3	100	3.9	415
5000	5/5	20.8	24.2	3.4	96	4.2	909
10000	5/5	21.0	19.9	-1.1	79	2.9	1362
FEMALE							
0	5/5	19.1	21.0	1.9		5.5	0
630	5/5	19.0	22.3	3.3	106	6.4	197
1250	5/5	19.0	22.1	3.1	105	5.4	326
2500	5/5	19.0	22.4	3.4	107	6.6	793
5000	5/5	19.0	18.8	-0.2	90	5.4	1399
10000	5/5	18.9	18.0	-0.9	86	4.1	2169

a Number surviving at 15 days/number of animals per dose group.
b Body weights were measured 4 days before the first dose.
c Mean weight change of the animals in each dose group.
d (Dosed group mean/Control group mean) x 100.
e Average water consumption in ml/animal/day.
f Diethanolamine consumption in mg/kg body weight/day.

TABLE 18 Liver Toxicity in B6C3F$_1$ Mice in the 2-Week Drinking Water Studies of Diethanolamine[a]

Dose (ppm)	0	630	1250	2500	5000	10000
MALE						
Necropsy weight	25.2	24.7	25.1	25.3	24.2	19.9
Liver weight	1.50 ± 0.16	1.61 ± 0.09	1.73 ± 0.12	1.94 ± 0.13**	2.18 ± 0.13*	1.86 ± 0.32*
Relative liver weight	59.8 ± 6.6	65.1 ± 4.8	69.1 ± 5.3	76.5 ± 4.2**	90.0 ± 4.4**	93.2 ± 11.8**
SDH, IU/L	84.5 ± 50.9	49.9 ± 4.9	50.0 ± 7.9	80.6 ± 17.4	127.2 ± 33.6	237.8 ± 163.6
Cytologic alteration	0/5	0/5	1/5 (1.0)[b]	4/5 (1.3)	5/5 (2.4)	5/5 (1.6)
FEMALE						
Necropsy weight	21.0	22.3	22.1	22.4	18.8	18.0
Liver weight	1.19 ± 0.10	1.34 ± 0.10	1.50 ± 0.26*	1.70 ± 0.16**	1.89 ± 0.12**	1.97 ± 0.12**
Relative liver weight.	56.5 ± 4.0	60.1 ± 2.1	67.4 ± 7.2*	75.8 ± 6.4**	100.4 ± 8.3**	109.0 ± 3.6**
SDH, IU/L	48.3 ± 11.0	43.8 ± 13.9	39.2 ± 4.7	44.0 ± 9.1	84.3 ± 29.4	105.7 ± 30.3*
Cytologic alteration	0/5	0/5	1/5 (1.0)	1/5 (1.0)	5/5 (1.8)	5/5 (1.4)

a Body weights and organ weights given in grams; ratio of organ-weight-to-body-weight given in mg organ weight/gram body weight. Values are means ± SD.
b Incidence and severity score( ) based on a scale of 1 (minimal) to 4 (marked). Severity scores are averages based on the number of animals with lesions from groups of 5.
* Significantly different from the control group, P 0.05 (ANOVA, Dunnett).
** Significantly different from the control group, P 0.01 (ANOVA, Dunnett).

13-Week Drinking Water Studies in B6C3F$_1$ Mice

All males and females in the 5000 and 10000 ppm dose groups and 3 females in the 2500 ppm dose group died before the end of the study (Table 19). Mice in the top dose group all died or were killed in a moribund condition by week 5; animals in the 5000 ppm groups died by week 10. Body weight gains were decreased in males that received 2500 ppm and in females that received 1250 or 2500 ppm diethanolamine (Figure 3). Water consumption was not affected in those groups of mice that survived until the end of the study. Based on water consumption and body weight data, the average daily doses of diethanolamine were estimated to range from about 100 to 1600 mg/kg in males and about 150 to 1120 mg/kg in females. Among animals that died early, and those in the 2500 ppm dose group, toxic signs included tremors, ruffled fur, emaciated appearance, abnormal posture, and hypoactivity.

TABLE 19 Survival, Weight Gain, and Water Consumption of Mice in the 13-Week Drinking Water Studies of Diethanolamine

		Mean Body Weight (grams)			Final Weight Relative	Average Water	Estimated DEA
Dose (ppm)	Survival[a]	Initial	Final	Change[b]	to Controls (%)[c]	Consumption[d]	Consumed[e]
MALE							
0	10/10	23.7	39.1	15.4		4.8	
630	10/10	23.5	37.6	14.1	96	5.1	104
1250	10/10	23.6	37.8	14.2	97	4.4	178
2500	10/10	23.6	35.5	11.9	91	5.1	422
5000	0/10[f]	23.5	g	g	g	4.0	807
10000	0/10[h]	23.7	g	g	g	3.9	1674
FEMALE							
0	10/10	20.0	32.7	12.7		6.9	
630	10/10	20.0	33.2	13.2	102	6.1	142
1250	10/10	20.0	29.8	9.8	91	6.9	347
2500	7/10[i]	20.0	26.7	6.7	82	8.2	884
5000	0/10[j]	20.0	g	g	g	4.8	1154
10000	0/10[k]	20.0	g	g	g	2.2	1128

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.
d Average water consumption in ml/animal/day.
e Chemical consumption in mg/kg body weight/day.
f Day of Death: 19, 24, 25 ,28, 30, 30, 35, 35, 52, 70.
g All animals in group died before scheduled termination.
h Day of Death: 24, 25, 25, 25, 25, 29, 30, 30, 32, 32.
i Day of Death: 30, 78, 82.
j Day of Death: 14, 15, 18, 20, 20, 22, 24, 27, 28, 31.
k Day of Death: 15, 17, 18, 20, 20, 21, 21, 21, 23, 27.

No significant gross findings were observed at necropsy in mice that died early or survived to study termination. Absolute and relative liver weights were increased markedly in a dose-dependent manner in male and female mice, with significant increases in both parameters at all exposure concentrations (Table 20). Liver weight changes were associated with increases in serum alanine aminotransferase and sorbitol dehydrogenase activities, as well as with microscopic changes diagnosed as hepatocellular cytologic alteration and necrosis (Table 20; Table 21). Cytologic alteration, as in the 2-week study, consisted of multiple hepatocytic

changes including hypertrophy (cellular enlargement) with increased eosinophilia and disruption of hepatic cords. These lesions were observed in early-death mice, as well as in mice that survived to the end of the study. In addition, there was increased nuclear pleomorphism and the frequent presence of large, multinucleated hepatocytes. These giant cells often contained 10 or more nuclei. Hepatocyte necrosis was randomly distributed and involved single cells or small foci.

Increases in absolute and relative kidney weights in male mice (Table 20) were associated with a dose-dependent increase in incidences of nephropathy among those mice that survived to the end of the study (Table 21). Nephropathy was minimal, and consisted of renal tubules lined by basophilic cells with high nuclear/cytoplasmic ratio; this was considered a regenerative response, although active tubular necrosis was observed only in a few early-death mice in the 2 highest dose groups.

Heart weight increased in female mice that received 2500 ppm diethanolamine, and relative heart weights increased in males (2500 ppm group) and females (1250 and 2500 ppm groups). This finding is noteworthy because minimal-to-marked degeneration and necrosis of cardiac myocytes was observed in male and female mice exposed to 2500 ppm or higher concentrations of diethanolamine (Table 21). This lesion was characterized by degenerated and fragmented myofibers, primarily in the ventricles, associated with inflammatory cells or mineralization (Plate 5). Patchy fibrosis in areas of myocyte loss was occasionally present, and ventricular chambers appeared dilated in more severely affected animals. Myocardial degeneration was generally more severe in the higher dose mice that died early than in those that survived to study termination; this may have caused early deaths of some of these animals.

Microscopic changes in the submandibular salivary gland, diagnosed as cytologic alteration, were treatment-related in the 3 highest dose groups of male and female mice (Table 21). This lesion was characterized by a reduction in size and loss of eosinophilic granules in the secretory duct cells of this gland. There was a concomitant hypertrophy of secretory acini due to an increased amount of lightly basophilic-staining cytoplasm. No effects were observed in parotid or sublingual salivary glands of these animals.

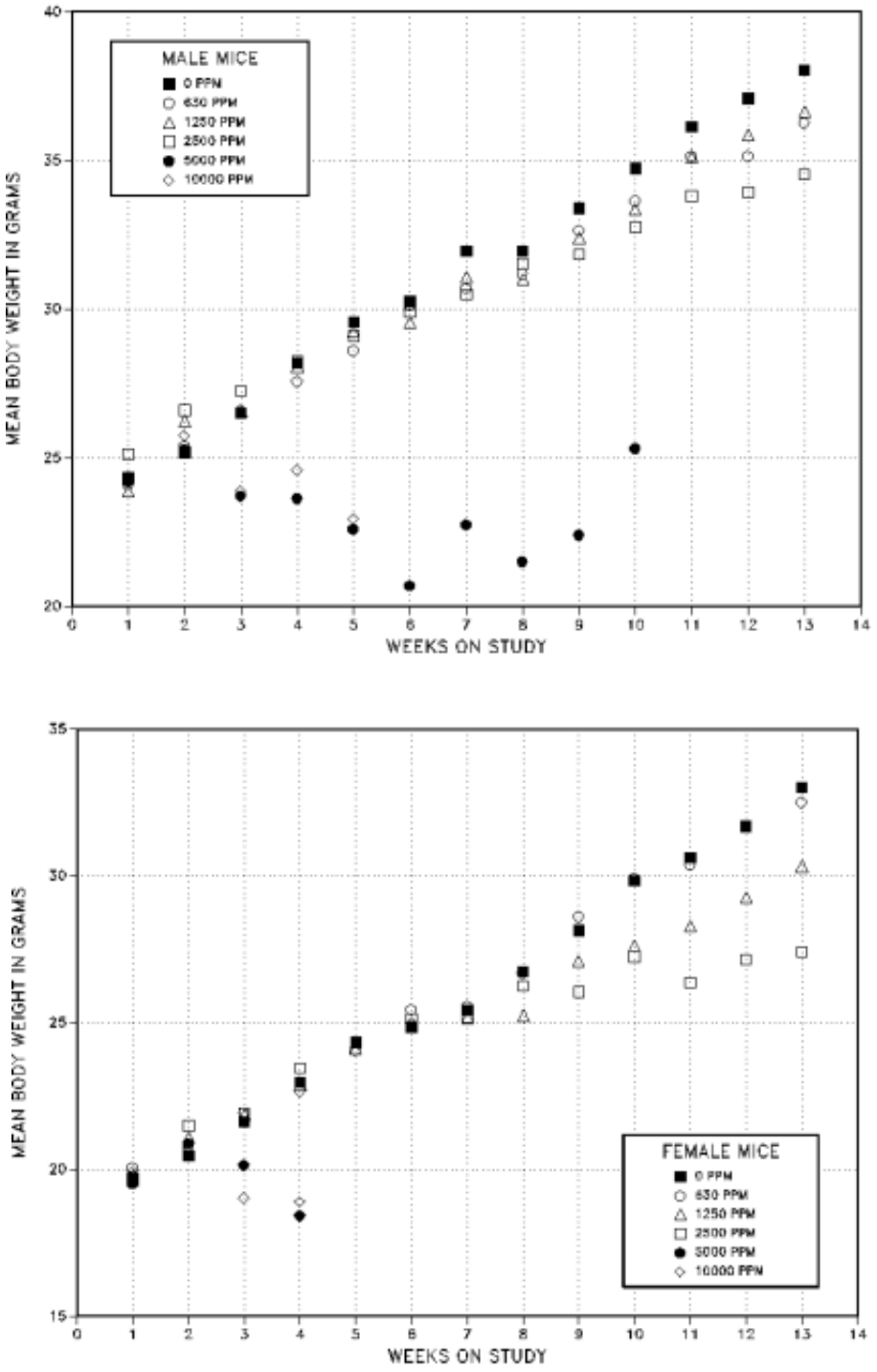

Figure 3 Body Weights of B6C3F$_1$ Mice Exposed to Diethanolamine by Drinking Water for 13 Weeks

TABLE 20 Selected Organ Weights and Clinical Pathology of B6C3F$_1$ Mice in the 13-Week Drinking Water Studies of Diethanolamine[a]

Dose (ppm)	0	630	1250	2500
MALE				
Body weight at necropsy	39.1	37.6	37.8	35.4
Kidney weight	0.321	0.329	0.354*	0.365**
Relative kidney weight	8.22	8.74	9.38**	10.35**
Heart weight	0.171	0.162	0.173	0.179
Relative heart weight	4.39	4.31	4.58	5.07**
Liver weight	1.67	1.89**	2.07**	2.36**
Relative liver weight	42.64	50.29**	54.89**	66.65**
Alanine aminotransferase (IU/L)	40	35	33	91‡
Sorbitol dehydrogenase (IU/L)	58	50	56	107‡
FEMALE				
Body weight at necropsy	32.7	33.2	29.8	26.7
Kidney weight	0.218	0.229	0.225	0.235
Relative kidney weight	6.71	6.93	7.57*	8.80**
Heart weight	0.144	0.146	0.152	0.161**
Relative heart weight	4.42	4.41	5.14**	6.03**
Liver weight	1.33	1.70**	1.85**	2.46**
Relative liver weight	41.01	51.17*	62.69**	91.93**
Alanine aminotransferase (IU/L)	25	25	32†	74‡
Sorbitol dehydrogenase (IU/L)	37	36	36	47

[a] Body weights and organ weights are given in grams; relative organ weights are given in mg organ weight/g body weight. All animals in the 5000 and 10000 ppm groups died prior to scheduled termination.

* Significantly different from the control group (P 0.05) by Williams' or Dunnett's test.

** Significantly different from the control group (P 0.01) by Williams' or Dunnett's test.

† Significantly different from the control group (P 0.05) by Dunn's or Shirley's test.

‡ Significantly different from the control group (P 0.01) by Dunn's or Shirley's test.

TABLE 21 Incidence and Severity of Liver, Kidney, Heart and Salivary Gland Lesions in B6C3F$_1$ Mice Administered Diethanolamine in Drinking Water for 13 Weeks[a]

Dose (ppm)	0	630	1250	2500	5000[b]	10000[b]
MALE						
Liver						
Cytologic alteration	0/10	9/10 (2.0)	10/10 (2.8)	10/10 (3.0)	10/10 (3.0)	10/10 (3.0)
Hepatocellular necrosis	0/10	0/10	0/10	9/10 (1.0)	7/10 (1.3)	9/10 (1.2)
Kidney						
Nephropathy	0/10	1/10 (1.0)	5/10 (1.0)	8/10 (1.0)	2/10 (1.0)	0/10
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	0/10	2/10 (1.0)
Heart						
Degeneration	0/10	0/10	0/10	1/10 (1.0)	10/10 (2.8)	10/10 (2.8)
Salivary gland						
Cytologic alteration	0/10	0/10	0/10	10/10 (1.0)	10/10 (2.7)	10/10 (3.0)
FEMALE						
Liver						
Cytologic alteration	0/10	10/10 (1.9)	10/10 (2.8)	10/10 (3.0)	10/10 (3.0)	10/10 (3.0)
Hepatocellular necrosis	0/10	0/10	1/10 (1.0)	4/10 (1.0)	8/10 (1.1)	7/10 (1.3)
Kidney						
Nephropathy	0/10	0/10	0/10	1/10 (1.0)	1/10 (1.0)	0/10
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	1/10 (1.0)	0/10
Heart						
Degeneration	0/10	0/10	0/10	9/10 (1.2)	10/10 (2.6)	10/10 (2.6)
Salivary gland						
Cytologic alteration	0/10	0/10	0/10	7/10 (2.9)	10/10 (4.0)	10/10 (4.0)

a Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.
b All animals at this dose level died prior to study termination.

2-Week Dermal Studies in B6C3F$_1$ Mice

Chemically-related deaths occurred only in the 2500 mg/kg dose group; all male mice and 3 females in this group died after day 11 of the study; 1 male was killed in a moribund condition (Table 22). There were no apparent body weight effects in male or female mice exposed to lower doses of diethanolamine (160 to 1250 mg/kg).

Ulceration, irritation, and crusting at the site of application were observed in male mice in the 1250 and 2500 mg/kg dose groups and in females in the 2500 mg/kg dose group. These lesions were characterized microscopically as epidermal ulceration and inflammation of moderate to marked severity (Table 23). Ulcerative necrosis of the epidermis extended into the underlying dermis to variable degrees. Inflammatory infiltrate was primarily neutrophilic; these cells exhibited degenerative changes, forming borders of cellular debris surrounding necrotic areas. Acanthosis (epidermal hyperplasia) of minimal severity and without inflammatory cell infiltrate was present in all mice in the 3 lowest dose groups (160, 320, and 630 mg/kg). At the 1250 mg/kg dose level, acanthosis was minimal to moderate in severity.

Dose-dependent increases in absolute and relative liver weights were observed in male and female mice (Table 24). Microscopic findings were limited to minimal cytologic alteration of

hepatocytes of all males and females at the highest dose level. The lesion consisted of slight enlargement, and increased eosinophilia of hepatocytes, with a periportalt to diffuse distribution.

No biologically significant changes in clinical chemistry parameters were detected in any dose group, and no conclusive cause of death could be established for the high-dose mice that died before the end of the study. Minimal cardiac degeneration was observed in 2 high-dose males that died early.

TABLE 22 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Dermal Studies of Diethanolamine

Dose (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[d]
		Initial[b]	Final	Change[c]	
MALE					
0	5/5	23.7	26.0	2.3	
160	5/5	23.6	25.9	2.3	100
320	5/5	23.9	27.0	3.1	104
630	5/5	23.7	26.3	2.6	101
1250	5/5	23.4	26.5	3.1	102
2500	0/5[e]	23.8	f	f	
FEMALE					
0	5/5	19.4	22.0	2.6	
160	5/5	19.3	22.5	3.2	102
320	5/5	19.3	22.2	2.9	101
630	5/5	19.3	23.1	3.8	105
1250	5/5	19.2	23.7	4.5	108
2500	2/5[g]	19.4	19.5	0.1	89

a Number surviving at 17 days/number of animals per dose group.
b Weight recorded four days prior to study start.
c Mean weight change of the animals in each dose group.
d (Dosed group mean/Control group mean) x 100.
e Day of Death: 11, 12, 14.
f All animals in group died before scheduled termination.
g Day of Death: 11, 11, 14.

TABLE 23 Incidence and Severity of Skin Lesions in B6C3F$_1$ Mice in the 2-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	160	320	630	1250	2500[b]
MALE						
Ulcer	0/5	0/5	0/5	0/5	3/5 (2.7)	5/5 (3.8)
Chronic active inflammation	0/5	0/5	0/5	0/5	5/5 (2.4)	5/5 (3.6)
Acanthosis	0/5	5/5 (1.0)	5/5 (1.0)	5/5 (1.0)	5/5 (2.8)	0/5
FEMALE						
Ulcer	0/5	0/5	0/5	0/5	0/5	5/5 (2.8)
Chronic active inflammation	0/5	0/5	0/5	0/5	0/5	5/5 (3.0)
Acanthosis	0/5	5/5 (1.0)	5/5 (1.0)	5/5 (1.0)	5/5 (1.4)	0/5

a Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions.
b All male animals in group died before scheduled termination.

TABLE 24 Liver Weights for B6C3F$_1$ Mice in the 2-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	160	320	630	1250	2500
MALE						
Necropsy weight	26.0	25.9	27.0	26.3	26.5	b
Liver weight	1.49 ± 0.10	1.59 ± 0.17	1.80 ± 0.10*	1.94 ± 0.22**	2.09 ± 0.23**	b
Relative liver weight	57.2 ± 2.8	61.3 ± 3.4	66.5 ± 2.2**	73.7 ± 4.3**	78.8 ± 8.9**	b
FEMALE						
Necropsy weight	22.0	22.5	22.2	23.1	23.7	19.5[c]
Liver weight	1.39 ± 0.11	1.51 ± 0.21	1.60 ± 0.11	1.75 ± 0.15*	2.06 ± 0.18**	2.06 ± 0.15**[c]
Relative liver weight	63.2 ± 3.1	67.0 ± 5.1	72.2 ± 5.2*	75.9 ± 4.4*	87.2 ± 3.7**	106.0 ± 6.0**

a Body weights and organ weights given in grams; ratio of organ-weight-to-body-weight given in mg organ weight/gram body weight. Values are means ± S.D.
b All animals in group died before scheduled termination.
c N = 2.
* Significantly different from the control group, P 0.05 (ANOVA, Dunnett).
** Significantly different from the control group, P 0.01 (ANOVA, Dunnett).

13-Week Dermal Studies in B6C3F$_1$ Mice

Two male mice administered 1250 mg/kg diethanolamine were killed in moribund condition during weeks 2 and 9 (Table 25); 4 top-dose female mice died or were killed in moribund condition during weeks 2 and 3. The final mean body weight of males that received 1250 mg/kg was lower that of controls (Figure 4). The primary clinical signs of toxicity were irritation, crust formation, and thickening of the skin at the site of diethanolamine application in the 2 highest dose groups of both sexes.

Dose-dependent increases in absolute and relative liver weights (Table 26) were associated with hepatocellular cytological changes, similar to those described in previous mouse studies and collectively termed cytologic alteration. The lesions consisted of enlarged hepatocytes with homogeneous eosinophilic cytoplasm, loss of normal lobular arrangement, increased nuclear pleomorphism, and, in the higher dose levels, the presence of multinucleated, giant hepatocytes. In the most marked cases, these giant cells appeared as syncytia resulting from the fusion of several hepatocytes, with numerous superimposed or confluent nuclei (Plate 4).

Syncytia formation was more prominent in males than females in the dermal study and was more extensive in the dermal study than in the drinking water study. Hepatocellular necrosis was observed in treated male mice, but not in treated females (Table 27); this lesion was usually seen as randomly distributed small foci of coagulative necrosis. Increases in serum alanine aminotransferase and sorbitol dehydrogenase activities were observed in male mice that received 320 mg/kg or higher doses of diethanolamine (Table 26). Activities of ALT were mildly increased in female mice receiving 1250 mg/kg.

TABLE 25 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Dermal Studies of Diethanolamine

Dose (mg/kg)	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
0	10/10	22.5	34.2	11.7	
80	10/10	23.1	34.1	11.0	100
160	10/10	23.0	33.1	10.1	97
320	10/10	22.6	34.0	11.4	99
630	10/10	23.0	33.0	10.0	96
1250	8/10[d]	23.2	31.3	8.1	92
FEMALE					
0	10/10	19.0	27.8	8.8	
80	10/10	18.6	29.5	10.9	106
160	10/10	19.3	29.7	10.4	107
320	10/10	19.2	29.3	10.1	105
630	10/10	19.5	28.5	9.0	103
1250	6/10[e]	18.9	28.4	9.5	102

a Number surviving at 13 weeks/number of animals per dose group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean/Control group mean) x 100.
d Day of Death: 9, 61 (Weeks 2, 9).
e Day of Death: 12, 21, 21 (Weeks 2, 3, 3).

Microscopically, skin lesions at the site of application were similar to those present in the 2-week study, and included ulceration, inflammation, and acanthosis (Table 27). Ulceration and inflammation were observed in the 2 highest dose groups of males and females (630 and 1250 mg/kg). Necrosis of epidermal and dermal cells was focally extensive in nature and accompanied invariably by an infiltration of inflammatory cells. Inflammation was characterized as chronic-active due to the frequent presence of fibrovascular proliferation, in addition to acute inflammatory cells at the edges of ulcerated areas. Acanthosis (epidermal hyperplasia) was observed in males and females in all treatment groups. Hyperkeratosis of minimal to mild severity was also observed in males at treatment levels of 320 mg/kg or higher and in females in the 1250 mg/kg dose group.

Increases in absolute and relative kidney weights were observed in male and female mice (Table 26); however, unlike the drinking water study, these changes were not associated with increased nephropathy. Minimal to mild renal tubular necrosis was observed in 4 male mice and 1 female mouse from the 1250 mg/kg dose group.

Heart weights were increased in male and female mice that were administered 1250 mg/kg (Table 26), and cardiac myocyte degeneration was observed at this dose level (Table 27). An effect in the submandibular salivary gland, termed cytologic alteration, and consisting of reduced size and eosinophilia of secretory duct cells and swelling of acinar cells, was found in most high dose males and females (Table 27), and was similar to that seen in the 13-week drinking water study.

Administration of diethanolamine to mice in their drinking water, or topically, did not affect any of the parameters measured in the sperm morphology/vaginal cytology evaluations (Appendix C).

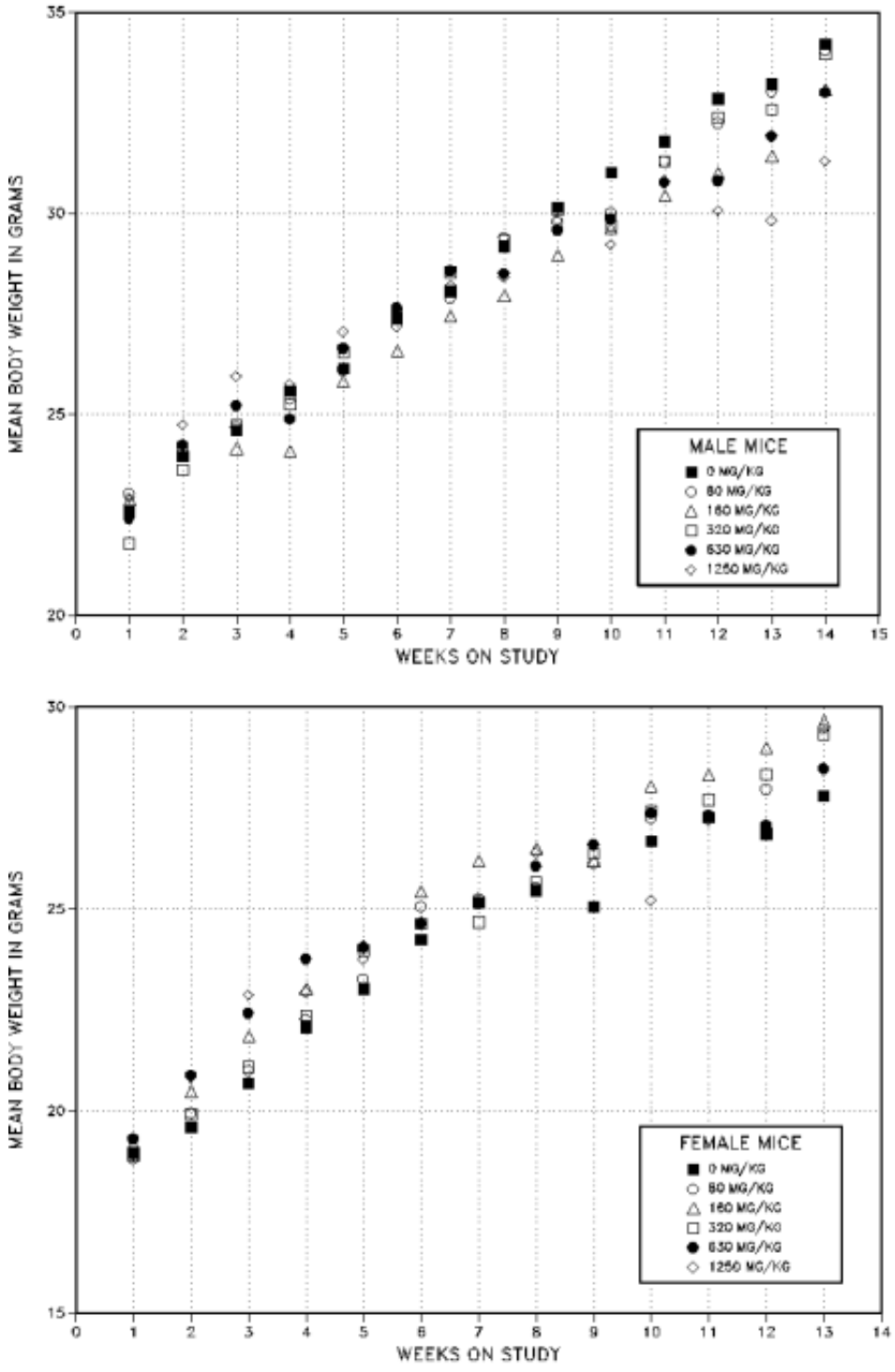

Figure 4 Body Weights of B6C3F$_1$ Mice Exposed Dermally to Diethanolamine for 13 Weeks

TABLE 26 Selected Organ Weights and Clinical Pathology of B6C3F$_1$ Mice in the 13-Week Dermal Studies of Diethanolamine[a]

Dose (mg/kg)	0	80	160	320	630	1250
MALE						
Necropsy weight	32.7	33.1	32.5	33.5	32.3	30.9
Kidney weight	0.308	0.340*	0.339*	0.364**	0.360**	0.399**
Relative kidney weight	9.47	1.03*	1.05**	1.09**	1.12**	1.29**
Heart weight	0.160	0.155	0.152	0.163	0.161	0.189**
Relative heart weight	4.93	4.69	4.70	4.86	4.98	6.12**
Liver weight	1.53	1.59	1.77**	2.06**	2.06**	2.27**
Relative liver weight	46.8	48.2	54.7**	61.5**	63.8**	73.3**
Alanine aminotransferase (IU/L)	36	33	39	47†	73†	102†
Sorbitol dehydrogenase (IU/L)	55	53	53	69†	97‡	105‡
FEMALE						
Necropsy weight	27.3	29.6	29.6	29.2	27.7	27.7
Kidney weight	0.201	0.215*	0.229**	0.223**	0.234**	0.250**
Relative kidney weight	7.38	7.31	7.79	7.65	8.47**	9.10**
Heart weight	0.135	0.136	0.136	0.146	0.145	0.167**
Relative heart weight	4.96	4.63	4.64	4.99	5.25	6.04**
Liver weight	1.36	1.67**	1.74**	1.94**	2.00**	2.61**
Relative liver weight	49.8	56.7**	59.2**	66.4**	72.0**	94.1**
Alanine aminotransferase (IU/L)	36	38	34	31	37	53‡
Sorbitol dehydrogenase (IU/L)	43	39	39	37	39	44

[a] Body weights and organ weights given in grams; organ-weight-to-body-weight ratios given in mg organ/gram body weight.
* Significantly different from the control group (P 0.05) by Williams' or Dunnett's test.
** Significantly different from the control group (P 0.01) by Williams' or Dunnett's test.
† Significantly different from the control group (P 0.05) by Dunn's or Shirley's test.
‡ Significantly different from the control group (P 0.01) by Dunn's or Shirley's test.

TABLE 27 Incidence and Severity of Liver, Kidney, Heart, Salivary Gland, and Skin Lesions in B6C3F$_1$ Mice Administered Diethanolamine Dermally for 13 Weeks[a]

Dose (mg/kg)	0	80	160	320	630	1250
MALE						
Liver						
Cytologic alteration	0/10	4/10 (1.0)	10/10 (1.0)	10/10 (1.4)	10/10 (2.0)	10/10 (2.5)
Hepatocellular necrosis	0/10	2/10 (1.0)	0/10	3/10 (1.3)	7/10 (1.1)	6/10 (2.0)
Kidney						
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	0/10	4/10 (1.3)
Heart						
Degeneration	0/10	0/10	0/10	0/10	0/10	4/10 (1.3)
Salivary gland						
Cytologic alteration	0/10	0/10	0/10	0/10	0/10	9/10 (1.2)
Skin						
Ulcer	0/10	0/10	0/10	0/10	2/10 (2.0)	10/10 (3.0)
Chr.-active inflammation	0/10	0/10	0/10	0/10	5/10 (1.2)	10/10 (2.7)
Acanthosis	0/10	10/10 (1.0)	9/10 (1.0)	10/10 (1.1)	10/10 (2.6)	10/10 (2.9)
Hyperkeratosis	0/10	0/10	0/10	2/10 (1.5)	5/10 (1.8)	10/10 (2.0)
FEMALE						
Liver						
Cytologic alteration	0/10	0/10	10/10 (1.0)	10/10 (1.1)	10/10 (1.2)	9/10 (1.3)
Kidney						
Tubular epithelial necrosis	0/10	0/10	0/10	0/10	0/10	1/10 (1.0)
Heart						
Degeneration	0/10	0/10	0/10	0/10	0/10	8/10 (1.6)
Salivary gland						
Cytologic alteration	0/10	0/10	0/10	0/10	0/10	8/10 (2.3)
Skin						
Ulcer	0/10	0/10	0/10	0/10	2/10 (1.0)	9/10 (3.3)
Chr.-active inflammation	0/10	0/10	0/10	1/10 (1.0)	1/10 (1.0)	9/10 (3.0)
Acanthosis	0/10	10/10 (1.0)	10/10 (1.0)	9/10 (1.0)	10/10 (1.3)	10/10 (2.9)
Hyperkeratosis	0/10	0/10	0/10	0/10	0/10	10/10 (2.0)

[a] Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

Genetic Toxicity

Diethanolamine (33-3333 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested with a preincubation protocol in the presence and absence of

Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Haworth *et al.*, 1983; Appendix D). No induction of trifluorothymidine resistance was observed in mouse L5178Y/tk$^{+/-}$ lymphoma cells treated with diethanolamine with or without Aroclor 1254-induced male Fisher rat liver S9 (Appendix D). In this assay, a shift in pH to the basic range was noted at all but 1 of the concentrations tested. Diethanolamine did not induce sister chromatid exchanges or chromosomal aberrations (ABS) in Chinese hamster ovary cells, with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Loveday *et al.*, 1989; Appendix D). The trial with S9 produced a dose-related increase in the percentage of cells with ABS; however, this increase was not large enough for a positive determination. As with the mouse lymphoma assay, pH shifts due to the presence of diethanolamine in the culture medium were noted. Peripheral blood

samples taken from male and female mice treated by topical application of diethanolamine (80-1250 mg/kg) in the 13-week study showed no increases in micronucleated normochromatic erythrocytes (Appendix D).

PLATES

PLATE 1. Kidney from a 10000 ppm dose group female rat in the 2-week drinking water study of diethanolamine. Tubular necrosis and loss of epithelium has resulted in a zone of staining pallor (arrows) between the cortex (C) and inner medulla (M). (H&E, 25X)

PLATE 2. Brain (coronal section at the levels of the pons) from a 10000 ppm dose group male rat in the 13-week drinking water study of diethanolamine. There is a circumscribed area of myelin loss (arrows) in the longitudinally sectioned white matter tract of the dorso-lateral medulla oblongata. (H&E, 50X)

PLATES 3a. and 3b. Liver from a male control (a) and 10000 ppm dose group (b) mouse in the 2-week dosed water study of diethanolamine. Hepatocytes in the treated animal are enlarged with darker staining cytoplasm. Many cells are binucleated. (H&E, 130X)

PLATE 4. Liver from a 1250 mg/kg dose group male mouse in the 13-week dermal study of diethanolamine. Several prominent multinucleated, syncytial hepatocytes (arrows) are seen in the centrilobular area. (H&E, 160X)

PLATE 5. Heart from a 10000 ppm dose group female mouse in the 13-week drinking water study of diethanolamine. There is widespread myofiber degeneration and mineralization (arrows) in the ventricular myocardium. (H&E, 100 X)

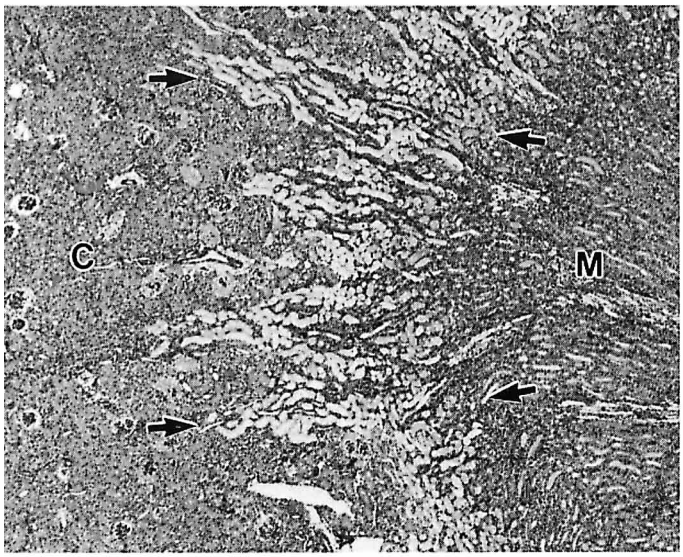

PLATE 1

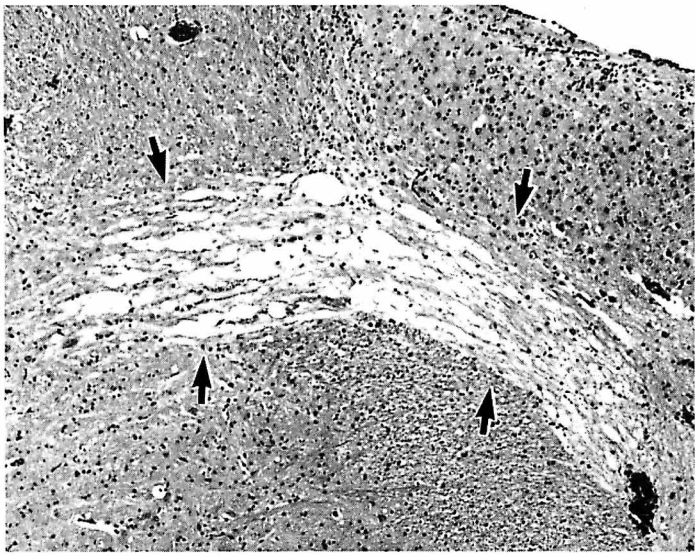

PLATE 2

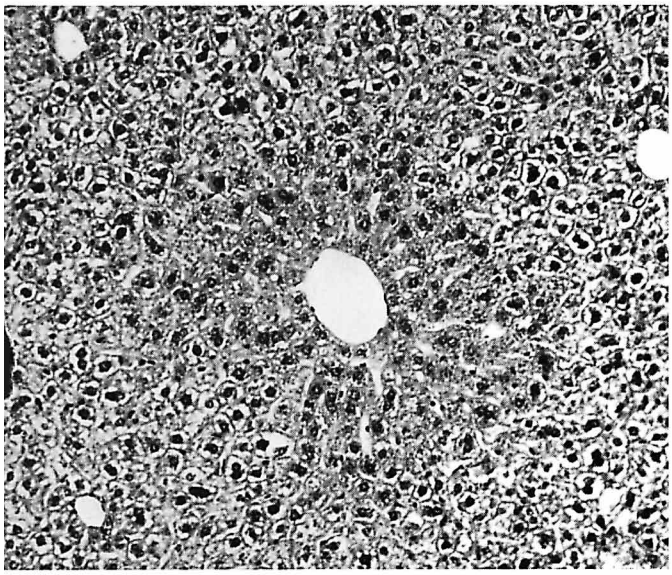

PLATE 3 (a)

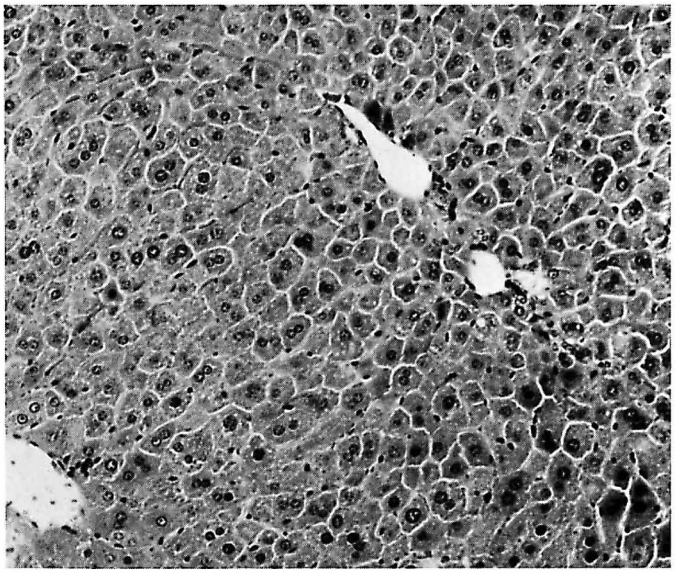

PLATE 3 (b)

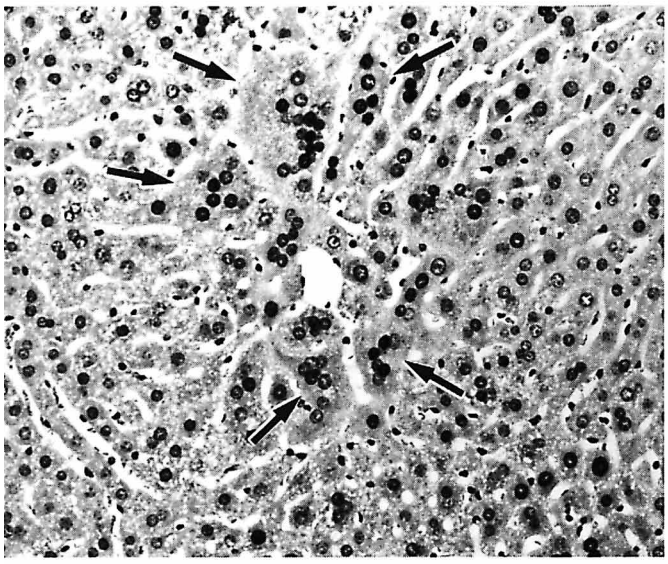

PLATE 4

PLATE 5

DISCUSSION

Two- and 13-week toxicology studies of diethanolamine were conducted in rats and mice to characterize and compare the effects of oral and dermal exposure. Chemically related changes were served in several organs; however, other than the development of skin lesions in the dermal studies, the sites affected in each species were identical for both routes of exposure. Target organs of diethanolamine toxicity included bone marrow, kidney, brain, testis, and skin in rats, and liver, kidney, heart, salivary gland, and skin in mice.

Preliminary results of disposition studies of diethanolamine in rats revealed that only 16% of a dose of 27.5 mg/kg was absorbed when applied over a skin area of 2 cm^2; at lower concentrations, the percentage of the applied dose that was absorbed was further decreased (RTI, 1991). For comparison to the dermal toxicology studies in which the dose of diethanolamine was applied over an area of about 6 cm^2, the treatment in the dermal absorption study (27.5 mg/kg applied over 2 cm^2) is approximately equal to a skin application of 83 mg/kg. This dose of diethanolamine did not cause ulceration or inflammation of the skin at the site of application in the 2- or 13-week studies. It is likely that the uptake of diethanolamine is greater than 16% at doses that cause ulceration. Oral administration of ^{14}C-diethanolamine resulted in nearly complete absorption of radiolabel from the gastrointestinal tract, and the tissue distribution of radioactivity was comparable in rats after intravenous or gavage administration (RTI, 1991). Thus, at equivalent administered doses, internal levels of diethanolamine would be much lower in rats that are exposed by topical application to non-ulcerative doses than in those given diethanolamine in the drinking water. The limited dermal absorption of diethanolamine in rats probably was the major reason why toxicologic effects were less prominent in rats exposed to equivalent total doses of diethanolamine by topical application than in those exposed via drinking water.

In mice, approximately 60% of a dose of 81 mg/kg diethanolamine was absorbed when applied over a skin area of 1 cm^2 (RTI, 1991). This dose is approximately equal to a skin application of 162 mg/kg when applied over an area of about 2 cm^2, as was done in the dermal toxicology studies in mice. This dose of diethanolamine did not cause ulceration or inflammation of the skin. A greater percentage of applied diethanolamine was absorbed from mouse skin compared to rat skin, which may be due to the fact that mouse skin is thinner than rat skin. However, this comparison of diethanolamine absorption may not be entirely valid, because a larger dose was used in the mouse study, and the absorption of diethanolamine from rat skin increased with dose. Because of the apparently higher dermal absorption of diethanolamine in rats probably was the major reason why toxicologic effects were less prominent in rats exposed to equivalent total doses of diethanolamine by topical application than in those exposed via drinking water.

Skin lesions at the site of application in the 2- and 13-week dermal studies in rats and mice included ulcerative lesions, characterized by necrosis of the epidermis and dermis that were accompanied by inflammatory cell infiltration and cell proliferative changes (hyperkeratosis and acanthosis). The no-observable-adverse-effect-level (NOAEL) for ulceration was 125 mg/kg for

male rats, 63 mg/kg for female rats, and 320 mg/kg for mice of either sex. Because the NOAEL for this lesion is higher in mice than in rats, it might seem that the rat is more susceptible than the mouse to diethanolamine-induced dermal toxicity. However, a species difference in the dermal toxicity of diethanolamine at the site of application is not evident when comparisons are based on the amount of diethanolamine applied over an equivalent surface area of skin. In Table 28 the average calculated daily doses of diethanolamine applied per animal are presented for some of the dose groups, based on the amount of diethanolamine applied per cm^2 of skin. On this basis, the species difference in diethanolamine-induced dermal toxicity is markedly diminished. For example, the skin exposure for male rats that received 250 mg diethanolamine per kg body weight is similar to that of male mice that received 630 mg per kg body weight (9.4 versus 8.8 mg/cm^2), and the incidence and severity of skin ulceration are similar in these 2 groups. Female rats were more susceptible than male rats to diethanolamine-induced skin lesions; whereas in mice, the severity and incidence of skin lesions were similar in males and females. Hyperkeratosis and acanthosis of minimal severity were observed in rats at doses lower than those that caused ulceration. A NOAEL for hyperkeratosis in female rats was not achieved in these studies. In mice, acanthosis (epidermal hyperplasia) of minimal severity was observed at lower doses than those concentrations that caused ulceration. A NOAEL for this lesion was not achieved in these studies.

TABLE 28 Daily Doses, Incidence, and Severity of Skin Ulcers in the 13-Week Dermal Studies of Diethanolamine in F344/N Rats and B6C3F$_1$ Mice

Species	Sex	Dose Group (mg/kg)	Mean Body Wt. (grams)	Avg. amount of Diethanolamine applied (mg)	Avg. Dose (mg/cm^2)	Ulcer incidence (severity)[a]
Rats	Male	250	226	56.5	9.4	3/10 (1.3)
		500	192	96.0	16.0	10/10 (2.6)
	Female	250	152	38.0	6.3	7/10 (1.9)
		500	138	69.0	11.5	10/10 (3.4)
Mice	Male	320	28.0	9.0	4.5	0/10 (NA)
		630	27.8	17.5	8.8	2/10 (2.0)
		1250	27.8	34.8	17.4	10/10 (3.0)
	Female	630	24.9	15.7	7.8	2/10 (1.0)
		1250	24.6	30.8	15.4	9/10 (3.3)

[a] Incidence and severity score based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions.

Regardless of the route or duration of exposure, diethanolamine produced a moderate, poorly regenerative, microcytic, normochromic anemia in rats. Review of preparations of peripheral blood failed to reveal any important morphologic changes in the red blood cells. Similarly, a review of histologic preparations of bone marrow showed no significant differences between control and treated animals. Based on hematologic (microcytic, poorly regenerative anemia) and histologic (lack of extramedullary hematopoiesis and hemosiderosis) findings, the cause of the anemia does not appear to involve hemolysis. Rather, the evidence is consistent with either a decreased proliferative response, or with an ineffective hematopoiesis. Causes of the former include decreased production of, or bone marrow response to, erythropoietin, and chemically induced damage to stem cells. In these situations, the anemia is generally normochromic and normocytic, and the bone marrow is hypocellular. Ineffective erythropoiesis generally results in microcytic anemia and hypercellular bone marrow, and causes include iron deficiencies, chronic disease, impaired globin chain synthesis (thalassemias), and impaired porphyrin synthesis. The

current findings are more consistent with a mechanism involving a maturation defect (ineffective erythropoiesis) rather than one in which stem cells were affected. Additional studies would be required to confirm this, however.

Although these studies did not identify a NOAEL for diethanolamine-induced hematological changes in rats, there were only minimal differences between males exposed to 32 mg/kg by topical application and controls, as well as minimal differences between females exposed to 160 ppm diethanolamine in their drinking water and their controls. These differences are considered to be chemical-related, because they were part of clear dose-response effects.

Hartung *et al.* (1970) reported that diethanolamine induced a normocytic anemia without bone marrow depletion in rats exposed to 4000 ppm in their drinking water for 7 weeks.

Rats were more susceptible than mice to diethanolamine-induced renal toxicity. In the 2-week studies there were no renal changes in mice; whereas, in rats, tubular epithelial necrosis accompanied by increases in urinary excretion of urea nitrogen, glucose, protein, and lactate dehydrogenase activity were indicative of degenerative changes in the structure and function of the proximal tubular epithelium. The no-observable-effect-levels for renal tubular necrosis in the 2-week drinking water studies in rats were 5000 ppm (620 mg/kg) in males, and 1250 ppm (160 mg/kg) in females. In the 2-week dermal study in rats, the NOAELs for renal tubular necrosis were 1000 mg/kg in males and 500 mg/kg in females. These results show that female rats are more susceptible than male rats to diethanolamine-induced nephrotoxicity. A sex difference in susceptibility to the renal toxicity of diethanolamine also was seen in the 13-week studies. Minimal renal tubular epithelial cell necrosis was observed in male rats given 5000 ppm (440 mg/kg) diethanolamine in their drinking water and in female rats given 1250 ppm (124 mg/kg) or 2,500 ppm (240 mg/kg) diethanolamine in their drinking water, or 250 mg/kg or 500 mg/kg by dermal application. The incidence and severity of renal tubular necrosis was lower in female rats exposed to 2500 ppm in the drinking water for 13 weeks than in female rats exposed to this concentration of diethanolamine for 2 weeks. Because renal tubular necrosis was less severe after 13 weeks of exposure and not accompanied by increases in urinary glucose, protein, or lactate dehydrogenase activity, as had been observed in the 2-week study, it is likely that a more active necrosis occurs early during exposure of rats to 2000 ppm or higher concentrations of diethanolamine. Increases in the incidence or severity of tubular mineralization and/or nephropathy in the 13-week studies in rats presumably resulted from earlier renal tubular epithelial cell necrosis. Renal tubular cell necrosis had been observed in single dose studies in male Sprague-Dawley rats (Grice *et al.*, 1971; Korsrud *et al.*, 1973). In mice, kidney changes were limited mainly to a dose-related increase in the incidence of nephropathy in males that survived until the end of the 13-week drinking water study, and, in the dermal study, to minimal tubular epithelial cell necrosis in the 1250 mg/kg dose groups.

The most prominent effects of diethanolamine in mice were seen in the liver. Marked increases in liver weight were accompanied by hepatocellular cytologic alteration that included hypertrophy, increased eosinophilia, individualization of hepatocytes, nuclear pleomorphism, the presence of multinucleated hepatocytes, and hepatocellular necrosis of single cells or small foci. A NOAEL was not achieved for cytologic alteration of the liver in male mice in either the drinking water or dermal studies, or in female mice in the drinking water study. Increased eosinophilic

staining is often associated with increased amounts of smooth endoplasmic reticulum; however, assays for the induction of microsomal enzymes were not included in these studies. Dilation and degranulation of the endoplasmic reticulum, and mitochondrial swelling, were noted in the liver of mice given a lethal dose of diethanolamine (Blum *et al.*, 1972). Although hepatocellular hypertrophy in mice has been observed to result from nearly 20% of the chemicals evaluated in NTP 13-week studies, the occurrence of multinucleated hepatocytes is much less common. Multinucleated hepatocytes were observed in male mice, but not in female mice, that were treated with either bisphenol A (NTP, 1982) or 1,4-dichlorophenol (NTP, 1989) for 13 weeks or 2 years. Neither of these chemicals caused an increase in the incidence of hepatocellular neoplasms in the 2-year studies. A causal association between multinucleated hepatocytes and liver neoplasia has not been established. The mechanism by which multinucleated hepatocytes are formed is unknown. 2,4-Dichlorophenol, an uncoupler of mitochondrial oxidative phosphorylation (Weinbach and Garbus, 1965), may block normal cell division in mouse hepatocytes by reducing intracellular levels of ATP. In contrast, diethanolamine does not directly affect energy-coupled processes in liver mitochondria (Barbee and Hartung, 1979a). As suggested below, cellular changes in animals dosed with diethanolamine may be due to alterations in membrane phospholipid structure. Liver weights also were increased in rats treated with diethanolamine; however, there were no corresponding histopathological changes seen in the liver. Increases in serum bile salt concentrations in exposed rats were indicative of a decreased functional ability of rat hepatocytes to reabsorb circulating bile acids.

Two unusual lesions caused by diethanolamine were demyelination in the brain (medulla oblongata) and spinal cord in rats, and cardiac degeneration in mice. Demyelination in the brain was observed in the 13-week drinking water and dermal application studies in rats, but not in the 2-week studies, and female rats appeared to be develop this lesion at lower oral and dermal doses than did males. Brain lesions were not seen in mice, even though that species received higher doses of diethanolamine than did rats. In another NTP study, demyelination in the medulla of the brain was observed in rats, but not in mice, administered glycidol for 13 weeks; in subsequent 2-year studies, glycidol caused uncommon gliomas of the brain in rats (NTP, 1990). It is difficult to predict the potential carcinogenicity of diethanolamine to the brain based on the findings with glycidol, because it is not known whether or not brain demyelination influences the outcome of chemically induced carcinogenicity. Some factors impacting on this consideration are a) demyelination by glycidol was not observed in the 2-year studies, b) unlike diethanolamine, glycidol is an alkylating agent, and c) most glial tumors induced by glycidol were not located in the part of the brain where demyelination had been seen in the 13-week studies. Other chemicals known to cause demyelination in the brain include hexachlorophene (Pleasure *et al.*, 1974), isonicotinic acid hydrazide (isoniazid) (Lampert and Schochet, 1968), and triethytin (Leow *et al.*, 1979). Long-term studies in rats have not revealed carcinogenic effects in the brain of animals exposed to isoniazid or hexachlorophene (IARC, 1974; IARC, 1979). Diethanolamine shows no structural relationship to these chemicals.

Heart lesions rarely have been seen in toxicology studies conducted by the NTP. The only other recent example of myocardial degeneration was observed in rats and mice exposed by inhalation to methyl bromide (NTP, in press). In those studies, heart lesions were prominent in both species, and were associated with a reduction in heart weight (Eustis *et al.*, 1988). In 2-year inhalation studies of methyl bromide in mice, myocardial degeneration occurred in animals that

died during the first six months of the study; whereas, mice that survived after that time showed a high incidence of chronic cardiomyopathy. Thus, chronic cardiomyopathy may result from the progression of early myocardial degenerative changes. Cardiac myocyte degeneration was observed in male and female mice exposed to 2500 ppm or higher concentrations of diethanolamine in their drinking water and in male and female mice exposed to 1250 mg/kg diethanolamine by dermal application; such degeneration probably was a major contributing factor to the early mortality of male and female mice that received 5000 or 10000 ppm diethanolamine in the 13-week drinking water study. Unlike the effect of methyl bromide, heart weights were increased in mice exposed to diethanolamine. Myocardial degeneration may occur by a different mechanism in animals exposed to methyl bromide, since in contrast to diethanolamine the later chemical is a highly toxic methylating agent. Heart lesions were not detected in rats exposed to diethanolamine; however, rats were not exposed to doses sufficient to cause more than a 10% incidence of cardiac degeneration in mice.

It is likely that testicular degeneration was a direct toxic effect of diethanolamine in male rats. In the 13-week drinking water study, degeneration of the seminiferous tubule epithelium was associated with dose-related decreases in testis and epididymis weights; in the 2500 ppm dose group, it was associated with reduced sperm motility and count in the cauda epididymis. Epididymis weight was the most sensitive endpoint for detecting reproductive toxicants from male reproductive organ weight measurements and from sperm motility examinations (Morrissey *et al.*, 1988). The effects of diethanolamine on the male reproductive system are indicative of a potential to impair reproductive capability. Based on the results of these studies, reproductive toxicity evaluations of diethanolamine, by a continuous breeding protocol, are planned by the NTP.

In 13-week studies, administration of diethanolamine by either the drinking water or dermal route resulted in salivary gland effects in both sexes of mice. Lesions were limited to the submandibular gland, in which microscopic changes were observed in both the acinar and ductal compartments. Acinar cells were swollen due to increased amounts of pale-staining cytoplasm, whereas duct cells were diminished in size, with loss of the normal granular, eosinophilic staining intensity. Acinar hypertrophy of salivary glands has been demonstrated in other subchronic toxicity studies; a recent example is that of glyphosate, in which submandibular and parotid effects were observed in rats and mice administered the chemical in dosed feed (NTP, 1991a). In this study, the salivary gland lesion was demonstrated to be at least partly mediated by an adrenergic mechanism; a similar mechanism was proposed for other chemicals which have induced enlargement of salivary gland acinar cells and which also have structural similarities to catecholamines, such as doxylamine and methapyrilene. Diethanolamine, a substituted alkylamine, also may fall into this category. Atrophic change of the submandibular granular duct cells, also evident in the diethanolamine study, was not present in these other studies and, therefore, probably is mediated through some other mechanism. Because these cells are the synthesis site for many bioactive peptides and are under the trophic influence of several hormones, it is possible that generalized debilitation and loss of trophic input resulted in atrophy of this compartment.

The mechanism of diethanolamine-induced toxicity is not known, but a number of possible contributing factors may be considered. Disposition studies have shown that urinary excretion

was the major route of elimination of radiolabel by rats and mice after i.v. or gavage administration of ^{14}C- labeled diethanolamine (RTI, 1991). Unexpectedly, only 30% of the total dose of this highly polar compound was eliminated by either species at 48 hours after administration; most of the administered dose was retained in tissues. The highest concentrations of radiolabel were found in liver and kidney, and tissue to blood ratios ranged from 100 to 200. In single dose experiments, concentrations of diethanolamine equivalents in liver and kidney of male F344 rats, following oral administration of 8 mg/kg or dermal administration of 27.5 mg/kg (spread over an area of 2 cm^2), were approximately 0.2-0.5 mM. In $B6C3F_1$ mice, the liver and kidney concentrations of diethanolamine equivalents were approximately 2 mM after dermal administration of 81 mg/kg (spread over an area of 1 cm^2). These high-tissue concentrations were achieved with an oral dose that was less than that used in the 13-week studies and with dermal doses that were approximately the same as the next-to-lowest doses used in the 13-week toxicology studies. In repeated dose studies, steady-state tissue levels of diethanolamine equivalents were approximately 6 to 12 times higher than those after a single oral administration. Thus, with repeated administration of higher doses of diethanolamine, even greater tissue concentrations than those described above would be expected. If diethanolamine equivalents in these tissues are present largely as the parent compound, then toxicity may somehow be related to the high intracellular transport of this positively charged chemical, which may hinder normal cellular functions. A direct interference by 5 mM diethanolamine on mitochondrial oxidative phosphorylation, however, was not observed in *in vitro* studies (Barbee and Hartung, 1979a).

It is doubtful that biotransformation of diethanolamine to a reactive intermediate occurs in the rat. HPLC analyses of rat urine samples collected after a single dose, or five repeated doses, of ^{14}C-diethanolamine showed the predominant radiolabeled urinary analyte (>90%) to be diethanolamine. Furthermore, chloroform-methanol-HCl extraction (100:100:1) of radioactivity from the liver of rats given a single i.v. injection of ^{14}C-diethanolamine led to recovery of 72% of the radioactivity in the aqueous phase and 6% in the organic phase. HPLC analysis of the aqueous phase yielded only a single peak, which corresponded to diethanolamine.

High tissue concentrations of diethanolamine equivalents may occur because the cell takes up diethanolamine by a mechanism intended to maintain intracellular ethanolamine. Eight hours after i.p. injection of ^{14}C-ethanolamine (0.52 µmoles) in Wistar rats, 50% of the injected radioactivity was found in the liver, with greater than 90% of the hepatic radioactivity in the lipid fraction (Taylor and Richardson, 1967). Twenty-four hours after dermal application of ^{14}C-ethanolamine to athymic nude mice, 26% of the applied radioactivity was found in the liver and 2.2% was in the kidneys (Klain *et al.*, 1985); radioactivity was found in hepatic phospholipids and in proteins and amino acids. The efficient accumulation of ethanolamine by the liver and the incorporation of radiolabel into hepatic phospholipids may be indicative of an active transport mechanism that maintains ethanolamine for phospholipid synthesis. If diethanolamine can substitute for ethanolamine, then it too would be actively accumulated. Such a mechanism would account for the high tissue to blood ratios for diethanolamine in rats and mice.

Inside the cell, diethanolamine may partially substitute for ethanolamine or choline in the synthesis of phosphatidyl-ethanolamine or phosphatidylcholine, or it could inhibit the synthesis of these membrane components. A number of experimental findings are pertinent to this

suggestion. Barbee and Hartung (1979b) reported that diethanolamine decreased the incorporation of choline and ethanolamine into phosphoglycerides in hepatic and renal tissues of Sprague-Dawley rats. These decreases were seen only after repeated exposure (1 to 3 weeks) to diethanolamine. The *in vitro* syntheses of phosphatidylcholine and phosphatidylethanolamine in liver tissue also were inhibited by diethanolamine (Ki = 3 mM). In addition, liver tissue was capable of incorporating diethanolamine into a phospholipid derivative; however, the effectiveness of diethanolamine as a substrate (Km = 12 mM) for this reaction was much less than that for choline (Km = 0.076 mM) or ethanolamine (Km = 0.054 mM). Jenkins and Melnick (1989) reported that hepatic lipid levels of phosphatidyl-ethanolamine and phosphatidylcholine were decreased in rats exposed to diethanolamine; whereas, in mice exposed to diethanolamine, HPLC analyses of hepatic lipid extracts revealed multiple peaks in the regions of phosphatidylethanolamine and phosphatidylcholine elution. These novel peaks may represent atypical phospholipids. Finally, the organic extract obtained from livers of rats given a single i.v. injection of ^{14}C-diethanolamine revealed radioactivity, detected by HPLC, in the region of phosphatidylethanolamine elution (RTI, 1991b). Thus, there is compelling evidence that diethanolamine may interfere with phosphatidylethanolamine or phosphatidylcholine synthesis, or even become incorporated into a phospholipid derivative. Although diethanolamine is neither a potent inhibitor of nor an effective substrate in phospholipid synthesis, the high accumulation of this chemical in various organs may lead to tissue levels which result in an adverse effect on subcellular membrane structure and function. Thus, it is suggested that the systemic toxicity of diethanolamine is related to its tissue accumulation and subsequent alteration of membrane phospholipid composition. In this regard, the requirement for repeated exposure of rats to diethanolamine to impair hepatic mitochondrial oxidative phosphorylation was attributed to an effect on phospholipid metabolism and subsequent alterations in mitochondrial membrane structure (Barbee and Hartung, 1979a).

Phospholipid synthesis occurs independently in most tissues except for plasma phospholipids, which are synthesized by the liver (Van Den Bosch, 1980). A combination of factors may influence the dose response and target sites of diethanolamine toxicity, including the efficiency of uptake of diethanolamine, relative tissue concentrations of ethanolamine and choline, and tissue differences in the kinetics of diethanolamine incorporation or of inhibition of phospholipid synthesis. Tissues with low phospholipid turnover rates, such as the myelin fraction of brain (Van Den Bosch, 1980), may require an extended exposure to diethanolamine to elicit toxicity. Toxicity resulting from direct contact with diethanolamine may, in part, be due to irritation (e.g., skin, eyes) associated with the alkalinity of this chemical.

In conclusion, diethanolamine is toxic at multiple organ sites in rats and mice, either by oral exposure in the drinking water or by dermal application. Other than the development of skin lesions in the dermal studies, the sites of diethanolamine toxicity were independent of the route of exposure. Target organs of diethanolamine toxicity identified in these studies included bone marrow, kidney, brain, spinal cord, testis, and skin in rats, and liver, kidney, heart, salivary gland, and skin in mice. A NOAEL was not achieved in the drinking water studies for hematological changes or nephropathy in rats, or for cytological alterations of liver in mice. In the dermal studies, a NOAEL was not achieved for hematological changes, nephropathy, or hyperkeratosis in rats, or for cytological alterations of liver or acanthosis in mice. Differences in dose-responses between the drinking water and dermal studies were due largely to the limited

dermal absorption of this chemical. Diethanolamine was absorbed to a greater extent by mouse skin than by rat skin. Other than dermal effects, the systemic toxicity of diethanolamine from dermal exposure can be estimated from oral exposure with adjustment for the extent of dermal absorption of the applied dose. The mechanism of diethanolamine toxicity is unknown, but may be related to its high tissue accumulation and effects on phospholipid metabolism, resulting in alterations in membrane structure and function.

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