FINAL

Report on Carcinogens Background Document for

Diethanolamine

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Diethanolamine. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Executive Summary

Introduction

Diethanolamine (DEA) is a secondary amine containing two molecules of ethanol linked through their beta carbons. It is used as an anticorrosion agent in metalworking fluids, in textile processing, and in soaps and surfactants used in consumer products. Diethanolamine was nominated for listing in the Report on Carcinogens by Dr. Frank Mirer, of the United Auto Workers, because a National Toxicology Program (NTP) two-year skin painting study of DEA concluded there was clear evidence of carcinogenic activity of DEA in male and female $B6C3F_1$ mice.

Human Exposure

Use. DEA is used both occupationally and by consumers. It is widely used as an intermediate in the production of fatty-acid condensates formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. DEA also is used as a surface-active agent and corrosion inhibitor in metalworking fluids and as a dispersant in agricultural chemical formulations. Other applications include use in adhesives; anti-static agents; cement and concrete work; coatings; electroplating; printing inks; metal cleaning and lubricating; mining; natural gas treatment; paint and pigments; paper, petroleum, and coal production; polymers and polymer production; rubber processing; soldering flux; textile finishing; and polyurethane production and use; and as an epoxy hardener, a fuel-gelling agent, a pharmaceutical intermediate, and an ointment-emulsifier.

Production. DEA usually is produced by reaction of ethylene oxide with ammonia in a 2:1 molar ratio. Estimated annual production of DEA in the United States in 1995 was 106 thousand tons, and production has been increasing since 1960.

Environmental exposure. The most probable route of environmental exposure to DEA is via dermal exposure to personal-care products (i.e., soaps, shampoos, and cosmetics), detergents, and other surfactants that contain DEA. Cosmetic formulations may have concentrations of DEA ranging from 1% to 25%.

Occupational exposure. Occupational exposure to DEA is most likely through inhalation during the use of lubricating liquids in various processes in machine building and metallurgy (e.g., cutting, die stamping, grinding, extrusion, and die casting). Dermal exposure also is expected to occur, though inhalation exposure is more probable. DEA was identified in Material Safety Data Sheets as a component of bulk cutting fluids, with concentrations ranging from 4% to 5% by weight in synthetic and semisynthetic fluids. The National Institute for Occupational Safety and Health (NIOSH) estimates that over 10 million workers in the United States are exposed to machining and grinding coolants and cutting fluids. Recent estimates from the 1981 to 1983 National Occupational Exposure Survey put the number potentially exposed to DEA at approximately 800,000 workers, many of them metalworkers. DEA has been detected in workplace air in the

metal manufacturing industry. DEA also has been detected in wetting fluids used in road paving, and levels of up to 0.05 mg/m^3 in air were detected in a stationary sample at a slurry machine.

Regulations. DEA is regulated by the United States Environmental Protection Agency (Clean Air Act; Federal Insecticide, Fungicide, and Rodenticide Act; Comprehensive Environmental Response, Compensation, and Liability Act; and Superfund Amendments and Reauthorization Act) and the Food and Drug Administration (Federal Food, Drug, and Cosmetic Act). The American Conference of Governmental Industrial Hygienists has established a threshold limit value for DEA of 0.46 ppm (1.99 mg/m^3) in air and 2 mg/m^3 for dermal exposure. NIOSH has established a recommended exposure limit of 3 ppm (15 mg/m^3).

Human Cancer Studies

No studies have been reported on the relationship between human cancer and exposure specifically to DEA. Nevertheless, ethanolamines commonly have been added to certain types of metalworking fluids since the 1950s, and numerous studies have evaluated cancer in workers exposed to metalworking fluids. These studies have reported small excesses of cancer from exposure to metalworking fluids; the most consistent finding was an excess of stomach cancers among workers exposed to synthetic fluids and among grinders using water-based (soluble, semisynthetic, or synthetic) fluids. Based on consistency between studies and strength of the risk estimate, the next strongest evidence of an association between cancer and exposure to soluble or synthetic metalworking fluids (after stomach cancer) is probably for esophageal cancer. Excesses of liver, pancreas, prostate, and laryngeal tumors and leukemia also were observed in some studies.

Metalworking fluids are complex mixtures. In addition to ethanolamines, they may contain biocides, chlorinated compounds, metals, sulfur compounds, and nitrites, which can interact with ethanolamines to form nitrosamines. (Recently, the United States Environmental Protection Agency prohibited the addition of nitrosating agents to metalworking fluids.) Moreover, workers at machining plants can be exposed to other agents, such as acid mists and asbestos. Thus, the specific effects of exposure to DEA cannot be separated from the effects of exposure to other components in metalworking fluids.

Studies in Experimental Animals

In $B6C3F_1$ mice, dermal application of DEA induced increased incidences of liver neoplasms in males (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma) and females (hepatocellular adenoma and hepatocellular carcinoma) and renal tubule adenoma in males. Liver tumors also were observed in $B6C3F_1$ mice in the NTP two-year dermal exposure bioassay of DEA and in concurrent bioassays of the coconut oil acid DEA condensate (containing 18.2% free DEA) and the lauric acid DEA condensate (containing 0.83% free DEA), but no significant increases in liver tumors were observed in the bioassay of the oleic acid DEA condensate (which contained only 0.19% free DEA). In male mice receiving the highest dose of coconut oil DEA

Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

condensate, the incidence of renal tubule neoplasms also was significantly increased. There was no evidence that DEA was carcinogenic when applied to the skin of Tg·AC transgenic mice for 20 weeks. No increased incidence of tumors was observed in male or female F344/N rats administered DEA topically five days/week for 103 weeks.

Genotoxicity

DEA did not induce reverse mutation in *Salmonella typhimurium* or *Escherichia coli*, had no effect on gene conversion in *Saccharomyces cerevisiae*, and did not induce micronuclei in larval newt blood cells. In mammalian *in vitro* systems, DEA did not induce chromosomal aberrations in rat liver cells, gene mutation in mouse lymphoma cells, or sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells. DEA did induce cell transformation in Syrian hamster embryo cells. DEA did not induce micronuclei *in vivo* in mice. The available data indicate that DEA is not mutagenic, nor is it metabolized to a mutagen.

Other Relevant Data

Absorption, distribution, and excretion. DEA is readily absorbed following oral administration and absorbed somewhat less efficiently following dermal administration. When applied dermally, DEA appears to facilitate its own absorption, as higher doses were more completely absorbed than lower doses. Distribution to the tissues was similar following administration by all routes. DEA is cleared from the tissues with a half-life of approximately 6 days; thus, it accumulates with repeated exposure. The highest concentrations are observed in liver and kidney. DEA is excreted primarily in urine as the parent molecule, with lesser amounts of *O*-phosphorylated and *N*-methylated metabolites. DEA is thought to be conserved by a mechanism that normally conserves ethanolamine, a normal constituent of phospholipids. DEA is incorporated as the head group to form aberrant phospholipids, presumably via the same enzymatic pathways that normally utilize ethanolamine. The presence of aberrant phospholipids and the disruption of choline utilization are thought to account for much of the observed toxicity of DEA.

Potential mechanisms of carcinogenicity. Potential mechanisms of DEA carcinogenicity include its conversion to a carcinogenic nitrosamine, *N*-nitrosodiethanolamine (NDELA), which occurred *in vivo* in rats simultaneously administered DEA dermally and nitrite orally. However, NDELA is not a hepatocarcinogen in these animals; thus, this mechanism probably does not explain hepatocarcinogenesis observed in $B6C3F_1$ mice. The second proposed mechanism involves the displacement of ethanolamine by DEA in phospholipids. Phosphatidyl DEA cannot serve as a precursor for synthesis of phosphatidyl choline, which is the only endogenous source of new molecules of choline in mammals. Lower levels of phosphocholine and glycerophosphocholine, which are biomarkers for choline deficiency, have been reported to be associated with chronic administration of DEA to mice. Additional observations in the Syrian hamster embryo cell culture model demonstrated that DEA can inhibit phosphatidyl choline synthesis and induce cell transformation by a mechanism that can be blocked by supplemental choline. Taken together, these observations on the effects of DEA on choline metabolism support the proposal that DEA-induced hepatocarcinogenesis may be related to choline deficiency.

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1 Introduction

Diethanolamine (DEA) is used in textile processing, in industrial gas purification to remove acid gases, as an anticorrosion agent in metalworking fluids, and in preparation of agricultural chemicals. Diethanolamine also is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. Aqueous DEA solutions are used as solvents for numerous drugs that are administered intravenously. Diethanolamine was nominated for listing in the Report on Carcinogens by Dr. Frank Mirer, of the United Auto Workers, because a National Toxicology Program (NTP) two-year skin painting study (TR-478) of DEA concluded there was clear evidence of carcinogenic activity of DEA in male and female B6C3F$_1$ mice, based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males (NTP 1999a).

1.1 Chemical identification

DEA ($C_4H_{11}NO_2$, mol wt 105.14, CASRN 111-42-2) is a secondary amine containing two molecules of ethanol linked through their beta carbons. It is a crystalline solid at room temperature and usually is offered commercially as a viscous liquid. DEA has a mild, ammonia-like odor. Synonyms for DEA include 2,2'-iminobis[ethanol], diethylolamine, bis(2-hydroxyethyl)amine, diolamine, *N,N*-diethanolamine, bis(hydroxyethyl)amine, 2,2'-dihydroxydiethylamine, and 2,2'-iminodiethanol. Its RTECS number is KL2975000. The structure of DEA is illustrated in Figure 1-1.

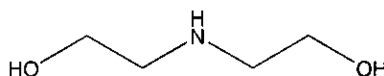


Figure 1-1. Structure of DEA

1.2 Physical-chemical properties

DEA melts at 28°C and is soluble in water, alcohol, ethanol, and benzene but insoluble in most other organic solvents. It is hygroscopic and reacts with the carbon dioxide in air. DEA is corrosive to copper, zinc, and galvanized iron. It reacts violently with oxidizers, strong acids, acid anhydrides, and halides (NTP 1999a, HSDB 2000). The physical and chemical properties of DEA are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of DEA

Property	Information	Reference
Molecular weight	105.14	ChemFinder 2001
Color	colorless to faintly colored	NTP 1999a
Odor	mild, ammonia-like	Budavari *et al.* 1996
Physical state	crystalline solid	Budavari *et al.* 1996
Melting point (°C)	28	Lide 1999
Boiling point (°C)	268.8	Lide 1999
Flash point (°C)	137	NTP 2001
Specific gravity 30°C/20°C	1.092	NTP 2001
Density at 20°C/4°C (g/cm^3)	1.0966	Lide 1999
Vapor pressure (mm Hg at 25°C)	0.00028	Syracuse Research Corp. 2001
Solubility:		
water at 14°C	> 100 mg/mL	NTP 2001
acetone at 14°C	> 100 g/mL	NTP 2001
benzene at 25°C	4.2%	NTP 2001
carbon tetrachloride	< 0.1%	Budavari *et al.* 1996
chloroform	miscible	NTP 2001
DMSO at 14°C	> 100 mg/mL	NTP 2001
ethanol	miscible	NTP 2001
95% ethanol at 14°C	> 100 mg/mL	NTP 2001
ether	slightly soluble	IARC 2000
n-heptane	< 0.1%	Budavari *et al.* 1996
methanol	miscible	NTP 2001
Log octanol-water partition coefficient (log P)	-1.43	NTP 2001
Negative log acid dissociation constant (pK_a) at 25°C	8.96	Syracuse Research Corp. 2001
Henry's Law constant at 25°C	3.87×10^{-11}	Syracuse Research Corp. 2001

1.3 Identification of metabolites and analogues

DEA has two analogues: ethanolamine and triethanolamine. Parallel metabolic pathways for DEA and ethanolamine are discussed in Section 6. Structures for ethanolamine and triethanolamine are provided in Figure 1-2.

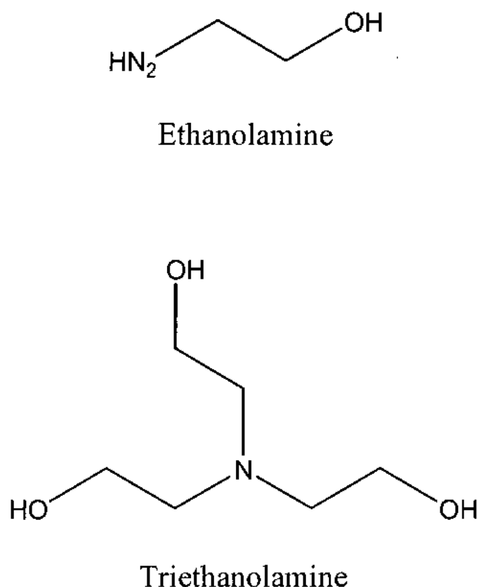


Figure 1-2. Structures of DEA analogues

Ethanolamine is a component of phosphatidylethanolamine, one of the four major phospholipids. Ethanolamine is not considered an essential nutrient for humans (Shenkin 2001). New molecules of ethanolamine can be synthesized by decarboxylation of phosphatidylserine to form phosphatidylethanolamine (Goodridge and Sul 2000).

It has been reported that when DEA was administered to Sprague-Dawley rats dermally in combination with sodium nitrite in the drinking water at 2,000 ppm, *N*-nitrosodiethanolamine (NDELA) was formed as a metabolite (Preussmann *et al.* 1981). NDELA (Figure 1-3) has been demonstrated to be a potent animal carcinogen in various models by a variety of exposure routes (see Section 6) (NTP 2001). Thus, there is concern that NDELA could be formed as a metabolite or degradation product of DEA. [However, it should be noted that in this study, DEA was administered to the skin undiluted at up to 400 mg/animal, and the site of administration was not protected to prevent grooming. Thus, it is possible that DEA was ingested and reacted with sodium nitrite to form NDELA in the acid environment of the stomach. It also should be noted that NDELA is found only when there is a supplemental source of nitrite.]

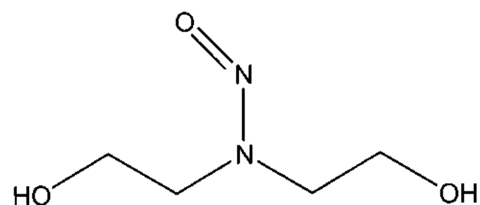


Figure 1-3. Structure of *N*-nitrosodiethanolamine

Urinary metabolites of DEA are discussed in Section 6.

2 Human Exposure

2.1 Use

There appears to be widespread occupational and consumer exposure to DEA. It is widely used as an intermediate in the production of fatty-acid condensates formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. DEA also is used as a surface-active agent and corrosion inhibitor in metalworking fluids and as a dispersant in agricultural chemical formulations. Other applications include use in adhesives; anti-static agents; cement and concrete work; coatings; electroplating; printing inks; metal cleaning and lubricating; mining; natural gas treatment; paint and pigments; paper, petroleum, and coal production; polymers and polymer production; rubber processing; soldering flux; textile finishing; and polyurethane production and use; and as an epoxy hardener, a fuel-gelling agent, a pharmaceutical intermediate, and an ointment-emulsifier (NTP 1999a, IARC 2000). Table 2-1 shows the estimated percentages DEA of production used in various products and processes in the United States.

Table 2-1. Major uses of DEA in the United States

Application	Percentage of DEA production[a]
Surfactants	39
Gas purification	30
Textile processing	15
Metalworking fluids	10
Miscellaneous	8
Laundry detergents	2
Agricultural chemicals	2

Source: Knaak *et al.* 1997.

[a]The percentages, which sum to 106%, are those listed in the source.

2.2 Production

DEA usually is produced by reaction of ethylene oxide with ammonia in a 2:1 molar ratio. Most production facilities react the ethylene oxide and ammonia in a bath process that yields a crude mixture of ethanolamine, DEA, and triethanolamine. The mixture is distilled to separate and purify the DEA (NTP 1999a). Estimated annual production of DEA in the United States is shown in Table 2-2.

Table 2-2. Estimated annual production of DEA in the United States (thousand tons)

Year	1960	1965	1970	1975	1980	1985	1980	1990	1995
Production	24	35	42	39	56	76	86	91	106

Source: Edens and Lochary 2000.

2.3 Analysis

DEA in workplace air is detected by ion chromatography, with a detection limit of 13 μg/sample (the minimum sample size for this method is 5 L, and the maximum is 300 L). DEA in water samples is determined by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with fluorescence detection. DEA in metalworking fluids is detected by GC–mass selective detection of silylated derivatives, isotachophoresis, capillary zone electrophoresis with indirect ultraviolet detection, and spectrophotometry. DEA in cosmetics and pharmaceuticals is detected by GC with flame ionization detection, ion-exclusive chromatography, and reversed-phase HPLC (IARC 2000). The Occupational Health and Safety Administration (OSHA) has partially validated an HPLC method for detecting DEA in air drawn through sampling tubes; the detection limit is 1 ng with a 15-μL injection volume, for an overall detection limit of 0.04 ppm based on a 10-L air volume (OSHA 1987).

2.4 Environmental occurrence

DEA is not known to occur in nature. However, because of its extensive use in industrial and consumer products, environmental releases are likely (IARC 2000).

2.5 Environmental fate

2.5.1 *Atmospheric fate*

DEA would be expected to remain almost entirely in the vapor phase in the atmosphere, where its reaction with photochemically generated hydroxyl radicals is thought to account for its relatively short half-life of four hours. Because it is soluble in water, DEA also may be removed from the atmosphere via precipitation (HSDB 2000).

2.5.2 *Aquatic fate*

DEA is expected to biodegrade in water with a half-life on the order of days to weeks, depending upon the degree of acclimation of the system. Bioconcentration in aquatic organisms and volatilization are not expected to be important aquatic-fate processes (HSDB 2000).

2.5.3 *Terrestrial fate*

DEA is expected to biodegrade in soil with a half-life on the order of days to weeks. Because DEA is soluble in water, it also may leach from soil when present in high concentrations. Volatilization is not expected to be an important terrestrial-fate process (HSDB 2000).

2.6 Environmental exposure

The most probable route of environmental exposure to DEA is via dermal exposure to personal-care products (i.e., soaps, shampoos, and cosmetics), detergents, and other surfactants that contain DEA (HSDB 2000). Cosmetic formulations may have concentrations of DEA ranging from 1% to 25% (IARC 2000).

2.7 Occupational exposure

Occupational exposure to DEA is most likely through inhalation during the use of lubricating liquids in various processes in machine building and metallurgy (e.g., cutting, die stamping, grinding, extrusion, and die casting). Ethanolamines, including DEA, often are used in soluble, synthetic, and semisynthetic metalworking fluids for pH adjustment or as corrosion inhibitors. Dermal exposure also is expected to occur, though inhalation exposure is more probable. The National Institute for Occupational Safety and Health (NIOSH) estimates that over 10 million workers in the United States are exposed to machining and grinding coolants and cutting fluids. Exposure to DEA may be lower, because not all metalworking fluids contain DEA. Estimates from NIOSH surveys for DEA exposure have varied from 573,025 to 1,284,534 workers potentially exposed to DEA (HSDB 2000). Recent estimates from the 1981 to 1983 National Occupational Exposure Survey put the number potentially exposed to DEA at approximately 800,000 workers, many of them metalworkers (IARC 2000, NTP 1999a). DEA was identified in Material Safety Data Sheets as a component of bulk cutting fluids, with concentrations ranging from 4% to 5% by weight in synthetic and semisynthetic fluids. DEA has been detected in workplace air in the metal manufacturing industry. Although it was speculated that metalworkers were exposed to DEA, DEA is too volatile to be collected on filters and thus was not measured in personal air samples (Kenyon *et al.* 1993).

DEA also has been detected in wetting fluids used in road paving. Levels up to 0.05 mg/m^3 in air were detected in a stationary sample at a slurry machine discharging a bitumen emulsion that contained 0.2% DEA. Personal exposure samples of DEA all were below the 0.02 mg/m^3 detection limit (IARC 2000).

2.8 Biological indices of exposure

No data were found on biological indices of human exposure to DEA. Mathews *et al.* (1995, 1997) proposed metabolic pathways for DEA based on studies with rats. DEA is excreted primarily in the urine as the parent molecule. It also is metabolized by biosynthetic pathways common to ethanolamine, a naturally occurring component of phospholipids. Thus, DEA is *O*-phosphorylated, *N*-methylated, and incorporated into phosphoglycerine and sphingomyelin analogues as the parent compound and as its *N*-methyl and *N,N*-dimethyl derivatives. It also is conserved, presumably by a mechanism that normally conserves ethanolamine. Conservation of DEA is thought to account for its bioaccumulation, which results in tissue levels much greater than would be anticipated for such a small polar molecule.

2.9 Regulations

The U.S. Environmental Protection Agency (EPA) regulates DEA under the Clean Air Act (CAA), listing national emission standards for DEA. Under the Federal Insecticide, Fungicide, and Rodenticide Act and the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, EPA mandates a reportable quantity of 100 lb (45.4 kg) for DEA. It also lists pesticide tolerances for DEA in several crops. Under section 313 of Title III of the Superfund Amendments and Reauthorization Act (SARA) of 1986, EPA sets forth requirements for the submission of information relating to the release of toxic chemicals.

The U.S. Food and Drug Administration (FDA) regulates DEA under the Federal Food, Drug, and Cosmetic Act. The FDA allows DEA to be used in several indirect food additives. DEA may be used in some adhesives and components of coatings and paper and paperboard components.

The American Conference of Governmental Industrial Hygienists (ACGIH) has established a threshold limit value (TLV) for DEA of 0.46 ppm (1.99 mg/m^3) in air and 2 mg/m^3 for dermal exposure. NIOSH has established a recommended exposure limit of 3 ppm (15 mg/m^3).

EPA regulations are summarized in Table 2-3 and FDA regulations in Table 2-4. No OSHA regulations were found in current literature.

Table 2-3. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR 60.660ff – Subpart NNN – Standards of Performance for Volatile Organic Compound (VOC) Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Promulgated: 55 FR 26942, 06/29/90.	The provisions of this subpart apply to each affected facility (where construction, modification, or reconstruction commenced after December 30, 1983) that is part of a process unit that produces DEA. Standards, monitoring of emissions and operations, and test methods and procedures are provided.
40 CFR 60.700ff – Subpart RRR – Standards of Performance for Volatile Organic Compound Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Reactor Processes. Promulgated: 58 FR 45962, 08/31/93.	The provisions of this subpart apply to each affected facility (where construction, modification, or reconstruction commenced after June 29, 1990) that is part of a process unit that produces DEA. Standards, monitoring of emissions and operations, and test methods and procedures are provided.
40 CFR 63 – PART 63 – NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Codes: 7401 et seq.; CAA.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
40 CFR 63.100ff – Subpart F – National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94.	This subpart applies to synthetic organic chemical manufacturing facilities. This subpart lists standards and general compliance, reporting, and recordkeeping provisions to determine hazardous air pollutant levels of compounds such as DEA.
40 CFR 63.640ff – Subpart CC – National Emission Standards for Hazardous Air Pollutants From Petroleum Refineries. Promulgated: 60 FR 43260, 08/18/95.	This subpart applies to petroleum refining process units and to related emission points specified in this section that are located at a plant site that emits DEA. DEA is classified as a hazardous air pollutant.
40 CFR 63.800ff – Subpart JJ – National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95.	This subpart applies to each facility that is engaged, either in part or in whole, in the manufacture of wood furniture or wood furniture components. This subpart lists compliance methods and testing procedures to determine hazardous air pollutant levels of compounds such as DEA.

Regulatory action	Effect of regulation or other comments
40 CFR 172.101ff – Subpart B – Table of Hazardous Materials and Special Provisions. Promulgated: 55 FR 52582, 12/21/90. DEA has a reportable quantity of 100 lb (45.4 kg).	The Hazardous Materials Table in this section designates the materials listed therein as hazardous materials for the purpose of their transportation. For each listed material, the table identifies the hazard class or specifies that the material is forbidden in transportation and gives the proper shipping name or directs the user to the preferred proper shipping name. In addition, the table specifies or references requirements in this subchapter pertaining to labeling, packaging, quantity limits aboard aircraft, and stowage of hazardous materials aboard vessels.
40 CFR 180 – PART 180 – TOLERANCES AND EXEMPTIONS FROM TOLERANCES FOR PESTICIDE CHEMICALS IN OR ON RAW AGRICULTURAL COMMODITIES. Promulgated: 36 FR 22540, 11/25/71. U.S. Codes: 21 U.S.C. 346a, 371a.	This part outlines tolerances for pesticide chemicals in or on raw agricultural commodities. It lists procedural regulations, specific tolerances, and exemptions from tolerances for various pesticide chemicals.
40 CFR 180.101ff – Subpart C – Specific Tolerances. Promulgated: 47 FR 620, 01/06/82. DEA residues are listed for several crops.	The tolerances established for pesticide chemicals in this subpart apply to residues resulting from their application prior to harvest or slaughter. Tolerances are expressed in terms of parts by weight of the pesticide chemical per one million parts by weight of the raw agricultural commodity.
40 CFR 180.1001ff – Subpart D – Exemptions from Tolerances. Promulgated: 36 FR 22540, 11/25/71. DEA is exempt from tolerances when used as a stabilizer or inhibitor for formulations used before crop emerges from soil.	An exemption from a tolerance shall be granted when it appears that the total quantity of the pesticide chemical in or on all raw agricultural commodities for which it is useful under conditions of use currently prevailing or proposed will involve no hazard to the public health.
40 CFR 302 – PART 302 – DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Codes: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361. DEA has a reportable quantity of 100 lb (45.4 kg).	This part identifies reportable quantities and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the Clean Water Act.
40 CFR 372PART 372TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11013, 11028. The effective date of this regulation for DEA is 1/1/87.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and to aid in the development of regulations, guidelines, and standards.
40 CFR 414 – PART 414 – ORGANIC CHEMICALS, PLASTICS, AND SYNTHETIC FIBERS. Promulgated: 52 FR 42568, 11/05/87. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, and 1361.	The provisions of this part are applicable to process wastewater discharges from all establishments or portions of establishments that manufacture organic chemical, plastic, and synthetic fiber products, such as DEA.

Source: The regulations in this table have been updated through the Code of Federal Regulations 40 CFR, 1 July 2002.

Table 2-4. FDA regulations

Regulatory action	Effect of regulation or other comments
21 CFR 175 – PART 175 – INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS. Promulgated: 42 FR 14534, 03/15/77. U.S. Codes: 21 U.S.C. 321, 342, 348, 379e.	DEA may be safely used in adhesive components of articles intended for use in packaging, transporting, or holding food.
21 CFR 176 – PART 176 – INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS. Promulgated: 42 FR 14554, 03/15/77. U.S. Codes: 21 U.S.C. 321, 342, 346, 348, 379e.	Substances identified in this section may be safely used as components of the uncoated or coated food-contact surface of paper and paperboard intended for use in producing, manufacturing, packaging, processing, preparing, treating, packing, transporting, or holding aqueous and fatty foods, subject to the provisions of this section.
21 CFR 176.170 – Sec. 176.170 – Components of paper and paperboard in contact with aqueous and fatty foods. Promulgated: 42 FR 14554, 03/15/77.	DEA may be used (1) as an adjuvant to control pulp absorbency and pitch content in the manufacture of paper and paperboard prior to the sheet-forming operation or (2) in paper mill boilers.
21 CFR 176.210 – Sec. 176.210 – Defoaming agents used in the manufacture of paper and paperboard. Promulgated: 42 FR 14554, 03/15/77.	Defoaming agents, such as DEA, may be safely used in the manufacture of paper and paperboard intended for use in packaging, transporting, or holding food.

Source: The regulations in this table have been updated through the Code of Federal Regulations 21 CFR, 1 April 2002.

3 Human Cancer Studies

No studies have been reported on the relationship between human cancer and exposure specifically to DEA. Nevertheless, ethanolamines commonly have been added to certain types of metalworking fluids since the 1950s, and numerous studies have evaluated cancer in workers exposed to metalworking fluids (also known as cutting fluids). There are four types of metalworking fluids: (1) straight oils, which contain mineral oil, fat, additives, and no water, (2) soluble fluids, which are mineral oil-based but also contain fat, emulsifiers (including amines), additives (rarely nitrites), and water, (3) semisynthetic fluids, which consist of mineral oil, a soluble base (usually amines), emulsifiers, and additives (usually nitrites), with large amounts of water, and (4) synthetic fluids, which consist of a soluble base (usually amines), additives (usually nitrites), and water (NIOSH 1976). Ethanolamines (mainly DEA and triethanolamine) are added to soluble, semisynthetic, and synthetic fluids as corrosion inhibitors or for pH adjustment. Kenyon *et al.* (1993) reported that DEA was present in bulk machining fluids at levels ranging from 1% to 4%. The combined presence of nitrites (often used as additives) and ethanolamines can lead to the formation of *N*-nitrosamines (mainly NDELA) (IARC 2000). The discussion in this section is limited to cancer studies of exposure to soluble, semisynthetic, and synthetic metalworking fluids. Studies of exposure to unclassified metalworking fluids are not included.

3.1 IARC evaluation

The International Agency for Research on Cancer (IARC) Working Group included cancer studies of metalworkers exposed to soluble and synthetic metalworking fluids in its 2000 evaluation of DEA. The IARC evaluation included one cohort study of bearing manufacturing workers (Järvholm and Lavenius 1987) and four studies whose populations were derived from three United Autoworkers/General Motors (UAW/GM) plants (two cohort studies [Eisen *et al.* 1992, Tolbert *et al.* 1992] and two nested case-control studies [Eisen *et al.* 1994, Sullivan *et al.* 1998]). Section 3.2 reviews the studies reported by IARC and two additional case-control studies from the same UAW/GM cohort, published before the 2000 IARC review (Bardin *et al.* 1997, Schroeder *et al.* 1997).

IARC evaluated the carcinogenicity of NDELA in the same volume (Some Industrial Chemicals) that included the monograph on DEA. For this evaluation, IARC considered all the studies reviewed in the DEA monograph and three studies described in the monograph on NDELA where the exposure assessment specifically stated that nitrites and ethanolamines were used together (Park and Mirer 1996, Park *et al.* 1988, Sullivan *et al.* 1998). These studies are included in Section 3.2 because they address worker exposure to soluble and synthetic metalworking fluids that are likely to contain ethanolamines. Two other studies of exposure to soluble and synthetic metalworking fluids not reviewed by IARC (Park 2001, Silverstein *et al.* 1988) also are discussed in Section 3.2. The IARC Working Group (2000) stated that it was difficult to draw conclusions regarding DEA from the metalworking studies, in which workers were exposed to complex mixtures, and concluded that there was inadequate evidence of carcinogenicity in humans.

3.2 Human cancer studies on exposure to metalworking fluids

Studies of human cancer associated with exposure to metalworking fluids are summarized in Table 3.1.

3.2.1 Studies related to the UAW/GM cohort

Three cohort and four case-control publications on cancer (laryngeal, lung, pancreatic, and esophageal) in the UAW/GM cohort have been published. The original report (Eisen *et al.* 1992) did not describe effects for specific metalworking fluids, so effects are not included in Table 3-1, but the study population is described, because it provides the population base for the nested case-control studies. The UAW/GM cohort consisted of over 45,000 workers who had worked at least three years (from 1920 to 1985) in one of three auto-part manufacturing facilities (Plants I, II, and III) in Michigan. Over 10,000 deaths occurred between 1941 and 1985 (almost 1 million person-years of follow-up), the causes of which were ascertained from UAW records and death certificates. Workers were exposed to metalworking fluids (straight oil, soluble, and synthetic). The use of synthetic fluids expanded in the 1970s. Overall mortality from all causes in white males at Plants I and II was similar to that of the U.S. population (standardized mortality ratio [SMR] ~ 1.0). However, there were fewer deaths from all causes in black males at Plant I (SMR = 0.8, 95% CI = 0.8 to 0.9) and in white males at Plant III (SMR = 0.8, 95% CI = 0.8 to 0.9) than in the U.S population, suggesting a healthy worker effect.

Eisen *et al.* (2001a) reported the findings of an extended follow-up (10 additional years) of the UAW/GM cohort discussed above. Between 1941 and 1994, more than 15,000 deaths occurred (more than 1.5 million person-years). Relative risks (RRs) were calculated for specific causes of death and levels of exposure to synthetic and soluble metalworking fluids with a Poisson regression model that adjusted for possible confounders. Exposure to grinding with soluble fluids was associated with cancer of the esophagus, larynx, skin, and brain, and exposure to synthetic fluids was associated with cancer of the esophagus, liver, and prostate.

Tolbert *et al.* (1992) conducted a cohort study of more than 33,000 workers at two of the three plants in the UAW/GM cohort. Mortality was followed from 1941 to 1984, and causes of death were known for 92% of the 9,349 deaths that occurred. Years of exposure and ever exposure to straight oil, soluble, and synthetic metalworking fluids were estimated from exposure matrixes and employee records. Ever exposure to soluble fluids was modestly associated with cancer of the stomach (SMR = 1.2, 95% CI = 1.0 to 1.5, 99 cases), larynx (SMR = 1.4, 95% CI = 1.0 to 2.0, 30 cases), and brain (SMR = 1.2, 95% CI = 0.9 to 1.7, 46 cases) and with leukemia (SMR = 1.3; 95% CI = 1.1 to 1.7, 75 cases) in white males and with cancer of the pancreas (SMR = 1.6, 95% CI = 1.0 to 2.5, 19 cases) and larynx (SMR = 1.5, 95% CI = 0.5 to 3.2, 6 cases) in black males. Ever exposure to synthetic fluids was associated with small excesses of cancer of the stomach (SMR = 1.3, 95% CI = 0.8 to 2.0, 21 cases) and larynx (SMR = 1.6, 95% CI = 0.7 to 3.1, 8 cases) and of leukemia (SMR = 1.2, 95% CI = 0.7 to 2.0, 16 cases) in white males. The exposure-response relationship for each cancer site was evaluated by Poisson regression analysis, which adjusted for plant, sex, race, length of follow-up, year of birth, and age at risk. A negative exposure response was observed for lung cancer and synthetic fluids (*P* value

for trend = 0.006) and soluble fluids (*P* value for trend = 0.09), and no exposure-response relationships were observed for cancers at other sites.

The relationship of cancer to exposure to specific types of metalworking fluids, estimated through the use of a job exposure matrix based on job processes and employee records, or to processes involving specific types of metalworking fluids was evaluated in four nested case-control studies of the UAW/GM (Eisen *et al.* 1992) cohort published between 1994 and 1998 (Bardin *et al.* 1997, Eisen *et al.* 1994, Schroeder *et al.* 1997, Sullivan *et al.* 1998). Studies of laryngeal, esophageal, and pancreatic cancer included small numbers of cases (53 to 108), whereas the lung-cancer study was relatively large, with 667 cases. Controls (5 per case of laryngeal or lung cancer and 20 per case of esophageal or pancreatic cancer) were selected by incidence density sampling and matched for age, gender, race, and plant site. Matched logistic regression analyses were performed to examine the relationship between cancer and cumulative exposure to each type of metalworking fluid. Cumulative exposure to soluble fluids was moderately associated with esophageal cancer (high exposure) (Sullivan *et al.* 1998), weakly associated with laryngeal cancer (Eisen *et al.* 1994), and not associated with lung or pancreatic cancer (Schroeder *et al.* 1997, Bardin *et al.* 1997). Cumulative exposure to synthetic fluids was significantly associated with increased risk of esophageal (Sullivan *et al.* 1998) and pancreatic cancer (Bardin *et al.* 1997), but an inverse exposure-response relationship with lung cancer risk was observed (Schroeder *et al.* 1997). Effects of exposure to synthetic fluids on laryngeal cancer risk were not reported (Eisen *et al.* 1994). Individuals exposed to synthetic fluids probably also were exposed to nitrosamines (Sullivan *et al.* 1998). For pancreatic cancer, addition of nitrosamines to the risk model decreased the risk estimate from 3.0 to 1.5 for grinding with synthetic fluids (Bardin *et al.* 1997), and exposure to nitrosamines was associated with an elevated risk of esophageal cancer (Sullivan *et al.* 1998).

3.2.2 Other human studies of occupational exposure to metalworking fluids

Other studies on human exposure to metalworking fluids reviewed in this section include studies of bearing manufacturing workers in Sweden (Järvholm and Lavenius 1987) and Connecticut (Park *et al.* 1988, Silverstein *et al.* 1988) and engine plant workers in Detroit, Michigan (Park and Mirer 1996) and Cleveland, Ohio (Park 2001).

Järvholm and Lavenius (1987) conducted a cohort study of cancer incidence that included 792 workers who had worked at least five years in a bearing manufacturing factory. Solvent-refined mineral oils had been used since 1975, and amines, emulsifiers, and bactericides were documented to have been components of cutting fluids since the mid 1950s. Between 1958 and 1983, 41 incident cases of cancer occurred in male grinders (N = 559) who were reported to have been exposed to soluble fluids (the expected number was 65.1). An excess of esophageal cancer and deficits of lung and prostate cancer cases were observed.

The remaining studies are industry-based proportional mortality studies. Proportional mortality ratios (PMRs) differ from standardized mortality ratios in that a set of age-specific proportions, rather than rates, is used as the standard in calculation of the expected numbers. The major problem with this measure is that the PMR for one cause is

not independent of the PMR for another cause. To alleviate some of the problems due to use of PMRs, the studies also reported mortality odds ratios (ORs) based on use of non-cases as controls.

Park *et al.* (1988) conducted a mortality study of all hourly employees with 10 or more years of service at a bearing manufacturing plant in Connecticut. Exposure to straight oil and water-based metalworking fluids, as well as processes involving these fluids, was estimated from the category of the last known job held 15 years before death. Work history information was not considered sufficient for estimating cumulative exposure. Plant records indicated that fluids contained organic amines and nitrite additives at certain times. The process of grinding was associated with a significantly increased risk of stomach cancer (PMR = 3.8, $P = 0.006$, 7 observed cases). Limitations of this study include the small number of exposed and non-exposed cancer cases, selection of controls, and the use of one job category to categorize each worker's exposure.

Silverstein *et al.* (1988) also observed an increased risk of stomach cancer (PMR = 4.0, $P = 0.08$, 3 exposed cases) among grinders exposed to water-based cutting fluids for at least 10 years (N = 70) at a similar type of plant (a ball-bearing manufacturing plant also in Connecticut). An elevated risk of digestive-system cancer also was observed (PMR = 2.2, $P = 0.06$, 9 exposed cases). Water-based fluids were defined as soluble, semisynthetic, or synthetic fluids; soluble fluids containing nitrites and ethanolamines had been in use since the 1940s. Mortality OR analyses of pancreatic and stomach cancer used an internal comparison group as controls. ORs were higher in workers with longer exposures (10 vs. 5 years) to grinding with water-based fluids (pancreatic and stomach cancer) and machining with water-based fluids (pancreatic cancer).

Park and Mirer (1996) conducted a study of workers employed for at least two years at two engine plants in Detroit. Exposure to operations involving metalworking fluids was assessed. Crankshaft and camshaft grinding involved exposure to synthetic or semisynthetic fluids containing nitrites. Records also documented the use of fluids that contained both ethanolamines and nitrites, and NDELA had been detected in metalworking fluids. PMRs were given only for the entire cohort. Mortality case-control analyses, using decedents with causes of death unrelated to the potential exposure as controls, evaluated cancer risk for different exposure categories. Camshaft/crankshaft operations were strongly associated with stomach cancer mortality (MOR = 5.1, 95% CI = 1.6 to 16.9, 3 exposed cases), and grinding with soluble fluids was associated with mortality from non-Hodgkin's lymphoma and multiple myeloma (MOR = 4.1, 95% CI = 1.1 to 15.4, 7 exposed cases).

Park (2001) conducted a mortality study of workers employed at least two years at an automobile and truck engine manufacturing complex consisting of a foundry and two machining/assembly plants in Cleveland, Ohio. This study was an extended follow-up (1968 to 1993) of a previous cohort study (with follow-up from 1970 to 1987) initiated because of a cluster of stomach and lung cancers observed among crankshaft grinders (Rotimi *et al.* 1993, cited in Park 2001). Cumulative exposure to soluble, semisynthetic, and synthetic metalworking fluids was estimated from plant records and interviews. Records indicated that fluids containing nitrites and alkanolamines had been used.

According to the authors, PMRs were not calculated because they were potentially confounded by multiple exposures and did not account for latency, exposure duration, or healthy worker or survivors bias. Mortality ORs were calculated with a logistic regression model that combined gender/race groups and incorporated as an offset the expected mortality odds derived from age-, gender-, race-, and year-specific U.S. reference rates. The model also included employment duration (latency weighted) to adjust for healthy worker bias. Controls were all deaths due to causes believed not to be work-related. Elevated risks for stomach cancer (OR = 2.4, 95% CI = 1.1 to 5.1, 7 exposed cases) and liver cancer (OR = 2.6, 95% CI = 1.2 to 5.8, 5 exposed cases) were observed.

3.3 Discussion and summary

Human epidemiological studies reviewed in this section have reported small excesses of cancer from exposure to metalworking fluids. Some of the studies evaluated effects associated with specific metalworking fluids (soluble or synthetic) or processes (grinding or machining) involving specific metalworking fluids, whereas others evaluated effects associated with processes (mainly grinding) involving exposure to either soluble or synthetic fluids.

The most consistent finding of the studies on exposure to metalworking fluids was an excess of stomach cancers among workers exposed to synthetic fluids and among grinders using water-based (soluble, semisynthetic, or synthetic) fluids. Stomach cancer was reported in the UAW/GM cohort (synthetic fluids) (Tolbert *et al.* 1992), engine workers in Detroit (crankshaft workers exposed to synthetic fluids) (Park and Mirer 1996) and Cleveland (grinding with semisynthetic fluids) (Park 2001), and bearing manufacturing workers in Sweden and two independent sites in Connecticut (grinders exposed to soluble, synthetic, or water-based fluids) (Järvholm *et al.* 1986, Park *et al.* 1988, Silverstein *et al.* 1988). Most of these associations occurred in workers involved in grinding. Conversely, no association of stomach cancer with exposure to synthetic fluids was observed in the extended follow-up of the UAW/GM cohort (Eisen *et al.* 2001a). Synthetic metalworking fluids often contain nitrites in addition to ethanolamines, and nitrites can interact with ethanolamines to form nitrosamines. The presence of nitrosamines was documented in several of these studies (Park 2001, Park and Mirer 1996); however, there is no way to determine the number of workers exposed or their level of exposure to nitrosamines. Recently, the U.S. EPA prohibited the addition of nitrosating agents to metalworking fluids (40 CFR 747.115).

In addition to stomach cancer, exposure to synthetic metalworking fluids was associated with moderate or weak risk of the following cancers:

- liver (moderate) — Cleveland engine workers (Park 2001) and UAW/GM workers (Eisen *et al.* 2001a)
- esophagus (moderate) — UAW/GM workers (Sullivan *et al.* 1998, Eisen *et al.* 2001a)
- pancreas (moderate) — UAW/GM workers (Bardin *et al.* 1997, Tolbert *et al.* 1992)
- prostate (weak) — UAW/GM workers (Eisen *et al.* 2001a)

- laryngeal cancer (weak) — UAW/GM workers (Tolbert *et al.* 1992)
- leukemia (weak) — UAW/GM workers (Tolbert *et al.* 1992).

Exposure to synthetic metalworking fluids was negatively associated with lung cancer among UAW/GM workers in a case-control study (Schroeder *et al.* 1997).

Exposure to soluble metalworking fluids was moderately associated with esophageal cancer (Sullivan *et al.* 1998) and weakly associated with cancer of the larynx (Eisen *et al.* 1994, Tolbert *et al.* 1992), brain, and pancreas and with leukemia (Tolbert *et al.* 1992) in GM/UAW workers. Grinding with soluble fluids was weakly associated with cancer of the esophagus, larynx, skin, and brain in UAW/GM workers (Eisen *et al.* 2001a) and strongly associated with non-Hodgkin's lymphoma in Detroit engine workers (Park and Mirer 1996). Exposure of bearing manufacturing workers to water-based fluids (including soluble and synthetic fluids) during grinding was associated with an excess of esophageal cancer in Swedish workers (Järvholm and Lavenius 1987) and pancreatic and digestive cancer in Connecticut workers (Silverstein *et al.* 1988) (in addition to stomach cancer) and with a deficit of lung and prostate cancer in Swedish workers (Järvholm and Lavenius 1987). Based on consistency between studies and strength of the risk estimate, the next strongest evidence of an association between cancer and exposure to soluble or synthetic metalworking fluids (after stomach cancer) is probably for esophageal cancer.

Metalworking fluids are complex mixtures. In addition to ethanolamines and nitrites, they may contain (1) biocides, added to control the growth of microorganisms (e.g., triazine, which releases formaldehyde), (2) chlorinated compounds, added to fluids containing mineral oil (including soluble fluids), (3) metals, which are trace contaminants of mineral oils, and (4) sulfur compounds, added to soluble fluids as emulsifiers (Eisen *et al.* 1994). While most of these other agents are more likely to be in soluble fluids than in synthetic fluids, exposure to synthetic fluids is complicated by the addition of nitrites and the potential formation of nitrosamines. Moreover, workers at machining plants can be exposed to other agents, such as acid mists and asbestos. Thus, the specific effects of exposure to DEA cannot be separated from the effects of exposure to other components in metalworking fluids.

Table 3-1. Human cancer studies

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Eisen *et al.* 1992 Michigan, USA	Historical cohort, UAW/GM. 1941–1985	> 45,000 workers from 3 auto-part manufacturing facilities (Plants I, II, and III) employed at least 3 years from 1920–1985. Vital status obtained through Social Security Administration and National Death Index; cause of death ascertained from UAW records and death certificates (> 10,000 deaths).	Metalworking fluids (straight oil, soluble, and synthetic); use of synthetic fluids expanded in mid 1970s.	No effects given for specific types of metalworking fluids.	Included in table because nested case-control studies are derived from this cohort. Information on smoking and alcohol use not available. Possible other occupational exposures include asbestos, nitrosamines, acid mist, sulfur compounds, and chlorinated compounds.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Eisen *et al.* 2001a Michigan, USA	Extended follow-up of UAW/GM cohort of Eisen *et al.* 1992 (10 years longer). 1940–1994	See Eisen *et al.* 1992 (above). Extended follow-up includes > 1.5 million person-years and > 15,000 deaths.	In mid 1970s, use of water-based fluids expanded, and polyaromatic hydrocarbons in straight oils were reduced. Semisynthetic and soluble fluids were combined. A type of fluid was assigned to each plant, department, and job-specific exposure category based on historical records. Cumulative exposure (mg/m^3) were calculated for each person.	Cumulative exposure analyses: RR for each cancer (Poisson regression) and exposure stratum. *Grinding with soluble fluids:* Elevated RRs (1.5–2.6) observed for esophageal*, laryngeal**, skin, and brain* cancer. *Synthetic fluids:* Elevated RRs (1.3–2.6) observed for esophageal*, liver*, and prostate cancer. *Significant associations observed in some exposure categories. **Test for trend, $P = 0.07$.	Stomach cancer elevated in entire cohort in last 10 years of follow-up but not associated with synthetic or soluble fluids. Poisson regression analysis model relating cumulative exposure to specific causes of death included plant, gender, decade of hire, race, and calendar year at risk (< 1950, 1950–1970, ≥ 1970). Proportional hazards models using exposure as a continuous variable also executed on a subcohort (not reported in table). Possible confounders: see Eisen *et al.* 1992.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Tolbert *et al.* 1992 Michigan, USA	Cohort study of 2 of the 3 plants in the UAW/GM cohort. 1941–1984	33,619 cohort members (see Eisen *et al.* 1992); 9,349 deaths, with causes known for 92%.	Years of exposure to straight oil, soluble, or synthetic fluids estimated from exposure matrix (based on industrial hygiene records), interviews, calendar year, and employees' records.	For ever exposed*, SMRs (95% CI); no. of exposed cases. *White males:* *Soluble fluids* (23,488 exposed): all causes 1.0 (1.0–1.0); 7,287 all cancers 1.0 (1.0–1.1); 1,479 stomach 1.2 (1.0–1.5); 99 larynx 1.4 (1.0–2.0); 30 brain 1.2 (0.9–1.7); 46 leukemia 1.3 (1.1–1.7); 75 *Synthetic fluids* (8,446 exposed): all causes 1.0 (1.0–1.1); 1,632 all cancers 1.0 (0.9–1.1); 333 stomach 1.3 (0.8–2.0); 21 larynx 1.6 (0.7–3.1); 8 brain 0.6 (0.2–1.3); 6 leukemia 1.2 (0.7–2.0); 16 *Black males:* *Soluble fluids* (4,964 exposed): all causes 0.8 (0.8–0.9); 922 all cancers 0.9 (0.8–1.0); 200 pancreas 1.6 (1.0–2.5); 19 colon 0.6 (0.2–1.1); 8 larynx 1.5 (0.5–3.2); 6 *Synthetic:* not given; only 30 deaths. *Exposure-response regression analysis* (years, four quartiles): lung cancer: decreased risk with increasing exposure (duration) to soluble or synthetic fluids.	*Only elevated or decreased SMRs reported in table (20% for white males, 35% for black males). Cancers reported include esophagus, stomach, colon, rectum, pancreas, larynx, lung, prostate, brain, and leukemia. Poisson regression analysis model included gender, race, age at risk, years at risk, length of follow-up, and each exposure category.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Eisen *et al.* 1994 Michigan, USA	Nested case-control study of laryngeal cancer, UAW/GM cohort. 1941–1990	*Cases:* 108 cohort members with cancer of the larynx listed as underlying cause or other significant condition on death certificate. *Controls:* 538 employees matched (5 per case) by year of birth, race, gender, and plant.	Cumulative exposure to straight oils and soluble fluids and exposure during grinding estimated by combining exposure tables and scale factors with employment records. Scale factors derived from historical records, past air sampling measurements, and exposure predictions based on models from exposure measurements.	OR for laryngeal cancer and cumulative exposure (mg/mg^3-yr); no. of exposed cases. *Soluble fluids:* 0 1.0 (ref); 9 > 0–2.0 1.3 (0.6–3.0); 41 > 2.0–6.0 1.2 (0.5–2.9); 29 > 6.0 1.2 (0.5–2.7); 29 Elevated risks not observed for grinding (not differentiated by type of fluid).	ORs for cumulative exposure involve models that lagged for 10 or 20 years; ORs adjusted for gender, race, age, and age at risk (matching factors). Possible confounders: see Eisen *et al.* 1992; also iron and steel. Main finding was a significant exposure-response relationship with straight oils.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Schroeder *et al.* 1997 Michigan, USA	Nested case-control study of lung cancer, UAW/GM cohort. 1941–1985	*Cases:* 667 cohort members with lung cancer listed as cause or underlying cause on death certificate. *Controls:* 3,041 employees matched (5 per case) by year of birth, race, gender, and plant.	Cumulative exposure to straight oils, soluble fluids, synthetic fluids, grinding, grinding with synthetic fluids, grinding with soluble fluids, and machining with soluble fluids estimated by exposure reconstruction based on historical data (e.g., industrial hygiene records) and current exposure levels.	OR for lung cancer and cumulative exposure (mg/m^3-yr); no. of exposed cases. *Soluble fluids:* > 0–5.77 0.7 (0.5–1.1); 152 5.78–15.55 0.8 (0.5–1.1); 151 15.56–31.84 0.9 (0.7–1.4); 151 31.85+ 0.7 (0.5–1.0); 152 *Grinding and machining with soluble fluids:* ORs not elevated. *Synthetic fluids:* > 0–0.092 1.1 (0.7–1.6); 40 0.09–0.57 1.0 (0.7–1.5); 39 0.58–1.84 0.9 (0.6–1.3); 41 1.85+ 0.6 (0.4–0.8); 40 No trend for increasing lag time. Inverse dose-response also observed for grinding with synthetic fluids.	ORs for nitrosamine and biocides consistent with those for synthetic fluids. Little evidence for confounding of exposure to synthetics by exposure to soluble fluids or straight oils. ORs adjusted for years since hire to adjust for healthy worker effect. Decision concerning confounding based on extent of change in ORs for primary exposure. Possible confounders: see Eisen *et al.* 1992.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Bardin *et al.* 1997 Michigan, USA	Nested case-control study of pancreatic cancer, UAW/GM cohort. 1941–1985	*Cases:* 97 cohort members with pancreatic cancer listed as underlying cause or other significant condition on death certificate. *Controls:* 1,825 employees matched (20 per case) by year of birth, race, gender, and plant.	Cumulative exposure to straight oil, soluble, and synthetic fluids and grinding or machining estimated by combining employment records with job exposure matrix created for each job/department and calendar period.	OR for pancreatic cancer by cumulative exposure (mg/m^3-yr); no. of exposed cases. *Synthetic fluids:* > 0–1.4 1.0 (0.4–2.4); 9 > 1.4 2.8 (1.1–6.9); 9 *Grinding with synthetic fluids:* > 0–1.4 1.0 (0.4–2.5); 9 > 1.4 3.0 (1.2–7.5); 9 *Soluble fluids or grinding or machining with soluble fluids:* no significant increase in risk.	For grinding with synthetic fluids, addition of nitrosamines to model decreased risk estimate, and addition of biocides increased risk estimate. ORs adjusted for years since hire (to adjust for healthy worker effect).

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Sullivan *et al.* 1998 Michigan, USA	Nested case-control study of esophageal cancer, UAW/GM cohort. 1941–1984	*Cases:* 53 cohort members with esophageal cancer listed as underlying cause or other significant condition on death certificate. *Controls:* 971 employees matched (20 per case) by year of birth, race, gender, and plant.	Cumulative exposure and duration of exposure to straight oil, soluble, and synthetic fluids and grinding or machining estimated by combining employment records with job exposure matrix created for each job/department and calendar period.	Adjusted OR for cumulative exposure (mg/m^3-yr); no. of exposed cases. *Synthetic fluids:* > 0 3.9 (1.1–14.3); 7 *Soluble fluids:* > 0 to < 3.3 2.1 (0.7–6.7); 10 ≥ 3.3 to < 12 2.1 (0.6–7.8); 10 ≥ 12 to < 22.5 3.5 (0.9–13.1); 9 ≥ 22.5 1.7 (0.4–6.7); 10 OR for esophageal cancer and 5 years' exposure to synthetic fluids: lag time (years) 10 20 *Synthetic fluids:* 1.5 (0.9–2.7) 3.3 (1.1–9.6) *Grinding:* 1.5 (0.9–2.7) 3.3 (1.1–9.5)	Adjusted ORs calculated by conditional logistic regression, which included number of years from hire, to control for healthy worker effect. ORs for synthetic fluids adjusted for cumulative exposure to soluble fluids, and ORs for soluble fluids adjusted for cumulative nitrosamine exposure. Confounding assessed for each exposure that suggested an elevated risk. Possible confounders: see Eisen *et al.* 1992.
Järvholm and Lavenius 1987 Gothenburg, Sweden	Cohort (incident), Gothenburg factory. 1958–1983	All who had worked at least 5 years in grinding (559) or turning (251) departments of a bearing-ring manufacturing factory between 1950–1966; 792 workers included in analysis; cancer morbidity identified from Swedish Cancer Registry.	Turners always exposed to straight oils; grinders exposed to soluble and synthetic fluids. Soluble fluids introduced in mid 1950s and synthetic fluids in 1970s. Amines reported to have been a component since mid 1950s. Exposure estimated from occupational titles.	Standardized incidence ratio (SIR); no. of exposed cases. *Male grinders:* esophagus [2.0]; 0.2–7.2; 2 stomach [1.5]; 0.7–3.0; 8 lung [0.2]; 0.0–0.9; 2 prostate [0.2]; 0.0–0.6; 2 No associations with gastrointestinal or bladder cancer; similar risks for 20 years' latency.	Cancer incidences for city of Gothenburg used for expected numbers. SIRs calculated from observed and expected numbers (not reported). Healthy worker effect for entire cohort: 209 observed deaths, 253 expected (SMR = [0.8], 95% CI = 0.7–0.9).

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Park *et al.* 1988 New Britain, Connecticut USA	PMR study (industry); case-control studies of stomach and lung cancer. 1969–1982	All hourly employees with ≥ 10 years' service at bearing manufacturing plant; death certificates and job histories available for 702 workers. Case-control analyses (for each disease outcome: stomach cancer, lung cancer, and non-malignant. Controls defined as non-cases (cohort members) with causes of death not plausibly associated with exposure of interest.	Exposure to straight oil or water-based fluids estimated from job histories (one job title). Exposure-based groups included comprehensive (more broadly defined) and restrictive (more definite exposure) categories for each type of exposure. Water-based fluids predominated in grinding. Fluids contained organic amines and nitrite additives at certain times.	Cohort: PMR for cancer; *P* value; no. of exposed cases. *Grinders:* stomach 3.8; 0.006; 7 30-year latency 4.2; 0.03; 4 lung (women) 2.7; 0.08; 5 Case-control studies of exposure to water-based fluids: mortality OR; *P* value; no. of exposed cases. *Stomach:* grinding 6.5; 0.01; 7 comprehensive 6.6; 0.02; 8 restricted 5.2; 0.13; 2 *Lung (women):* grinding 19.3; 0.008; NR	U.S. reference rates through 1980 used to calculate PMRs. Stomach cancer higher in CT than U.S., but this should not affect case-control study findings. For comprehensive exposure, crude mortality OR or Mantel-Haenszel summary OR across age strata. All lung cancer in women occurred in grinders.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Silverstein *et al.* 1988 Connecticut, USA	PMR study (industry); case-control studies of stomach and pancreatic cancer. 1950–1982	All union members who had worked at ball-bearing manufacturing plant ≥ 5 years; cause of death and work history available for 1,766 workers. Case-control analyses of stomach and pancreatic cancer: controls all non-cases except those dying of causes suspected or known to be associated with exposure of interest.	Exposure assessed for straight oils and water-based fluids and grinding or machining, using seniority list (process-related job grouping). Exposure categories weighted for latency. Soluble fluids containing nitrites and ethanolamine in use in late 1940s. *Air sample for oil mist:* 1949–1961: 15.7 mg/m^3 (machining areas) 1977–1979: 1.7 mg/m^3 (grinders)	Cohort: PMR for cancer and ≥ 10 years' experience; *P* value; no. of exposed cases. *Water-based grinding* (n = 70): digestive 2.2; 0.06; 9 stomach 4.0; 0.08; 3 colon 1.4; NR; 2 pancreas 2.5; NR; 2 lung 0.6; NR; 3 lymphopoietic 0.8; NR; 1 Case-control for 5 and 10 years' exposure: mortality OR; *P* value; no. of exposed cases. 5 + / 10 + *Water-based grinding:* stomach 1.8; NR; 3 / 2.7; 0.14, 3 pancreas 2.2; 0.31; 2 / 3.5; 0.16; 2 *Water-based machining:* pancreas 5.0; 0.10; 2 / 8.4; 0.15; 1	PMRs determined for populations defined by latency-weighted employment duration and unweighted duration with ≥ 5 years. Only 20 deaths (9 cancer) for machining with water-based fluids; elevated risk for all cancers, digestive tract, colon, pancreas, and lung, but few deaths. ORs adjusted for age, year of death, and place of birth. Water-based fluids include soluble, semisynthetic, and synthetic.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Park and Mirer 1996 Detroit, Michigan USA	PMR study (industry). 1970–1989	All workers employed at two engine plants (Plants I and Plant II) for at least 2 years from 1966–1987; 1,870 deaths available. Case-control analyses: 802 of deaths qualified as controls.	Exposure to machining and grinding with straight oil, soluble, and synthetic fluids estimated by exposure matrix based on records, plant inspection, and interviews. Synthetic or semisynthetic fluids containing nitrites used in Plant I crankshaft and camshaft grinding. Fluids containing nitrites and ethanolamines used (1977), and nitrosamine and NDELA identified in fluids.	PMR only for plant, not specific exposure. Mortality OR (95% CI); no. of exposed cases. *Stomach cancer:* Camshaft/crankshaft, synthetic: 5.1 (1.6–16.9); 3 *Non-Hodgkin's lymphoma and multiple myeloma:* Grinding, soluble: 4.1 (1.1–15.4); 7 Mortality ORs not reported for exposure (or related exposures) to soluble or synthetic fluids and cancer of pancreas, lung, prostate, or bladder.	PMRs potentially confounded by multiple exposures; did not account for latency, exposure duration, or healthy worker or survivors bias; underestimated effects for more than one cause of death associated with exposure. Mortality OR analyses performed by logistic regression model that included age, gender, race, and year-specific mortality odds.

Reference	Study design and follow-up	Population	Exposure	Effects	Comments
Park 2001 Cleveland, Ohio, USA	PMR study (industry). 1968–1993	All workers (20,959) employed at automobile and truck engine manufacturing complex ≥ 2 years from 1966–1983; additional 8 years of follow-up of previous cohort mortality study initiated because of a cluster of stomach and lung cancer among crankshaft grinders (1979). 2,456 deaths identified from death records. Case-control analysis: controls were all deaths due to causes not believed to be work-related.	Cumulative exposure to operations involving soluble, semisynthetic, or synthetic fluids calculated by summing duration in departments, using latency weighting. Exposure in departments assessed by industrial hygiene records and interviews. Records indicated that coolant contained nitrites, alkanolamines (ethanolamines), and nitrosamines.	OR (95% CI); no. of exposed cases. *Grinding, semisynthetic fluids:* stomach 2.4 (1.1–5.1), 7 liver 2.6 (1.2–5.8), 5 ORs not given for grinding and other cancer or machining or grinding with soluble fluids. Attributable deaths (AD) and attributable fraction (AF). *Grinding, semisynthetic fluids:* AD AF stomach 3.5 0.5 liver 2.2 0.4	PMRs not used (see Park and Mirer, 1996). Mortality ORs calculated by logistic regression models that combined gender/race groups and incorporated as an offset the expected mortality odds derived from age-, gender-, race-, and year-specific U.S. reference rates. Cluster-initiated study.

4 Studies of Cancer in Experimental Animals

Very few studies have evaluated the carcinogenicity of DEA in experimental animals. The IARC Working Group (2000) reviewed the available data for DEA and concluded that there was limited evidence for the carcinogenicity of DEA in experimental animals (see Appendix A). This conclusion was based largely on the NTP's two-year dermal carcinogenicity study of DEA in $B6C3F_1$ mice and F344/N rats (NTP 1999a) (see Appendix B). In addition, DEA was tested in a transgenic (Tg·AC) mouse model. Results of these and a few other pertinent studies are summarized for mice and rats in Sections 4.1 and 4.2, respectively.

4.1 Mice

4.1.1 Subchronic toxicity

The NTP conducted subchronic studies of DEA administered to mice in drinking water or by topical application (NTP 1992, Melnick *et al.* 1994a). The NTP (1992) report is included as Appendix C. In the drinking-water studies, groups of 10 $B6C3F_1$ mice of each sex were exposed to DEA at 0, 630, 1,250, 2,500, 5,000, or 10,000 ppm for 13 weeks. Other groups of mice received topical applications of DEA in 95% ethanol to provide daily doses of 0, 80, 160, 320, 630, or 1,250 mg/kg body weight (b.w.) for 13 weeks. In the drinking-water study, all mice given the two highest concentrations (5,000 and 10,000 ppm) and 3 female mice in the 2,500-ppm group died before the end of the study. In the topical application study, 2 males and 4 females in the high-dose group (1,250 mg/kg b.w.) were found in moribund condition before the end of the study and sacrificed.

Both of these studies indicated adverse effects of DEA exposure on liver (cytologic alterations and hepatocellular necrosis), kidney (nephropathy and tubular epithelial cell necrosis), and heart (degeneration). Effects specific to the administration route included salivary gland lesions (cytologic alterations) in the drinking-water study and skin lesions at the exposure site (ulcers, chronic active inflammation, acanthosis, and hyperkeratosis) in the topical application study. Dose-dependent liver effects were particularly prominent and included hypertrophy, eosinophilia, disruption of hepatic cords, nuclear pleomorphism, multinucleated hepatocytes, and necrosis. A no-observed-effect level was not identified for hepatocellular cytological alterations or for acanthosis. Subchronic toxicity results are summarized in Tables 4-1 and 4-2a and b.

Table 4-1. Incidence and severity of non-neoplastic lesions in B6C3F$_1$ mice following exposure to DEA in drinking water for 13 weeks

Sex	Exposure conc. (ppm)	Survival (%)	Liver		Kidney nephropathy[a]	Heart degeneration[a]
			Cytological alterations[a]	Hepatocellular necrosis[a]		
Male	0	100	0	0	0	0
(n = 10)	630	100	9 (2.0)	0	1 (1.0)	0
	1,250	100	10 (2.8)	0	5 (1.0)	0
	2,500	100	10 (3.0)	9 (1.0)	8 (1.0)	1 (1.0)
	5,000	0	10 (3.0)	7 (1.3)	2 (1.0)	10 (2.8)
	10,000	0	10 (3.0)	9 (1.2)	0	10 (2.8)
Female	0	100	0	0	0	0
(n = 10)	630	100	10 (1.9)	0	0	0
	1,250	100	10 (2.8)	1 (1.0)	0	0
	2,500	70	10 (3.0)	4 (1.0)	1 (1.0)	9 (1.2)
	5,000	0	10 (3.0)	8 (1.1)	1 (1.0)	10 (2.6)
	10,000	0	10 (3.0)	7 (1.3)	0	10 (2.6)

Source: NTP 1992, Melnick *et al.* 1994a. No statistical analysis was included.

[a]Severity score (in parentheses) is an average based on the following scale: (1) minimal, (2) mild, (3) moderate, and (4) marked.

Table 4-2a. Incidence and severity of non-neoplastic lesions in $B6C3F_1$ mice following dermal exposure to DEA for 13 weeks

Sex	Dose (mg/kg)	Survival (%)	Liver		Kidney tubular epithelial necrosis[a]	Heart degeneration[a]
			Cytological alterations[a]	Hepatocellular necrosis[a]		
Male	0	100	0	0	0	0
(n = 10)	80	100	4 (1.0)	2 (1.0)	0	0
	160	100	10 (1.0)	0	0	0
	320	100	10 (1.4)	3 (1.3)	0	0
	630	100	10 (2.0)	7 (1.1)	0	0
	1,250	80	10 (2.5)	6 (2.0)	4 (1.3)	4 (1.3)
Female	0	100	0	0	0	0
(n = 10)	80	100	0	0	0	0
	160	100	10 (1.0)	0	0	0
	320	100	10 (1.1)	0	0	0
	630	100	10 (1.2)	0	0	0
	1,250	60	9 (1.3)	0	1 (1.0)	8 (1.6)

Source: NTP 1992, Melnick *et al.* 1994a. No statistical analysis was included.

[a]Severity score (in parentheses) is an average based on the following scale: (1) minimal, (2) mild, (3) moderate, and (4) marked.

Table 4-2b. Incidence and severity of non-neoplastic skin lesions in $B6C3F_1$ mice following dermal exposure to DEA for 13 weeks

Sex	Dose (mg/kg)	Survival (%)	Ulcer[a]	Chronic active inflammation[a]	Acanthosis[a]	Hyper-keratosis[a]
Male	0	100	0	0	0	0
(n = 10)	80	100	0	0	10 (1.0)	0
	160	100	0	0	9 (1.0)	0
	320	100	0	0	10 (1.1)	2 (1.5)
	630	100	2 (2.0)	5 (1.2)	10 (2.6)	5 (1.8)
	1,250	80	10 (3.0)	10 (2.7)	10 (2.9)	10 (2.0)
Female	0	100	0	0	0	0
(n = 10)	80	100	0	0	10 (1.0)	0
	160	100	0	0	10 (1.0)	0
	320	100	0	1 (1.0)	9 (1.0)	0
	630	100	2 (1.0)	1 (1.0)	10 (1.3)	0
	1,250	60	9 (3.3)	9 (3.0)	10 (2.9)	10 (2.0)

Source: NTP 1992, Melnick *et al.* 1994a. No statistical analysis was included.

[a]Severity score (in parentheses) is an average based on the following scale: (1) minimal, (2) mild, (3) moderate, and (4) marked.

4.1.2 NTP carcinogenicity bioassay

A two-year carcinogenicity bioassay of DEA was conducted in $B6C3F_1$ mice. Dose levels were based on the results of the 13-week topical application study, in which doses ≥ 320 mg/kg resulted in increasingly severe toxic effects in the liver, kidney, and skin. Groups of six-week-old mice (50 of each sex) received dermal applications of DEA in 95% ethanol to provide daily doses of 0, 40, 80, or 160 mg/kg b.w., five days/week for 103 weeks (NTP 1999a). Dose volumes were continuously adjusted based on the group mean body weights. The overall purity of the test chemical was > 99%. Animals were observed twice daily, body weights were recorded weekly for the first 13 weeks and monthly thereafter, and clinical findings were recorded monthly. All animals were necropsied and given a complete histopathological examination. Animals dying of accidental causes were censored from the survival analysis. The probability of survival was similar in all male groups but was significantly lower in all DEA-exposed female groups than in controls (see Appendix B, pp. B-32 and B-33, Table 10 and Figure 3 in NTP 1999a). Reduced survival of female mice was attributed to liver neoplasms. Mean body weight was lower in high-dose females than in controls after week 53 and in low- and mid-dose females than in controls after week 73. Mean body weight was lower in high- and mid-dose males than in controls after weeks 77 and 88, respectively (see Appendix B, pp. B-34 to B-36, Figure 4 and Tables 11 and 12 in NTP 1999a).

Incidences of liver neoplasms showed positive dose-related trends and were significantly higher in all DEA-exposed groups (combined tumors) than in controls (Table 4-3). Hepatocellular carcinomas and hepatoblastomas metastasized to the lung in 3, 4, 9, and 7 males and in 0, 3, 6, and 1 females in the control and 40-, 80-, and 160-mg/kg groups, respectively. Both the size and multiplicity of liver neoplasms were greater in DEA-exposed mice than in vehicle controls. In addition, incidence of renal tubule adenoma in male mice showed a significant positive dose-related trend; however, the increased incidences were not statistically significant for any of the exposed groups (Table 4-4). Incidences of renal tubule hyperplasia or carcinoma did not show a positive dose-related trend. The kidneys were step sectioned, and an extended analysis of proliferative lesions revealed additional adenomas in all DEA-exposed groups, but not in the controls. The combined analysis of single and step sections indicated a significant dose-related trend and significantly increased incidences of renal tubule adenoma in the two highest dose groups (80 and 160 mg/kg). However, the number of malignant kidney tumors did not change. The data for the single sections and single and step sections combined are shown in Table 4-4.

Table 4-3. Liver tumor incidence in $B6C3F_1$ mice following dermal exposure to DEA for up to two years

Sex	Dose (mg/kg)	Liver tumor incidence[a] (%)[b]			
		Hepatocellular adenoma	Hepatocellular carcinoma	Hepatoblastoma	Combined
Male	0	31 (65.0%)	12 (25.1%)	0 (0.0%)	39 (79.0%)
	40	42 (86.5%)**	17 (34.9%)	2 (4.2%)	47 (95.3%)*
	80	49 (98.0%)***	33 (66.9%)***	8 (17.5%)**	50 (100.0%)***
	160	45 (93.5%)***	34 (72.3%)***	5 (11.3%)*	49 (99.9%)***
	Hist. control	38%–62%[c]	18%–24%[c]	0%–2%	56%–78%
	Trend	$P < 0.001$	$P < 0.001$	$P = 0.018$	$P < 0.001$
Female	0	32 (66.1%)	5 (10.4%)	0 (0%)	33 (68.2%)
	40	50 (100.0%)***	19 (43.4%)***	2 (4%)[d]	50 (100.0%)***
	80	48 (96.4%)***	38 (77.9%)***	1 (2%)[d]	50 (100.0%)***
	160	48 (96.4%)***	42 (84.9%)***	1 (2%)[d]	50 (100.0%)***
	Hist. control	38%–64%[c]	6%–23%[c]	0%–2%	52%–66%
	Trend	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$

Source: NTP 1999a.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Poly-3 test). NS = not significant ($P \geq 0.05$).

[a]Overall rate, 50 animals per group.

[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality unless otherwise noted.

[c]The incidences of hepatocellular adenomas and carcinomas for ethanol controls in four other NTP bioassays (TR-438, 479, 480, and 481) in $B6C3F_1$ mice were as follows: adenomas – 44% (males), 54% (females); carcinomas – 21% (males), 15% (females).

[d]Overall rate; adjusted rate not provided.

Table 4-4. Kidney tumor incidence in male B6C3F$_1$ mice following dermal exposure to DEA for up to two years

Sections	Dose (mg/kg)	Renal tubule tumor incidence[a] (%)[b]		
		Adenoma	Carcinoma	Combined
Single	0	1 (2.2%)	2 (4%)[c]	3 (6.6%)
	40	4 (8.3%)	1 (2%)[c]	5 (10.4%)
	80	6 (13.1)	0 (0%)	6 (13.1%)
	160	6 (13.3%)	2 (4%)[c]	8 (17.8%)
	Hist. control	0.7% (0%–2%)	0.7% (0%–4%)	1.3% (0%–6%)
	Trend	$P < 0.05$	NS	$P = 0.064$
Single and step combined	0	1 (2.2%)	2 (4%)[c]	3 (6.6%)
	40	6 (12.5%)	1 (2%)[c]	7 (14.5%)
	80	8 (17.5)*	0 (0%)	8 (17.5%)
	160	7 (15.5%)*	2 (4%)[c]	9 (20.0%)
	Trend	$P < 0.05$	NS	$P = 0.056$

Source: NTP 1999a.

*$P < 0.05$ (Poly-3 test). NS = not significant ($P \geq 0.05$).

[a]Overall rate, 50 animals per group.

[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality unless otherwise noted.

[c]Overall rate; adjusted rate not provided.

The kidney tumor incidence observed in the concurrent control group (3/50, including two carcinomas) was unusually high and fell outside the range (0% to 2%) from other studies in the historical control database reported by the NTP (1999a). It should be noted that this database was limited to six dermal exposure studies and did not include step-section data. However, the incidences of renal tubule neoplasms reported in seven other NTP studies that used step sections (Eustis *et al.* 1994) also were quite low (1/350, or 0.3%; range 0% to 2%). The step sections identified no new kidney tumors in the control groups from these studies. Finally, in a recently published tabulation of NTP feeding studies carried out at approximately the same time as the DEA study, the incidences of renal tubule tumors (based on single sections) in male B6C3F$_1$ mouse vehicle controls also were quite low (3/1,351, or 0.2%; range 0% to 2%; Haseman *et al.* 1998).

The original and extended (step-section) analyses considered together indicated an exposure- and dose-related increase in the incidence of renal tubule adenoma, an increase that the NTP considered to be due to DEA. There was no indication of nephrotoxicity, and neither the incidence nor the severity of nephropathy was increased in DEA-exposed mice.

The NTP (1999a) concluded that there was clear evidence of carcinogenicity in mice based on dose-related increased incidences of liver neoplasms in both males and females and increased incidences of renal tubule neoplasms in males.

4.1.3 Transgenic mice

In recent years, transgenic mouse models have been investigated as an alternative to the conventional rodent carcinogenicity bioassay. Transgenic mice that carry an inducible v-Ha-*ras* gene (Tg·AC) are expected to have increased susceptibility to carcinogenic agents. Furthermore, tests with transgenic mice are completed before any significant numbers of strain-related spontaneous tumors occur. To test transgenic mouse models further, Spalding *et al.* (2000) selected a group of nine agents, including DEA, being tested in the conventional bioassay and prospectively evaluated the specificity of the responses of this transgenic mouse line. Homozygous female Tg·AC transgenic mice, which carry a zetaglobin v-Ha-*ras* gene on an FVB background, were exposed to DEA at dose levels higher than the maximum tolerated dose used in the conventional bioassays. DEA in 95% ethanol at daily doses of 0, 5, 10, or 20 mg/mouse was topically applied to the dorsal interscapular region five days/week for 20 weeks. This study included both a vehicle control group and a positive control group (exposed to 12-*O*-tetradecanoyl-phorbol-13-acetate [TPA]). Transgenic mice treated with DEA did not have an increased incidence of tumors (Table 4-5).

Table 4-5. Tumor incidence in female Tg·AC transgenic mice following dermal exposure to DEA for 20 weeks

Dose (mg)	Number of mice	Survival (%)	Tumor incidence (%)	Mean weeks to first tumor	Mean weeks to maximum tumor burden
0 (95% ethanol)	19	17 (89.5)	1 (5.3)	19	19
5	15	14 (93.3)	1 (6.7)	18	18
10	15	14 (93.3)	0 (0.0)	–	–
20	15	12 (80.0)	1 (6.7)	18	18
TPA	20	18 (90.0)	18 (90.0)	12.7	19

Source: Spalding *et al.* 2000.

4.2 Rats

4.2.1 Subchronic toxicity

The NTP conducted subchronic toxicity studies of DEA administered to F344/N rats in drinking water or by topical application (1992, Melnick *et al.* 1994b) (see Appendix C). In the drinking-water studies, groups of 10 rats of each sex were exposed to DEA for 13 weeks at concentrations of 0, 320, 630, 1,250, 2,500, or 5,000 ppm for males and 0, 160, 320, 630, 1,250, or 2,500 ppm for females. In the dermal exposure studies, groups of 10 rats of each sex received topical applications of DEA in 95% ethanol to provide daily doses of 0, 32, 63, 125, 250, or 500 mg/kg b.w. for 13 weeks. In the drinking-water study, 2 male rats in the high-dose group died before the end of the experiment; 1 female in the

low-dose group died, but this death was not considered treatment-related. In the topical application study, 1 male and 2 females in the high-dose group died or were found in moribund condition before the end of the study and sacrificed.

Results of these studies indicated adverse effects of DEA exposure on the hematopoietic system (microcytic anemia), kidney (increased weight, tubular necrosis, decreased renal function, and tubular mineralization), brain and spinal cord (demyelination), testis (degeneration of the seminiferous tubules), and skin (ulcers, inflammation, acanthosis, and hyperkeratosis) (Tables 4-6 and 4-7). A moderate, poorly regenerative, normochromic anemia occurred at the lowest dose levels tested. Other than skin lesions at the site of topical application and testicular degeneration following drinking-water exposure, the toxicological effects did not differ by route of exposure. Differences in dose response were attributed to lower absorption by the dermal route than by the oral route.

Table 4-6. Incidence and severity of non-neoplastic lesions in F344/N rats following exposure to DEA in drinking water for 13 weeks

Sex	DEA conc. (ppm)	Survival (%)	Kidney[a]			Demyelination[a]		Testis[a,b]
			Nephro-pathy	Necrosis	Tubular mineralization	Brain	Spinal cord	
Male	0	100	6 (1.0)	0	0	0	0	0
(n = 10)	320	100	2 (1.0)	0	0	0	0	0
	630	100	2 (1.0)	0	0	0	0	0
	1,250	100	3 (1.0)	0	1 (1.0)	0	0	0
	2,500	100	6 (1.0)	0	10 (1.8)	10 (1.7)	10 (1.9)	3 (1.3)
	5,000	80	10 (2.4)	10 (1.0)	10 (1.7)	10 (2.0)	10 (2.0)	10 (2.1)
Female	0	100	2 (1.0)	0	10 (1.3)	0	0	NA
(n = 10)	160	90	9 (1.0)	0	10 (2.0)	0	0	
	320	100	10 (1.5)	0	10 (2.5)	0	0	
	630	100	10 (1.4)	0	10 (3.0)	0	0	
	1,250	100	9 (1.0)	1 (1.0)	10 (2.4)	10 (1.5)	10 (1.0)	
	2,500	100	2 (1.0)	3 (1.0)	10 (1.7)	10 (1.9)	10 (1.9)	

Source: NTP 1992, Melnick *et al.* 1994b. No statistical analysis was reported.

[a]Severity score (in parentheses) was an average based on the following scale: (1) minimal, (2) mild, (3) moderate, and (4) marked. NA = not applicable.

[b]Seminiferous tubule degeneration.

Table 4-7. Incidence and severity of non-neoplastic lesions in F344/N rats following dermal exposure to DEA for 13 weeks

Sex	Dose (mg/kg)	Survival (%)	Kidney[a]			Skin[a]			
			Nephropathy	Necrosis	Tubular mineralization	Ulcer	CAI[b]	Acanthosis	Hyperkeratosis
Male	0	100	9 (1.0)	0	0	0	0	0	0
(n = 10)	32	100	6 (1.0)	0	0	0	0	0	0
	63	100	5 (1.0)	0	0	0	0	3 (1.0)	5 (1.0)
	125	100	6 (1.0)	0	0	0	0	6 (1.0)	10 (1.1)
	250	100	4 (1.0)	0	0	3 (1.3)	3 (1.3)	6 (1.5)	10 (1.4)
	500	90	5 (1.0)	0	9 (1.9)	10 (2.6)	10 (1.7)	10 (2.2)	10 (1.9)
Female	0	100	3 (1.0)	0	4 (1.0)	0	0	0	0
(n = 10)	32	100	9 (1.3)	0	9 (1.0)	0	0	0	5 (1.0)
	63	100	10 (1.4)	0	10 (1.6)	0	0	1 (1.0)	6 (1.0)
	125	100	10 (1.7)	0	10 (1.9)	1 (1.0)	3 (1.0)	6 (1.2)	9 (1.2)
	250	100	7 (1.1)	2 (1.0)	10 (1.1)	7 (1.9)	7 (1.6)	7 (2.0)	10 (1.7)
	500	80	4 (1.0)	10 (1.0)	10 (1.0)	10 (3.4)	10 (2.5)	10 (2.6)	10 (2.1)

Source: NTP 1999a, Melnick *et al.* 1994b. No statistical analysis was reported.

[a]Severity score (in parentheses) was an average based on the following scale: (1) minimal, (2) mild, (3) moderate, and (4) marked.

[b]CAI = chronic active inflammation.

4.2.2 NTP carcinogenicity bioassay

A two-year dermal carcinogenicity bioassay of DEA was conducted in F344/N rats. Exposure concentrations were based on the subchronic toxicity study (see Section 4.2.1), in which doses ≥ 250 mg/kg were clearly toxic, various skin lesions occurred at 125 mg/kg, and lesions were more severe in females than males at the lower dose levels. Groups of six-week-old rats (50 of each sex) received dermal applications of DEA in 95% ethanol, five days/week for 103 weeks. Daily doses were 0, 16, 32, or 64 mg/kg b.w. for males and 0, 8, 16, or 32 mg/kg b.w. for females (NTP 1999a). Dose volumes were continuously adjusted based on the group mean body weights. The overall purity of the test chemical was > 99%. Animals were observed twice daily, body weights were recorded weekly for the first 13 weeks and monthly thereafter, and clinical findings were recorded monthly. All animals were necropsied and given a complete histopathological examination. Animals dying of accidental causes were censored from the survival analysis. The probability of survival was not significantly affected by exposure to DEA (see Appendix B, pp. B-25 and B-26, Table 6 and Figure 1 in NTP 1999a). Mean body weights were lower in high-dose females than in controls after week 97 and lower in high-dose males than in controls from week 8 to week 89 (see Appendix B, pp. B-27 to B-29, Tables 7 and 8 and Figure 2 in NTP 1999a).

Exposure to DEA induced dose-related skin irritation at application site in both males and females and increased the incidence and severity of nephropathy in females. There was no evidence of carcinogenic activity of DEA in male or female rats by either route of exposure (NTP 1999a).

4.3 Related studies – DEA condensates

DEA was one of four chemically related compounds investigated by the NTP for carcinogenicity in F344/N rats and B6C3F_1 mice. The other three test materials were coconut oil acid DEA condensate, lauric acid DEA condensate, and oleic acid DEA condensate (NTP 1999b, 1999c, 1999d). Each of these compounds also was administered dermally in 95% ethanol. There was clear evidence of carcinogenicity in male and female mice, equivocal evidence in female rats, and no evidence in male rats exposed to coconut oil acid DEA condensate. Male mice had increased incidences of liver and kidney neoplasms, female mice had increased incidences of liver neoplasms, and female rats had marginally increased incidences of kidney neoplasms. There was some evidence of carcinogenicity in female mice exposed to lauric acid DEA condensate, based on increased incidence of liver neoplasms; however, there was no evidence of carcinogenicity in male mice, male rats, or female rats. There was no evidence of carcinogenicity in mice or rats of either sex exposed to oleic acid DEA condensates. The tumor incidences in B6C3F_1 mice following dermal exposure to DEA condensates are shown in Table 4-8.

Unreacted (free) DEA was present in a different concentration in each of the three DEA condensates evaluated in this class study (see Appendix B, p. B-46, in NTP 1999a). Although it is recognized that other factors may have influenced tumor occurrence, observation of tumors in the coconut oil acid and lauric acid DEA condensate studies indicates a strong association between the concentration of free DEA present in each condensate and the incidences of hepatocellular neoplasms in male and female mice and renal tubule neoplasms in male mice (see Appendix B, p. B-47, Figure 5 in NTP 1999a).

Table 4-8. Tumor incidence in B6C3F$_1$ mice following dermal exposure to DEA condensates for up to two years

DEA condensate	Dose (mg/kg)	Free DEA (mg/kg)	Tumor incidence[a] (%)[b]		
			Total liver tumors[c]		Kidney tumors[d]
			Males	Females	Males
Coconut oil acid	0	0	29 (59.8%)	33 (70.9%)	1 (2.2%)
(18.2% free DEA)	100	18.2	39 (82.4%)*	46 (94.4%)***	1 (2.3%)
	200	36.4	49 (99.3%)***	48 (99.0%)***	9 (19.6%)**
		Trend	$P < 0.001$	$P < 0.001$	$P < 0.001$
Lauric acid	0	0	28 (59.1%)	28 (59.3%)	0
(0.83% free DEA)	100	0.83	29 (62.1%)	40 (84.7%)**	1 (2%)[e]
	200	1.66	32 (65.6%)	36 (80.3%)*	1 (2%)[e]
		Trend	$P = 0.288$	$P = 0.009$	NS
Oleic acid	0	0	29 (59.3%)	28 (61.4%)	0
(0.19% free DEA)	15	0.028	27 (58.4%)	35 (74.3%)	0
	30	0.056	30 (65.2%)	29 (65.2%)	0
		Trend	$P = 0.321$	$P = 0.385$	NS

Source: NTP 1999b, c, d.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Poly-3 test). NS = not significant ($P \geq 0.05$).

[a]Overall rate, 50 animals per group.

[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality unless otherwise noted.

[c]Includes hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma.

[d]Includes renal tubule adenoma or carcinoma.

[e]Overall rate; adjusted rate not provided.

4.4 Summary

In B6C3F$_1$ mice, dermal application of DEA induced increased incidences of liver neoplasms in males (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma) and females (hepatocellular adenoma and hepatocellular carcinoma) and renal tubule adenoma in males. Liver tumors also were observed in B6C3F$_1$ mice in the NTP two-year dermal exposure bioassay of DEA and in concurrent bioassays of the coconut oil acid DEA condensate (containing 18.2% free DEA) and the lauric acid DEA condensate (containing 0.83% free DEA). In the bioassay of the oleic acid DEA condensate (which contained only 0.19% free DEA), the incidence of liver tumors was not significantly increased. In male mice receiving the highest dose of coconut oil DEA condensate, the incidence of renal tubule neoplasms also was significantly increased. There was no evidence that DEA was carcinogenic when applied to the skin of Tg·AC transgenic mice for 20 weeks. No evidence of an increased incidence of tumors was

observed in male or female F344/N rats administered DEA topically five days/week for 103 weeks.

5 Genotoxicity

The genotoxicity of DEA has been tested in bacteria, yeast, larval newts, and mammalian *in vitro* and *in vivo* systems. The NTP (1999a) and the IARC Working Group (2000) recently reviewed the genetic toxicity of DEA. Based on the available data, IARC (2000) concluded that DEA was not genotoxic. This section reviews available genetic toxicology data for DEA. Results are summarized in Table 5-1.

5.1 Prokaryotic systems

5.1.1 Reverse mutation in Salmonella typhimurium

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 were exposed to DEA at concentrations of 33, 100, 333, 1,000, and 3,333 μg/plate (Haworth *et al.* 1983). Results were negative in all strains, with or without S9 metabolic activation from rat or hamster liver. Dean *et al.* (1985) also reported that DEA did not induce reverse mutation in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538, with or without rat liver S9, at concentrations up to 4,000 μg/plate.

5.1.2 Reverse mutation in Escherichia coli

Dean *et al.* (1985) reported that DEA did not induce reverse mutation in *Escherichia coli* WP_2 or WP_2 *uvr*A strains, with or without rat liver S9.

5.2 Plants

No information on the genotoxicity of DEA in plants was found in the published literature.

5.3 Non-mammalian eukaryotic systems

DEA did not induce gene conversion at the histidine-4 or tryptophan-5 loci in *Saccharomyces cerevisiae* in the presence or absence of rat liver S9 (Dean *et al.* 1985).

Micronucleus tests were conducted with newt larvae (*Pleurodeles waltl*). After the larvae had developed to the appropriate stage for testing (about 8 weeks after the eggs were laid), DEA (75 ppm) was added to their aquaria for 12 days. Blood was collected by heart puncture, and red blood cells were examined for micronuclei. Micronuclei were not induced by exposure to DEA (Fernandez *et al.* 1993, L'Haridon *et al.* 1993).

5.4 Mammalian *in vitro* systems

DEA was tested for genotoxic effects in cultured rat liver epithelium-like RL_1 and RL_4 cells (Dean *et al.* 1985), mouse lymphoma L5178Y cells (NTP 1999a), Chinese hamster ovary (CHO) cells (Sorsa *et al.* 1988, Loveday *et al.* 1989), and Syrian hamster embryo (SHE) cells (Inoue *et al.* 1982, Kerchaert *et al.* 1996, Lehman-McKeeman and Gamsky 2000). Results were negative for chromosomal aberrations in RL or CHO cells, gene mutation in mouse lymphoma cells, and sister chromatid exchange in CHO cells. Results for cell transformation in SHE cells were mixed. No transformed colonies were observed when SHE cells were exposed for eight days to DEA at 25 to 500 μg/mL (Inoue *et al.* 1982). However, positive results were reported for the SHE cell transformation assay

following a 24-hour exposure at 3,000 or 4,500 μg/mL or a 7-day exposure at 250 to 1,500 μg/mL (Kerchaert *et al.* 1996). Lehman-McKeeman and Gamsky (2000) also reported positive results in this assay following a 7-day exposure at 500 μg/mL; however, no morphological transformation occurred in cultures treated with excess choline. DEA has been shown to interfere with the normal metabolism of ethanolamine and choline (Barbee and Hartung 1979a, 1979b, Mathews *et al.* 1995, 1997). Further, choline deficiency has been associated with an increase in liver tumors in the absence of carcinogens (see Section 6.3.2). Therefore, the addition of excess choline may have protected against this effect (see Section 6.3.3).

5.5 Mammalian *in vivo* systems

Blood samples were collected from mice at the end of the NTP 13-week toxicity study (NTP 1992; see Section 4.1). Smears were prepared from the peripheral blood samples and fixed in absolute methanol, and the slides were scanned to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes from each dose group. DEA did not increase the frequency of micronuclei in any dose group of either sex (NTP 1999a).

Table 5-1. Summary of genotoxicity studies of DEA

Test system	End point (dose)	Results[a]		Reference
		+S9	-S9	
Prokaryote				
S. typhimurium (TA98, TA100, TA1535, TA1537)	reverse mutation (33–3,333 μg/plate)	–	–	Haworth *et al.* 1983
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538)	reverse mutation (125–4,000 μg/plate)	–	–	Dean *et al.* 1985
E. coli (WP_2 and WP_2 *uvrA*)	reverse mutation (125–4,000 μg/plate)	–	–	Dean *et al.* 1985
Non-mammalian eukaryotes				
S. cerevisiae (JD1)	mitotic gene conversion in stationary and log-phase cultures (10–5,000 μg/mL)	–	–	Dean *et al.* 1985
Pleurodeles waltl (larval newt blood cells *in vivo*)	micronucleus formation (75 ppm)	–	NA	Fernandez *et al.* 1993, L'Haridon *et al.* 1993
Mammalian *in vitro*				
Rat liver RL cells	chromosomal aberrations (0.5 GI_{50})[b]	–	–	Dean *et al.* 1985
Chinese hamster ovary cells	sister chromatid exchange (150–2,176 mg/L)	–	–	Sorsa *et al.* 1988, Loveday *et al.* 1989
Chinese hamster ovary cells	chromosomal aberrations (101–3,010 mg/L)	–	–	Loveday *et al.* 1989
Syrian hamster embryo cells	cell transformation (25–500 μg/mL for 8 d)	–	NT	Inoue *et al.* 1982
Syrian hamster embryo cells	cell transformation (3,000 or 4,500 μg/mL for 24 h; 250–1,500 μg/mL for 7 d)	+	NT	Kerchaert *et al.* 1996
Syrian hamster embryo cells	cell transformation (500 μg/mL for 7 days)	+	NT	Lehman-McKeeman and Gamsky 2000
Mouse lymphoma L5178Y cells	gene mutation *tk* locus (25–400 μL/L)	–	–	NTP 1999a
Mammalian *in vivo*				
Mouse peripheral erythrocytes	micronuclei (80–1,250 mg/kg b.w. for 13 wk)	–	NA	NTP 1999a

[a]+ = positive; – = negative; NA = not applicable; NT = not tested.

[b]GI_{50} = concentration resulting in 50% growth inhibition.

5.6 Summary

DEA did not induce reverse mutation in *S. typhimurium* or *E. coli*, had no effect on gene conversion in *S. cerevisiae*, and did not induce micronuclei in larval newt blood cells. In

mammalian *in vitro* systems, DEA did not induce chromosomal aberrations in rat liver cells, gene mutation in mouse lymphoma cells, sister chromatid exchange in CHO cells, or chromosomal aberrations in CHO cells. Cell transformation in SHE cells occurred in two studies but not in a third. Finally, peripheral blood samples collected from male and female mice at the end of a subchronic toxicity study did not contain an increased frequency of micronuclei. The available data indicate that DEA is not mutagenic, nor is it metabolized to a mutagen.

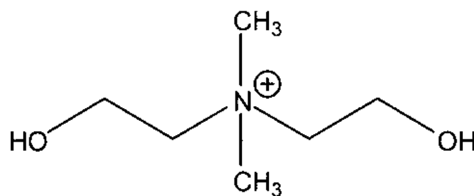
6 Other Relevant Data

DEA is a small polar molecule that accumulates in tissues following repeated exposure. In rats, DEA is excreted primarily in the urine as the parent molecule. It also is metabolized by biosynthetic pathways common to ethanolamine, a naturally occurring component of phospholipids. Thus, DEA is *O*-phosphorylated, *N*-methylated, and incorporated into phosphoglycerine and sphingomyelin analogues as the parent compound and as its *N*-methyl and *N,N*-dimethyl derivatives. It also is conserved, presumably by a mechanism that normally conserves ethanolamine. Conservation of DEA is thought to account for its bioaccumulation, which results in tissues levels much greater than would be anticipated for such a small polar molecule. Numerous studies have reported that DEA is absorbed from both the gastrointestinal tract and the skin in a number of laboratory animal species following oral or dermal administration. Consumer exposure to DEA is primarily via the dermal route, whereas occupational exposure is primarily by inhalation (see Section 2). No data are available to confirm absorption following inhalation, but it is likely that DEA would be absorbed following inhalation as well.

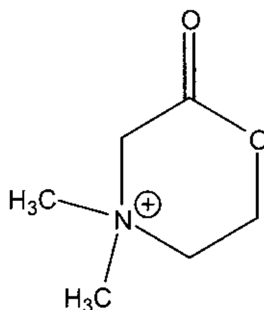
The IARC Working Group (2000) reviewed the literature on absorption, distribution, metabolism, and excretion of DEA and reported that dermal absorption occurred in both rats and mice. However, *in vitro* studies with full-thickness skin preparations from rats, mice, rabbits, and humans demonstrated that penetration of human skin was less that that of rat, rabbit, or mouse. DEA also was absorbed after oral exposure of male Fischer 344 rats. Once absorbed, DEA was detected in blood, urine, feces, liver, kidney, lung, spleen, heart, brain, and muscle.

The IARC Working Group (2000) also reported that DEA was metabolized by the same biosynthetic routes used by the naturally occurring ethanolamine. *O*-Phosphorylated, *N*-methylated, and *N,N*-dimethylated DEA derivatives were incorporated into phospholipids as the polar head groups. Displacement of phosphatidyl ethanolamine by these aberrant phospholipids was associated with functional and structural alterations in rat liver mitochondria and with decreases in choline, phosphocholine, and glycerophosphocholine levels in mouse liver.

DEA was excreted mainly as the unchanged molecule but also as the metabolic products *N*-methyldiethanolamine and *N,N*-dimethyl-2-oxomorpholinium (see Figure 6-1). The half-life of radiolabeled DEA in rats was reported to be about six days (Mathews *et al.* 1997). Another metabolite, *N*-nitrosodiethanolamine (NDELA), was detected in urine of Sprague-Dawley rats but not of B6C3F_1 mice exposed both dermally to DEA and orally to sodium nitrite (Preussmann *et al.* 1981) (see Section 1-1).



DMDEA
N,N'-Dimethyldiethanolamine



N,N-Dimethyl-2-oxomorpholinium

Figure 6-1. Structures of proposed cationicmetabolites of DEA

6.1 Mammalian absorption, distribution, and excretion

Once absorbed, DEA is distributed to the tissues in a similar manner regardless of the route of administration. In tissues, DEA is found primarily as the parent compound, with the highest concentrations in liver, kidney, spleen, and brain (Mathews *et al.* 1995, 1997). For unknown reasons, DEA has a particular affinity for liver and kidney. Following a single administration to rats, concentrations of DEA in these tissues were 150 to 250 times those observed in blood. Because DEA has a longer half-life in blood than in liver, these ratios decreased with repeated administration. However, even after rats were exposed for 8 weeks, the concentrations in liver were 50 times those in blood (Mathews *et al.* 1997). This is very unusual for such a small polar molecule and suggests that DEA may be conserved by some biological mechanism developed for the conservation of the closely related ethanolamine, which is a normal constituent of phospholipids.

6.1.1 Human studies

Except for the *in vitro* studies with human skin described below, no data were available on absorption, distribution, metabolism, or excretion of DEA by humans.

6.1.2 Animal studies

Sun *et al.* (1996) compared the permeability constant and skin penetration rate for DEA through mouse, rat, rabbit, and human skin *in vitro* using full-thickness skin preparations obtained from female CD rats, female CD-1 mice, female New Zealand White rabbits, and female mammoplasty patients. These investigators measured the penetration of both an undiluted and a 37% (w/w) aqueous solution of [^{14}C]DEA (96.5% purity by HPLC) through the skin into a physiological solution circulating on the flesh side of the skin during a six-hour sampling period. The fraction of the dose recovered in the effluent physiological solution represented the portion of the dose absorbed across the skin preparation. The fraction recovered after application of undiluted DEA was similar for rat, rabbit, and human skin (0.04%, 0.02%, and 0.08%, respectively), whereas that for mouse skin was at least 16 times as great (1.30%). When the 37% aqueous solution of DEA was applied to skin preparations, the fraction recovered in the effluent was 3 to 140 times as great as with undiluted DEA. Skin penetration was in the following order: mouse (6.68%) > rabbit (2.81%) > rat (0.56%) > human (0.23%). The authors concluded that the potential for percutaneous absorption of DEA is significantly less in humans than in rats, rabbits, or mice.

Mathews *et al.* (1995) characterized metabolism and tissue distribution of [^{14}C]DEA administered to male F-344 rats and incorporation of [^{14}C]DEA into phospholipids in human liver slices. HPLC analysis of the aqueous extract of rat livers collected 48 hours after oral administration of [^{14}C]DEA revealed a large peak of unmetabolized DEA and smaller peaks identified as *N*-methylDEA, *N,N*-dimethylDEA, and phosphates of DEA and these two metabolites. HPLC separation of the organic extract from the same livers produced peaks of radioactivity co-eluting with phosphatidyl ethanolamine and phosphatidyl choline. When the organic extract was digested with sphingomyelinase, which cleaves sphingolipids but not phosphatidyl ethanolamine or phosphatidyl choline, 30% of the phospholipids were identified as ceramides (a combination of sphingosine with a fatty acid) and the remaining 70% as phosphoglycerides. Incubation of human liver slices with [^{14}C]DEA demonstrated similar incorporation of DEA into ceramides, followed by methylation.

In a later study, Mathews *et al.* (1997) determined the disposition of [^{14}C]DEA in male Fischer 344 rats after oral, intravenous (i.v.), and dermal administration and in B6C3F$_1$ mice after dermal administration. The authors covered the dermal application site with a hemispherical wire mesh dome. Very similar patterns of tissue distribution and retention of [^{14}C]DEA were observed 48 hours after i.v. and oral administration to rats. The total percentage of the dose present in tissues (adipose, blood, brain, heart, kidney, liver, lung, muscle, skin, and spleen) was 53.7% after i.v. administration and 57.1% after oral administration. In both rats and mice, the highest tissue accumulation occurred in liver. The total percentage of the dose recovered in excreta (urine, feces, carbon dioxide, and volatiles) was 29.1% after i.v. administration and 24.4% after oral administration to rats; the primary route of excretion was in the urine. DEA was excreted slowly in the urine, and repeated oral doses administered five days/week for two, four, or eight weeks resulted in bioaccumulation that reached steady-state at approximately four weeks. The concentration of DEA in blood, however, continued to increase throughout the exposure

period. Although single oral or i.v. doses of DEA were recovered from the urine predominately as unmetabolized DEA, repeated oral administration resulted in excretion of more cationic molecules, which were tentatively identified as *O*-phosphorylated DEA, *N*-methylated DEA, and a product resulting from oxidation of dimethylated DEA (see Figure 6-1). Absorption of DEA was much slower through the skin than by the i.v. or oral route. DEA may facilitate its own absorption; in rats, 3% and 16% of the applied doses (in 95% ethanol) of 2 and 28 mg/kg b.w. were absorbed during 48 hours, and in mice, 27% and 58% of the doses of 8 and 81 mg/kg b.w. were absorbed during 48 hours.

Mendrala *et al.* (2001) characterized the pharmacokinetics of DEA in female Sprague-Dawley rats injected i.v. with either 10 or 100 mg/kg of [^{14}C]DEA. Urine and feces were collected at 12-hour intervals for 96 hours, and blood samples were collected at 5, 10, 15, and 30 minutes and at 1, 2, 4, 6, 12, 24, 36, 48, 60, 72, and 84 hours after the injection. Tissues, including liver, kidneys, heart, brain, stomach, perirenal fat, and skin, were collected at 96 hours after administration. The tissues contained 69% of the administered radioactivity at the low dose and 57% at the high dose. The largest portion (35%, low dose; 28%, high dose) was detected in the carcass. In the tissues examined, the highest levels were retained in liver (21%, high dose; 17%, low dose) and kidneys (7%, high dose; 5%, low dose). Red blood cells also showed a tendency for a gradual accumulation of radioactivity between 6 and 96 hours after administration. About 25% (low dose) and 36% (high dose) of the administered radioactivity was excreted in the urine as the parent compound. The calculated clearance of DEA from blood was 84 mL/h per kilogram b.w. for the low dose and 242 mL/h per kilogram b.w. for the high dose. The authors concluded that the dose dependency of the distribution and elimination of DEA likely represented a saturation of the processes of bioaccumulation at the higher dose level of 100 mg/kg b.w.

Barbee and Hartung (1979b) determined inhibition of phosphatidyl choline and phosphatidyl ethanolamine synthesis (Figure 6-2) by DEA both *in vitro* and *in vivo*. The coefficient of inhibition (K_i) *in vitro* was approximately 3 mM DEA for both phosphatidyl choline and phosphatidyl ethanolamine synthesis.

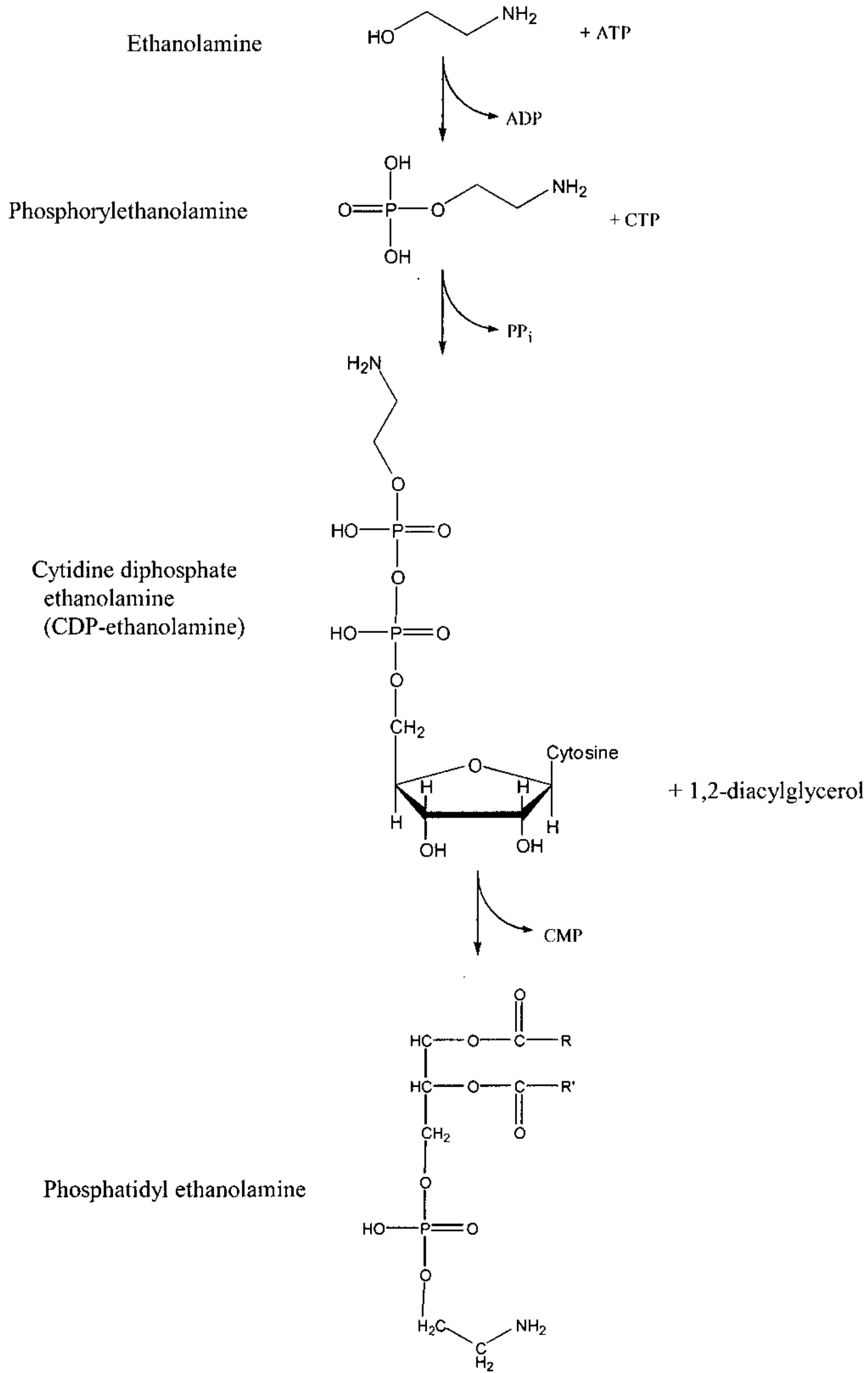


Figure 6-2. Phosphatidyl ethanolamine biosynthesis

Inhibition of incorporation of choline into phosphatidyl choline was consistent with a competitive mechanism, whereas inhibition of incorporation of ethanolamine into phosphatidyl ethanolamine indicated a mixed type of inhibition. DEA also could be incorporated into a phospholipid derivative; however, the rate constant was 11.6 mM for DEA, compared with 75.5 μM for phophatidyl choline and 53.5 μM for phophatidyl ethanolamine synthesis.

The authors also examined inhibition of phospholipid synthesis by DEA *in vivo*. Acute oral administration of 250 mg DEA/kg b.w. did not decrease incorporation of [^{3}H]choline or [^{3}H]ethanolamine into phospholipids; in fact, DEA significantly increased the radioactivity incorporated into phosphatidyl ethanolamine in renal tissue. In contrast, chronic administration of DEA at 330 mg/kg per day in drinking water for one, two, or three weeks significantly inhibited incorporation of radiolabel into phospholipids. Incorporation of tritiated ethanolamine was inhibited by 73% after one week of DEA administration, while incorporation of tritiated choline declined by only 18%, but the difference was significant from the control value at $P < 0.05$. Finally, *in vivo* elimination of phospholipids containing DEA was compared with that of phosphatidyl choline. The half-lives for disappearance of DEA-containing phospholipids from liver and kidney (3.5 days and 4.2 days, respectively) were longer than the corresponding values for phosphatidyl choline (1.7 days, liver; 2.1 days, kidney) and phosphatidyl ethanolamine (1.6 days, liver; 2.3 days, kidney). The authors concluded that the combination of inhibited synthesis of the natural phospholipids and slower clearance of the DEA-containing phospholipids could result in alterations in membrane structure and in the function of membrane-associated enzymes.

In a related paper, Barbee and Hartung (1979a) reported that administration of DEA to male Sprague-Dawley rats at 42, 160, or 490 mg/kg per day in drinking water for up to five weeks altered hepatic mitochondrial function. Mitochondrial state 4 activity (the slower rate of respiration in isolated mitochondria after all adenosine diphosphate has been phosphorylated to adenosine triphosphate [ATP]) and magnesium ion–dependent ATPase activity were significantly increased after two and three weeks, respectively.

6.2 Toxicity

6.2.1 Human studies

An incident of possible human sensitization to DEA was reported by Piipari *et al.* (1998). A 39-year-old male metalworker experienced asthmatic symptoms approximately one to two years after a cutting fluid containing DEA and triethanolamine was introduced into his work place. Bronchial provocation tests with DEA were performed on the patient with two concentrations that were below the ACGIH TLV of 2.0 mg/m^3. Exposure to DEA aerosols at 0.75 or 1.0 mg/m^3 for 15 minutes caused maximal forced expiratory volume in one second to decrease by up to 14% and 27%, respectively. The authors concluded that this reaction represented sensitization, but the limited number of reported cases available to the authors suggested that the proportion of exposed individuals affected by this phenomenon is small. Because the test concentrations were below the TLV, the authors also felt that the effect did not involve a mechanism of toxicity.

Adverse nonmalignant respiratory effects, including nonspecific respiratory symptoms and acute impairment of lung function, asthma, and hypersensitive pneumonitis, have been associated with exposure to metalworking fluids (discussed in Eisen *et al.* 2001b). However, the components and types of fluids responsible for the symptoms have not been clearly defined. Surveillance of occupational illness by the Michigan Department of Public Health found that metalworking fluids were the second most common cause of work-related asthma reported in the state from 1988 to 1994 (Rosenman *et al.* 1997). Workers exposed to soluble, semisynthetic, or synthetic machining coolants were more likely to have chronic bronchitis, to visit doctors for sinus problems or shortness of breath, and to be bothered at work by respiratory symptoms (e.g. nasal stuffiness, runny nose, sore throat) than workers exposed to mineral oil metalworking fluids. In contrast, decreased lung function, as measured by forced ventilatory capacity, was associated with lifelong exposure to straight oil but not synthetic metalworking fluids in a study of automobile workers (Eisen *et al.* 2001b). Hypersensitive pneumonitis may be due to microbial contamination of water-based fluids (Kreiss and Cox-Ganser 1997, as cited by Eisen *et al.* 2001b). Exposure to semisynthetic metalworking fluids also has been associated with contact dermatitis (Sprince *et al.* 1996).

6.2.2 Animal studies

Beyer *et al.* (1983) reviewed the literature on DEA, monoethanolamine, and triethanolamine to assess their safety for use in cosmetic formulations. They reported that LD_{50} values for DEA in rats ranged from 0.77 to 2.83 g/kg and concluded that DEA was slightly toxic and showed little potential to irritate rabbit skin in either acute or subchronic skin irritation tests. However, the reported potential for formation of a known carcinogen, NDELA, led them to recommend that DEA not be used in products together with *N*-nitrosating agents.

In a review of the metabolism and toxicity of DEA, Melnick and Tomaszewski (1990) reported that the oral LD_{50} of DEA in rats was 1,820 mg/kg b.w. in one study and 780 mg/kg in another, the intraperitoneal LD_{50} in mice was 2,300 mg/kg b.w., and the subcutaneous LD_{50} in mice was 3,553 mg/kg b.w. The fatal dose of DEA in humans was estimated to be 20 g (~286 mg/kg b.w).

Melnick *et al.* conducted 2- and 13-week toxicology studies of DEA administered either in drinking water or by topical application to F344 rats (1994b) and B6C3F$_1$ mice (1994a). The results are summarized in Section 4.1.1. Briefly, dose-dependent toxic effects included hematological changes and toxic effects on kidney, brain and spinal cord, testis, and skin in rats and toxic effects on liver, kidney, heart, and skin in mice.

DEA acted as a competitive inhibitor of hepatic lysosomal glycosidases α-glucosidase and β-glucuronidase *in vivo* and *in vitro* (Balbaa *et al.* 1999). The inhibition of both enzymes was competitive and reversible, with a K_i value for α-glucosidase of 1.3×10^{-4} M and for β-glucuronidase of 5×10^{-5} M. These effects of DEA were not linked to any known toxic effect.

6.3 Potential mechanisms of carcinogenicity

6.3.1 NTP reports

The NTP conducted subchronic toxicity studies of DEA (NTP 1992, Melnick *et al.* 1994a, b). The toxic effects of DEA administered by topical application to B6C3F$_1$ mice and F344/N rats are reviewed in Sections 4.1.1 and 4.2.1, respectively. Melnick *et al.* (1994a) concluded that there was "compelling evidence that DEA may interfere with phosphatidylethanolamine or phosphatidylcholine synthesis, or even become incorporated into a phospholipid derivative."

The NTP also conducted two-year carcinogenicity bioassays in B6C3F$_1$ mice and F344/N rats (see Sections 4.1.2 and 4.2.2 for summaries) and proposed two potential mechanisms of DEA carcinogenicity. The first involves reaction of DEA with nitrite to form NDELA, a known carcinogen. The second potential mechanism is based on the structural similarity of DEA and ethanolamine, one of four endogenous amines incorporated as head groups in phospholipids. The displacement of ethanolamine by DEA, which cannot be converted into choline, may disrupt choline metabolism and promote carcinogenesis by the same mechanism(s) responsible for the hepatocarcinogenesis of choline deficiency. These two potential mechanisms are discussed below.

The first potential mechanism is based on the possible conversion of DEA to NDELA, a carcinogenic nitrosamine, by a nonenzymatic reaction between nitrous acid and DEA. Nitrous acid forms when nitrates react with the acid environment of the stomach. However, administration of NDELA to F344/N rats and B6C3F$_1$ mice caused liver neoplasms in rats but not mice, while the opposite pattern was observed for DEA (NTP 2001). Thus, the NTP did not consider this potential mechanism to be a plausible explanation of hepatocarcinogenesis in mice.

The second proposed mechanism is based on the potential effects on choline metabolism of DEA's incorporation as a head group in phospholipids. Although DEA can be a precursor for phospholipid biosynthesis by the same pathways that use ethanolamine as a substrate (see Figure 6-2), the resulting phospholipids are not chemically identical. DEA is a larger molecule than ethanolamine and has an additional hydroxyl group (see Figure 1-1), increasing the potential for hydrogen bonding. As a result, molecules of phosphatidyl DEA are likely to alter the properties of the membranes into which they are incorporated. Phosphatidyl ethanolamine also participates in important biosynthetic pathways, including successive methylation reactions involving *S*-adenosyl-methionine (SAM) to form phosphatidyl choline (see Figure 6-3). Cleavage of phosphatidyl choline to choline and diacylglyerol is the only pathway yielding new molecules of choline in mammals. If incorporation of DEA into phospholipids reduces the availability of choline indirectly by reducing the concentration of phosphatidyl ethanolamine and directly by competitively inhibiting incorporation of choline into phosphatidyl choline (Barbee and Hartung 1979b), choline deficiency could result. Choline deficiency and the corresponding reduction in availability of phosphatidyl choline lead to accumulation of fat in the liver and eventually to hepatocarcinogenesis in Fischer 344 male rats (da Costa *et al.* 1993, Mikol *et al.* 1983, Ghoshal and Farber 1984, Newberne and Rogers 1986). Newberne and Rogers (1986) also reported that the cirrhosis resulting from a choline-

deficient diet could be induced in several outbred and inbred strains of rats and in inbred strains of mice. Other choline-related mechanisms include reduction of the availability of SAM, since the methyl groups donated to DEA cannot return to the 1-carbon pool. In addition, biosynthesis of sphingomyelin molecules containing DEA may alter the second messenger pathways that utilize ceramides. The NTP concluded that DEA's toxic effects in mice and rats and carcinogenic effects in mice may be extrapolated to humans, because DEA is incorporated into phospholipids in human liver slices (Mathews *et al.* 1995). [It should be noted that the majority of the choline-deficiency carcinogenesis studies have been conducted with rats; however, in the two-year NTP DEA bioassays, liver tumors were observed in mice but not in rats (see Section 4).]

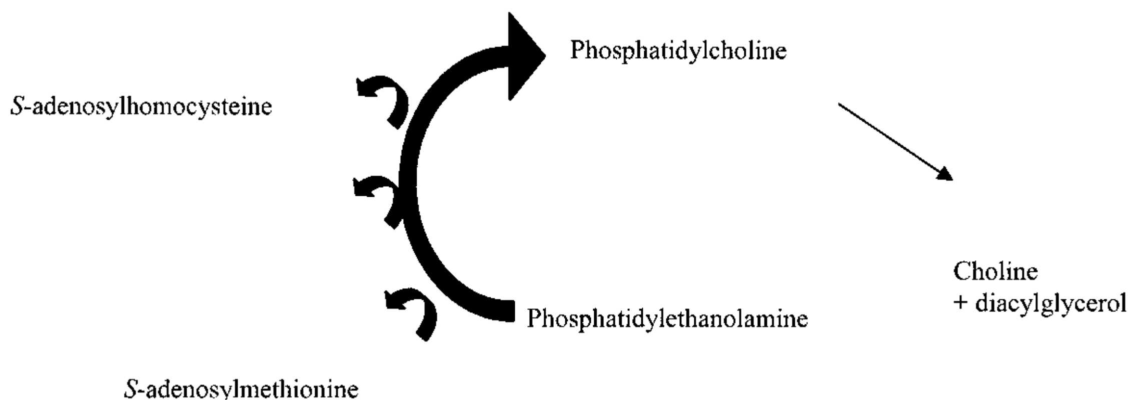


Figure 6-3. Synthesis of phosphatidyl choline from phosphatidylethanolamine and hydrolysis of phosphatidyl choline to choline and diacylglcyerol

The two-year NTP bioassay of DEA in rats and mice was based on dermal application of DEA in a 95% ethanol vehicle. The question of the potential for promotional or carcinogenic effects of ethanol itself was raised in the Review Subcommittee comments on the technical report. The potentiating effect of ethanol on carcinogenesis in rats has been investigated by several researchers. Radike *et al.* (1981) administered 5% ethanol in drinking water and 600 ppm vinyl chloride by inhalation to male Sprague-Dawley rats for one year. The incidence of liver neoplasms was higher in rats exposed to ethanol plus vinyl chloride (50% incidence of angiosarcoma and 60% incidence of hepatocellular carcinoma) than in rats exposed only to vinyl chloride (23% angiosarcoma and 44% hepatocellular carcinoma). Ethanol-exposed rats also had an excess of tumors (8 carcinomas) relative to controls (1 carcinoma); however, no statistical analysis was reported. The potential promoting effect of ethanol also was examined in studies by Driver and McLean (1986) and Takada *et al.* (1986) and compared with that of phenobarbitone. Male Wistar rats initiated with a single dose of diethylnitrosamine (DEN) at 30 mg/kg b.w. were given 5% ethanol in drinking water for one year (Driver

and McLean 1986). Hepatocellular carcinomas occurred in 25% of the rats exposed to DEN plus ethanol, but in none of the rats exposed only to DEN (no statistical analysis was reported). Takada *et al.* (1986) performed 70% partial hepatectomy on male Wistar rats followed by a single dose of DEN at 10 mg/kg b.w. Rats then received 20% ethanol–10% sucrose or 10% sucrose as drinking water, and one group of rats not administered DEN received 20% ethanol–10% sucrose. After 32 weeks, the average number of visible hepatic nodules was significantly greater ($P < 0.05$, Student's t test) in rats receiving DEN plus ethanol than in rats receiving DEN alone or DEN plus sucrose. Ethanol alone produced no nodules.

The studies described above all used rats in a model with ethanol as a promoter following an initiator. The IARC Working Group (1988) reviewed eight studies of oral administration of ethanol or alcoholic beverages to mice. Although several types of tumors were found, an increased incidence of liver sarcomas, but not of hepatocellular carcinomas, was reported for only one study. No studies of topical application of ethanol reported an association with liver tumors.

Ethanol may also affect the metabolism of choline. Ethanol was reported by Barak *et al.* (1973, 1985) to increase the uptake of choline by isolated perfused livers of rats, a process that they concluded was the result of choline oxidase activity. Sidransky and Farber (1960) investigated the choline oxidase activity of several species, including rat, mouse, and human. They found that the enzyme activity varied widely from 2,408 ± 121 (microliters oxygen uptake/hour per gram wet weight of liver ± standard error of the mean) in rat liver, to 895 ± 72 in mouse liver and 40 ± 7 in human liver. The authors proposed that the level of hepatic choline oxidase activity could be correlated with the ease of induction of choline-deficiency fatty liver. No data directly relevant to the potential effect of dermal application of ethanol on choline metabolism were found.

6.3.2 *Animal models of choline deficiency and hepatocarcinogenesis*

Choline deficiency is unique among nutrient deficiencies in leading directly to liver tumor formation in experimental rodents fed semisynthetic choline-deficient diets that did not include exposure to any known carcinogen (da Costa *et al.* 1993, Mikol *et al.* 1983, Ghoshal and Farber 1984, Newberne and Rogers 1986). The first three publications were based on studies in F344 male rats, whereas Newberne and Rogers (1986) reviewed literature on susceptibility of outbred and inbred rats and inbred mice to carcinogenesis. Zeisel (1996) reviewed the mechanisms proposed for carcinogenesis mediated by choline deficiency. Choline deficiency may damage DNA through increased free radical generation, as reflected by 8-oxydeoxyguanosine residues when the diet contains saturated fat (Lombardi and Smith 1994). Choline deficiency also is associated with significant increases in the diacylglycerol content of hepatic plasma membranes and overexpression of protein kinase C, which has been proposed to promote carcinogenesis by increasing expression of oncogenes (da Costa *et al.* 1993, 1995).

Stott *et al.* (2000) administered DEA with or without supplemental sodium nitrite to B6C3F$_1$ mice for two weeks. No NDELA was detected in the urine, blood, or gastric contents of any group of treated mice. The liver contents of choline, phosphocholine, and

glycerophosphocholine, however, were reduced by up to 84% and were in inverse proportion to blood DEA levels. The authors concluded that the marked depletion of choline and other biomarkers for choline deficiency was consistent with a potential mechanism for DEA-mediated tumorigenesis in the mouse. They proposed that preservation of phophatidyl choline, which did not decrease in DEA-treated animals, might place a higher demand on dietary sources of choline.

Choline deficiency has been induced in humans during periods of total parenteral nutrition (TPN) (Buchman *et al.* 2001). Buchman *et al.* (2001) reported that addition of choline to the usual TPN solution of adult subjects on long-term TPN significantly increased liver density and decreased serum concentrations of liver enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase). It has not been shown in humans that changes in liver function resulting from choline deficiency lead to the liver cell death, liver cell proliferation, and liver cell cancer described in rodents (Ghoshal and Farber 1993).

6.3.3 Cell culture models

Lehman-McKeeman and Gamsky (1999) added DEA at concentrations ranging from 20 to 1,000 μg/mL to cultured Chinese hamster ovary cells and measured incorporation of phosphorus-33 into phospholipid pools. DEA had no effect on cell number or total phospholipid biosynthesis, but separation of lipids by thin-layer chromatography revealed a significant decrease in phosphatidyl choline synthesis, from 51% to 27% of total phospholipids. The inhibitory effect on phospholipid synthesis was completely reversed by supplementation of the cell culture with 30 mM choline. DEA also inhibited uptake of choline at all concentrations; the maximum inhibition was 95% at concentrations of 250 or 500 μg/mL.

Lehman-McKeeman and Gamsky (2000) also tested the effects of DEA on morphologic transformation of Syrian hamster embryo cells. DEA inhibited both phosphatidyl choline synthesis and choline uptake by SHE cells and induced cell transformation at concentrations of 10 to 500 μg/mL. All three effects could be blocked by excess choline (30 mM) in the culture medium.

6.4 Summary

DEA is readily absorbed following oral administration and absorbed somewhat less efficiently following dermal administration. When applied dermally, DEA appears to facilitate its own absorption, as higher doses were more completely absorbed than lower doses. Distribution to the tissues was similar following administration by all routes. DEA is cleared from the tissues with a half-life of approximately 6 days; thus, it accumulates with repeated exposure. The highest concentrations are observed in liver and kidney. DEA is excreted primarily in urine as the parent molecule, with lesser amounts of *O*-phosphorylated and *N*-methylated metabolites.

The mechanism that accounts for accumulation of DEA at high levels in liver and kidney is unknown, but it is speculated that DEA is conserved by a mechanism that normally conserves ethanolamine, a normal constituent of phospholipids. DEA is incorporated as

the head group to form aberrant phospholipids, presumably via the same enzymatic pathways that normally utilize ethanolamine. The presence of aberrant phospholipids and the disruption of choline utilization are thought to account for much of the observed toxicity of DEA.

Potential mechanisms of DEA carcinogenicity include its conversion to a carcinogenic nitrosamine, NDELA, which occurred *in vivo* in rats simultaneously administered DEA dermally and nitrite orally. The NTP, however, concluded that this mechanism did not explain hepatocarcinogenesis observed in B6C3F_1 mice, because NDELA is not a hepatocarcinogen in these animals. The second proposed mechanism involves the displacement of ethanolamine by DEA in phospholipids. Phosphatidyl DEA cannot serve as a precursor for synthesis of phosphatidyl choline, which is the only endogenous source of new molecules of choline in mammals. Lower levels of phosphocholine and glycerophosphocholine, which are biomarkers for choline deficiency, have been reported to be associated with chronic administration of DEA to mice. Additional observations in the SHE cell culture model demonstrated that DEA can inhibit phosphatidyl choline synthesis and induce cell transformation by a mechanism that can be blocked by supplemental choline. Taken together, these observations on the effects of DEA on choline metabolism support the proposal that DEA-induced hepatocarcinogenesis may be related to choline deficiency.

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Appendix A: IARC (2000). Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Industrial Chemicals. V. 77. pp 349 - 379.

DIETHANOLAMINE

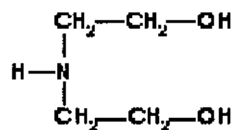
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 111-42-2
Deleted CAS Reg. No.: 8033-73-6
Chem. Abstr. Name: 2,2′-Iminobis[ethanol]
IUPAC Systematic Name: 2,2′-Iminodiethanol
Synonyms: Bis(hydroxyethyl)amine; bis(2-hydroxyethyl)amine; *N,N*-bis(2-hydroxyethyl)amine; DEA; *N,N*-diethanolamine; 2,2′-dihydroxydiethylamine; di-(β-hydroxyethyl)amine; di(2-hydroxyethyl)amine; diolamine; 2-(2-hydroxyethyl-amino)ethanol; iminodiethanol; *N,N*′-iminodiethanol; 2,2′-iminodi-1-ethanol

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_{11}NO_2$ Relative molecular mass: 105.14

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Deliquescent prisms; colourless, viscous liquid with a mild ammonia odour (Budavari, 1998; Dow Chemical Company, 1999)
(*b*) *Boiling-point*: 268.8 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: 28 °C (Lide & Milne, 1996)
(*d*) *Density*: 1.0966 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (proton [5830]; grating [33038]), nuclear magnetic resonance (proton [6575]; C-13 [2936]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Very soluble in water (954 g/L) and ethanol; slightly soluble in benzene and diethyl ether (Lide & Milne, 1996; Verschueren, 1996)

(*g*) *Volatility*: Vapour pressure, < 0.01 mm Hg [1.33 Pa] at 20 °C; relative vapour density (air = 1), 3.6; flash-point, 149 °C (Verschueren, 1996)
(*h*) *Stability*: Incompatible with some metals, halogenated organics, nitrites, strong acids and strong oxidizers (Dow Chemical Company, 1999)
(*i*) *Octanol/water partition coefficient (P)*: log P, –2.18 (Verschueren, 1996)
(*j*) *Conversion factor*[1]: mg/m^3 = 4.30 × ppm

1.1.4 *Technical products and impurities*

Diethanolamine is commercially available with the following specifications: purity, 99.3% min.; monoethanolamine, 0.45% max.; triethanolamine (see monograph in this volume), 0.25% max.; and water content, 0.15% max. (Dow Chemical Company, 1998a). Diethanolamine is also available as a blend of 85% diethanolamine and 15% deionized water which is a low freeze-grade product for use in colder temperatures (Dow Chemical Company, 1998b).

1.1.5 *Analysis*

Diethanolamine can be determined in workplace air by drawing the air sample through aqueous hexanesulfonic acid and analysing by ion chromatography. The limit of detection for this method is 13 µg per sample (Eller, 1994).

Diethanolamine can be determined in water samples by gas chromatography (GC) and by high-performance liquid chromatography (HPLC) with fluorescence detection (Melnick *et al.*, 1994a,b; Pietsch *et al.*, 1997); in metalworking and cutting fluids by GC–mass selective detection of silylated derivatives, by isotachophoresis, by capillary zone electrophoresis with indirect ultraviolet detection, and by spectrophotometry (Kenyon *et al.*, 1993; Fernando, 1995; Schubert *et al.*, 1996; Sollenberg, 1997); and in cosmetics and pharmaceuticals by GC with flame ionization detection, by ion-exclusive chromatography, and by reversed-phase HPLC (Fukui *et al.*, 1992; Maurer *et al.*, 1992; Chou, 1998).

1.2 Production

Ethanolamines became available commercially in the early 1930s; they assumed steadily growing commercial importance as intermediates after 1945, because of the large-scale production of ethylene oxide. Since the mid-1970s, economical production of very pure, colourless ethanolamines has been possible. Ethanolamines are produced on an industrial scale exclusively by reaction of ethylene oxide (see IARC, 1994) with excess ammonia. This reaction takes place slowly but is accelerated by water. An

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

anhydrous procedure uses a fixed-bed ion-exchange resin catalyst (Hammer *et al.*, 1987).

Worldwide production of ethanolamines in 1985 was approximately (thousand tonnes per year): United States, 220; western Europe, 145; south-east Asia, 40; South America, 18; eastern Europe, 4. About 50% of world production of ethanolamines in 1985 was monoethanolamine, 30–35% diethanolamine and 15–20% triethanolamine (Hammer *et al.*, 1987). Estimated annual production of diethanolamine in the United States is presented in Table 1.

Table 1. Estimated annual production of diethanolamine in the USA (thousand tonnes)

Year	1960	1965	1970	1975	1980	1985	1989[a]
Production	24	35	42	39	56	76	92

From Bollmeier (1992)
[a] National Toxicology Program (1992)

Information available in 1999 indicated that diethanolamine was manufactured by seven companies in the United States, three companies each in China and Germany, two companies each in France and India, and one company each in Belgium, Brazil, Canada, Iran, Japan, Mexico, Netherlands, the Russian Federation, Spain, Sweden and the United Kingdom (Chemical Information Services, 1999).

1.3 Use

Diethanolamine is used as surface-active agent in metal-cutting fluids and oils (see General Remarks), as a corrosion inhibitor, as a dispersant in agricultural chemical formulations, and as an intermediate in the production of other compounds such as fatty acid condensates of diethanolamine which are extensively used in soaps and cosmetics as emulsifiers, thickeners, wetting agents and detergents (Beyer *et al.*, 1983). In the cosmetic formulations, the concentration of diethanolamine may range from 1 to 25% (National Toxicology Program, 1999a).

Other applications of diethanolamine are in adhesives, antistatic agents, cement and concrete work, coatings, electroplating, an epoxy hardener, a fuel-gelling agent, printing inks, metal cleaning and lubricating, mining, natural gas treatment, paint and pigments, paper, petroleum and coal production, a pharmaceutical intermediate and an ointment-emulsifier, polymers and polymer production, rubber processing, soldering flux, textile finishing and polyurethane production and use (Hammer *et al.*, 1987; Bollmeier, 1992; Knaak *et al.*, 1997; Dow Chemical Company, 1998b). Table 2 presents estimates of percentages used in major applications in the United States (Knaak *et al.*, 1997).

Table 2. Major uses of diethanolamine in the United States

Applications	Percentage of production
Surfactants	39
Gas purification	30
Textile processing	15
Metalworking fluids	10
Miscellaneous	8
Laundry detergents	2
Agricultural chemicals	2

From Knaak *et al.* (1997)

Free diethanolamine is reported to be a contaminant in fatty acid-diethanolamine condensates (amides of coconut oil acid, oleic acid and lauric acid) at levels ranging from < 1% to nearly 10% (National Toxicology Program, 1999b,c,d). Diethanolamine also occurs as a contaminant in triethanolamine products (National Toxicology Program, 1999e).

1.4 Occurrence

1.4.1 *Natural occurrence*

Diethanolamine is not known to occur as a natural product.

1.4.2 *Occupational exposure*

Diethanolamine is present in machining and grinding fluids and has been detected in workplace air in the metal manufacturing industry. It was present in bulk cutting fluids at levels ranging from 4 to 5% (Kenyon *et al.*, 1993). Diethanolamine has also been reported to be present in wetting fluids used in road paving. A level of 0.05 mg/m^3 was detected in a stationary sample at a slurry machine discharging a bitumen emulsion containing 0.2% of the amine. All personal exposures were below the detection limit (0.02 mg/m^3) (Levin *et al.*, 1994). In a German study (1992–94), diethanolamine was measured in samples of metalworking fluids in a range of 0–44% ($n = 69$). The number of samples with diethanolamine present steadily declined from 90% to 60% over the study period (Pfeiffer *et al.*, 1996).

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 800 000 workers (many of whom were metalworkers) in the United States were potentially exposed to diethanolamine (see General Remarks).

1.4.3 *Environmental occurrence*

Production of diethanolamine and its wide use in industrial and consumer products may result in its release to the environment (Yordy & Alexander, 1981; Beyer *et al.*, 1983; Environment Canada, 1995; Mathews *et al.*, 1995; Knaak *et al.*, 1997).

(*a*) *Air*

According to the Environmental Protection Agency Toxics Release Inventory, air emissions of diethanolamine from 358 industrial facilities in 1994 were approximately 149 200 kg in the United States (Environmental Protection Agency, 1996). According to the National Pollutant Release Inventory (NPRI) of Canada, on-site releases of diethanolamine to air from 74 facilities amounted to about 40 000 kg (Environment Canada, 1995).

(*b*) *Water*

Surface water discharges of diethanolamine from 358 industrial facilities in 1994 in the United States amounted to 100 350 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1996). On-site releases of diethanolamine (and its salts) to water from 74 facilities in Canada amounted to about 26 000 kg, as reported to the NPRI (Environment Canada, 1995).

Because of the spectrum of industrial and consumer uses of diethanolamine and its miscibility with water, large amounts of the chemical can be discharged into wastewater and sewage in an unaltered form (Yordy & Alexander, 1981; Mathews *et al.*, 1995).

(*c*) *Soil*

Releases of diethanolamine to land and underground from 358 industrial facilities in the United States in 1994 (as reported to the Toxics Release Inventory) amounted to 77 050 kg and 36 850 kg respectively (Environmental Protection Agency, 1996). Canadian on-site releases of diethanolamine (and its salts) to land and underground amounted to about 118 000 kg and 497 000 kg, respectively, as reported to the NPRI (Environment Canada, 1995).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for diethanolamine are presented in Table 3.

The Food and Drug Administration permits the use of diethanolamine as a component of adhesives in food packaging, as an indirect food additive, as a component of the uncoated or coated food contact surface of paper and paperboard for use with dry solid foods with no free fat or oil on the surface, and for use only as an adjuvant to control pulp absorbance and pitch content in the manufacture of paper and paperboard or for use only in paper mill boilers in the United States (Food and Drug Administration, 1999).

Table 3. Occupational exposure limits and guidelines for diethanolamine[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Australia	1993	15	TWA
Belgium	1993	15	TWA
Denmark	1993	15	TWA
France	1993	15	TWA
Ireland	1997	15	TWA
Netherlands	1997	2	TWA
Russian Federation	1993	5 (sk)	STEL
Switzerland	1993	15	TWA
United Kingdom	1997	15	TWA
United States			
ACGIH[c]	1999	2	TWA
NIOSH	1999	15	TWA

[a] From American Conference of Governmental Industrial Hygienists (1999); National Library of Medicine (1999)
[b] TWA, time-weighted average; STEL, short-term exposure limit; sk, skin notation
[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

2. Studies of Cancer in Humans

The Working Group was not aware of any study that specifically examined the risk of cancer among persons exposed to diethanolamine. However, ethanolamines have been used as additives for metalworking fluids since the 1950s and are present in wetting fluids used in asphalt paving. Results from cohort and case–control studies of asphalt and road-maintenance workers suggest elevations in the risk of several cancers, including lung, stomach, non-melanoma skin cancer and leukaemia (reviewed by Partanen & Boffetta, 1994). These groups of workers are also exposed to known or suspected carcinogens present in road paving and roofing materials (see Table 4). These compounds include benzene (Group 1) (IARC, 1987a), 1,3-butadiene (Group 2A) (IARC, 1999) and coal-tar pitches (Group 1) (IARC, 1987b). In the light of these concomitant exposures, any observed risk elevations cannot be specifically attributed to diethanolamine or to any other constituent of the complex mixtures. The Working Group, therefore, did not make a detailed evaluation of these studies.

There are three major types of metalworking fluid; straight (generally mineral oils), soluble (straight oils diluted with water and additives) and synthetic (water and addi-

Table 4. Degrees of evidence for carcinogenicity in humans and experimental animals and overall evaluation of carcinogenicity to humans for agents to which asphalt workers and roofers may be or may have been exposed, as evaluated by IARC as of 1993[a]

Agent [CAS No.]	Human	Animal	Overall evaluation
Asbestos [1332-21-4]	S	S	1
Benzene [71-43-2]	S	S	1
Bitumens [8052-42-4], undiluted, steam-refined (straight-run)	I	L	3
Bitumens [92062-05-0], undiluted, cracking-residue	I	L	3
Bitumens [64742-93-4], undiluted, air refined (air-blown)	I	L	3
Extracts of steam-refined bitumens	I	S	2B
Extracts of air-refined bitumens	I	S	2B
1,3-Butadiene [106-99-0]	L	S	2A
Coal-tars [8007-45-2]	S	S	1
Coal-tar pitches [65996-93-2]	S	S	1
Diesel engine exhaust	L	S	2A
Gasoline	I	L	2B
Gasoline engine exhaust	I	I	2B
Kerosene [8008-20-6]	I	I	3
Petroleum solvents	I	I	3
Polyaromatic hydrocarbons			
Anthracene [120-12-7]	I	I	3
Phenanthrene [85-01-8]	I	I	3
Fluoranthene [206-44-0]	I	I	3
Pyrene [129-00-0]	I	I	3
Chrysene [218-01-0]	I	L	3
Benzo[*a*]pyrene [50-32-8]	I	S	2A
Benz[*a*]anthracene [56-55-3]	I	S	2A
Perylene [198-55-0]	I	I	3
Benzo[*b*]fluoranthene	I	S	2B
Benzo[*j*]fluoranthene	I	S	2B
Benzo[*k*]fluoranthene [207-08-9]	I	S	2B
Anthanthrene [191-26-4]	I	L	3
Silica, crystalline [7631-86-9]	L	S	2A
Solar radiation	S	S	1
Styrene [100-42-5]	I	L	2B

From Partanen and Boffetta (1994)

[a] I, inadequate evidence; L, limited evidence; S, sufficient evidence. Overall evaluation: 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, not classifiable as to its carcinogenicity to humans.

tives) (see General Remarks). Ethanolamines, either diethanolamine or triethanolamine, are very common additives to both soluble and synthetic metalworking fluids (see Sections 1.3 and 1.4.2). Metalworking fluids are complex mixtures that may vary considerably depending on the type of fluid and the additives used. These mixtures may contain many potential carcinogens and, in particular, there is potential for exposure to *N*-nitrosodiethanolamine (see monograph in this volume) in all of the studies considered. A number of studies have examined the risk of cancer among workers exposed to metalworking fluids. Only studies which stated that ethanolamines (no studies indicated diethanolamine alone) were used as additives or that presented results for workers primarily exposed to soluble or synthetic fluids were considered by the Working Group. The characteristics of these studies are presented in Table 5 and a summary of the results for specific cancer sites is presented in Table 6. The use of ethanolamines and nitrites together as additives to metalworking fluids can lead to the formation of *N*-nitrosodiethanolamine. Studies stating that ethanolamines and nitrites were used as additives or which presented results for exposure to nitrosamines are described in detail in the monograph in this volume on *N*-nitrosodiethanolamine. The other studies are described in detail below.

Järvholm and Lavenius (1987) examined the risk for cancer among Swedish men employed for at least five years and any time between 1950 and 1966 in the grinding or turning departments of a company producing bearing rings. This was an extension of an earlier study reported by Järvholm *et al.* (1981) in which a two-fold excess of stomach cancer morbidity was reported among workers in the grinding department during 1958–76. A total of 792 employees met the entrance criteria (4.4% were lost to follow-up). Of these, 559 men had been employed in the grinding department where soluble and some synthetic oils (acid-refined from 1940–75 and solvent-refined mineral oils from 1975) were used. Ethanolamines were introduced as additives in the metalworking fluids used in the department in the mid-1950s. Mortality and cancer incidence follow-up was conducted from 1958 until 1983 and expected numbers were calculated using reference rates from the same city. There were 209 deaths (standardized mortality ratio (SMR), [0.83]; 95% confidence interval (CI), 0.71–0.94) and 67 incident cancers (standardized incidence ratio (SIR), [0.69]; 95% CI, 0.54–0.87) in the full cohort. Among the subcohort of 559 workers in grinding departments, there were 41 incident cancers (SIR, [0.63]; 95% CI, 0.45–0.86), with the only notable excess being for stomach cancer (SIR, [1.5]; 95% CI, 0.7–3.0). [The Working Group noted that part of this cohort was also studied in relation to exposure to *N*-nitrosodiethanolamine. The results of this investigation (Järvholm *et al.*, 1986) are reported in the monograph on *N*-nitrosodiethanolamine in this volume.]

Eisen *et al.* (1992) performed a cohort mortality study of 46 384 workers employed for three or more years before 1985 in three United States auto parts manufacturing facilities. Exposure to all three types of metalworking fluid (straight oils (insoluble or cutting oils), soluble oils (water-miscible or emulsifier oils) and synthetic oils (chemical fluids, containing ethanolamines)), the last two introduced in the 1940s, existed and no

Table 5. Characteristics of studies on diethanolamine exposure

Study/country	Study design	Study population	Follow-up period	Potential exposures
Järvholm & Lavenius (1987) Sweden	Cohort	792 men employed > 5 years, any time 1950–66 in the grinding and turning departments of a bearing rings company (may overlap with Järvholm *et al.*, 1986)	1958–83	Analysis of the subgroup of 559 grinders exposed to soluble or synthetic oils
Eisen *et al.* (1992)[a] USA	Cohort	46 384 employed for > 3 years before 1985 at three auto parts manufacturing facilities	1941–84	All three types of metalworking fluid; no analysis by sub-group
Tolbert *et al.* (1992) USA	Cohort	33 619 (two of the three facilities in Eisen *et al.*, 1992)	1941–84	Analysis of three sub-groups exposed to each type of metalworking fluid by years of exposure
Eisen *et al.* (1994) USA	Nested case–control of laryngeal cancer	108 fatal and incident cases; 538 controls (study base: Eisen *et al.*, 1992 cohort)	1941–84	Cumulative exposure to straight and soluble types of metalworking fluid and metalworking fluid particulate exposure during grinding; duration of exposure to metalworking fluid and other components.
Sullivan *et al.* (1998) USA	Nested case–control of oesophageal cancer	53 fatal cases; 971 controls (study base: Eisen *et al.*, 1992 cohort)	1941–84	Cumulative exposure to the three types of metalworking fluid; duration of exposure to metalworking fluid and other components, incl. nitrosamines.

[a] The results of this study were not considered by the Working Group, but it is included because it forms the study base of the nested case–control studies considered.

Table 6. Results of epidemiological studies of cohorts exposed to soluble and synthetic metalworking fluids

Reference	Stomach		Oesophagus		Larynx		Leukaemia		Pancreas		All cancer		All mortality	
	Obs.	SMR/ PMR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR
Järvholm & Lavenius (1987) (incidence)														
All grinders (SIR)	8	[1.5] (0.7–3.0)	2	[2.0] (0.2–7.2)	NR		NR		NR		41	[0.63] (0.45–0.86)		
> 20 years latency (SIR)	7	[1.7] (0.7–3.5)	2	[2.4] (0.3–8.8)	NR		NR		NR		33	[0.66] (0.46–0.93)		
Tolbert *et al.* (1992) (mortality)														
Synthetic oils														
White males	21	1.3 (0.8–2.0)	8	0.99 (0.4–1.9)	8	1.6 (0.7–3.1)	16	1.2 (0.7–2.0)	19	1.03 (0.6–1.6)	333	0.97 (0.87–1.1)	1632	1.01 (0.96–1.1)
Soluble oils														
White males	99	1.2 (1.0–1.4)	35	1.03 (0.7–1.4)	30	1.4 (1.0–2.0)	75	1.3 (1.0–1.7)	61	0.8 (0.6–1.0)	1479	1.02 (0.97–1.1)	7287	1.00 (0.98–1.03)
Black males	17	1.0 (0.6–1.6)	10	0.7 (0.3–1.3)	6	1.5 (0.5–3.2)	4	0.7 (0.2–1.9)	19	1.6 (1.0–2.5)	200	0.90 (0.78–1.0)	922	0.81 (0.76–0.87)
Eisen *et al.* (1994) (mortality)														
Soluble fluids (mg/m^3–years)														
0	NA		NA		9	1.00	NA		NA		NA		NA	
0.1–2.0					41	1.34 (0.6–3.0)								
> 2 0–6.0					29	1.22 (0.5–2.9)								
> 6.0					29	1.16 (0.5–2.7)								
Sullivan *et al.* (1998) (20-year lag) (mortality)														
5 mg/m^3–years synthetic oil	NA		–	2.8 (1.1–7.5)	NA		NA		NA		NA		NA	
5 mg/m^3–years soluble oil			–	1 0 (1.0–1 1)										

NA, not applicable; NR, not reported

separate analyses for subgroups were presented (Tolbert *et al.*, 1992). This cohort formed the study base for the three subsequent studies in this monograph.

Tolbert *et al.* (1992) reported the results of a cohort study of 33 619 persons who had worked for at least three years before 1985 in two of the three facilities studied by Eisen *et al.* (1992) where metalworking fluids were used extensively. Mortality was followed from 1941 to 1984 and vital status could be determined for 94% of the cohort at the end of follow-up. In total, 9349 deaths were identified and death certificates were obtained for 92%. Plant records and industrial hygiene data were used in combination with detailed work history records to identify which persons were exposed to different types of machining fluid and their duration of exposure. Among white men exposed to soluble oils (n = 23 488), there were 7287 deaths (SMR, 1.00) and small excesses were observed for cancers of the stomach (SMR, 1.2; 95% CI, 1.0–1.4), larynx (SMR, 1.4; 95% CI, 1.0–2.0) and brain (SMR, 1.2; 95% CI, 0.9–1.7) and leukaemia (SMR, 1.3; 95% CI, 1.0–1.7). Among white men exposed to synthetic fluids (n = 8446), there were 1632 deaths (SMR, 1.01) and small excesses were observed for cancers of the stomach (SMR, 1.3; 95% CI, 0.8–2.0) and larynx (SMR, 1.6; 95% CI, 0.7–3.1) and leukaemia (SMR, 1.2; 95% CI, 0.7–2.0). Among black men exposed to soluble oils (n = 4964), there were 922 deaths (SMR, 0.81) and small excesses were observed for pancreatic cancer (SMR, 1.6; 95% CI, 1.0–2.5) and laryngeal cancer (SMR, 1.5; 95% CI, 0.5–3.2). Results for black men exposed to synthetic fluids or women exposed to any fluids were not presented because of small numbers. Poisson regression analyses were performed to examine the relationships between duration of exposure to each of the three types of metalworking fluid and specific cancer sites after adjustment for plant, sex, race, length of follow-up, year of birth and age at risk. With the exception of statistically significantly negative associations between lung cancer and synthetic fluids (p = 0.006) (for soluble oils, p = 0.09), no strong dose–response relationship was observed. Mild excesses were observed among persons exposed to soluble fluids for 20 or more years for stomach cancer (rate ratio, 1.2; 95% CI, 0.7–2.1) and pancreatic cancer (rate ratio, 1.4; 95% CI, 0.5–3.7). Slightly larger excesses were observed among persons exposed to synthetic fluids for eight or more years for colon cancer (rate ratio, 1.6; 95% CI, 0.8–3.4) and pancreatic cancer (rate ratio, 2.0; 95% CI, 0.9–4.7).

Eisen *et al.* (1994) reported the results of a nested case–control study of laryngeal cancer among the members of the cohort studied by Eisen *et al.* (1992). Potential cases were individuals who had, or died from, laryngeal cancer between 1941 and 1984 and people with laryngeal cancer identified using regional tumour registries or based on other information included on death certificates. Cases were verified using tumour registry or hospital records and a total of 108 cases were eligible for inclusion (all but one being squamous-cell carcinomas). Incidence density sampling was used to select five controls for each case matched on the basis of year of birth, plant, race and sex. Exposure was assessed based on air sampling data, plant records and interviews with plant personnel. Indices of exposure were developed for duration and cumulative

exposure to the straight and soluble metalworking fluids and duration of exposure to biocides, sulfur and various metals. Matched analyses were performed using conditional logistic regression models with additional adjustment for time since hire. The risk for laryngeal cancer was not found to be associated with either cumulative level (mg/m^3–years) or duration of exposure to soluble metalworking fluids. The relationship with exposure to synthetic fluids or ethanolamines was not presented.

Sullivan *et al.* (1998) conducted a nested case–control study of oesophageal cancer among the members of the cohort studied by Eisen *et al.* (1992). Potential cases were 60 individuals who died of oesophageal cancer between 1941 and 1984. Incidence density sampling was used to select 20 controls for each case matched on the basis of year of birth, plant, race and sex, but because of missing data, 53 cases and 971 controls remained. Work history data and an exposure matrix developed for the study were used to assign exposure. The same indices of exposure were used as those described for Eisen *et al.* (1994), with the addition of duration and cumulative exposure to synthetic fluids and duration of exposure to nitrosamines. Matched analyses were performed using conditional logistic regression with additional adjustment for time since hire. Lagging was used to account for latency. After allowing for a 20-year latency, oesophageal cancer was associated with cumulative exposure to synthetic fluids (odds ratio, 2.8; 95% CI, 1.1–7.5 for 5 mg/m^3–years) and duration of exposure to synthetic fluids (odds ratio, 3.3; 95% CI, 1.1–9.6 for five years). Analyses for exposure specifically to ethanolamines and the risk for oesophageal cancer were not presented. [The Working Group noted that in the last studies, data on tobacco smoking and alcohol drinking were not directly presented.]

The Working Group was aware of several other cohort and proportionate mortality studies which included workers exposed to metalworking fluids but did not include analyses of sub-groups of workers exposed to soluble or synthetic fluids. The Working Group was also aware of a number of population-based case–control studies that reported risks associated with exposure to unspecified metalworking fluids or employment in occupations with potential exposure to metalworking fluids. However, these studies were not considered informative for the evaluation because of the unknown probability of exposure to ethanolamines and the potential for confounding from exposure to other known or suspected carcinogens.

[The Working Group noted that the mixed and varying exposures may explain the variability of the results of the different studies and also make it very difficult to ascribe the excesses of cancer observed to any single agent.]

3. Studies of Cancer in Experimental Animals

3.1 Skin application

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were administered 0, 40, 80 or 160 mg/kg bw diethanolamine (purity, > 99%) in 95% ethanol by dermal application on five days per week for two years. Survival of dosed male mice was similar to that of the vehicle control group, but survival of dosed female mice was reduced (44/50, 33/50, 33/50 and 23/50 for the control, low-, mid- and high-dose groups, respectively). The mean body weights of the mid- and high-dose males were lower than those of the vehicle controls after weeks 88 and 77, respectively. The mean body weights of the low- and mid-dose females were lower than those of the vehicle controls from week 73, but those of the high-dose females were reduced compared with the vehicle controls from week 53. In male mice, the incidences of hepatocellular adenoma and of hepatocellular adenoma and carcinoma (combined) in all dosed groups were significantly greater than those in the vehicle control group (hepatocellular adenoma: 31/50, 42/50, 49/50 and 45/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 12/50, 17/50, 33/50 and 34/50 ($p < 0.001$, Poly-3 trend test), for the control, low-, mid- and high-dose groups, respectively). In addition, the incidences of hepatoblastoma in the mid- and high-dose groups were significantly increased compared with the vehicle control (0/50, 2/50, 8/50 ($p = 0.004$) and 5/50 ($p = 0.028$, pairwise comparisons) in the control, low-, mid- and high-dose groups, respectively). In the female mice, the incidences of hepatocellular neoplasms were significantly higher than those in the vehicle control group (hepatocellular adenoma: 32/50, 50/50, 48/50 and 48/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 5/50, 19/50, 38/50 and 42/50 ($p < 0.001$, Poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). Renal tubule adenomas in males showed a marginal increase after standard single-section examination (1/50, 4/50, 6/50 and 6/50 ($p = 0.05$, Poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). When combining single with extended step-sectioning, the incidences were: 1/50, 6/50, 8/50 and 7/50 ($p = 0.055$, Poly-3 trend test) for the control, low-, mid- and high-dose groups, respectively (National Toxicology Program, 1999a).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were administered diethanolamine (purity, > 99%) in 95% ethanol by dermal application on five days per week for two years. Males received 0, 16, 32 or 64 mg/kg bw and females 0, 8, 16 or 32 mg/kg bw. Survival rates for dosed male and female groups were similar to those of corresponding vehicle control groups. The mean body weight

of the high-dose male group was lower than that of the vehicle controls from week 8 and the mean body weight of the high-dose female group was lower than that of the vehicle controls from week 97. There were no increases in tumours in treated groups compared with the vehicle controls (National Toxicology Program, 1999a).

3.2 Genetically modified mouse

Groups of 15–20 female Tg.AC mice, which carry a zeta-globin promoted v-Ha-*ras* gene on an FVB background, 14 weeks of age, were administered diethanolamine topically in 95% ethanol (the diethanolamine used was from the same chemical batch as that used in the mouse National Toxicology Program study (National Toxicology Program, 1999a). The diethanolamine was administered in 200-μL volumes, five times per week for 20 weeks. The concurrent negative control groups were treated with 200 μL 95% ethanol. The positive control group was treated with 1.25 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA; approximately 99% pure) twice per week for 20 weeks. The doses of diethanolamine selected were based on the maximum tolerated dose used earlier (National Toxicology Program, 1999a) and were 5, 10 or 20 mg diethanolamine per mouse per application (higher than the MTD). Survival was high in both the control (90%) and treated groups (80–95%). Lesions were diagnosed as papillomas when they reached at least 1 mm in diameter and persisted for three weeks. Animals that did not survive until the end of week 10 were not included in the data summaries or calculations. Six weeks after the last application, all surviving mice were killed. There was no evidence of chronic irritation or ulceration at the site of application. In contrast to the positive controls, which developed multiple papillomas in 18/20 animals, there was no increase in the incidence of skin tumours in diethanolamine-treated animals in this model (Spalding *et al.*, 2000).

[The Working Group was aware of three carcinogenicity bioassays (dermal application studies) in $B6C3F_1$ mice and Fischer 344/N rats of fatty acid-diethanolamine condensates conducted by the National Toxicology Program. These were coconut oil acid, lauric acid and oleic acid diethanolamine condensates (National Toxicology Program, 1999b,c,d). The same three condensates were also tested in the transgenic Tg.AC and $p53^{+/-}$ mouse models (Spalding *et al.*, 2000). The Working Group concluded that these studies could not be used in the evaluation of the carcinogenicity of diethanolamine *per se*. This judgement was based on the fact that the substances tested were complex mixtures of imprecise composition, that the actual diethanolamine content had not been measured in any of the three studies and therefore the precise levels of exposure were indeterminable, and the fact that these studies were not designed as, and did not represent, conventional or adequate carcinogenesis bioassays of diethanolamine.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

(*a*) *Absorption and distribution*

Data on the toxicokinetics of diethanolamine have been reviewed (Beyer *et al.*, 1983; Melnick & Tomaszewski, 1990; Gillner & Loeper, 1995; Knaak *et al.*, 1997).

Evidence of dermal absorption and the effect of grooming were reported by Stott *et al.* (2000). Diethanolamine was administered (160 mg/kg bw per day) to $B6C3F_1$ mice by dermal application (with or without access to the application site) or by oral gavage for two weeks. After the final dose (1–2 h), the blood levels of diethanolamine were 5, 6.6 and 7.7 μg/g for dermal (collared mice), oral + dermal (grooming allowed) and oral gavage treatment, respectively. The dermal dosing method for diethanolamine (90 mg/mL in ethanol; 1.78 mL/kg bw per 4-cm^2 area) was the same as in the carcinogenicity bioassay (Section 3.1.1; National Toxicology Program, 1999a).

Skin penetration rates and permeability constants (k_p) for ^{14}C-labelled diethanolamine (Table 7) were determined *in vitro* using full-thickness skin preparations from rats, mice, rabbits and humans (female mammoplasty patients). Human skin proved to be the best barrier against aqueous diethanolamine (37%, w/w) followed by rat, rabbit and mouse skin when the chemical was applied as an 'infinite dose' (20 mg/cm^2 to cm^2 of skin for 6 h). The total absorbed dose from aqueous diethanolamine was greater (0.23–6.68%) than that from undiluted material (0.02–1.3%) (Sun *et al.*, 1996).

Dermal doses of [^{14}C]diethanolamine applied in 95% ethanol (for 48 h) to a 1-cm^2 area of $B6C3F_1$ mouse skin (8–81 mg/kg bw, 15 μL volume, and protected non-occlusively by a dome of wire mesh) were more efficiently absorbed (27–58%) than the test doses (2–28 mg/kg bw, 25 μl volume) applied to a 2-cm^2 area of Fischer 344 rat skin (3–16%) (Mathews *et al.*, 1997).

Dermal absorption was also studied in rats. [^{14}C]Diethanolamine was applied to 19.5 cm^2 of the dorsal skin (20 mg/cm^2, 1500 mg/kg bw) and covered for 48 h (no washing) or for 6 h before it was removed by washing. Absorbed [^{14}C]diethanolamine was determined in 48-h urine and faeces and from sampled tissues. Unwashed rats absorbed 1.4% and washed animals 0.64% of the dose, while the majority of [^{14}C]diethanolamine was recovered in the occlusive wrappings (80%) and in skin of the dose site (3.6%). The radioactivity was found in carcass, liver or kidneys but very little in urine (0.11%), faeces or blood (Waechter *et al.*, 1995, cited by Knaak *et al.*, 1997).

Table 7. Skin penetration characteristics of undiluted and aqueous solutions of [^{14}C]diethanolamine

Species	Cumulative dose absorbed (%)	Lag time (h)[a]	Steady-state penetration rate[b] (μg/cm²/h)	Permeability constant, k_P[c] (cm/h × 10^{-4})
Undiluted diethanolamine				
Rat	0.04 ± 0.01[d]	0.6	1.8	0.02
Mouse	1.30 ± 1.15[d]	0.9	46.3	0.42
Rabbit	0.02 ± 0.01[d]	1.3	0.9	0.01
Human	0.08 ± 0.03[e]	3.2	5.7	0.05
Aqueous diethanolamine (37% w/w)				
Rat	0.56 ± 0.43[d]	0.8	23.0	0.60
Mouse	6.68 ± 5.28[d]	0.8	294.4	7.62
Rabbit	2.81 ± 2.39[d]	1.5	132.2	3.42
Human	0.23 ± 0.09[e]	2.4	12.7	0.34

From Sun *et al.* (1996)
[a] Extrapolated from the intercept of the linear segment (regression) line with the abscissa
[b] Penetration rate at steady state, derived from the slope of the linear segment of a plot of the cumulative mg/cm² absorbed versus time
[c] $k_P = \frac{\text{Steady-state penetration rate (mg/cm}^2\text{/h)}}{\text{Initial concentration (mg/cm}^3\text{)}}$
[d] Mean ± SE ($n = 3$)
[e] Mean ± SE ($n = 6$)

Combined data from several studies (cited above) showed that in rats the absorption rate increased linearly with the [^{14}C]diethanolamine dose (single), and that a 100-fold increase in the dose of diethanolamine (188–19 720 μg/cm²) resulted in a 450-fold increase in absorption rate (0.113–45.0 μg/cm² per h) (Knaak *et al.*, 1997).

Non-radiolabelled diethanolamine was applied to the dorsal skin of rats (1500 mg/kg bw, *ca.* 20 mg/cm² to 25 cm² of skin and covered) once per day for 6 h per day for three or six days. [^{14}C]Diethanolamine (1500 mg/kg bw) was then applied to the skin for a 48-h penetration test. Animals in the three-day and six-day pretreatment groups absorbed 21% and 41% of the applied dose, respectively. Liver, kidney or carcass contained the majority of absorbed radioactivity, urine from three-day and six-day groups contained 4.3% and 13%, respectively, and less than 0.3% was found in brain, fat or heart (Waechter *et al.*, 1995, cited by Knaak *et al.*, 1997).

[^{14}C]Diethanolamine (7 mg/kg bw) was given orally to male Fischer 344 rats once or by daily repeat dosing for up to eight weeks. Single oral doses (0.7–200 mg/kg bw) were well absorbed but excreted very slowly. About 20–30% of oral and intravenous doses (7 mg/kg bw) was found in urine (mainly as unchanged diethanolamine), with less than 3% in faeces and only 0.2% or less was exhaled (CO_2) within 48 h. Most of the diethanolamine was retained in tissues at high concentrations. The tissue-to-blood

ratios were 150–200 for the liver and kidney, 30–40 for the lung and spleen and 10–20 for the heart, brain and muscle. Tissue radioactivity was found mainly in aqueous extracts (up to 90%) and 5–10% was organic-extractable (Mathews *et al.*, 1995, 1997).

(*b*) *Metabolism and excretion*

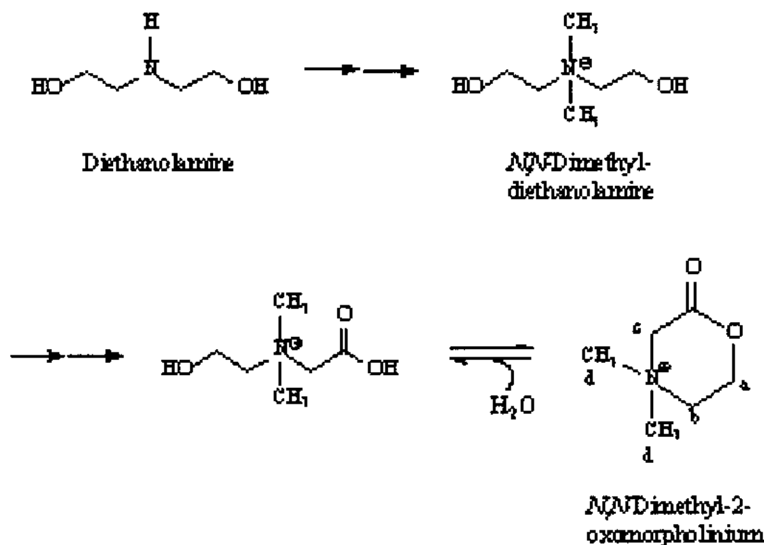
Diethanolamine is incorporated into membrane phospholipids (Artom *et al.*, 1949, 1958) and interacts with lipid metabolism *in vivo*, for example by inhibiting incorporation of ethanolamine and choline into phospholipids in rat liver and kidney. The synthesis of liver phospholipids *in vitro* was competitively inhibited by diethanolamine ($K_i \sim 3$ mM). Diethanolamine was a less effective precursor ($K_m = 12$ mM) in phospholipid synthesis than the natural substrates choline ($K_m = 0.076$ mM) and ethanolamine ($K_m = 0.054$ mM) (Barbee & Hartung, 1979a). The catabolism of diethanolamine-containing lipids was slower than that of the corresponding choline- and ethanolamine-containing derivatives (Artom *et al.*, 1958; Barbee & Hartung, 1979a). Diethanolamine is conserved and metabolized by biosynthetic routes common to ethanolamine, resulting in *O*-phosphorylated, *N*-methylated and *N*,*N*-dimethylated derivatives that are incorporated as polar head groups into aberrant phospholipids which are, in turn, incorporated into critical membranes (Mathews *et al.*, 1997). Functional and structural alterations induced by diethanolamine in liver mitochondria may ensue from its adverse effects on lipid metabolism in subcellular membranes (Barbee & Hartung, 1979b). About 30% of the diethanolamine-derived phospholipids in rat liver were ceramides (sphingomyelins) and about 70% were phosphoglycerides following a single oral dose of diethanolamine (7 mg/kg bw). After repeated administration (7 mg/kg bw on five days per week for eight weeks), the bioaccumulation of diethanolamine to plateau levels at between four and eight weeks was accompanied by an increasing degree of methylation and accumulation of aberrant sphingomyelinoid lipids in tissues. The highest concentrations of diethanolamine-associated radioactivity measured 72 h after the final dose given in the eight-week period were found in the liver (0.3 mg equivalent/g). The blood was a notable exception in that it continued to bioaccumulate diethanolamine throughout the eight-week dosing period. Uptake, retention and metabolism of diethanolamine in human and rat liver slices are reported to be similar (Mathews *et al.*, 1995, 1997).

Hepatic levels of choline, phosphocholine and glycerophosphocholine were reduced as much as 64, 84 and 70%, respectively in male B6C3F$_1$ mice after two weeks' administration of diethanolamine (160 mg/kg bw per day) via oral gavage or skin painting. These levels were inversely related to the blood diethanolamine levels (uptake) after the final dose. In contrast, the hepatic levels of sphingomyelin were increased relative to those in control mice, and were directly correlated with blood diethanolamine levels (Stott *et al.*, 2000).

The metabolism of diethanolamine leading to urinary elimination is illustrated in Figure 1. After single oral and intravenous administrations of diethanolamine to Fischer 344 rats, the compound is excreted predominantly unchanged in urine, only a

small portion being found as its mono- and dimethylated derivatives. As a result of progressive methylation after repeated oral administration (eight weeks), the relative amounts of *N*-methyldiethanolamine and *N*,*N*-dimethyl-2-oxomorpholinium appearing together with unchanged diethanolamine in urine increased markedly. The quaternized lactone was formed from *N*,*N*-dimethyldiethanolamine *in vivo* and in incubations with rat liver microsomes *in vitro* (Mathews *et al.*, 1995, 1997).

Figure 1. Proposed pathway of diethanolamine metabolism in the rat, based on urinary excretion data from an eight-week oral dosing study (7 mg/kg bw per day, five days per week for eight weeks)



From Mathews *et al.* (1997)

After four weeks' repeated administration of [^{14}C]diethanolamine (7 mg/kg bw per day on five days per week) to rats, the urinary excretion of radiolabel (during the 'washout phase') was followed for another four weeks. The log-linear response with time was a first-order process with a whole-body elimination half-life of about six days (Mathews *et al.*, 1997).

Excretion of *N*-nitrosodiethanolamine in urine was evident in Sprague-Dawley rats receiving diethanolamine via the skin (100–400 mg/animal) and sodium nitrite in the drinking water (2000 ppm [mg/L]) for six days but not in the absence of sodium nitrite (Preussmann *et al.*, 1981). *N*-Nitrosodiethanolamine was detected in a gastric rinse of rats treated with 100 μmol diethanolamine [59 mg/kg bw] and 400 μmol sodium nitrite [153 mg/kg bw] by gavage (Konishi *et al.*, 1987). No evidence of formation of *N*-nitrosodiethanolamine *in vivo* was found, however, in B6C3F$_1$ mice treated with diethanolamine (160 mg/kg bw per day) and sodium nitrite (140 ppm in drinking-water; 40 mg/kg bw) (Stott *et al.*, 2000).

4.2 Toxic effects

4.2.1 *Humans*

The only experimental data available on human exposure to airborne diethanolamine come from clinical provocation tests. Diethanolamine-induced occupational asthma was diagnosed following specific bronchial provocation tests in an exposure chamber. The positive reaction was observed in a 39-year-old male metal worker after a 30-min or 45-min inhalation exposure to aerosols from a warmed cutting fluid (40 °C) containing 0.15% diethanolamine and 0.32% triethanolamine, as well as after a 15-min exposure to pure diethanolamine at aerosol concentrations of 0.75 and 1.0 mg/m^3 (Piipari *et al.*, 1998).

4.2.2 *Experimental systems*

The toxicity of diethanolamine (as well as of mono- and triethanolamine) has been reviewed (Knaak *et al.*, 1997).

In Swiss Webster mice, the LD_{50} for diethanolamine (by intraperitoneal injection) was 2.3 g/kg bw. At this dose, marked liver changes, including extensive vacuolization and fat droplets, were observed 4 h after dosing. By 24 h, no vacuoles were visible in hepatocytes and fatty droplets were reduced in number (Blum *et al.*, 1972).

Extensive information is available on toxic effects following oral and dermal application of diethanolamine in a 13-week subchronic study (National Toxicology Program, 1992; Melnick *et al.*, 1994a,b). Groups of 10 male Fischer 344/N rats were given 0, 320, 630, 1250, 2500 or 5000 ppm [mg/L] diethanolamine in the drinking-water (equivalent to 25–440 mg/kg bw per day), while groups of 10 females were given 0, 160, 320, 630, 1250 or 2500 ppm (equivalent to 15–240 mg/kg bw per day). Two male rats died in the highest-dose group; both male and female rats lost weight in a dose-dependent fashion. Poorly regenerative microcytic anaemia developed within two weeks, without observed changes in bone marrow. Moreover, increased kidney weight, tubular necrosis and loss of kidney function occurred after two weeks. Epithelial cell necrosis in kidney tubules was seen only at the highest dose in both sexes. Some mild changes in the liver were observed, such as weight increase. Demyelination in the medulla oblongata (brain) and spinal cord was found after 13 weeks in both males and females (Melnick *et al.*, 1994a).

In a concurrent study, $B6C3F_1$ mice were given to 0, 630, 1250, 2500, 5000 and 10 000 ppm [mg/L] in the drinking-water; exposures were equivalent to 100–1700 mg/kg bw per day for males and 140–1100 mg/kg bw per day for females. At the three higher dose levels, the mice lost weight and all males and females in the two highest-dose groups died before the end of the study. In both males and females, a dose-dependent increase in liver weight was observed after two weeks; the effect was present even at the lowest dose after 13 weeks in both males and females. Hepatocellular necrosis was found with doses ≥ 2500 ppm. Cytological changes in hepatocytes were

found at all doses after 13 weeks. Kidney toxicity, including tubular necrosis, was seen only in male mice after 13 weeks. In both males and females, degeneration of cardiac myocytes was seen at doses of 2500 ppm and above (Melnick *et al.*, 1994b).

In the same study (Melnick *et al.*, 1994a,b), the effects of dermal exposure were observed during a 13-week study. Groups of 10 male and 10 female rats received applications of 32–500 mg/kg bw on five days per week. At the highest dose, some rats died during the study period. Ulcerative skin lesions at the site of application developed, accompanied by inflammation, hyperkeratinosis and acanthosis of the epidermis. Microcytic anaemia also developed, similarly to that observed after oral exposure. Kidney toxicity, including tubular necrosis and mineralization, was observed, especially in females. Liver weights were increased in both males and females, but no histopathological changes were observed in the liver. Demyelination in the medulla oblongata (brain) and spinal cord also occurred. In mice, after skin application of doses of 80–1250 mg/kg bw on five days per week, the highest dose induced a decrease in body weight compared with controls. Skin toxicity was observed at the site of application and liver weight increased, but hepatocellular necrosis occurred only in male mice. Kidney toxicity, including tubular necrosis, and cardiac myocyte degeneration were found in both males and females.

Irritation of the eye and skin after application of pure (98%) diethanolamine was investigated in New Zealand White rabbits. After 72 h, irritation of the skin was moderate, whereas irritation of the eye was severe (Dutertre-Catella *et al.*, 1982).

Diethanolamine has been shown to inhibit choline uptake into cultured Syrian hamster embryo (SHE) and Chinese hamster ovary cells and to inhibit the synthesis of phosphatidylcholine in in-vitro systems in a concentration-dependent, competitive and reversible manner (Lehman-McKeeman & Gamsky, 1999, 2000). Diethanolamine treatment caused a marked reduction in hepatic choline metabolite concentrations in mice following two weeks of dermal dosing. The most pronounced reduction was in the hepatic concentration of phosphocholine, the intracellular storage form of choline (Stott *et al.*, 2000). Moreover, the pattern by which choline metabolites were altered was similar to the pattern of change that has been observed following dietary choline deprivation in rodents (Pomfret *et al.*, 1990). Excess choline also prevented diethanolamine-induced inhibition of phosphatidylcholine synthesis and incorporation of diethanolamine into SHE cell phospholipids (Lehman-McKeeman & Gamsky, 2000).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The reproductive and developmental toxicity of diethanolamine tested has been

reviewed (Knaak *et al.*, 1997).

Diethanolamine was administered by gavage to Sprague-Dawley rats on days 6–15 of gestation at dose levels of 0, 50, 200, 500, 800 or 1200 mg/kg bw per day. The rats were killed on day 20 and the uteri examined for number of implantation sites and for live and dead implantations. Rats receiving 500 mg/kg bw or higher doses either died or were in a moribund condition and were killed. Maternal body weight gain was reduced in the 200-mg/kg bw group, but none of the gestational parameters in the treated groups was significantly different from those of the controls (Environmental Health Research & Testing, 1990; cited by Knaak *et al.*, 1997).

Diethanolamine was painted as an aqueous solution on the skin of CD rats on days 6–15 of gestation at dose levels of 0, 150, 500 and 1500 mg/kg bw per day. The two higher dose levels produced severe skin irritation. There was no effect of any treatments on fetal weight or on the incidence of external, visceral or skeletal abnormalities, but delayed ossification of the axial skeleton and distal appendages was observed in fetuses of the 1500-mg/kg bw group (Marty *et al.*, 1999).

Diethanolamine was applied as an aqueous solution to the skin of New Zealand White rabbits on days 6–18 of gestation at dose levels of 0, 35, 100 or 350 mg/kg bw per day. The highest dose level produced marked skin irritation. There was no effect of any treatments on development or on the incidence of external, visceral or skeletal abnormalities (Marty *et al.*, 1999).

In a 13-week subchronic study in male Fischer 344/N rats, testis and epididymis weights were decreased at diethanolamine doses of 1200 ppm or more in the drinking water (Melnick *et al.*, 1994a). Reduced sperm count and motility as well as degeneration of the seminiferous tubules were found at a dose of 2500 ppm.

Inhalation exposure of pregnant Wistar rats to 0.2 mg/m^3 diethanolamine aerosols for 6 h per day on days 6–15 of gestation caused an increased incidence of cervical ribs in the fetuses. No treatment-related malformations were observed (Gamer *et al.*, 1993, cited in Marty *et al.*, 1999).

4.4 Genetic and related effects

The genetic toxicity of diethanolamine has been reviewed by an expert panel for the cosmetic ingredient review (Beyer *et al.*, 1983) and by Knaak *et al.* (1997).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 8 for references)

Diethanolamine was not mutagenic to *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98 in three studies, or to *Escherichia coli* WP2 *uvrA*

Table 8. Genetic and related effects of diethanolamine

Test system	Result[a]		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	–	–	3333 μg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3333 μg/plate	National Toxicology Program (1999a)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	4000 μg/plate	Dean *et al.* (1985)
Escherichia coli WP2/WP2*uvr*A, reverse mutation	–	–	4000 μg/plate	Dean *et al.* (1985)
Saccharomyces cerevisiae JD1, mitotic gene conversion in stationary and log-phase cultures	–	–	5000	Dean *et al.* (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	330	National Toxicology Program (1999a)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	2176	Sorsa *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1500	National Toxicology Program (1999a)
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	3010	National Toxicology Program (1999a)
Chromosomal aberrations, rat liver RL cells *in vitro*	–	–	$0.5 \times GI_{50}$	Dean *et al.* (1985)
Cell transformation, Syrian hamster embryo cells (8-day treatment)	–	NT	500	Inoue *et al.* (1982)
Cell transformation, Syrian hamster embryo cells (24-h treatment)	+	NT	4500	Kerckaert *et al.* (1996)
Cell transformation, Syrian hamster embryo cells (7-day treatment)	+	NT	250	Kerckaert *et al.* (1996)
Cell transformation, Syrian hamster embryo cells (7-day treatment)	+[c]	NT	500	Lehman-McKeeman & Gamsky (2000)

Table 8 (contd)

Test system	Result[a]		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, newt larvae (*Pleurodeles waltl*) blood cells *in vivo*	–		75 ppm; 12 d	Fernandez *et al.* (1993)
Micronucleus formation, newt larvae (*Pleurodeles waltl*) blood cells *in vivo*[d]	–		75 ppm; 12 d	L'Haridon *et al.* (1993)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day
[c] Negative in the presence of 30 mM choline
[d] In the presence of sodium nitrite or nitrate at pH 8.6 and 5
GI_{50}, concentration causing 50% growth inhibition

in a single study, in the presence or absence of exogenous metabolic activation. It did not induce gene conversion in *Saccharomyces cerevisiae* strain JD1 in the presence or absence of exogenous metabolic activation. Exposure of the larvae of the newt *Pleurodeles waltl* to diethanolamine did not induce micronuclei in their blood cells and this result remained unaffected by changing the pH or by the addition of sodium nitrite or nitrate.

Diethanolamine did not induce mutations in mouse lymphoma L5178Y cells at the *Tk* locus in the presence or absence of exogenous metabolic activation in one study. It did not induce sister chromatid exchanges in Chinese hamster ovary cells in two studies with or without exogenous metabolic activation. A single study using cultured rat liver cells found no induction of chromosomal aberrations and one study in Chinese hamster ovary cells also found no induction of chromosomal aberrations in either the presence or absence of exogenous metabolic activation.

In one study of cell transformation in the Syrian hamster embryo clonal assay, diethanolamine had no effect after an eight-day treatment. A much larger study revealed induction of cell transformation at a similar dose after a seven-day treatment and at a much higher dose after a 24-h treatment with diethanolamine.

A further seven-day treatment cell transformation study demonstrated a positive dose-related response to diethanolamine up to 500 μg/mL that was abolished by co-administration with 30 mM choline.

4.5 Mechanistic considerations

In mice, diethanolamine alters choline homeostasis in a manner resembling choline deficiency. Stott *et al.* (2000) showed that diethanolamine induced choline deficiency and depleted several choline-containing compounds in B6C3F$_1$ mice, while Lehman-McKeeman & Gamsky (1999, 2000) found that diethanolamine inhibited the uptake of choline into mammalian cells.

It is known that deprivation of choline in the diet of rodents predisposes to the appearance of hepatocellular carcinomas (Zeisel, 1996). Diethanolamine-induced choline deficiency thus provides a mechanism for the tumorigenesis noted in mice but not in rats.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Diethanolamine is a viscous liquid widely used as a chemical intermediate and as a corrosion inhibitor and surface-active agent in various products including metal-working fluids, oils, fuels, paints, inks, cosmetic formulations and agricultural products. Occupational exposure may occur by inhalation and dermal contact, particularly

in metal-machining occupations. No data were available on environmental exposure to this substance. The general population may be exposed through contact with a variety of personal care products.

5.2 Human carcinogenicity data

Two cohort studies and two nested case–control studies looked at cancer mortality or incidence among workers using metalworking fluids with ethanolamines as additives, with or without sodium nitrite. Small excesses were observed for cancers at various sites, in particular the stomach, oesophagus and larynx. In most of these studies, only associations with use of soluble oils or synthetic fluids were presented and no results were given specifically in relation to diethanolamine exposure. It is difficult to draw conclusions regarding diethanolamine using data from studies of exposures to these complex mixtures.

5.3 Animal carcinogenicity data

Diethanolamine was tested for carcinogenicity by dermal application in one study in mice and in one study in rats. In the mouse study, there was a treatment-related increase in the incidences of both hepatocellular adenomas and carcinomas in both males and females, as well as an increase in the incidence of hepatoblastomas in males. There was also a marginal increase of renal tubule adenomas in males. In rats, no treatment-related increase in the incidence of tumours was seen in either males or females.

In a Tg.AC transgenic mouse model using similar doses to the first mouse study, there was no treatment-related increase in the incidence of skin tumours after skin application.

5.4 Other relevant data

Diethanolamine is metabolized by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) and incorporated into phospholipids. It is excreted predominantly unchanged with a half-life of approximately one week in urine. In the absence of sodium nitrite, no conversion to *N*-nitrosodiethanolamine is observed. Diethanolamine competitively inhibits the cellular uptake of choline *in vitro* and hepatic changes in choline homeostasis, consistent with choline deficiency, are observed *in vivo*.

No data on reproductive and developmental effects in humans were available.

Oral or dermal exposure of rats to diethanolamine during organogenesis was not associated with any sign of developmental toxicity, while inhalation exposure to diethanolamine aerosols caused signs of developmental toxicity. Dermal exposure of rabbits during organogenesis caused no sign of developmental toxicity.

Testicular effects have been found after exposure of rats to diethanolamine in the drinking water.

No data on genetic and related effects of diethanolamine in humans were available to the Working Group.

Diethanolamine induced cell transformation in Syrian hamster embryo cells *in vitro* in two studies but not in another. It did not induce gene mutations, sister chromatid exchanges or chromosomal aberrations. Diethanolamine did not induce micronucleus formation in larval newt blood cells in either the absence or presence of sodium nitrite or nitrate. It was without effect on gene conversion in yeast and was not mutagenic in bacteria.

The limited data available to the Working Group do not indicate that diethanolamine is genotoxic.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of diethanolamine.

There is *limited evidence* in experimental animals for the carcinogenicity of diethanolamine.

Overall evaluation

Diethanolamine is *not classifiable as to its carcinogenicity to humans (Group 3)*[1].

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[1] Dr Mirer dissociated himself from the conclusions of the Working Group.

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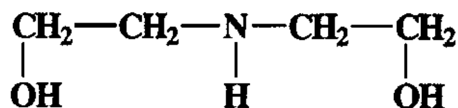
Appendix B: NTP (1999). Toxicology and Carcinogenesis Studies of Diethanolamine in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). TR 478. pp 5 - 60.

ABSTRACT



DIETHANOLAMINE

CAS No. 111-42-2

Chemical Formula: C H NO Molecular Weight: 105.14 4 11 2

Synonyms: Bis-2-hydroxyethylamine; DEA; diethylolamine; 2,2'-dihydroxydiethylamine; diolamine; 2,2'-iminobisethanol; iminodiethanol; 2,2'-iminodiethanol

Diethanolamine is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. Diethanolamine is also used in textile pro-cessing, in industrial gas purification to remove acid gases, as an anticorrosion agent in metalworking fluids, and in preparations of agricultural chemicals. Aqueous diethanolamine solutions are used as solvents for numerous drugs that are administered intravenously. Diethanolamine was selected for evaluation because its large-scale production and pattern of use indicate the potential for widespread human exposure. Male and female F344/N rats and B6C3F$_1$ mice received dermal applications of diethanolamine in 95% ethanol for 2 years. Genetic toxicology studies were performed in *Salmonella typhimurium*, L5178Y were performed in Salmonella typhimurium, L5178Y ovary cells, and B6C3F1 mouse peripheral blood erythrocytes.

RATS

Groups of 50 male rats were administered 0, 16, 32, or 64 mg diethanolamine/kg body weight in ethanol dermally for 2 years. Groups of 50 female rats were administered 0, 8, 16, or 32 mg/kg in ethanol dermally for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of vehicle control and dosed male and female rats was similar. Mean body weights of 64 mg/kg males were less than those of the vehicle controls beginning week 8, and mean body weights of females were generally similar to those of the vehicle control group. The only clinical finding attributed to diethanolamine administration was irritation of the skin at the site of application.

Pathology Findings

Minimal to mild nonneoplastic lesions occurred at the site of application in the epidermis of dosed male and female rats. The incidence of acanthosis in 64 mg/kg males, the incidences of hyperkeratosis in 32 and 64 mg/kg males and in all dosed female groups, and the incidences of exudate in 64 mg/kg males and in all dosed female groups were greater than those in the controls.

The incidences and severities of nephropathy were significantly increased in dosed female rats compared to the vehicle controls.

MICE

Groups of 50 male and 50 female mice were administered 0, 40, 80, or 160 mg diethanolamine/kg body weight in ethanol dermally for 2 years.

Survival, Body Weights, and Clinical Findings

Survival of dosed male groups was similar to that of the vehicle control group; survival of dosed female groups was significantly less than that of the vehicle control group. Mean body weights of 80 and 160 mg/kg males were less than those of the vehicle controls after weeks 88 and 77, respectively. Mean body weights of dosed groups of females were generally less than those of the vehicle controls during the second year of the study.

Pathology Findings

In male mice, the incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in all dosed groups and of hepatocellular carcinoma and hepatoblastoma in 80 and 160 mg/kg males were significantly increased compared to the vehicle controls. The incidences of hepatocellular neoplasms were significantly greater in dosed groups of female mice than in the vehicle control group. The incidences of hepatocellular neoplasms in all dosed groups of males and females exceeded the historical control ranges. Nonneoplastic hepatocyte changes were seen only in dosed male and female mice. Changes consisted of cytoplasmic alteration and syncytial alteration.

The incidences of renal tubule adenoma in males occurred with a positive trend; however, the incidences of carcinoma and hyperplasia did not follow this pattern. An extended evaluation of kidney step sections revealed additional adenomas and hyperplasias in all dosed groups. The combined analysis of single and step sections indicated a dose-related increase in the incidences of renal tubule hyperplasia and renal tubule adenoma or carcinoma (combined), and an increase in the incidences of renal tubule adenoma in male mice.

Incidences of thyroid gland follicular cell hyperplasia were increased in dosed male and female mice compared to vehicle controls.

Hyperkeratosis, acanthosis, and exudate were treatment-related changes in the skin at the site of application. The incidences of hyperkeratosis were significantly greater than those in the vehicle control groups in all dosed groups except 40 mg/kg females.

GENETIC TOXICOLOGY

Diethanolamine was not mutagenic in any of four strains of *Salmonella typhimurium*, in the presence or absence of S9 metabolic activation enzymes. No induction of trifluorothymidine resistance was observed in L5178Y mouse lymphoma cells treated with diethanolamine with or without S9. Diethanolamine did not induce significant sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9. Peripheral blood samples collected from male and female mice exposed to 80 to 1,250 mg/kg diethanolamine dermally for 13 weeks showed no increase in micronucleated normochromatic erythrocytes.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of diethanolamine in male F344/N rats administered 16, 32, or 64 mg/kg diethanolamine or in female F344/N rats administered 8, 16, or 32 mg/kg. There was *clear evidence of carcinogenic activity** of diethanolamine in male and female B6C3F$_1$ mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males.

Dermal administration of diethanolamine to rats was associated with increased incidences of acanthosis (males only), hyperkeratosis, and exudate of the skin and increased incidences and severities of nephropathy in females. Dermal administration of diethanolamine to mice was associated with increased incidences of cytoplasmic alteration (males only) and syncytial alteration of the liver, renal tubule hyperplasia (males only), thyroid gland follicular cell hyperplasia, and hyperkeratosis of the skin.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethanolamine

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in ethanol by dermal application	0, 16, 32, or 64 mg/kg	0, 8, 16, or 32 mg/kg	0, 40, 80, or 160 mg/kg	0, 40, 80, or 160 mg/kg
Body weights	64 mg/kg groups generally less than vehicle control groups	Dosed groups generally similar to vehicle control group	80 and 160 mg/kg groups less than vehicle control group	Dosed groups generally less than vehicle control group
Survival rates	14/50, 10/50, 21/50, 22/50	25/50, 29/50, 29/50, 24/50	40/50, 43/50, 34/50, 30/50	44/50, 33/50, 33/50, 23/50
Nonneoplastic effects	Skin: acanthosis (0/50, 2/50, 4/50, 10/50); hyperkeratosis (0/50, 3/50, 5/50, 11/50); exudate (0/50, 3/50, 2/50, 7/50)	Skin: hyperkeratosis (3/50, 13/50, 23/50, 23/50); exudate (1/50, 7/50, 7/50, 7/50) Kidney: nephropathy (40/50, 47/50, 48/50, 48/50); severity (1.2, 1.5, 1.9, 2.7)	Liver: cytoplasmic alteration (1/50, 17/50, 17/50, 12/50); syncytial alteration (0/50, 28/50, 38/50, 23/50) Kidney: renal tubule hyperplasia (standard and extended evaluation combined (3/50, 7/50, 7/50, 10/50) Thyroid gland: follicular cell hyperplasia (18/50, 22/49, 30/50, 42/50) Skin: hyperkeratosis (0/50, 13/50, 10/50, 17/50)	Liver: syncytial alteration (0/50, 2/50, 17/50, 18/50) Thyroid gland: follicular cell hyperplasia (18/50, 28/49, 32/50, 39/50) Skin: hyperkeratosis (1/50, 3/50, 8/50, 16/50)
Neoplastic effects	None	None	Liver: hepatocellular adenoma (31/50, 42/50, 49/50, 45/50); hepatocellular carcinoma (12/50, 17/50, 33/50, 34/50); hepatoblastoma (0/50, 2/50, 8/50, 5/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (39/50, 47/50, 50/50, 49/50) Kidney: adenoma (standard evaluation - 1/50, 4/50, 6/50, 6/50; standard and extended evaluation combined - 1/50, 6/50, 8/50, 7/50); adenoma or carcinoma (combined) (standard evaluation -3/50, 5/50, 6/50, 8/50; standard and extended evaluation combined - 3/50, 7/50, 8/50, 9/50)	Liver: hepatocellular adenoma (32/50, 50/50, 48/50, 48/50); hepatocellular carcinoma (5/50, 19/50, 38/50, 42/50); hepatocellular adenoma or carcinoma (33/50, 50/50, 50/50, 50/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Diethanolamine

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Level of evidence of carcinogenic activity	No evidence	No evidence	Clear evidence	Clear evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA98, TA100, TA1535, and TA1537
Mouse lymphoma gene mutations:	Negative
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on diethanolamine on 9 December 1997 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 9 December 1997, the draft Technical Report on the toxicology and carcinogenicity studies of diethanolamine received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of diethanolamine by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male and female F344/N rats and *clear evidence of carcinogenic activity* in male and female B6C3F_1 mice.

Dr. Goldsworthy, a principal reviewer, agreed with the proposed conclusions. He said that, because a majority of neoplasm responses observed in the companion studies of the fatty acid/diethanolamine condensates were concluded to result from the presence of free diethanolamine, some of his comments would also pertain to the condensates. Dr. Goldsworthy commented that the report should address if and how the distribution and metabolism of diethanolamine would be altered at various test concentrations and by the potential interactions with the different condensates. He said that, besides trying to link diethanolamine concentrations with neoplastic responses, it would be useful to chart comparative toxicities between the condensates and diethanolamine concentrations, as well as the potential for nitrosamine formation. Dr. Goldsworthy asked about the significance of the hepatoblastomas in treated male mice. Dr. J.R. Hailey, NIEHS, said that hepatoblastoma is a neoplasm with a fairly distinct morphology composed of primitive-appearing cells and appears to be part of the spectrum of the progression of liver neoplasms in the mouse; as such, with the higher background rate of liver neoplasms in mice, there is a concomitant increase in the incidence of hepatoblastoma.

Dr. Bailer, the second principal reviewer, agreed in principle with the proposed conclusions. He said the conclusions should be modified to note the significant negative trend in female rat mammary gland fibroadenomas and the increased survival experienced by rats administered diethanolamine. Dr. J.K. Haseman, NIEHS, said that a decrease in the incidence of mammary gland neoplasms is often associated with reduced body weight, although not in this case; therefore, more discussion might be merited. Dr. Bailer commented that the high liver neoplasm rates in control mice emphasize the importance of the concurrent controls in these studies, especially since the historical control database is so small for dermal studies using an ethanol vehicle.

Dr. Chatman, the third principal reviewer, did not agree with the conclusions for mice. She stated that diethanolamine is not a mutagen and is not metabolized to a reactive intermediate but can be converted to a carcinogenic nitrosamine. She felt that the potential for N-nitrosodiethanolamine formation should have been evaluated. Dr. Chatman referred to a letter received by the reviewers from the Alkanolamines Panel of the Chemical Manufacturers Association (CMA), which reported that rodent feed during some weeks of the studies was contaminated with high bacterial counts. She thought this could have enhanced N-nitroso-diethanolamine formation. Dr. Irwin responded that published studies with N-nitrosodiethanolamine given in drinking water show it to be a potent liver carcinogen in F344/N rats but a noncarcinogen in B6C3F_1 mice.

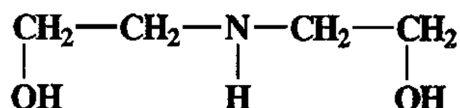
There were questions about the possible impact of *Helicobacter hepaticus* on the incidence of liver neoplasms in mice. Dr. Hailey said that in frozen tissues from about 20 animals, 10 males and 10 females, polymerase chain reaction analysis for *H. hepaticus* was negative. Dr. Goldsworthy asked for comment on the impact of increased liver neoplasm rates in control mice relative to interpretation of bioassay results. Dr. Hailey replied that, in view of higher background incidence, other components have to be assessed, especially progression to a malignant state and increases in numbers or multiplicity; both were

dramatically increased in these studies. Dr. Hecht agreed that formation of nitrosamines was not likely, but he was disappointed with the lack of detail in the analytical methods description so that contamination of diethanolamine with N-nitroso-diethanolamine could not be ruled out. Dr. Irwin said he would increase the detail in the analytical methods. Dr. G.N. Rao, NIEHS, stated that standards for the NIH-07 diet used since 1984 are much more stringent than those of most commercially available diets with regard to allowable bacterial counts.

Dr. W. Stott, Dow Chemical Company, representing the Alkanolamines Panel of the CMA, said that their major concerns with the study were questions about technical aspects of the bioassay and the inconsistency between the genotoxicity and carcinogenicity findings. Among technical questions which he thought should have been better discussed in the report were the choice of a dermal rather than an oral route of administration, the use of an ethanol vehicle, which has potential promo-tional/carcinogenic effects in itself, the potential for nitrosamine formation *in vivo*, and high liver neoplasm incidence in control mice. Dr. Stott reported that the Alkanolamines Panel plans to conduct mechanistic studies to help understand the NTP mouse bioassay results and their relevance to humans.

Dr. Goldsworthy moved that the Technical Report on diethanolamine be accepted with the revisions discussed and the conclusions as written for male and female rats, *no evidence of carcinogenic activity*, and for male and female mice, *clear evidence of carcinogenic activity*. Dr. Bailer seconded the motion, which was accepted with six yes votes to one no vote (Dr. Chatman) and one abstention (Dr. Bus).

INTRODUCTION



DIETHANOLAMINE

CAS No. 111-42-2
Chemical Formula: C H NO Molecular Weight: 105.14 4 11 2

Synonyms: Bis-2-hydroxyethylamine; DEA; diethylolamine; 2,2'-dihydroxydiethylamine; diolamine; 2,2'-iminobisethanol; iminodiethanol; 2,2'-iminodiethanol

CHEMICAL AND PHYSICAL PROPERTIES

Diethanolamine is a secondary amine in which two molecules of ethanol are linked through their beta carbons to a common nitrogen. It is a colorless or faintly colored crystalline solid at room temperature but melts at 28° C. It is soluble in water, alcohol, ethanol, and benzene but insoluble in most other organic solvents. In aqueous solutions, the pK of the a secondary amine is 8.88 at 25° C (Merck Index, 1989; *Hazardous Chemicals Desk Reference,* 1993).

PRODUCTION, USE, AND HUMAN EXPOSURE

Diethanolamine is produced by reacting two moles ethylene oxide with one mole of ammonia. In most production facilities, ethylene oxide and ammonia are are reacted in a bath process that yields a crude mixture of ethanolamine, diethanolamine, and triethanolamine. The mixture is then distilled to separate and purify the individual compounds (*Kirk-Othmer,* 1985). Diethanolamine is a high-production chemical; the worldwide production capacity for ethanolamines was estimated at 300,000 metric tons in 1992. United States production of ethanolamines was 447,727 metric tons in 1995; diethanolamine represented approximately one third of the total production (SRI, 1995).

There is potentially widespread occupational and consumer exposure to diethanolamine. It is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. Diethanolamine is also used in textile processing, in industrial gas purification to remove acid gases, as an anticorrosion agent of in metalworking fluids, and in preparations of agricultural chemicals. Aqueous diethanolamine solutions used as solvents for numerous drugs administered intravenously (*Merck Index,* 1989; *Hazardous Chemicals Desk Reference*, 1993). A review by Knaak *et al.* (1997) contains an excellent summary of additional uses of diethanolamine. The National Occupational Exposure Survey estimated that, during 1981 through 1983, 828,450 workers were occupationally exposed to diethanolamine (NIOSH, 1990).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Diethanolamine produces toxic responses after oral, dermal, or inhalation exposure; however, dermal exposure is the most important route of both occupational and consumer exposure. The percutaneous absorption of diethanolamine has been evaluated in F344/N rats and B6C3F$_1$ mice (NTP, 1991). Forty-eight hours after application of 2.1, 7.6, or 27.5 mg ^{14}C-labeled diethanolamine per kg body weight in 95% ethanol (total volume applied 25 μL) on a 2 cm^2 area of the intrascapular region of male F344/N rats, 2.9%, 10.5%, or 16.2%, respectively, of the applied dose had been absorbed. Based on diethanolamine recovered from tissues and excreta, 1.2%, 4.3%, or 4.5% was present in skin (washed to remove unabsorbed material) at the site of application. Male B6C3F$_1$ mice received a single dose of 81.1 mg/kg in 15 μL on a 1 cm^2 area of the intrascapular region; 48 hours after application, 58.1% of the dose was recovered in tissues or excreta of which 2.2% was present in skin at the site of application. In a separate study by Waechter, reported in Knaak *et al.* (1997), 1,500 mg/kg ^{14}C-labeled diethanolamine was applied to the intrascapular region of male F344 rats. Excluding skin from the site of application, 1.4% of the applied dose had been absorbed after 48 hours. Knaak *et al.* (1997) plotted absorption rates (μg/cm^2 per hour) as a function of applied dose determined from the NTP (1991) data and that determined from the study by Waechter and found that the rate of percutaneous absorption of diethanolamine increases linearly with the applied dose.

Diethanolamine was eliminated very slowly in the urine and feces of rats and mice following single intravenous, oral, or dermal administrations (Table 1; NTP 1991). Only trace amounts of radioactivity were detected in volatile metabolites (Matthews *et al.*, 1995).

TABLE 1
Elimination of ^{14}C-Diethanolamine in F344/N Rats and B6C3F$_1$ Mice[a]

Dose	Collection Period (hours)	Percentage[b] of Dose in:	
		Urine	Feces
Rats			
7.5 mg/kg intravenous	24	16.5 ± 1.9	0.35 ± 0.02
	48	28.3 ± 2.5	0.60 ± 0.03
7.9 mg/kg oral	24	9.0 ± 2.5	1.60 ± 0.20
	48	22.00 ± 1.80	2.42 ± 0.33
7.6 mg/kg dermal	48	16.2[c]	1.9[c]
Mice			
14.9 mg/kg intravenous	24	11.5 ± 7.0	1.6 ± 0.36
	48	25.5 ± 5.0	2.98 ± 0.86
81.1 mg/kg dermal	48	28.3[c]	4.4[c]

[a] These data are presented by NTP, 1991.
[b] Mean ± standard error
[c] Calculated from dermal absorption data

With repeated oral dosing, diethanolamine accumulated in tissues and eventually reached a steady state within 4 to 8 weeks (NTP, 1991). In F344/N rats administered ^{14}C-diethanolamine at daily oral doses of 7 mg/kg for 14 days followed by a 14-day washout period, elimination of the radiolabel in the urine and feces was consistent with an approximate half-life of 1 week (NTP, 1991).

Administration of ^{14}C-diethanolamine to F344/N rats by the intravenous, oral, or dermal routes led to similar tissue distribution; the greatest number of diethanolamine equivalents present 48 hours after a single dose were found in the liver and kidney (NTP, 1991; Matthews *et al.*, 1995). For the oral and dermal routes, lesser amounts of diethanolamine were also found in the brain and heart; with the intravenous route, lesser amounts were found in the spleen and lung. Extraction with phosphate-buffered saline and chloroform/methanol indicated that 87% to 89% of the radioactivity in the liver and brain partitioned into the aqueous phase, whereas 6% to 9% was removed by the organic extraction. Identification of the radioactive species present revealed that 70% to 80% of the radioactivity present in aqueous extracts of liver and brain was the parent compound, diethanolamine. Two minor metabolites, N-methyl-diethanolamine and N,N-dimethyl-diethanolamine, and several phosphorylated forms accounted for the remainder of the water-soluble radioactivity found in the liver. Diethanolamine-phosphate accounted for 95% of the phosphorylated metabolites present, and N,N-dimethyldiethanolamine-phosphate and N-methyldiethanolaminephosphate each constituted 2% of the phosphorylated metabolites extracted.

The chloroform/methanol extracts of liver contained two radioactive components associated with the phosphatidylethanolamine and phosphatidylcholine fractions. Upon incubation of the phosphatidylethanolamine fraction with phospholipase D, the radiolabel was liberated quantitatively as diethanolamine, whereas phospholipase D treatment of the phosphatidylcholine fraction yielded N-methyldiethanolamine (15%) and N,N-dimethyldiethanolamine (85%). Incubation of chloroform/methanol extracts from brain tissue with phospholipase D yielded only radioactive diethanolamine. Further analysis revealed that approximately 30% of diethanolamine-containing phospholipids in liver were ceramides and 70% were phosphoglycerides. These results indicated that diethanolamine was incorporated into phospholipid headgroups by the same pathway that led to the incorporation of ethanolamine, the natural substrate for these reactions (NTP, 1991; Matthews *et al.*, 1995).

Analysis of aqueous and organic extracts from the livers and brains of rats receiving daily oral doses of ^{14}C-diethanolamine for 8 weeks revealed that 97% of the radioactivity in the liver and 77% of the radioactivity in the brain were present in the aqueous extract and represented primarily free diethanolamine. Analysis of the organic extract from liver indicated that all radioactivity was associated with the phosphatidylcholine fraction and was present as N,N-dimethyldiethanolamine headgroups in ceramide derivatives. Analysis of the organic extract from brain revealed that all radioactivity was associated with the phosphatidylethanolamine fraction and that approximately 65% was present in ceramide derivatives (Matthews *et al.*, 1995). In human liver slices incubated with ^{14}C-diethanolamine, diethanolamine was readily incorporated into ceramide containing phospholipids in the form of phosphodiethanolamine headgroups, which were then slowly methylated (Matthews *et al.*, 1995).

TOXICITY

Experimental Animals

The acute toxicity of diethanolamine is summarized in Table 2.

The prechronic toxicology of diethanolamine has been evaluated in rats and mice in 13-week studies in which diethanolamine was administered by dermal application or in drinking water (Melnick *et al.*, 1994a,b). In the dermal study, solutions containing 0, 32, 63, 125, 250 or 500 mg/kg diethanolamine in 95% ethanol were applied to the shaved interscapular region of groups of 10 male and 10 female F344 rats 5 days per week for 13 weeks. One male rat and two female rats receiving 500 mg/kg died before the end of the study, and mean body weights of males that received 250 or 500 mg/kg and of females that received 125, 250, or 500 mg/kg were significantly less than those of the vehicle controls during the study.

TABLE 2
Acute Toxicity of Diethanolamine

Route	Species	LD_{50} (mg/kg)	Reference
Oral	rat	710; 1,820	HSDB, 1997
	rat, male	1,700 — 2,800	Knaak *et al.*, 1997
	rat, female	700 — 1,700	Knaak *et al.*, 1997
	mouse	3,300	Knaak *et al.*, 1997
Intraperitoneal	mouse	2,300	HSDB, 1997
Subcutaneous	mouse	3,553	HSDB, 1997
Dermal	rabbit	8,100 — 12,200	Knaak *et al.*, 1997

Hematology evaluations conducted at study termination revealed the presence of a normochromic, microcytic anemia in males and females, characterized by dose-dependent decreases in erythrocyte count, hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin, and reticulocyte counts in females but not males. In males, decreases were observed primarily at doses of 125 mg/kg and greater, whereas decreases were observed in all dosed groups of females.

The histopathologic lesions associated with dermal administration of diethanolamine to rats are shown in Table 3. Skin was the major site affected in dosed males, with hyperkeratosis and acanthosis apparent in groups that received 63 mg/kg and above. Skin was also affected in dosed females; however, incidences of nephropathy were increased in all dosed groups except the 500 mg/kg group, incidences of mineralization were increased in all dosed groups, and renal tubule epithelial necrosis was present in the 250 and 500 mg/kg female groups. Demyelination of the medulla occurred in males receiving 500 mg/kg and females receiving 250 or 500 mg/kg.

In the drinking water study, groups of 10 male F344/N rats received drinking water containing 0, 320, 630, 1,250, 2,500, or 5,000 ppm diethanolamine, and groups of 10 female F344/N rats received drinking water containing 0, 160, 320, 630, 1,250, or 2,500 ppm diethanolamine continuously for 13 weeks. Actual estimated daily intakes were 0, 25, 48, 97, 202, or 436 mg/kg (males) and 0, 14, 32, 57, 124, or 242 mg/kg (females). Two males exposed to 5,000 ppm died before the end of the study. Mean body weights of males exposed to 630 ppm or greater and females exposed to 320 ppm or greater were less than those of the controls during the study. Hematology evaluations conducted at study termination indicated the presence of a normochromic, microcytic anemia similar to that observed in the dermal study. Histopathologic lesions associated with exposure to diethanolamine included exposure-related increases in the severities of nephropathy and incidences of renal tubule mineralization in males and females, demyelination of the brain and spinal cord in females exposed to 1,250 or 2,500 ppm and males exposed 2,500 or 5,000 ppm, and degeneration of the seminiferous tubules in males exposed to 2,500 ppm or greater.

TABLE 3
Incidence of Selected Nonneoplastic Lesions in Rats in the 13-Week Dermal Study of Diethanolamine[a]

	Vehicle Control		32 mg/kg		63 mg/kg		125 mg/kg		250 mg/kg		500 mg/kg	
Male												
Kidney[b]	10		10		10		10		10		10	
Nephropathy[c]	9	(1.0)[d]	6	(1.0)	5	(1.0)	6	(1.0)	4	(1.0)	5	(1.0)
Renal Tubule Epithelial Necrosis	0		0		0		0		0		0	
Renal Tubule Mineralization	0		0		0		0		0		9**	(1.9)
Brain, Medulla	10		10		10		10		10		10	
Demyelination	0		0		0		0		0		10**	(1.0)
Skin	10		10		10		10		10		10	
Ulcer	0		0		0		0		3	(1.3)	10**	(2.6)
Chronic Active Inflammation	0		0		0		0		3	(1.3)	10**	(1.7)
Acanthosis	0		0		3	(1.0)	6**	(1.0)	6**	(1.5)	10**	(2.2)
Hyperkeratosis	0		0		5*	(1.0)	10**	(1.1)	10**	(1.4)	10**	(1.9)
Female												
Kidney	10		10		10		10		10		10	
Nephropathy	3	(1.0)	9**	(1.3)	10**	(1.4)	10**	(1.7)	7*	(1.1)	4	(1.0)
Renal Tubule Epithelial Necrosis	0		0		0		0		2	(1.0)	10**	(1.0)
Renal Tubule Mineralization	4	(1.0)	9*	(1.0)	10**	(1.6)	10**	(1.9)	10**	(1.1)	10**	(1.0)
Brain, Medulla	10		10		10		10		10		10	
Demyelination	0		0		0		0		7**	(1.0)	10**	(1.0)
Skin	10		10		10		10		10		10	
Ulcer	0		0		0		1	(1.0)	7**	(1.9)	10**	(3.4)
Chronic Active Inflammation	0		0		0		3	(1.0)	7**	(1.6)	10**	(2.5)
Acanthosis	0		0		1	(1.0)	6**	(1.2)	7**	(2.0)	10**	(2.6)
Hyperkeratosis	0		5*	(1.0)	6**	(1.0)	9**	(1.2)	10**	(1.7)	10**	(2.1)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] These data are presented by Melnick *et al.*, 1994a.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In companion studies (Melnick *et al.*, 1994b), groups of 10 male and 10 female B6C3F$_1$ mice received doses of 0, 80, 160, 320, 630, or 1,250 mg/kg by dermal application to the shaved interscapular region 5 days per week for 13 weeks. Two males and four females administered 1,250 mg/kg died before the end of the study. Final mean body weights of males receiving 1,250 mg/kg were slightly less than that of the vehicle controls, but final mean body weights of other dosed groups were similar to those of the

vehicle controls. Liver and kidney weights were significantly increased compared to the vehicle controls in groups of males administered 160 mg/kg or greater and females receiving 80 mg/kg or greater. Serum alanine aminotransferase and sorbitol dehydrogenase activities were significantly increased in males that received 630 or 1,250 mg/kg, and serum alanine aminotransferase activity was increased in females that received 1,250 mg/kg.

Histopathologic lesions associated with dermal administration of diethanolamine to mice are shown in Table 4 (Melnick *et al.*, 1994b). Acanthosis occurred at the site of application in all animals administered diethanolamine but was not observed in the vehicle controls. Cytologic alteration of the liver was observed in all groups of male mice administered diethanolamine and in females receiving 160 mg/kg or greater. Hepatocellular necrosis was also present in several dosed groups of males, especially groups receiving 320 mg/kg or greater. Renal tubule necrosis and cardiac degeneration were observed in males and females receiving 1,250 mg/kg.

In the mouse drinking water study, groups of 10 male and 10 female $B6C3F_1$ mice were given drinking water containing 0, 630, 1,250, 2,500, 5,000 or 10,000 ppm diethanolamine. Actual estimated daily intake was 0, 104, 178, 422, 807, or 1,674 mg/kg (males) and 0, 142, 347, 884, 1,154, or 1,128 mg/kg (females). All groups exposed to 5,000 or 10,000 ppm died before the end of the study; mean body weights of males and females exposed to 2,500 ppm were less than those of the controls during the study. Liver weights were significantly increased in groups of males and females exposed to 630, 1,250, or 2,500 ppm. Kidney weights were also increased in males exposed to 2,500 ppm. Histopathologic lesions observed in the drinking water study included exposure-related increases in the incidences and severities of cytologic alteration of the liver in males and females, the incidence of nephropathy in groups of males that survived to study termination, and cardiac degeneration in males and females exposed to 2,500 ppm or greater.

Humans

No references to human toxicity were found in a review of the current literature on diethanolamine.

CARCINOGENICITY

Experimental Animals

The carcinogenic potential of diethanolamine has not been previously evaluated; however, the carcinogenic potential of N-nitrosodiethanolamine has been examined in several studies. Lijinsky *et al.* (1980) administered N-nitrosodiethanolamine in drinking water at concentrations of 3,900, 7,800, 15,600, or 31,250 ppm to groups of 10 male and 10 female F344 rats, 5 days per week for 34 weeks. All exposed animals developed hepatocellular carcinomas, and the incidences of cholangiocarcinoma were increased in groups exposed to 7,800 ppm or greater. A number of studies (Preussmann *et al.*, 1981; Konishi *et al.*, 1987) demonstrated that N-nitrosodiethanolamine formed *in vivo* in rats coadministered diethanolamine and nitrite. Yamamoto *et al.* (1995) examined the ability of N-ni-trosodiethanolamine formed *in situ* to initiate hepatocytes *in vivo*. Groups of 11 male Wistar rats were exposed to feed containing 0.5% diethanolamine and drinking water containing 0.3% sodium nitrite. Control groups received either no exposure to diethanolamine, sodium nitrite only, or diethanolamine only. After 2 weeks, the animals underwent partial hepatectomy and were then maintained on the same diet for another week, after which they were exposed to diets containing 0.02% 2-acetylaminofluorene for 2 weeks, with a single injection of carbon tetrachloride administered between the first and second weeks. One week later the animals were killed and the number of γ-glutamyltranspeptidase-positive hepatic foci was determined to assay for initiation activity. The numbers of γ-glutamyltranspeptidase-positive foci per cm^2 in groups administered diethanolamine alone or nitrite alone were the same as in the control group. The number of positive foci per cm^2 was significantly increased in the group exposed to diethanolamine and nitrite, suggesting that N-nitrosodiethanolamine formed *in situ* could initiate rat liver.

Humans

No references to carcinogenicity in humans were found in a review of the current literature on diethanolamine.

TABLE 4
Incidence of Selected Nonneoplastic Lesions in Mice in the 13-Week Dermal Study of Diethanolamine[a]

	Vehicle Control	80 mg/kg	160 mg/kg	320 mg/kg	630 mg/kg	1,250 mg/kg
Male						
Liver[b]	10	10	10	10	10	10
Cytologic Alteration[c]	0	5* (1.0)[d]	10** (1.0)	10** (1.4)	10** (2.0)	10** (2.5)
Hepatocellular Necrosis	0	2 (1.0)	0	3 (1.3)	7** (1.1)	6** (2.0)
Kidney	10	0	0	0	10	10
Renal Tubule Epithelial Necrosis	0	0	0	0	0	4* (1.3)
Heart	10	0	0	0	10	10
Degeneration	0	0	0	0	0	1* (2.0)
Skin	10	10	10	10	10	10
Ulcer	0	0	0	0	2 (2.0)	10** (3.0)
Chronic Active Inflammation	0	0	0	0	5* (1.2)	10** (2.7)
Acanthosis	0	10** (1.0)	9** (1.0)	9** (1.1)	10** (2.6)	10** (2.9)
Hyperkeratosis	0	0	0	2 (1.5)	5* (1.8)	10** (2.0)
Female						
Liver	10	10	10	10	10	10
Cytologic Alteration	0	0	10** (1.0)	9** (1.1)	10** (1.3)	9** (1.3)
Kidney	10	0	0	0	10	10
Renal Tubule Epithelial Necrosis	0	0	0	0	0	1 (1.0)
Heart	10	0	10	0	10	10
Degeneration	0	0	0	0	0	8** (1.6)
Skin	10	10	10	10	10	10
Ulcer	0	0	0	0	1 (1.0)	9** (3.3)
Chronic Active Inflammation	0	0	0	1 (1.0)	1 (1.0)	9** (3.0)
Acanthosis	0	10** (1.0)	10** (1.0)	9** (1.0)	10** (1.3)	10** (2.9)
Hyperkeratosis	0	0	0	0	0	10** (2.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] These data are presented by Melnick *et al.*, 1994b.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Genetic Toxicity

Diethanolamine has been tested for mutagenicity in several short-term tests, and in general, the data indicate little evidence for activity. No mutagenic activity was noted in bacterial assays (Haworth *et al.*, 1983; Dean *et al.*, 1985) or in the yeast, *Saccharomyces cerevisiae* (Dean *et al.*, 1985). Diethanolamine did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells (Inoue *et al.*, 1982; Sorsa *et al.*, 1988; Loveday *et al.*, 1989). In addition, no cell transformation occurred in cultured Chinese hamster ovary cells treated with diethanolamine *in vitro* (Inoue *et al.*, 1982). Positive results were reported in an *in vitro* assay for induction of DNA single-strand breaks in freshly isolated hepatocytes from rats, hamsters, and pigs (Pool *et al.*, 1990).

Triethanolamine, a structural analogue of diethanolamine, was studied in several genetic toxicity tests and was found to be negative in bacterial mutagenicity assays (Inoue *et al.*, 1982; Dean *et al.*, 1985; Mortelmans *et al.*, 1986) and in the *Drosophila melanogaster* sex-linked recessive lethal mutation assay (Yoon *et al.*, 1985). It did not induce gene conversion in *S. cereviseae* (Dean *et al.*, 1985) or DNA damage in *Escherichia coli* (Inoue *et al.*, 1982). No induction of sister chromatid exchanges was noted in cultured Chinese hamster ovary cells treated with triethanolamine (Galloway *et al.*, 1987), and tests for induction of chromosomal aberrations in cultured rat liver cells (Dean *et al.*, 1985) and cultured Chinese hamster ovary cells (Inoue *et al.*, 1982; Galloway *et al.*, 1987) also gave negative results.

Study Rationale

Diethanolamine was selected for evaluation because its large-scale production and pattern of use indicate the potential for widespread human exposure. In addition, the toxicity and carcinogenic potential associated with long-term exposure had not been examined. Based on the pattern of occupational and consumer exposure, dermal administration was considered the most appropriate route for the 2-year study.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Diethanolamine

Diethanolamine was obtained from Kodak Laboratory and Specialty Chemicals (Rochester, NY) in one lot (A16) which was used during the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix F). Reports on analyses performed in support of the diethanolamine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless, viscous liquid, was identified as diethanolamine by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy and by boiling point and density. The purity of lot A16 was determined by elemental analyses, Karl Fischer water analysis, functional group titration, thin-layer chromatography, and gas chromatography. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for diethanolamine. Karl Fischer water analysis indicated 0.10% $\pm$ 0.02% water. Functional group titration indicated a purity of 100.1% $\pm$ 0.7%. Analysis by thin-layer chromatography indicated a major spot and one trace impurity by one system and a major spot and two trace impurities by a second system. Gas chromatography by two systems indicated no impurities equal to or greater than 0.1% relative to the major peak. The overall purity of lot A16 was determined to be greater than 99%.

An accelerated stability study was performed by the analytical chemistry laboratory using gas chromatography. This study indicated that diethanolamine was stable as a bulk chemical for 2 weeks when protected from light and stored at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in sealed amber glass containers in a metal drum, at room temperature. Stability was monitored by the study laboratory using gas chromatography. No degradation of the bulk chemical was detected.

Ethanol

Ethanol (95%) was obtained from Aaper Alcohol and Chemical Company (Shelbyville, KY). The purity was monitored by the study laboratory throughout the study by gas chromatography. United States Pharmacopeia ethanol reference standard samples were analyzed concomitantly. Purity of the bulk ethanol ranged from 98.7% to 101.3% that of the reference standard during the studies. No volatile impurities were detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared every 3 weeks by mixing diethanolamine with 95% ethanol to give the desired concentration (Table F1). The dose formulations were stored at room temperature, protected from light, in amber glass bottles for up to 28 days.

Stability studies of a 0.5 mg/mL formulation were performed by the analytical chemistry laboratory using gas chromatography. The formulation had only small losses of diethanolamine ($<5\%$) when stored at room temperature, protected from light, for up to 28 days; it was stable for 3 hours when stored open to air and light.

Periodic analyses of the dose formulations of diethanolamine were conducted at the study laboratory using gas chromatography. Dose formulations were analyzed approximately every 9 weeks. All dose formulations and animal room samples for rats and mice were within 10% of the target concentrations.

2-YEAR STUDIES

Study Design

Groups of 50 male rats were administered dermal doses of 0, 16, 32, or 64 mg diethanolamine per kilogram body weight by the application of 0, 27.5, 55, or 110 mg diethanolamine/mL ethanol solutions, 5 days per week for 103 weeks. Groups of 50 female rats were administered dermal doses of 0, 8, 16, or 32 mg/kg by the application of 0, 13.8, 27.5, or 55 mg/mL solutions, 5 days per week for 103 weeks. Groups of 50 male and 50 female mice were administered dermal doses of 0, 40, 80, or 160 mg/kg by the application of 0, 22.5, 45, or 90 mg/mL solutions, 5 days per week for 103 weeks. Dose volumes were adjusted to provide the appropriate mg/kg dose based on group mean body weights.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) for use in the 2-year studies. Animals were quarantined for 11 days (rats) or 13 days (mice) before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix H).

Animal Maintenance

Rats and mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated twice weekly. Further details of animal maintenance are given in Table 5. Information on feed composition and contaminants is provided in Appendix G.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly; body weights were recorded weekly for the first 13 weeks and monthly thereafter.

A complete necropsy and microscopic examination were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Step sections were made from the residual kidney wet tissue of male mice because of a slightly increased trend of proliferative lesions in the standard evaluation. Eight additional kidney sections taken at 1 mm intervals were prepared for each male and female. Tissues examined microscopically are listed in Table 5.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. 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 evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the skin (overall) from the site of application for all rats and mice, the kidney for control and 32 mg/kg female rats, and the kidney (males), liver, and thyroid gland for mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory

pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 5
Experimental Design and Materials and Methods in the 2-Year Dermal Studies of Diethanolamine

Study Laboratory
Battelle Columbus Laboratories (Columbus, OH)

Strain and Species
Rats: F344/N
Mice: $B6C3F_1$

Animal Source
Taconic Farms (Germantown, NY)

Time Held Before Studies
Rats: 11 days
Mice: 13 days

Average Age When Studies Began
6 weeks

Date of First Dose
Rats: 8 October 1990
Mice: 22 October 1990

Duration of Dosing
5 doses per week for 103 weeks

Date of Last Dose
Rats: 25 September 1992
Mice: 9 October 1992

Necropsy Dates
Rats: 5-7 October 1992
Mice: 19-23 October 1992

Average Age at Necropsy
111 weeks

Size of Study Groups
50 males and 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
1

Method of Animal Identification
Tail tattoo

TABLE 5
Experimental Design and Materials and Methods in the 2-Year Dermal Studies of Diethanolamine

Diet
NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly

Water
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

Cages
Polycarbonate (Lab Products Inc., Maywood, NJ), changed weekly

Bedding
Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly

Cage Filters
DuPont 2024 spun-bonded polyester filters (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks

Racks
Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks

Animal Room Environment
Temperature: 17.8°-25.6° C (rats)
20.6°-25.0° C (mice)
Relative humidity: 36%-69% (rats)
35%-69% (mice)
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Doses
Rats: 0, 16, 32, or 64 mg/kg (0, 27.5, 55, or 110 mg/mL; males); and 0, 8, 16, or 32 mg/kg (0, 13.8, 27.5, or 55 mg/mL; females) administered in 95% ethanol; dose volumes were adjusted to provide the appropriate mg/kg dose based on group mean body weights
Mice: 0, 40, 80, or 160 mg/kg (0, 22.5, 45, or 90 mg/mL) administered in 95% ethanol; dose volumes were adjusted to provide the appropriate mg/kg dose based on group mean body weights

Type and Frequency of Observation
Observed twice daily; animals were weighed weekly through week 13 and monthly thereafter; clinical findings were recorded monthly.

Method of Sacrifice
CO_2 anesthetization

Necropsy
Necropsy was performed on all animals.

Histopathology
Complete histopathologic examinations were performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (except male mice), nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B5, C1, C5, D1, and D5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of lesion incidence, and reported P values are one sided. Values of P greater than 0.5 are presented as 1-P with the letter N added to indicate a lower incidence or negative trend in neoplasm occurrence relative to the control group (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for

evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

Quality Assurance Methods

The 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of diethanolamine was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, mutations in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and increases in the frequency of micronucleated normochromatic erythrocytes in peripheral blood of mice. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of diethanolamine are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the molecular structure and the effects of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemical-induced DNA damage and to predict carcinogenicity in animals, based on the electrophilicity theory of chemical mutagenesis and the somatic mutation theory of cancer (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone.

The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests appears to be less than the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). Positive responses in long-term peripheral blood micronucleus tests have not been formally evaluated for their predictivity for rodent carcinogenicity. But, because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 1). Survival of dosed male and female rats was similar to that of the vehicle control groups.

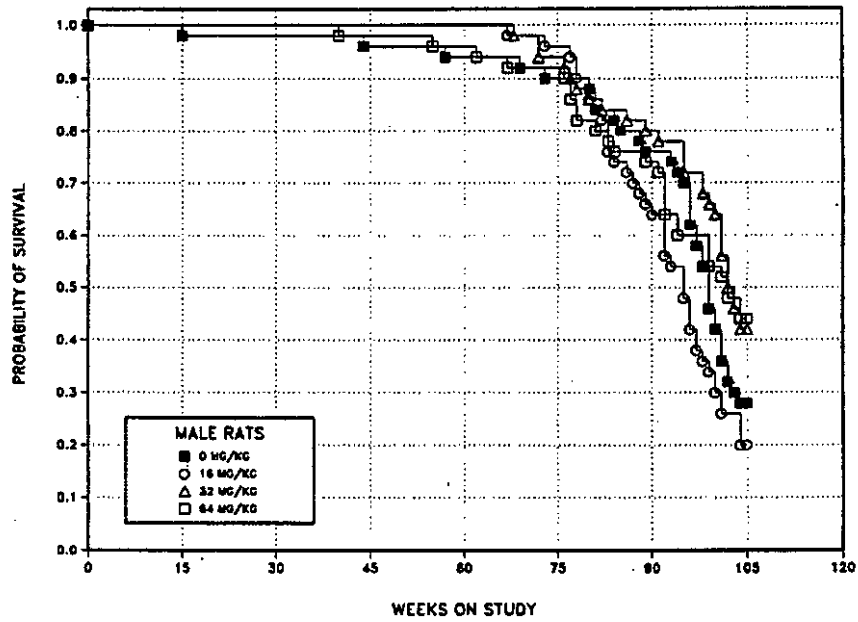
Body Weights and Clinical Findings

Mean body weights of 64 mg/kg males were less than those of the vehicle controls from week 8 to week 89. Mean body weights of 32 mg/kg females were less than those of the vehicle control group after week 97 (Tables 7 and 8 and Figure 2). The only clinical finding attributed to diethanolamine administration was irritation of the skin at the site of application. This effect was dose related (males: vehicle control, 0/50; 16 mg/kg, 1/50; 32 mg/kg, 0/50; 64 mg/kg, 4/50; females: vehicle control, 2/50; 8 mg/kg, 2/50; 16 mg/kg, 2/50; 32 mg/kg, 8/50).

TABLE 6
Survival of Rats in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	16 mg/kg	32 mg/kg	64 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	31	31	25	22
Natural deaths	5	9	4	6
Animals surviving to study termination	14	10	21	22
Percent probability of survival at end of study[a]	28	20	42	44
Mean survival (days)[b]	651	648	678	655
Survival analysis[c]	P=0.066N	P=0.204	P=0.125N	P=0.279N
	Vehicle Control	**8 mg/kg**	**16 mg/kg**	**32 mg/kg**
Female				
Animals initially in study	50	50	50	50
Moribund	11	16	12	13
Natural deaths	14	5	9	13
Animals surviving to study termination	25	29	29	24
Percent probability of survival at end of study	50	58	58	48
Mean survival (days)	669	689	679	665
Survival analysis	P=0.709	P=0.337N	P=0.448N	P=0.982

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.



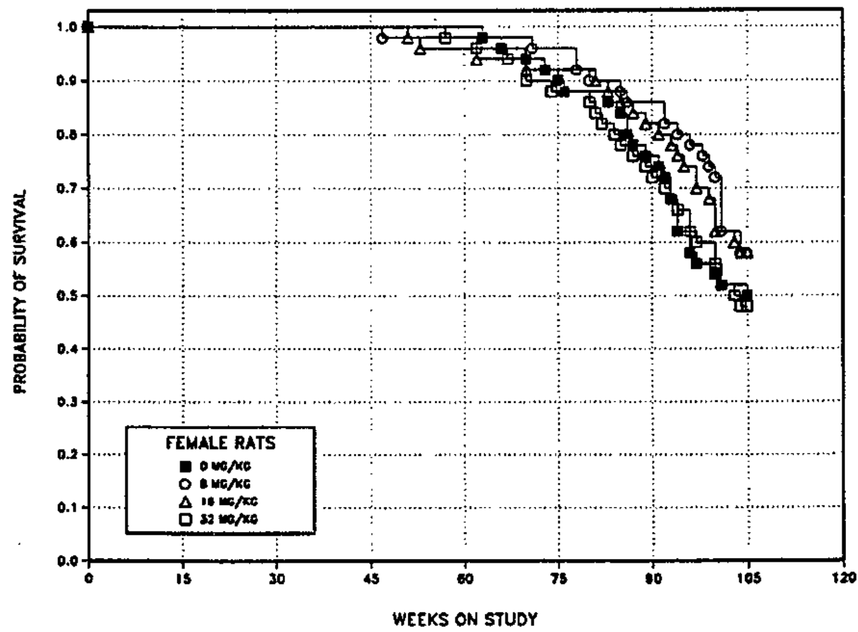


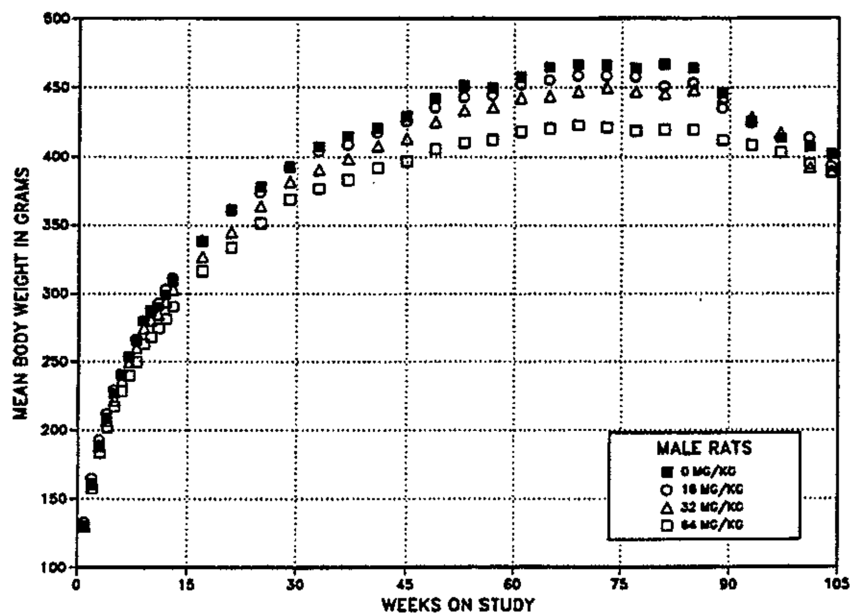
FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered Diethanolamine Dermally for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study of Diethanolamine

Weeks	Vehicle Control		16 mg/kg			32 mg/kg			64 mg/kg		
on Study	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	131	50	133	102	50	130	100	50	130	99	50
2	161	50	165	102	50	161	100	50	158	98	50
3	189	50	193	102	50	188	100	50	183	97	50
4	209	50	212	102	50	207	99	50	202	97	50
5	227	50	229	101	50	222	98	50	218	96	50
6	241	50	240	100	50	236	98	50	229	95	50
7	253	50	253	100	50	250	99	50	240	95	50
8	265	50	267	101	50	260	98	50	250	94	50
9	280	50	280	100	50	274	98	50	263	94	50
10	287	50	287	100	50	280	97	50	268	93	50
11	291	50	293	101	50	285	98	50	275	95	50
12	299	50	303	101	50	294	98	50	281	94	50
13	309	50	312	101	50	303	98	50	291	94	50
17	338	49	339	100	50	327	97	50	317	94	50
21	362	49	360	100	50	345	96	50	334	92	50
25	378	49	374	99	50	364	96	50	352	93	50
29	393	49	394	100	50	382	97	50	369	94	50
33	407	49	404	99	50	391	96	50	377	93	50
37	415	49	409	99	50	399	96	50	384	92	50
41	421	49	418	99	50	408	97	50	392	93	49
45	430	48	426	99	50	413	96	50	397	93	49
49	442	48	436	99	50	426	96	50	406	92	49
53	451	48	443	98	50	434	96	50	411	91	49
57	450	48	445	99	50	436	97	50	413	92	48
61	457	47	452	99	50	443	97	50	418	92	48
65	465	47	456	98	50	444	96	50	421	91	47
69	467	47	459	98	49	447	96	49	423	91	46
73	466	46	459	98	49	450	97	47	422	91	46
77	464	45	458	99	48	447	96	46	419	90	44
81	467	43	451	97	43	446	95	43	420	90	40
85	464	41	453	98	37	448	97	42	420	91	38
89	446	39	436	98	34	443	99	40	412	93	38
93	426	37	425	100	28	429	101	39	409	96	32
97	414	31	414	100	21	418	101	36	403	98	30
101	408	21	414	102	15	392	96	32	395	97	26
104	402	15	393	98	13	390	97	23	389	97	23
Mean for weeks											
1-13	242		244	101		238	98		230	95	
14-52	398		396	99		384	96		370	93	
53-104	446		440	99		433	97		413	93	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study of Diethanolamine

Weeks on Study	Vehicle Control		8 mg/kg			16 mg/kg			32 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	105	50	105	100	50	106	101	50	105	101	50
2	123	50	123	100	50	123	100	50	124	100	50
3	135	50	137	101	50	134	99	50	136	100	50
4	144	50	144	100	50	142	99	50	143	99	50
5	150	50	149	100	50	148	99	50	147	98	50
6	156	50	155	99	50	154	99	50	152	98	50
7	160	50	159	99	50	158	98	50	156	97	50
8	165	50	165	100	50	163	99	50	161	98	50
9	168	50	168	100	50	165	98	50	164	98	50
10	172	50	171	99	50	168	97	50	167	97	50
11	173	50	173	100	50	170	98	50	168	97	50
12	175	50	174	99	50	172	98	50	170	97	50
13	178	50	177	100	50	175	98	50	174	98	50
17	190	50	189	99	50	187	99	50	185	97	50
21	196	50	195	99	50	191	97	50	190	97	50
25	204	50	204	100	50	200	98	50	198	97	50
29	212	50	214	101	50	209	98	50	208	98	50
33	217	50	219	101	50	214	99	50	213	98	50
37	223	50	224	100	50	219	99	50	217	98	50
41	230	50	232	101	50	226	99	50	226	99	50
45	235	50	239	102	50	232	99	50	234	99	50
49	247	50	251	101	49	244	99	50	245	99	50
53	256	50	258	101	49	254	99	48	254	99	50
57	261	50	263	101	49	258	99	48	255	98	50
61	270	50	272	101	49	267	99	48	267	99	49
65	277	49	280	101	49	276	100	47	274	99	48
69	282	48	283	101	49	280	99	47	279	99	47
73	281	47	287	102	48	283	101	46	283	101	45
77	285	44	289	101	48	284	99	46	282	99	44
81	291	44	293	101	45	287	99	45	280	96	43
85	292	42	293	100	44	290	99	44	281	96	39
89	291	39	296	102	43	289	99	42	282	97	38
93	292	36	296	102	41	289	99	40	282	97	34
97	300	29	294	98	39	292	97	37	278	93	31
101	306	26	291	95	35	297	97	31	266	87	28
104	301	26	293	97	30	292	97	30	257	85	25
Mean for weeks											
1-13	154		154	100		152	99		151	98	
14-52	217		219	101		214	99		213	98	
53-104	285		285	100		281	99		273	96	



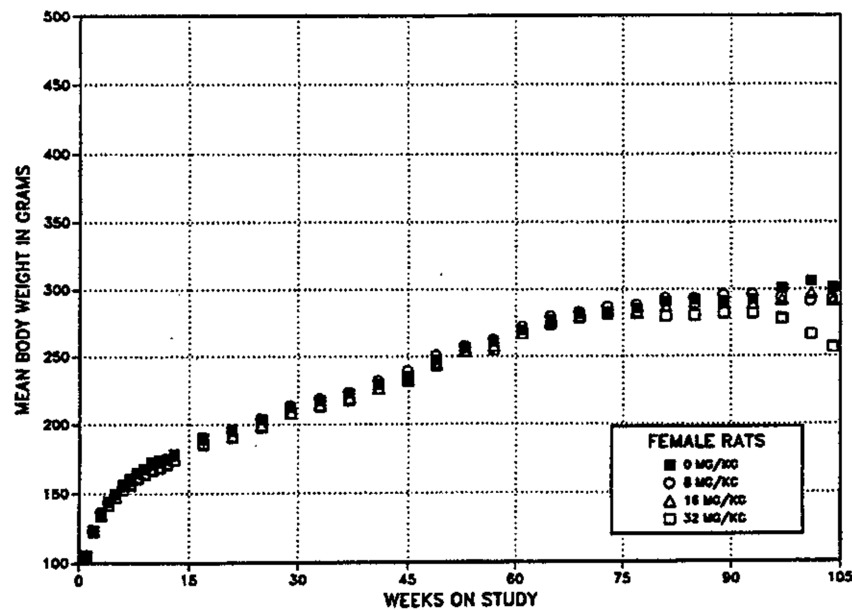


FIGURE 2
Growth Curves for Male and Female Rats Administered Diethanolamine Dermally for 2 Years

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the skin, kidney, liver, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Skin: No biologically significant incidences of skin or subcutaneous neoplasms occurred in the dosed groups. Minimal to mild nonneoplastic lesions occurred at the site of application in the epidermis of dosed male and female rats. One of the most common effects was thickening of the epidermis, or acanthosis. In 64 mg/kg males, the incidence of acanthosis was significantly greater than that in the vehicle control group (Tables 9 and A4). Hyperkeratosis, consisting of an increased amount of keratin on the surface of the skin, was more common in treated females than in males. This lesion was of minimal severity. The incidences of hyperkeratosis in the 32 and 64 mg/kg male groups and in all dosed female groups significantly exceeded those in the vehicle control groups (Tables 9, A4, and B5). Exudate, consisting of focal accumulations of serum and cellular debris on the epidermal surface, occurred at significantly increased incidences in 64 mg/kg males and in all dosed female groups.

TABLE 9
Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Rats in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	16 mg/kg	32 mg/kg	64 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Dermis, Ulcer[a]	0	0	0	2 (1.0)[b]
Epidermis, Acanthosis	0	2 (1.0)	4 (1.0)	10** (1.1)
Epidermis, Exudate	0	3 (1.0)	2 (1.0)	7* (1.0)
Epidermis, Hyperkeratosis	0	3 (1.0)	5* (1.0)	11** (1.0)
	Vehicle Control	**8 mg/kg**	**16 mg/kg**	**32 mg/kg**
Female				
Number Examined Microscopically	50	50	50	50
Dermis, Ulcer	2 (2.0)	1 (1.0)	1 (3.0)	1 (3.0)
Epidermis, Acanthosis	1 (2.0)	1 (1.0)	4 (1.0)	6 (1.2)
Epidermis, Exudate	1 (1.0)	7* (1.0)	7* (1.0)	7* (1.0)
Epidermis, Hyperkeratosis	3 (1.0)	13* (1.0)	23** (1.0)	23** (1.0)

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
a Number of animals with lesion
b Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Kidney: The incidences (vehicle control, 40/50; 8 mg/kg, 47/50; 16 mg/kg, 48/50; 32 mg/kg, 48/50; Table B1) and severities (1.2, 1.5, 1.9, 2.7) of nephropathy in dosed female groups were significantly greater than those in the vehicle controls; however, neither the incidences nor the severities of nephropathy in dosed male rats were significantly different from those in the vehicle controls. Minimal nephropathy consisted of a few scattered tubules with small, basophilic epithelial cells. More severe nephropathy included interstitial fibrosis and loss of nephrons. The severity grades were based on extent of kidney involvement as well as the amount of fibrosis and tubule/nephron loss. One 64 mg/kg male rat had a single renal tubule carcinoma (Table A1).

Liver: There was no neoplastic response in the liver associated with diethanolamine exposure (Tables A1 and B1). The incidences of basophilic foci were significantly decreased in all dosed groups of males and females (males: vehicle control, 15/50; 16 mg/kg, 5/50; 32 mg/kg, 1/50; 64 mg/kg, 2/50; females: 40/50, 31/50, 20/50, 7/50; Tables A4 and B5). The incidences of eosinophilic foci in dosed males were marginally less than that in the vehicle controls (4/50, 2/50, 2/50, 2/50), and the incidences of mixed cell foci in dosed females were somewhat variable (0/50, 3/50, 6/50, 1/50).

Mammary Gland: The incidence of fibroadenoma in 32 mg/kg females was significantly decreased compared to the vehicle control incidence (14/50, 8/50, 9/49, 5/50; Table B3). Incidences of fibroadenoma occurred with a negative trend. The incidences in all dosed groups were less than the historical control range (Table B4).

MICE

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 10 and in the Kaplan-Meier survival curves (Figure 3). Survival was similar in dosed male groups and vehicle controls. Survival of dosed female groups was significantly lower than that of the vehicle control group and decreased significantly with increasing dose.

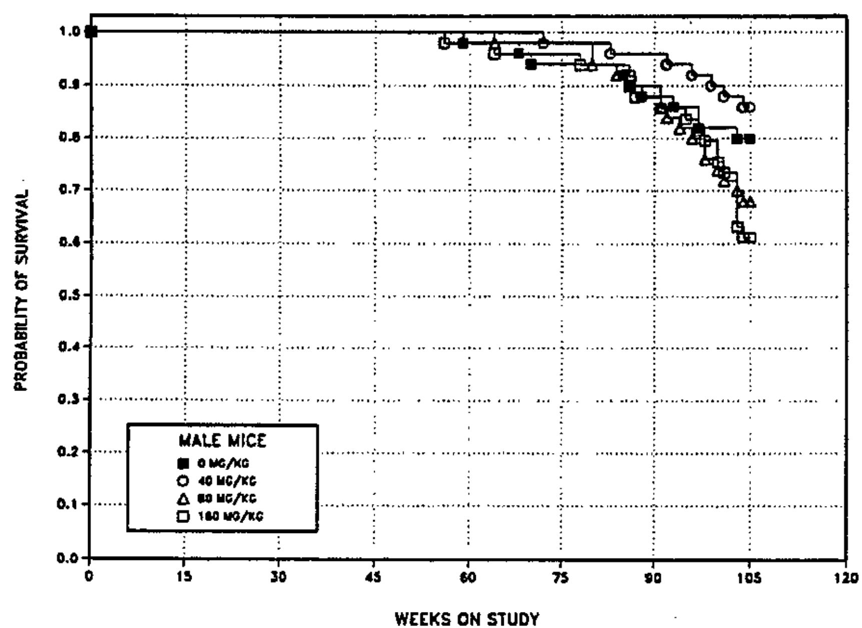
Body Weights

Mean body weights of 80 and 160 mg/kg males were less than those of the vehicle controls after weeks 88 and 77, respectively (Figure 4 and Table 11). Mean body weights of 40 and 80 mg/kg females were less than those of vehicle controls after week 73; mean body weights of 160 mg/kg females were less than those of the vehicle controls after week 53 (Figure 4 and Table 12).

TABLE 10
Survival of Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	3	1	6	10
Natural deaths	7	6	10	9
Animals surviving to study termination	40	43[e]	34	30
Percent probability of survival at end of study[b]	80	86	68	61
Mean survival (days)[c]	701	717	699	687
Survival analysis[d]	P=0.015	P=0.539N	P=0.289	P=0.097
Female				
Animals initially in study	50	50	50	50
Moribund	4	8	9	13
Natural deaths	2	9	8	14
Terminal sacrifice	44	33	33	23[f]
Percent probability of survival at end of study	88	66	66	46
Mean survival (days)	720	669	695	691
Survival analysis	P<0.001	P=0.012	P=0.016	P<0.001

a Censored from survival analyses
b Kaplan-Meier determinations
c Mean of all deaths (uncensored, censored, and terminal sacrifice)
d The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by N.
e Includes two animals that died during the last week of the study
f Includes one animal that died during the last week of the study



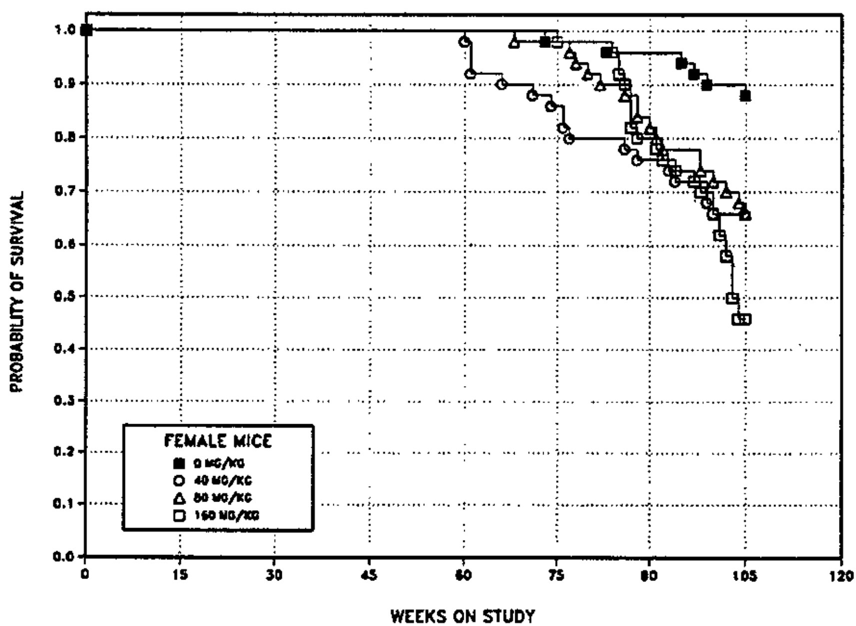
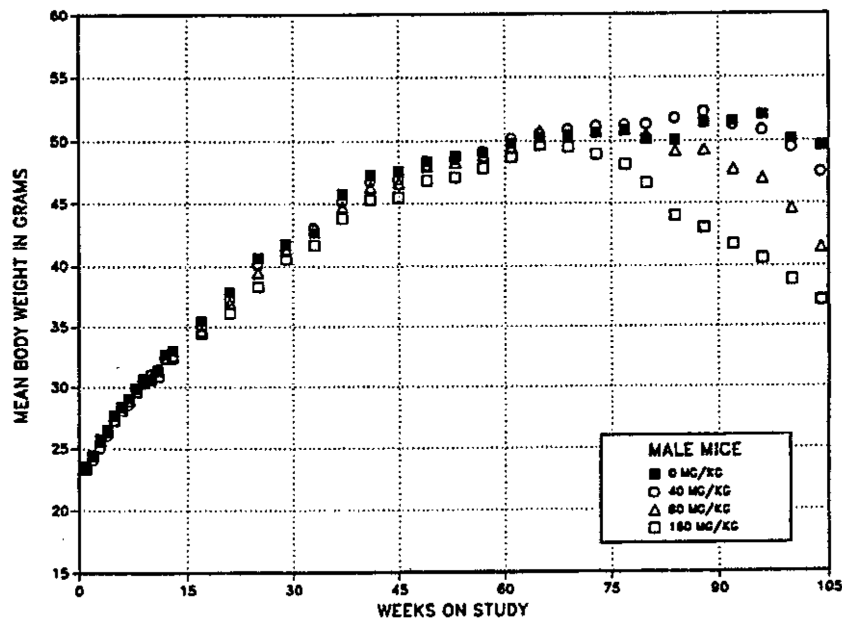


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered Diethanolamine Dermally for 2 Years



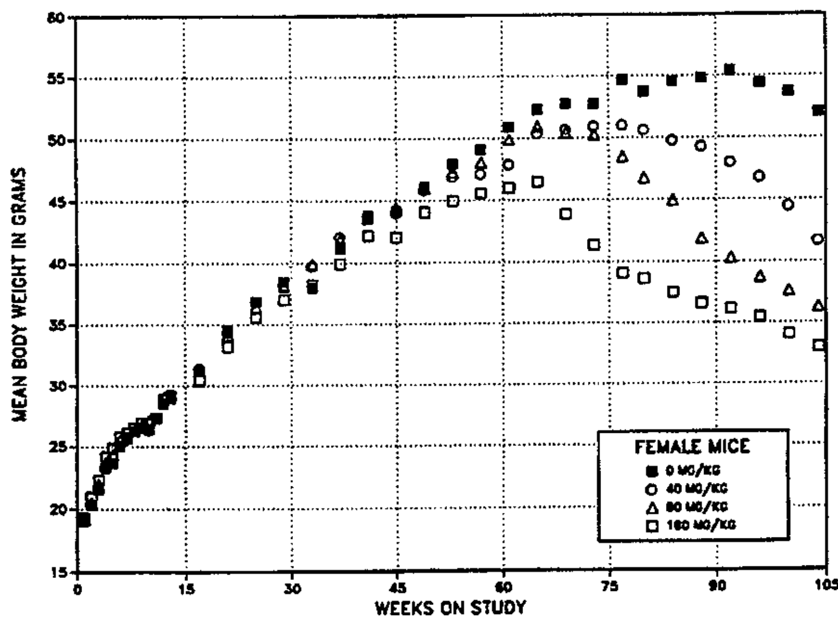


FIGURE 4
Growth Curves for Male and Female Mice Administered Diethanolamine Dermally for 2 Years

TABLE 11
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study of Diethanolamine

Weeks on Study	Vehicle Control Av. Wt. (g)	Vehicle Control No. of Survivors	40 mg/kg Av. Wt. (g)	40 mg/kg Wt. (% of controls)	40 mg/kg No. of Survivors	80 mg/kg Av. Wt. (g)	80 mg/kg Wt. (% of controls)	80 mg/kg No. of Survivors	160 mg/kg Av. Wt. (g)	160 mg/kg Wt. (% of controls)	160 mg/kg No. of Survivors
1	23.6	50	23.4	99	50	23.5	100	50	23.3	99	50
2	24.4	50	24.1	99	50	24.4	100	50	24.4	100	50
3	25.8	50	25.1	97	50	25.6	99	50	25.7	100	50
4	26.4	50	26.1	99	50	26.4	100	50	26.5	100	50
5	27.7	50	27.3	99	50	27.3	99	50	27.3	99	50
6	28.4	50	28.3	100	50	28.3	100	50	28.1	99	50
7	29.0	50	28.5	98	50	28.9	100	50	28.9	100	50
8	29.9	50	29.9	100	50	29.6	99	50	29.7	99	50
9	30.4	50	30.5	100	50	30.4	100	50	30.6	101	50
10	30.6	50	31.1	102	50	30.8	101	50	30.7	100	50
11	31.3	50	31.5	101	50	31.4	100	50	30.9	99	50
12	32.7	50	32.4	99	50	32.5	99	50	32.4	99	50
13	33.0	50	32.5	99	50	32.6	99	50	32.4	98	50
17	35.5	50	35.1	99	50	34.6	98	50	34.4	97	50
21	37.9	50	37.3	98	50	37.0	98	50	36.2	96	50
25	40.7	50	40.1	99	50	39.5	97	50	38.3	94	50
29	41.7	50	41.3	99	50	41.3	99	50	40.6	97	50
33	42.7	50	43.1	101	50	43.0	101	50	41.7	98	50
37	45.8	50	45.2	99	50	44.8	98	50	43.9	96	49
41	47.2	50	46.8	99	50	46.3	98	50	45.3	96	49
45	47.5	50	47.0	99	50	46.7	98	50	45.5	96	49
49	48.4	50	48.0	99	50	48.0	99	50	46.9	97	49
53	48.8	50	48.6	100	50	48.3	99	50	47.1	97	49
57	49.0	50	49.1	100	50	48.8	100	50	47.8	98	48
61	49.8	49	50.2	101	50	49.5	99	50	48.7	98	48
65	50.3	49	50.6	101	50	50.8	101	49	49.7	99	47
69	50.5	48	51.0	101	50	50.3	100	49	49.5	98	47
73	50.7	47	51.2	101	49	50.7	100	49	49.0	97	47
77	50.8	47	51.3	101	49	50.9	100	49	48.1	95	47
80	50.2	47	51.4	102	49	50.5	101	48	46.6	93	46
84	50.1	47	51.8	103	48	49.2	98	46	44.0	88	46
88	51.5	44	52.3	102	48	49.3	96	45	43.1	84	43
92	51.6	44	51.3	99	48	47.7	92	43	41.7	81	42
96	52.1	43	50.9	98	47	47.0	90	40	40.6	78	41
100	50.2	41	49.5	99	45	44.6	89	38	38.9	78	38
104	49.6	40	47.5	96	43	41.5	84	35	37.2	75	30
Mean for weeks											
1-13	28.7		28.5	99		28.6	100		28.5	99	
14-52	43.0		42.7	99		42.4	99		41.4	96	
53-104	50.4		50.5	100		48.5	96		45.1	89	

TABLE 12
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study of Diethanolamine

Weeks on Study	Vehicle Control		40 mg/kg			80 mg/kg			160 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.0	50	19.2	101	50	19.2	101	50	19.3	102	50
2	20.4	50	20.4	100	50	20.5	101	50	21.0	103	50
3	21.6	50	21.8	101	50	21.9	101	50	22.3	103	50
4	23.3	50	23.3	100	50	23.7	102	50	24.2	104	50
5	23.7	50	24.1	102	50	24.4	103	50	24.8	105	50
6	25.1	50	25.3	101	50	25.5	102	50	25.8	103	50
7	25.7	50	25.6	100	50	25.7	100	50	26.1	102	50
8	26.2	50	26.2	100	50	26.3	100	50	26.5	101	50
9	26.5	50	26.6	100	50	26.7	101	50	26.9	102	50
10	26.4	50	26.3	100	50	27.1	103	50	26.9	102	50
11	27.2	50	27.3	100	50	27.4	101	50	27.4	101	50
12	28.5	50	28.6	100	50	29.0	102	50	28.9	101	50
13	28.9	50	29.3	101	50	29.2	101	50	29.1	101	50
17	31.2	50	31.4	101	50	31.3	100	50	30.4	97	50
21	34.5	50	34.3	99	50	34.2	99	50	33.2	96	50
25	36.9	50	36.8	100	50	36.4	99	50	35.5	96	50
29	38.4	50	38.2	100	50	38.1	99	50	37.0	96	50
33	38.0	50	39.7	105	50	39.9	105	50	38.2	101	50
37	41.1	50	42.1	102	50	41.9	102	50	39.9	97	50
41	43.8	50	43.8	100	50	43.6	100	50	42.2	96	50
45	44.1	50	44.0	100	50	44.4	101	50	42.1	96	50
49	46.1	50	45.8	99	50	46.0	100	50	44.1	96	50
53	47.9	50	46.9	98	50	47.3	99	50	44.9	94	50
57	49.1	50	47.1	96	50	48.0	98	50	45.6	93	50
61	50.9	50	47.9	94	48	49.9	98	50	46.0	90	50
65	52.3	50	50.3	96	46	50.9	97	50	46.5	89	50
69	52.8	50	50.7	96	45	50.4	96	49	43.9	83	50
73	52.7	50	50.9	97	44	50.3	95	49	41.3	78	50
77	54.6	49	51.0	93	41	48.5	89	49	39.1	72	49
80	53.7	49	50.6	94	40	46.7	87	46	38.6	72	49
84	54.5	48	49.8	91	40	44.9	82	45	37.5	69	49
88	54.8	48	49.3	90	38	41.9	77	44	36.6	67	40
92	55.4	48	48.0	87	38	40.3	73	39	36.1	65	39
96	54.4	47	46.8	86	36	38.7	71	39	35.4	65	37
100	53.6	45	44.4	83	33	37.6	70	36	34.1	64	33
104	52.0	45	41.6	80	33	36.3	70	34	33.0	64	24
Mean for weeks											
1-13	24.8		24.9	100		25.1	101		25.3	102	
14-52	39.3		39.6	101		39.5	101		38.1	97	
53-104	52.8		48.2	91		45.1	85		39.9	76	

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, kidney, thyroid gland, skin, and salivary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analysis of primary neoplasms that occurred with an incidence of at least 5% in at least one group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: The incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in all dosed groups of male mice were significantly greater than those in the vehicle control group (Tables 13 and C3). The incidences of hepatocellular carcinoma and hepatoblastoma in 80 and 160 mg/kg males were also significantly increased compared to the vehicle controls. In dosed groups of female mice, the incidences of hepatocellular neoplasms were significantly greater than those in the vehicle control group (Tables 13 and D3). The incidences of hepatocellular neoplasms in all dosed groups of males and females exceeded the historical control ranges (Tables C4a and D4a).

The microscopic appearance of these liver neoplasms was typical of that usually observed in $B6C3F_1$ mice. There was a morphologic continuum from adenoma to carcinoma, with less differentiation and typical trabecular formations in the carcinomas. Carcinomas were often a centimeter or more in diameter, whereas adenomas were generally smaller and more discrete. Both adenomas and carcinomas displaced normal liver parenchyma and neither contained the normal liver lobular architecture. Lung metastases were seen in mice with hepatocellular carcinomas or hepatoblastomas (males: 3/50, 4/50, 9/50, 7/50; females: 0/50, 3/50, 6/50, 1/50; Tables C1 and D1).

Hepatoblastomas often originated within hepatocellular carcinomas and were characterized by well-demarcated, focal areas composed of bundles of deeply basophilic spindle-shaped cells. These cells were presumably poorly differentiated cells, and hepatoblastoma probably was a primitive variant of hepatocellular carcinoma.

The size and multiplicity of neoplasms in treated animals was considerably greater than in the vehicle controls. In many instances, a diagnosis of multiple adenomas in vehicle control mice was indicative of two neoplasms, whereas in dosed mice (especially 160 mg/kg mice), multiple adenomas corresponded to five or more separate neoplasms.

Nonneoplastic lesions were seen only in dosed male and female mice and consisted of cytoplasmic alteration, characterized by mild to moderate enlargement of centrilobular hepatocytes, and syncytial alteration, characterized by scattered hepatocytes with three or more small nuclei (Table C5).

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Cytoplasmic Alteration[a]	1 (2.0)[b]	17** (1.6)	17** (1.9)	12** (2.0)
Syncytial Alteration	0	28 (1.3)	38** (1.7)	23** (2.0)
Hepatocellular Adenoma, Multiple	12/50 (24%)	36/50 (72%)**	47/50 (94%)**	41/50 (82%)**
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	31/50 (62%)	42/50 (84%)	49/50 (98%)	45/50 (90%)
Adjusted rate[e]	65.0%	86.5%	98.0%	93.5%
Terminal rate[f]	28/40 (70%)	40/43 (93%)	33/34 (97%)	28/30 (93%)
First incidence (days)	411	641	445	386
Poly-3 test[g]	P<0.001	P=0.009	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	2/50 (4%)	5/50 (10%)	14/50 (28%)**	17/50 (34%)**
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	12/50 (24%)	17/50 (34%)	33/50 (66%)	34/50 (68%)
Adjusted rate	25.1%	34.9%	66.9%	72.3%
Terminal rate	6/40 (15%)	13/43 (30%)	20/34 (59%)	20/30 (67%)
First incidence (days)	485	576	445	446
Poly-3 test	P<0.001	P=0.206	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma (includes multiple)				
Overall rate	39/50 (78%)	47/50 (94%)	50/50 (100%)	49/50 (98%)
Adjusted rate	79.0%	95.3%	100.0%	99.9%
Terminal rate	31/40 (78%)	41/43 (95%)	34/34 (100%)	30/30 (100%)
First incidence (days)	411	576	445	386
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001
Hepatoblastoma[i]				
Overall rate	0/50 (0%)	2/50 (4%)	8/50 (16%)	5/50 (10%)
Adjusted rate	0.0%	4.2%	17.5%	11.3%
Terminal rate	0/40 (0%)	2/43 (5%)	4/34 (12%)	2/30 (7%)
First incidence (days)	—[j]	729 (T)	633	684
Poly-3 test	P=0.018	P=0.249	P=0.004	P=0.028
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma (includes multiple)[k]				
Overall rate	39/50 (78%)	47/50 (94%)	50/50 (100%)	49/50 (98%)
Adjusted rate	79.0%	95.3%	100.0%	99.9%
Terminal rate	31/40 (78%)	41/43 (95%)	34/34 (100%)	30/30 (100%)
First incidence (days)	411	576	445	386
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Female				
Number Examined Microscopically	50	50	50	50
Syncytial Alteration	0	2 (1.5)	17** (1.1)	18** (1.2)
Hepatocellular Adenoma, Multiple	16 (32%)	43 (86%)**	46 (92%)**	45 (90%)**
Hepatocellular Adenoma (includes multiple)[l]				
Overall rate	32/50 (64%)	50/50 (100%)	48/50 (96%)	48/50 (96%)
Adjusted rate	66.1%	100.0%	96.4%	96.4%
Terminal rate	30/44 (68%)	33/33 (100%)	33/33 (100%)	23/23 (100%)
First incidence (days)	674	418	474	522
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	0/50 (0%)	6/50 (12%)*	21/50 (42%)**	26/50 (52%)**
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	5/50 (10%)	19/50 (38%)	38/50 (76%)	42/50 (84%)
Adjusted rate	10.4%	43.4%	77.9%	84.9%
Terminal rate	4/44 (9%)	12/33 (36%)	26/33 (79%)	18/23 (78%)
First incidence (days)	729	423	474	522
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma (includes multiple)				
Overall rate	33/50 (66%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	68.2%	100.0%	100.0%	100.0%
Terminal rate	31/44 (71%)	33/33 (100%)	33/33 (100%)	23/23 (100%)
First incidence (days)	674	418	474	522
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatoblastoma[n]	0/50 (0%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[o]				
Overall rate	33/50 (66%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	68.2%	100.0%	100.0%	100.0%
Terminal rate	31/44 (71%)	33/33 (100%)	33/33 (100%)	23/23 (100%)
First incidence (days)	674	418	474	522
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Dermal Study of Diethanolamine

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* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year dermal studies with vehicle control groups (mean ± standard deviation): 118/249 (47.4% ± 8.9%); range, 38%-62%
[d] Number of animals with neoplasm per number of animals with liver examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the vehicle control column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to the pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: 54/249 (21.7% ± 2.5%); range, 18%-24%
[i] Historical incidence: 1/249 (0.4% ± 0.9%); range, 0%-2%
[j] Not applicable; no neoplasms in animal group
[k] Historical incidence: 154/249 (61.8% ± 9.1%); range, 56%-78%
[l] Historical incidence: 133/252 (52.8% ± 11.4%); range, 38%-64%
[m] Historical incidence: 35/252 (13.9% ± 7.3%); range, 6%-23%
[n] Historical incidence: 1/252 (0.4% ± 0.9%); range, 0%-2%
[o] Historical incidence: 149/252 (59.1% ± 6.4%; range, 52%-66%

Kidney: The incidences of renal tubule adenoma in males occurred with a positive trend; however, the incidences of carcinoma and hyperplasia did not follow this pattern (Tables 14, C3, and C5). The incidences of adenoma or carcinoma (combined) in male mice exceeded the historical control range (Table C4b). To determine whether additional hyperplasias or neoplasms were present, the kidneys were step sectioned and an extended analysis of proliferative lesions in the kidney was conducted. The combined analysis of single and step sections indicated a dose-related increase in the incidences of renal tubule hyperplasia and renal tubule adenoma in male mice. Two additional adenomas were found in the 40 mg/kg group, four in the 80 mg/kg group, and one in the 160 mg/kg group. Additional incidences of hyper-plasia were found in all groups, and the single- and step-section (combined) incidence in the 160 mg/kg group was significantly greater than that in the vehicle control group. Adenomas were focal compressive masses approximately the size of five tubule diameters or greater. Carcinomas were similar morphologically but were relatively large and often showed cellular debris and/or mineralization. Renal tubule neoplasms were located in the cortex and/or outer medulla.

Focal proliferative masses less than the diameter of five tubules, observed in vehicle control and dosed males, were classified as focal hyperplasia (Table 14).

Thyroid Gland: Incidences of follicular cell hyperplasia were significantly increased in dosed groups (males: vehicle control, 18/50; 40 mg/kg, 22/49; 80 mg/kg, 30/50; 160 mg/kg, 42/50; females: 18/50, 28/50, 32/50, 39/49; Tables C5 and D5). This lesion consisted of focal areas of thyroid gland follicles lined by increased numbers of epithelial cells, which in some instances formed papillary projections. The severity grade for follicular cell hyperplasia (males: 1.6, 1.5, 1.6, 2.2; females: 1.9, 2.0, 1.7, 1.9) was based on the size of the lesion. Incidences of thyroid gland follicular cell adenomas were not increased relative to vehicle controls (males: 4/50, 5/50, 4/50, 2/50; females: 4/50, 9/50, 5/50, 3/49; Tables C3 and D3), and no thyroid gland follicular cell carcinomas were detected in this study.

TABLE 14
Incidences of Renal Tubule Neoplasms and Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Renal Tubule Hyperplasia[a]	1 (3.0)[b]	2 (2.5)	0	3 (2.7)
Renal Tubule Adenoma, Multiple[c]	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Renal Tubule Adenoma (includes multiple)[d]				
Overall rate	1/50 (2%)	4/50 (8%)	6/50 (12%)	6/50 (12%)
Adjusted rate[e]	2.2%	8.3%	13.1%	13.3%
Terminal rate[f]	1/40 (3%)	3/43 (7%)	3/34 (9%)	2/30 (7%)
First incidence (days)	729 (T)	692	654	540
Poly-3 test[g]	P=0.049	P=0.196	P=0.056	P=0.053
Renal Tubule Carcinoma (Includes multiple)	2/50 (4%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Renal Tubule Adenoma or Carcinoma (includes multiple)[h]				
Overall rate	3/50 (6%)	5/50 (10%)	6/50 (12%)	8/50 (16%)
Adjusted rate	6.6%	10.4%	13.1%	17.8%
Terminal rate	3/40 (8%)	4/43 (9%)	3/34 (9%)	4/30 (13%)
First incidence (days)	729 (T)	692	654	540
Poly-3 test	P=0.064	P=0.386	P=0.242	P=0.093
Step Sections (Extended Evaluation)				
Renal Tubule Hyperplasia	2 (1.5)	5 (2.2)	7 (1.3)	7 (2.3)
Renal Tubule Adenoma	0/50 (0%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Renal Tubule Carcinoma	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Renal Tubule Adenoma or Carcinoma	0/50 (0%)	2/50 (4%)	4/50 (8%)	2/50 (4%)

TABLE 14
Incidences of Renal Tubule Neoplasms and Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Number Examined Microscopically	50	50	50	50
Single Sections and Step Sections (Combined)				
Renal Tubule Hyperplasia	3 (2.0)	7 (2.3)	7 (1.3)	10* (2.4)
Renal Tubule Adenoma, Multiple	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Renal Tubule Adenoma (includes multiple)				
Overall rate	1/50 (2%)	6/50 (12%)	8/50 (16%)	7/50 (14%)
Adjusted rate	2.2%	12.5%	17.5%	15.5%
Terminal rate	1/40 (3%)	5/43 (12%)	5/34 (15%)	3/30 (10%)
First incidence (days)	729 (T)	692	654	540
Poly-3 test	P=0.046	P=0.065	P=0.016	P=0.028
Renal Tubule Carcinoma (includes multiple)	2/50 (4%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Renal Tubule Adenoma or Carcinoma (includes multiple)				
Overall rate	3/50 (6%)	7/50 (14%)	8/50 (16%)	9/50 (18%)
Adjusted rate	6.6%	14.5%	17.5%	20.0%
Terminal rate	3/40 (8%)	6/43 (14%)	5/34 (15%)	5/30 (17%)
First incidence (days)	729 (T)	692	654	540
Poly-3 test	P=0.056	P=0.180	P=0.098	P=0.055

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Number of animals with neoplasm per number of animals with kidney examined microscopically
[d] Historical incidence for 2-year dermal studies with vehicle control groups: 2/299 (0.7% ± 1.0%); range, 0%-2%
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the vehicle control column are the P values associated with the trend test. In the dosed group columns are the P values corresponding to the pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical incidence: 4/299 (1.3% ± 2.4%); range, 0%-6%

Skin (Site of Application): Hyperkeratosis, acanthosis, and exudate were treatment-related changes in the skin at the site of application. The incidences of hyperkeratosis in all dosed groups except 40 mg/kg females were significantly greater than those in the vehicle control groups (Table 15). Hyperkeratosis was of minimal to mild severity and consisted of increased keratin on the surface. Acanthosis and exudate were observed in a few dosed mice. Acanthosis consisted of increased thickness of the epidermis. Exudate was a mixture of cellular debris and inflammatory cells on the surface of the epidermis, usually mixed with the thickened keratin layer.

Salivary Gland: A significant number of 80 and 160 mg/kg male mice showed a loss of the normal granularity of the cells lining the secretory ducts, which was termed cytoplasmic alteration (1/50, 2/50, 8/50, 23/50; Table C5). The biologic significance of this change is unknown.

TABLE 15
Incidences of Nonneoplastic Lesions of the Epidermis at the Site of Application in Mice in the 2-Year Dermal Study of Diethanolamine

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Acanthosis[a]	0	0	2 (2.0)[b]	4 (1.8)
Exudate	0	0	0	3 (1.3)
Hyperkeratosis	0	13** (1.0)	10** (1.0)	17** (1.1)
Female				
Number Examined Microscopically	50	50	50	50
Acanthosis	0	2 (2.0)	1 (1.0)	2 (2.0)
Exudate	0	1 (4.0)	1 (1.0)	3 (1.7)
Hyperkeratosis	1 (1.0)	3 (2.0)	8* (1.0)	16** (1.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

GENETIC TOXICOLOGY

Diethanolamine (33 to 3,333 μg/plate) was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 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; Table E1). No induction of trifluorothymidine resistance was observed in L5178Y mouse lymphoma cells treated with diethanolamine with or without Aroclor 1254-induced male Fisher 344 rat liver S9 (Table E2). In the assay, an increase in pH was noted at all but one (25 nL/mL) of the concentrations tested. Diethanolamine did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Loveday *et al.*, 1989; Tables E3 and E4). In the chromosomal aberration assay, the trial with S9 produced a dose-related increase in the percentage of cells with chromosomal aberrations; however, this increase was not large enough for a positive determination. As with the mouse lymphoma assay, pH increases due to the presence of diethanolamine in the culture medium were noted. Peripheral blood samples collected from male and female mice exposed to 80 to 1,250 mg/kg diethanolamine dermally for 13 weeks showed no increase in the frequency of micronucleated normochromatic erythrocytes (Table E5).

DISCUSSION AND CONCLUSIONS

Diethanolamine is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos, and hair conditioners. Because of the extensive human exposure to diethanolamine and diethanolamine condensates of fatty acids and the absence of information concerning the consequences of long-term exposure, diethanolamine, lauric acid diethanolamine condensate, oleic acid diethanolamine condensate, and coconut oil acid diethanolamine condensate were selected for evaluation of carcinogenic potential. The primary route of human exposure to products containing diethanolamides is by contact with the skin. Therefore, this series of studies was conducted by dermal administration.

Diethanolamine administered by topical application to rats at doses of 32, 63, 125, 250, or 500 mg/kg for 13 weeks produced nonneoplastic lesions at several sites (NTP, 1992; Melnick *et al.*, 1994a; Table 3). Minimal nephropathy was present in all groups of rats but was somewhat more severe in dosed females. Renal tubule epithelial cell necrosis was present in the 250 and 500 mg/kg females but was not observed in males, and the incidences of renal tubule mineralization were increased in all groups of dosed females, but this lesion was present only in 500 mg/kg males. Demyelination in the medulla oblongata occurred in males and females that received 500 mg/kg and in seven females that received 250 mg/kg. Acanthosis and hyperkeratosis of the skin at the site of application were present in males administered 63 mg/kg and increased in both incidence and severity at higher doses; however, hyperkeratosis was also present in 32 mg/kg females. Ulcer and chronic inflammation were present in males administered 250 or 500 mg/kg and females administered 125 mg/kg or greater. The toxic response also included a moderate, poorly regenerative, normochromic anemia involving significant changes in red cell parameters at the lowest dose administered (32 mg/kg).

Based on these results, doses of 250 or 500 mg/kg were clearly too high for use in a 2-year study. At 125 mg/kg, acanthosis and hyperkeratosis were present in the skin at the site of application in males and females, but, in addition, females exhibited ulceration and chronic inflammation. Because of the presence of more severe lesions in females at this dose and in males that received higher doses and the potential for skin lesions to progress to ulceration and chronic inflammation over the duration of a 2-year study, the high dose chosen for males was 64 mg/kg. Because females exhibited greater skin sensitivity to diethanolamine than males, 32 mg/kg was selected as the high dose for female rats.

Diethanolamine was also toxic to mice that received 80, 160, 320, 630, or 1,250 mg/kg by topical application for 13 weeks (NTP, 1992; Melnick *et al.*, 1994b; Table 4). The most significant responses occurred in the liver, kidney, and skin. Liver weights were significantly increased in all dosed groups of females and in males treated with 160 mg/kg or greater. Associated with the increase in liver weights were increases in the incidences and severities of cytological alteration of hepatocytes, more so in males than females. Kidney weights were significantly increased in all dosed groups of males and females, and renal tubule epithelial necrosis was present in a few animals treated with 1,250 mg/kg. Acanthosis was present at the site of application in almost all animals treated with diethanolamine. At doses of 320 mg/kg or greater, this was accompanied in some animals by hyperkeratosis and/or chronic inflammation. At 630 mg/kg or greater, ulceration was also present. Based on these results, doses of 320 mg/kg or greater were considered too high for a 2-year study. At 160 mg/kg, acanthosis of the skin and cytological alteration of the liver were minimal in males and females. Therefore, 160 mg/kg was selected as the high dose for the 2-year mouse study.

No neoplasms of the skin associated with exposure to diethanolamine occurred in rats or mice during the 2 year studies. The most significant toxic response in

skin at the site of application was an increased incidence of hyperkeratosis. In rats, the highest incidence (46%) occurred in the 16 and 32 mg/kg groups of females, but the severity was minimal even at 32 mg/kg. In mice, the highest incidence (34%) occurred in the 160 mg/kg group of males. The severity of hyperkeratosis in this group was moderate; however, minimal severity was observed in other groups of males and the 160 mg/kg group of females.

Exposure of mice to diethanolamine for 2 years produced a marked neoplastic response in the liver characterized by significant increases in the incidences and multiplicity of hepatocellular adenoma and hepatocellular carcinoma in males and females. The average size of hepatocellular neoplasms in dosed mice was considerably larger than that of neoplasms in the vehicle controls, and dosed animals with multiple adenomas typically had five or more separate neoplasms, whereas vehicle controls with multiple adenomas usually had only two separate neoplasms. Reduced survival of dosed female mice was considered to be a consequence of the presence of liver neoplasms. The incidences of hepatoblastomas, uncommon phenotypic variants of hepatocellular carcinoma which arise within existing hepatocellular carcinomas, were significantly increased in male mice, but not in females. The incidences of syncytial alteration, a nonneoplastic lesion characterized by the presence of hepatocytes containing multiple (three or more) nuclei, were increased in all groups of dosed mice; this lesion was not present in the controls. A similar lesion was observed in mouse livers during 13-week studies (Melnick *et al.*, 1994b). Centrilobular cytoplasmic alteration was increased in treated males but was not present in females.

The incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) occurred with a positive trend in male mice, but renal tubule carcinoma did not follow the same pattern. An extended evaluation of kidney sections revealed the presence of additional hyperplasias and adenomas in all dosed groups of males and one additional carcinoma in the 160 mg/kg group. Therefore, the extended analysis in male mice confirmed the original observations and, taken together, indicated a treatment- and dose-related increase in the incidences of renal tubule adenoma and adenoma or carcinoma (combined). Although diethanolamine is eliminated in urine as the parent compound, there were no indications that it was nephrotoxic, and neither the incidence nor severity of nephropathy in diethanolamine-treated male mice was increased.

No neoplastic response associated with diethanolamine exposure occurred in rats. In view of the strong toxic response observed in rats during the 13-week studies (NTP, 1992; Melnick *et al.*, 1994a), the absence of response in the 2-year studies is somewhat surprising. However, in rats in the 13-week drinking water and dermal studies, toxic responses at sites other than the site of application (liver, kidney, brain) occurred at doses higher than those used in the 2-year study. In 13-week studies, rat skin was clearly more sensitive to diethanolamine than mouse skin, and doses as low as 125 mg/kg produced ulceration and chronic inflammation. Therefore, it was necessary to use lower doses in the 2-year rat study to avoid excessive dermal toxicity. In addition, the percutaneous absorption of diethanolamine is more rapid through mouse skin than through rat skin. Because the rate of elimination of diethanolamine from rats and mice is very similar, the systemic exposure experienced by rats during the 2-year study was considerably less than that by mice.

Mean body weights of female mice were depressed more than those of male mice, and hepatocellular neoplasms may have contributed to the reduced survival of 160 mg/kg female mice. In addition to the neoplastic response in the liver, increased incidences of renal tubule neoplasms occurred in male mice.

The neoplastic response to diethanolamine exposure involving the liver and kidney of male mice and liver of female mice in the present study is similar to that observed in mice in the 2-year studies of other diethanolamine condensates (NTP 1999a,b). Unreacted diethanolamine was present in varying concentrations in each of the three diethanolamine condensates evaluated in this class study and, therefore, animals in these studies were exposed to a wide range of diethanolamine concentrations. Comparison of the results of these studies reveals a strong association between the concentration of free diethanolamine contaminant present in the different diethanolamide preparations and the incidences of hepatocellular neoplasms in male and female mice and renal tubule neoplasms in male mice. The comparison also reveals a clear gender difference in the response of male and female mice to diethanolamine exposure.

The strongest response occurred in the present study (100% diethanolamine) and involved male and female mice. In addition to increased incidences of liver neoplasms, diethanolamine administration was also associated with significant increases in the multiplicity and size of hepatocellular adenomas and carcinomas.

The next strongest response was observed in the coconut oil acid diethanolamine condensate study (NTP, 1999a) and involved significant increases in hepatocellular neoplasm incidences in mice, but no corresponding increase in the multiplicity or size of neoplasms, as was observed in the diethanolamine study. The incidences of hepatoblastoma were significantly increased in male mice, but not in females. Mean body weights and survival of 200 mg/kg female mice were less than those of the vehicle controls. In addition, the incidences of renal tubule neoplasms were increased in male mice. Based on data provided by the manufacturer, the coconut oil acid diethanolamine condensate contained 18.2% free diethanolamine by weight. Therefore, mice in this study were exposed to 18.2 or 36.4 mg/kg free diethanolamine.

The weakest positive response occurred in the lauric acid diethanolamine condensate (NTP, 1999b) study, in which hepatocellular neoplasms were increased only in female mice. Moreover, although the combined incidence of hepatocellular neoplasms was significantly greater than the combined incidence in the vehicle controls, the incidences of adenomas or carcinomas alone were not significantly increased. Survival of female mice was similar to survival of the vehicle controls, and no response was observed in the kidney of male mice. Based on data provided by the manufacturer, lauric acid diethanolamine condensate contained 0.83% free diethanolamine by weight and, therefore, mice in this study were exposed to 0.83 or 1.66 mg/kg free diethanolamine.

No carcinogenic response occurred in the oleic acid diethanolamine condensate study (NTP, 1999c). Data provided by the manufacturer indicated a free diethanolamine content of 0.19%, less than the 0.83% content for lauric acid diethanolamine condensate. However, in the oleic acid diethanolamine condensate study, mice were given doses of only 15 or 30 mg/kg oleic acid diethanolamine condensate, compared to the other studies in which mice were given doses of 100 or 200 mg/kg of the diethanolamide. Therefore, mice in the oleic acid diethanolamine condensate study were exposed to 0.028 or 0.056 mg/kg of free diethanolamine, the lowest concentration in any of the four studies.

The neoplastic response associated with diethanolamine exposure includes hepatocellular neoplasms in male and female mice and renal tubule neoplasms in male mice. The liver is clearly the most responsive site, and female mice are more sensitive than males. To quantify the association between the incidences of hepatocellular neoplasms and diethanolamine concentration, a logistic regression model was fitted to individual animal neoplasm incidence and survival data. The model predicts the incidence of hepatocellular neoplasms as a function of diethanolamine dose (mg/kg) and survival. This analysis compares the observed liver neoplasm rates in female mice with the rates predicted by the logistic regression model (Figure 5). The close agreement between observed and predicted rates strongly supports the conclusion that the liver neoplasm responses in the diethanolamine study and the three diethanolamine condensate studies are determined primarily by the concentrations of free diethanolamine.

The composition and purity of the bulk diethanolamide preparations used in these studies varied considerably. Lauric acid diethanolamine condensate was approximately 90% lauric acid diethanolamine, 0.83% free diethanolamine, and 9.17% other organic impurities. Oleic acid diethanolamine condensate was 47.5% oleic acid diethanolamine, 0.19% free diethanolamine, approximately 30% other fatty acid alkanolamides, and 22.31% other organic impurities (most probably unreacted fatty acids). Coconut oil itself is a mixture of fatty acids that typically contains as much as 40% lauric acid.

This variable composition was reflected in the composition of coconut oil acid diethanolamine

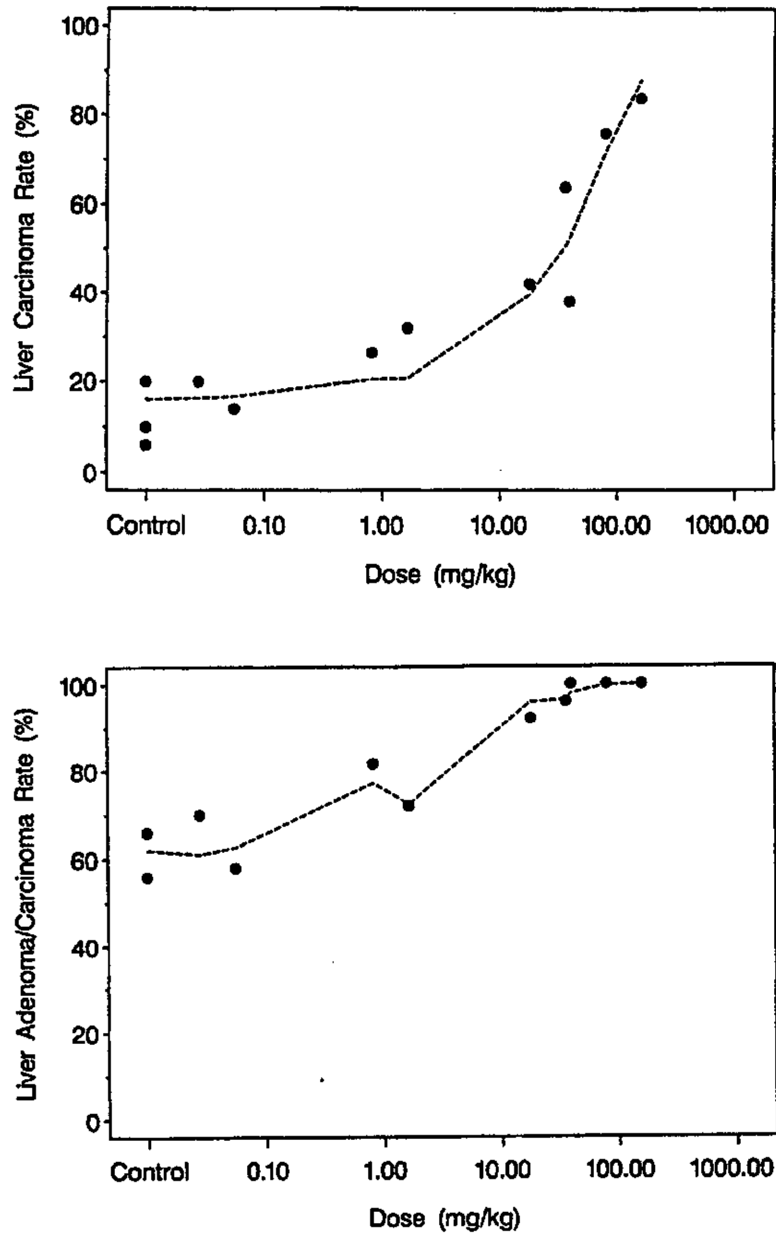

FIGURE 5

Observed and Predicted Liver Neoplasm Incidences in Female B6C3F$_1$ Mice as a Function of Dose and Survival (• = Observed, —— = Predicted). Predicted rates are based on the logistic regression model, P = 1/[1+exp(T)], where P is the probability of observing a neoplasm. For carcinoma, T = 3.2425 – 0.2920D – 0.00226S, and for adenoma/carcinoma, T = 6.3820 – 0.6822D – 0.00979S, where D = dose$^{1/2}$ in mg diethanolamine/kg body weight and S = survival in days.

condensate in which lauric acid diethanolamine condensate was the major constituent. With animals administered preparations of such widely varying diethanolamide of such widely varying diethanolamide composition, it seems improbable that chance alone could explain the strong correlation between liver neoplasm response and diethanolamine content or the similarity in species, gender, and target tissues specificity observed in the studies with 100% diethanolamine.

The incidences of thyroid gland follicular cell hyperplasia were increased in dosed male and female mice compared to the vehicle controls. Similar increased incidences of follicular cell hyperplasia occurred in male and female mice in the coconut oil acid diethanolamine condensate study and in male mice in the lauric acid diethanolamine condensate study; no increase in the incidences of follicular cell hyperplasia occurred in either male or female mice in the oleic acid diethanolamine condensate study. The incidence of follicular cell hyperplasia appears to be related to the concentration of diethanolamine, although not as strongly as the association between hepatocellular neoplasms and diethanolamine. It is unlikely that the incidences of follicular cell hyperplasia are related to exposure to the diethanolamides because of their varying composition. The lack of an increased incidence of thyroid gland follicular cell neoplasms associated with the increased incidence of follicular cell hyperplasia may be an indication that the increase in the incidence of follicular hyperplasia is a late event occurring near the end of the study.

Absorption, disposition, and metabolism studies of lauric acid diethanolamine condensate indicate that it is well absorbed after dermal or oral administration and is eliminated primarily in the urine as the half amides of succinic and adipic acid (Matthews *et al.*, 1996). No parent diethanolamide, diethanolamine, or diethanolamine-derived metabolites were detected in urine even after oral doses of 1,000 mg/kg. This indicates that there is no metabolic cleavage of the amide linkage of lauric acid diethanolamine and that the metabolism of this compound involves ω-hydroxylation followed by β-oxidation to half amides that are eliminated in urine. Therefore, the source of free diethanolamine in lauric acid diethanolamine condensate and probably also in coconut oil diethanolamine condensate and oleic acid diethanolamine condensate is unreacted material present in the original preparation. No additional bioavailable diethanolamine is released during metabolism of lauric acid diethanolamide, which is quite likely also the case for coconut oil diethanolamine condensate and oleic acid diethanolamine condensate.

Diethanolamine is not a mutagen and is not metabolized to a reactive intermediate; however, it can be converted to a carcinogenic nitrosamine. Nitrosamine formation *in vivo* is thought to occur as a result of a nonenzymatic reaction between an amine and nitrous acid, formed from nitrate in the acid environment of the stomach. The formation of N-nitrosodiethanolamine *in vivo* was demonstrated by Preussmann *et al.* (1981) in rats simultaneously administered sodium nitrate in drinking water (2,000 ppm) and diethanolamine by dermal application of doses comparable to those used in the present rat study (100 to 400 mg/kg). However, N-nitrosodiethanolamine was not detected in groups of rats administered dermal doses of diethanolamine up to 300 mg/kg without nitrite supplementation. Dietary administration of 5,000 ppm diethanolamine, along with 3,000 ppm sodium nitrite, caused a significant increase in the number of γ-glutamyl-transpeptidase (GGT) positive foci in the liver of Wistar rats dosed for 8 weeks. However, 5,000 ppm diethanolamine, without nitrite supplementation, was not associated with any increase in GGT-positive foci (Yamamoto *et al.*, 1995).

Nitrosodiethanolamine was not identified as a urinary metabolite in F344/N rats administered oral (gavage) doses of 200 mg/kg ^{14}C-diethanolamine in repeated-dose studies of 2, 4, or 8 weeks duration, nor was there any significant binding to liver proteins (Matthews *et al.*, 1995). All administered radioactivity was accounted for in excreta as parent compound, free N-methylated diethanolamines, or diethanolamine incorporated into phospholipids. Therefore, nitrosamine formation was not detected in rats given oral doses of diethanolamine twice as large as those administered dermally to high dose rats (64 mg/kg in males; 32 mg/kg in females) in the present study. Because diethanolamine was applied dermally without nitrite supplementation in this study, and dietary nitrate levels were kept within strict limits, it is likely that the concentration of diethanolamine

and nitrite in the stomach contents of rats and mice was too low to support the formation of biologically meaningful quantities of nitrosamine.

N-nitrosodiethanolamine, administered in drinking water at concentrations of 3,900 to 62,500 ppm, produced hepatocellular carcinomas in all male and female F344/N rats after 34 weeks of exposure (Lijinsky *et al.*, 1980). However, no neoplastic response was noted in groups of male and female B6C3F$_1$ mice exposed to the same concentrations of N-nitrosodiethanolamine in drinking water, indicating that F344/N rats are more responsive to the hepatocarcinogenic activity of N-nitrosodiethanolamine than are B6C3F$_1$ mice (Lijinsky *et al.*, 1980). In contrast, in the present studies, exposure to diethanolamine was associated with a strong neoplastic response in the liver of mice but not rats. Moreover, during 13-week drinking water studies (Melnick *et al.*, 1994a), no microscopic lesions were present in the liver of rats administered 5,000 ppm diethanolamine in drinking water for 13 weeks. However, cytologic alteration was present in B6C3F$_1$ mice that received doses as low as 630 ppm, and hepatocellular necrosis was present in groups that received 2,500 ppm or greater for 13 weeks. A similar result was observed in 13-week dermal studies (Melnick *et al.*, 1994b); no microscopic lesions were present in the liver of F344/N rats, even at the highest dose administered (500 mg/kg), while cytologic alteration and hepatocellular necrosis were present in B6C3F$_1$ mice at doses of 80 mg/kg or greater. Therefore, the pattern of response associated with exposure to diethanolamine is distinctly different from that observed after exposure to N-nitrosodiethanolamine.

Diethanolamine, as a structural analogue of ethanolamine, is incorporated into phospholipid head groups by the same biosynthetic pathways as ethanolamine, leading to the formation of diethanolamine-containing phospholipids. Phospholipids are vital structural components of cell membranes and have a profound effect on membrane properties such as fluidity. Diethanolamine is a longer molecule than ethanolamine and carries an additional hydroxyl group not present in an ethanolamine-containing phospholipid. Both the increased size and additional interaction (hydrogen bonding) through the extra hydroxyl group have the potential to alter the structure and properties of membranes containing diethanolamine phospholipids.

The primary amino group of the ethanolamine portion of phosphatidyl ethanolamine is methylated to form phosphatidylcholine in a reaction catalyzed by phosphatidylethanolamine N-methyltransferase, an enzymatic activity found uniquely in the liver. The source of methyl groups for this reaction is *S*-adenosyl-methionine (SAM). Methylation of phosphatidyl ethanolamine to phosphatidylcholine followed by phospholipase cleavage of phosphatidylcholine to choline and diacylglycerol represents the only pathway of *de novo* choline synthesis in mammals. The presence of free N-methyl and N,N-dimethyl-diethanolamine in liver extracts of exposed rats, as well as the presence of methylated diethanolamine head groups in liver phospholipids (Matthews *et al.*, 1995), is an indication that phosphatidyl diethanolamine is methylated and cleaved by the same pathway.

Choline enters hepatocytes by a specific carrier-mediated process (Moseley *et al.*, 1996). The close structural similarity between diethanolamine, methylated diethanolamine, and choline may explain the strong retention of diethanolamine noted by Matthews *et al.* (1995); 70% of diethanolamine equivalents in tissues of animals given daily oral doses for 8 weeks was present as the parent compound. Barbee and Hartung (1979) demonstrated that diethanolamine was a competitive inhibitor of choline incorporation into phosphatidylcholine and a mixed inhibitor of ethanol-amine incorporation into phosphatidylethanolamine in rat liver homogenates. In male Sprague-Dawley rats that consumed 320 mg diethanolamine/kg body weight per day in drinking water, ethanolamine incorporation into phospholipid in liver was reduced to 27% of the control value after 1 week of treatment and choline incorporation was reduced to 41% of the control value after 3 weeks of treatment. This suggests that animals receiving diethanolamine over a long period of time could become choline deficient. Choline deficiency significantly reduces the synthesis of phospholipids in rodents and humans. Since phosphatidylcholine is a major constituent of lipoprotein envelopes, the inability to form these structures inhibits the secretion of triglycerides and leads to the accumulation of fat in the liver, which is easily identified by histologic

examination. Although liver weights were increased in both rats and mice after 13 weeks of exposure to diethanolamine (Melnick *et al.*, 1994a,b), no evidence of fat accumulation was observed in the livers of rats or mice in either the 13-week studies or the present 2-year studies. Therefore, it would appear that exposure to diethanolamine did not produce notable choline deficiency.

Although N,N-dimethyl-diethanolamine can substitute for choline in the biosynthesis of choline-containing phospholipids, it is unclear whether it can substitute for choline in other reactions. Choline is oxidized to betaine in the liver and kidney, and betaine, in turn, serves as a methyl group donor in the conversion of homocysteine to methionine. This reaction establishes a pathway between the methyl groups of choline and the 1-carbon pool. If methylated diethanolamine cannot substitute for choline in methyl group donation, then methylation of diethanolamine would serve to remove methyl groups from the 1-carbon pool. This has the potential of reducing the availability of SAM, the source of methyl groups for the methyltransferases that methylate DNA. Undermethylation of critical genes as a result of reduced availability of SAM could be a factor in the carcinogenic response observed in mice.

Diethanolamine incorporation into phospholipids could also induce toxicity or influence carcinogenic response through its effect on the generation of lipid second messengers from diethanolamine-containing phospholipids. Ceramide is a second messenger generated by the action of sphingomyelinase, a sphingomyelin-specific form of phospholipase C that, upon activation, hydrolyzes phosphocholine from sphingomyelin to yield free phosphocholine and ceramide. Activation of sphingomyelinase is coupled to certain cell surface receptors. Ceramide in turn interacts with specific targets involved in initiating a wide variety of cellular responses (see reviews by Spiegel and Merrill, 1996, and Spiegel *et al.*, 1996). In diethanolamine-treated animals, Matthews *et al.* (1995) found that 93% of diethanolamine incorporated into liver phospholipid was present as ceramide derivatives in which diethanolamine and/or phosphodiethanolamine was incorporated into "sphingomyelin" in place of phosphocholine. The presence of diethanolamide-containing phospholipids in the phospholipid signaling pool used for second messenger generation could have a significant effect on the ability of cells to respond to activation of the sphingomyelin pathway as well as on the character of the response.

Diethanolamine is not a mutagen, it is not metabolized to a mutagen, and it does not interact with DNA. All its biologic effects appear to be associated with its incorporation into phospholipids in place of ethanolamine. The pathways of phospholipid biosynthesis using ethanolamine and choline are highly conserved and essentially the same in all mammals, as is the function of phospholipids as structural components of cell membranes and their role as second messengers. Therefore, it is likely that incorporation of diethanolamine into phospholipids would occur in any suitably exposed mammalian species. Matthews *et al.* (1995) have demonstrated the incorporation of diethanolamine into phospholipids in human liver slices. Therefore, the toxic responses observed in the 13-week studies in rats and mice, as well as the carcinogenic responses that occurred in the 2-year study in mice, indicate potential hazards to humans exposed to diethanolamine.

Conclusions

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of diethanolamine in male F344/N rats administered 16, 32, or 64 mg/kg diethanolamine or in female F344/N rats administered 8, 16, or 32 mg/kg. There was *clear evidence of carcinogenic activity* of diethanolamine in male and female B6C3F$_1$ mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males.

Dermal administration of diethanolamine to rats was associated with increased incidences of acanthosis (males only), hyperkeratosis, and exudate of the skin and increased incidences and severities of nephropathy in females. Dermal administration of diethanolamine to mice was associated with increased incidences of cytoplasmic alteration (males only) and syncytial alteration of the liver, renal tubule hyperplasia (males only), thyroid gland follicular cell hyperplasia, and hyperkeratosis of the skin.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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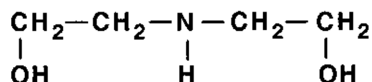
Diethanolamine



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 (92×10^6 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-nitrosodiethanolamine (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

		Mean Body Weight (grams)			Final Weight Relative	Average Water	Estimated DEA
Dose (ppm)	Survival[a]	Initial[b]	Final	Change[c]	to Controls (%)[d]	Consumption[e]	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.

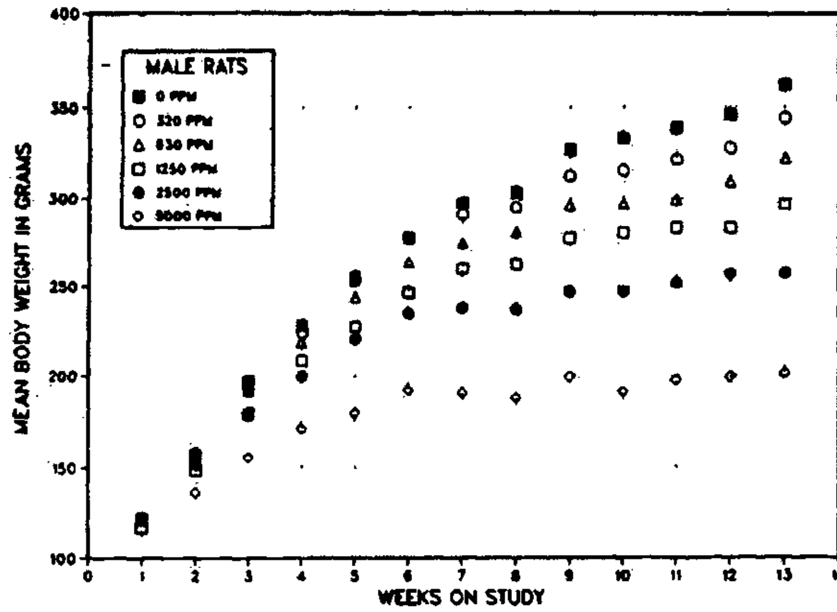
ABLE 4 Renal Toxicity in F344/N Rats in the 2-Week Drinking Water Studies of Diethanolamine[a]

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 ($P \leq 0.05$).
** Significantly different from control ($P \leq 0.01$).

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.



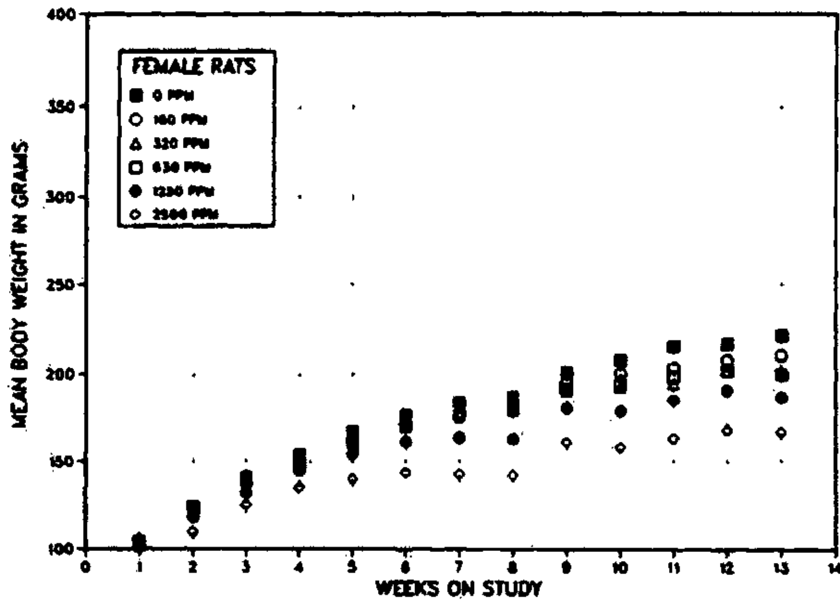


Figure 1 Body Weights of F344/N Rats Exposed to Diethanolamine by Drinking Water for 13 Weeks

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/µL)	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/µL)	0.23	--	0.23	0.23	0.24	0.14**	0.16**
FEMALE							
RBC (10^6/µL)	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/µL)	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 ($p \le 0.05$) by Dunn's or Shirley's test.
** Significantly different from control ($p \le 0.01$) 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 ($P \le 0.05$).
** Significantly different from the control group by Williams' or Dunnett's test ($P \le 0.01$).

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) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]
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 ($p \leq 0.05$).
** Significantly different from the control group ($p \leq 0.01$).

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

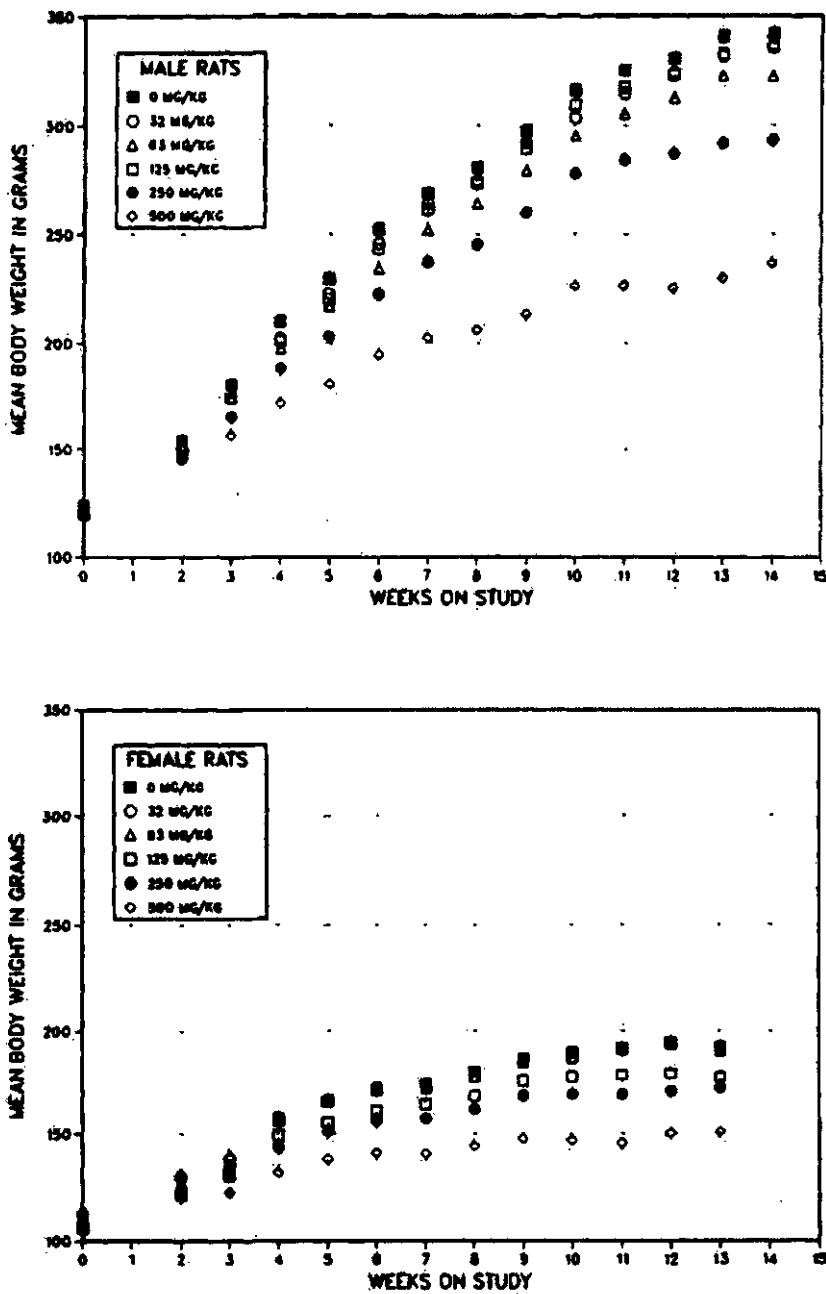


Figure 2 Body Weights of F344/N Rats Exposed Dermally to Diethanolamine for 13 Weeks

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 B6C3F1 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 B6C3F1 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

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	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.

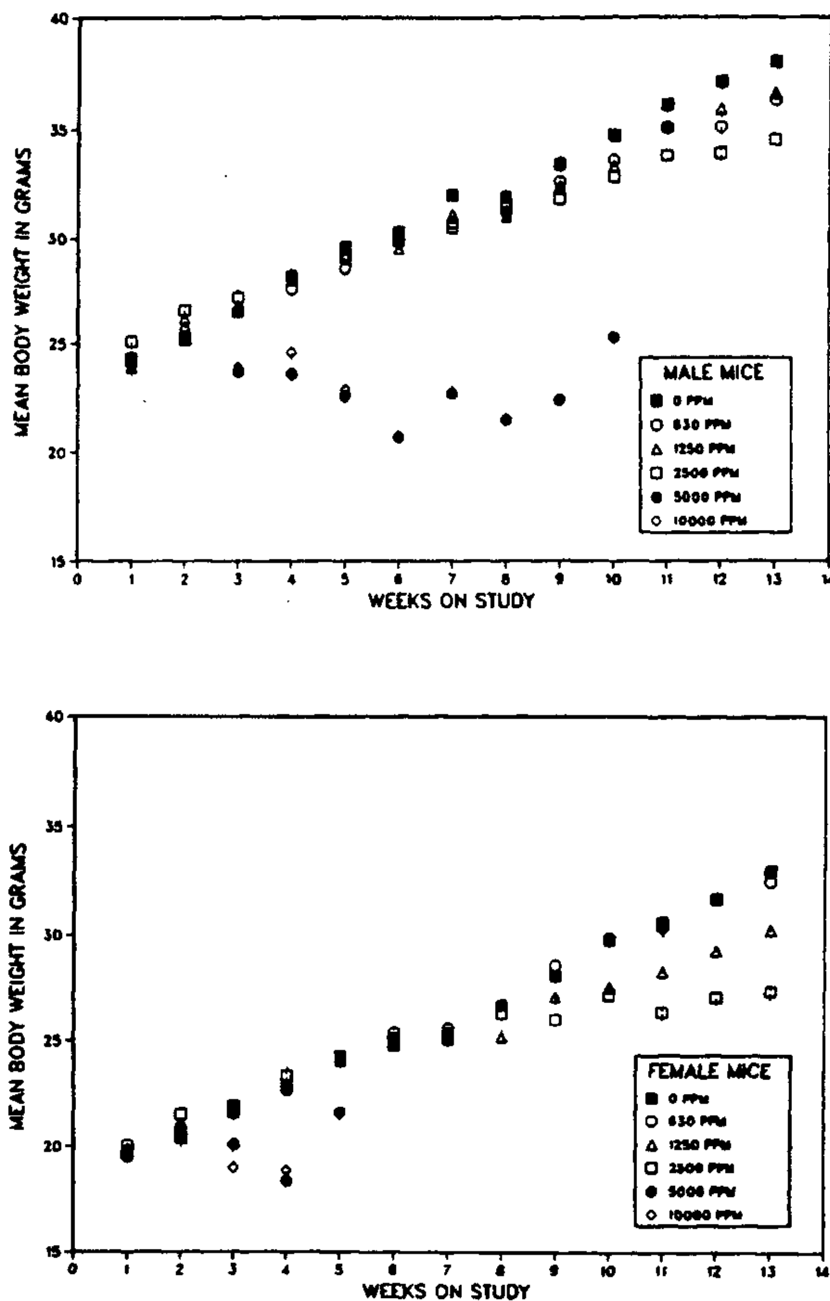


Figure 3 Body Weights of B6C3F$_1$ Mice Exposed to Diethanolamine by Drinking Water for 13 Weeks

TABLE 20 Selected Organ Weights and Clinical Pathology of B6C3F1 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 B6C3F1 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 B6C3F1 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, $P \leq 0.05$ (ANOVA, Dunnett).
** Significantly different from the control group, $P \leq 0.01$ (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) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]
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).

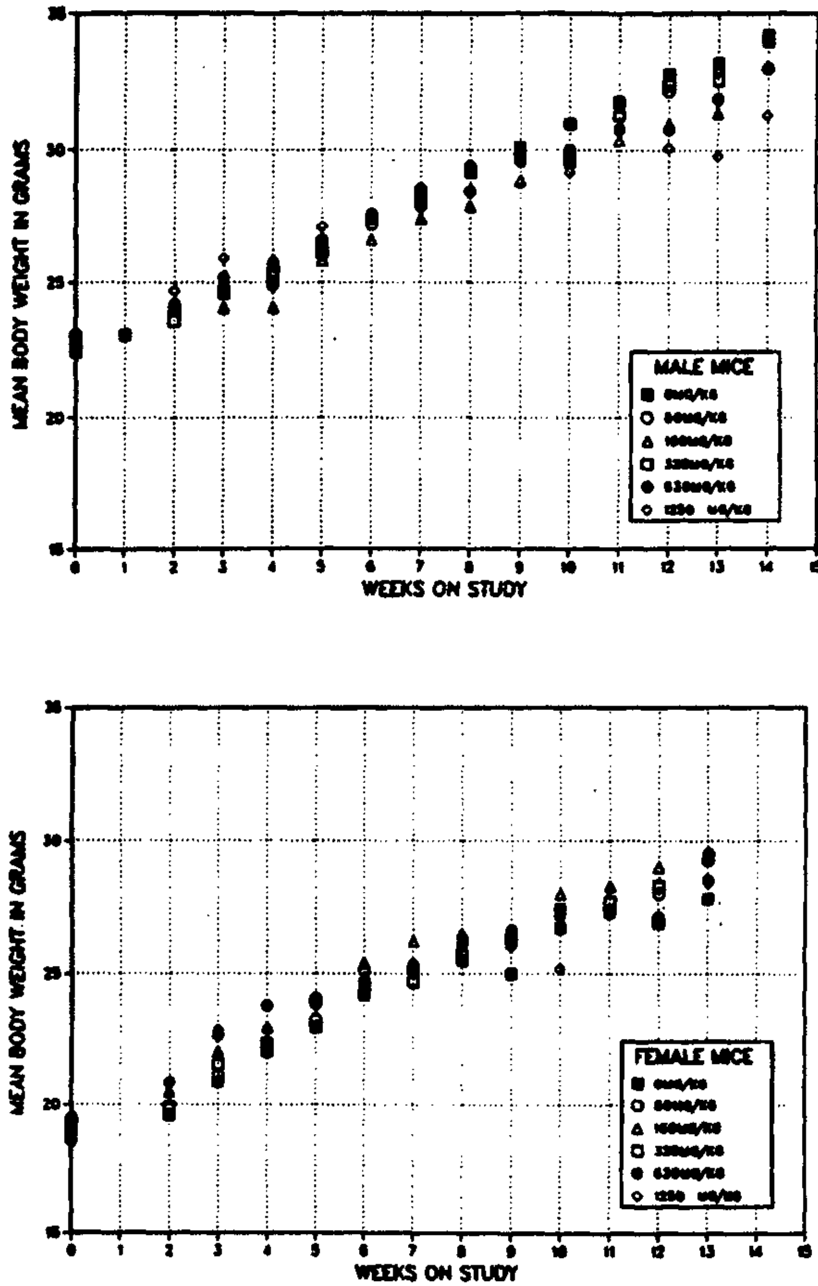


Figure 4 Body Weights of B6C3F$_1$ Mice Exposed Dermally to Diethanolamine for 13 Weeks

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 ($P \leq 0.05$) by Williams' or Dunnett's test.
** Significantly different from the control group ($P \leq 0.01$) by Williams' or Dunnett's test.
† Significantly different from the control group ($P \leq 0.05$) by Dunn's or Shirley's test.
‡ Significantly different from the control group ($P \leq 0.01$) 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).

APPENDIX D

Genetic Toxicology

Table D1 Mutagenicity of Diethanolamine in *Salmonella typhimurium* D-2

Table D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Diethanolamine D-3

Table D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Diethanolamine D-7

Table D4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Diethanolamine D-8

Table D5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Exposed Dermally for 13 Weeks to Diethanolamine D-9

TABLE D1 Mutagenicity of Diethanolamine in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2]					
		-S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	131 ± 6.7	124 ± 3.8	206 ± 7.2	198 ± 4.2	173 ± 16.0	163 ± 5.0
	33	143 ± 4.3	127 ± 6.6	211 ± 7.3	189 ± 7.5	203 ± 6.3	172 ± 14.2
	100	132 ± 5.3	130 ± 3.9	211 ± 12.3	194 ± 6.9	219 ± 11.0	160 ± 4.7
	333	141 ± 5.9	125 ± 6.1	239 ± 6.1	198 ± 15.6	216 ± 7.1	180 ± 13.0
	1,000	144 ± 3.2	121 ± 7.8	233 ± 1.8	178 ± 9.2	228 ± 15.4	153 ± 6.4
	3,333	122 ± 3.0	119 ± 3.7	208 ± 4.7	180 ± 12.9	203 ± 7.9	167 ± 12.9
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control[3]		527 ± 16.6	365 ± 3.5	808 ± 44.8	651 ± 112.2	442 ± 47.1	365 ± 15.9
TA1535	0	8 ± 1.7	8 ± 0.7	10 ± 1.2	10 ± 0.7	12 ± 2.3	12 ± 0.6
	33	11 ± 2.3	8 ± 1.7	15 ± 0.7	11 ± 2.3	14 ± 2.4	12 ± 1.2
	100	5 ± 0.7	6 ± 0.7	17 ± 2.2	13 ± 2.5	11 ± 2.1	9 ± 1.5
	333	9 ± 2.7	9 ± 0.3	14 ± 3.2	10 ± 1.9	19 ± 1.5	11 ± 4.0
	1,000	8 ± 1.9	8 ± 0.9	18 ± 2.7	13 ± 3.0	15 ± 2.1	12 ± 1.7
	3,333	9 ± 1.8	7 ± 0.9	19 ± 1.8	11 ± 4.7	17 ± 4.1	12 ± 2.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		380 ± 30.1	227 ± 11.8	40 ± 12.1	33 ± 7.7	30 ± 7.0	25 ± 4.4
TA1537	0	9 ± 1.5	8 ± 2.1	10 ± 2.0	8 ± 1.2	11 ± 1.5	9 ± 1.9
	33	7 ± 1.2	4 ± 1.2	10 ± 2.3	7 ± 0.7	14 ± 1.5	8 ± 1.5
	100	6 ± 0.3	5 ± 1.5	8 ± 2.9	6 ± 0.9	12 ± 1.5	8 ± 1.0
	333	4 ± 0.7	3 ± 0.9	9 ± 1.7	7 ± 0.6	14 ± 1.8	5 ± 3.0
	1,000	8 ± 0.3	5 ± 1.5	12 ± 2.2	7 ± 1.2	10 ± 1.2	7 ± 2.1
	3,333	6 ± 0.3	7 ± 2.5	12 ± 1.9	6 ± 1.5	8 ± 0.6	7 ± 0.7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		380 ± 78.5	119 ± 20.5	59 ± 5.5	65 ± 3.2	33 ± 4.1	25 ± 4.4
TA98	0	17 ± 2.5	16 ± 0.7	24 ± 5.0	35 ± 5.8	20 ± 3.8	30 ± 5.2
	33	14 ± 3.0	14 ± 0.9	21 ± 1.5	37 ± 3.1	27 ± 3.5	41 ± 2.9
	100	17 ± 1.5	18 ± 0.9	18 ± 2.6	38 ± 5.8	22 ± 3.7	36 ± 1.8
	333	17 ± 1.5	16 ± 1.7	17 ± 2.5	25 ± 0.3	24 ± 1.7	41 ± 2.6
	1,000	11 ± 2.7	20 ± 4.7	19 ± 0.6	33 ± 3.5	21 ± 2.0	45 ± 6.4
	3,333	15 ± 0.9	18 ± 2.1	20 ± 4.1	33 ± 3.0	20 ± 1.8	31 ± 1.8
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		600 ± 94.4	190 ± 1.5	812 ± 54.4	677 ± 103.6	303 ± 10.1	322 ± 54.7

[1] The detailed protocol and the data from Case Western Reserve University are presented in Haworth *et al.* (1983). Cells and diethanolamine or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose did not exceed 10 µg/plate; 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Diethanolamine[1]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol		92	91	66	24	
		75	80	51	23	
		96	108	65	23	
		113	121	84	25	24
Methyl methanesulfonate						
	5	55	27	578	352	
		48	42	582	403	
		48	28	472	329	361[3]
Diethanolamine (nL/mL)						
	25[4]	53	57	39	24	
		75	70	54	24	
		85	91	49	19	23
	50	93	90	44	16	
		66	92	33	17	
		98	85	47	16	16
	100	70	68	48	23	
		80	62	46	19	
		68	66	53	26	23
	200	66	35	59	30	
		75	42	50	22	
		61	43	31	17	23
	300	47	12	47	33	
		70	14	46	22	27
		Lethal				
	400	Lethal				
		Lethal				
		Lethal				

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Diethanolamine (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9						
Trial 2						
Ethanol		95	109	81	28	
		111	102	80	24	
		114	117	34	10	
		75	73	37	16	20
Methyl methanesulfonate						
	5	69	57	277	134	
		56	49	390	233	
		59	55	448	252	206[3]
Diethanolamine (nL/mL)						
	50[4]	89	91	66	25	
		109	86	87	27	
		100	108	83	28	26
	100	98	96	115	39	
		105	100	127	40	
		110	102	102	31	37[3]
	150	110	98	108	33	
		108	58	91	28	
		93	96	87	31	31[3]
	200	117	104	93	26	
		118	89	93	26	
		89	99	88	33	29
	300	96	78	74	26	
		85	64	67	26	
		116	59	81	23	25
	400	98	51	59	20	
		87	38	78	30	
		93	38	65	23	24
	600	Lethal				
		Lethal				
		Lethal				

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Diethanolamine (continued)

Compound	Concentration µg/mL	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9[5]						
Trial 1						
Ethanol		80	111	103	43	
		81	114	102	42	
		76	92	117	52	
		62	83	105	56	48
Methylcholanthrene						
	2 5	58	21	710	412	
		47	20	622	438	
		50	52	621	413	421[3]
Diethanolamine (nL/mL)						
	25	58	109	94	54	
		55	75	82	50	
		70	106	96	46	50
	50[4]	65	90	131	67	
		91	120	122	45	
		66	93	97	49	54
	100	64	91	104	54	
		80	114	130	54	
		74	108	120	54	54
	200	49	96	72	49	
		76	93	130	57	
		60	94	99	55	54
	300	56	86	94	56	
		70	99	92	44	
		73	90	101	46	49
	400	63	67	106	56	
		80	83	115	48	
		53	72	54	34	46
	600	Lethal				
		Lethal				
		Lethal				

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Diethanolamine (continued)

Compound	Concentration μg/mL	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 2						
Ethanol		76	96	120	53	
		85	117	68	27	
		68	95	92	45	
		72	91	83	38	41
Methylcholanthrene						
	2 5	60	63	651	363	
		74	61	716	323	
		62	68	482	260	315[3]
Diethanolamine (nL/mL)						
	100[4]	57	83	60	35	
		68	67	63	31	33
	200	72	62	66	31	
		62	69	72	39	
		81	78	72	30	33
	300	72	37	77	36	
		67	13	71	35	
		67	27	75	37	36
	400	Lethal				
		Lethal				
		Lethal				

[1] *Study performed at Litton Bionetics, Inc The experimental protocol is presented in detail by Myhr et al (1985)* All doses are tested in triplicate, the average of the three tests is presented in the table Cells (6×10^5/mL) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated)

[3] Significant positive response

[4] Basic pH shift at this concentration

[5] Tests conducted with metabolic activation were performed as described in [1] except that S9, prepared from the livers of Aroclor 1254-induced Fischer 344/N rats, was added at the same time as the diethanolamine and/or solvent

TABLE D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Diethanolamine[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary Negative								
Medium		50	1,049	465	0 44	9 3	26 5	
Mitomycin C	0 002	50	1,044	727	0 69	14 5	26 5	57 10
	0 010	10	207	319	1 54	31 9	26 5	247 66
Diethanolamine	150	50	1,046	459	0 43	9 2	26 5	1 01
	500[4]	50	1,046	487	0 46	9 7	26 5	5 03
	1 500	50	1 037	469	0 45	9 4	26 5	2 03
								P=0 277[5]
+S9[6]								
Trial 1								
Summary Negative								
Medium		50	1 006	518	0 51	10 4	25 5	
Cyclophosphamide	0 5	50	1 016	1 053	1 03	21 1	25 5	101 28
	2 5	10	201	592	2 94	59 2	25 5	472 00
Diethanolamine	150	50	1,018	505	0 49	10 1	25 5	3 66
	500	50	1,011	539	0 53	10 8	25 5	3 54
	1,500[4]	50	1 003	565	0 56	11 3	25 5	9 40
								P=0 038

[1] SCE = sister chromatid exchange, BrdU = bromodeoxyuridine Study performed at Bioassay Systems Corporation A detailed description of these data is presented by Loveday *et al*, 1989 Briefly, Chinese hamster ovary cells were incubated with diethanolamine or solvent (medium) as described in [3] and [6] below, and cultured for sufficient time to reach second metaphase division Cells were then collected by mitotic shake-off, fixed, air-dried, and stained

[2] SCEs/chromosome of culture exposed to diethanolamine relative to those of culture exposed to solvent

[3] In the absence of S9, cells were incubated with diethanolamine or solvent for 2 hours at 37° C Then BrdU was added and incubation was continued for 24 hours Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 5 hours

[4] Alkaline pH shift was noted at this concentration, for the culture with S9, the pH change was noted at the time of washing and addition of fresh medium, and pH returned to normal by the time of cell harvest

[5] Significance of relative SCEs/chromosome tested by the linear regression trend test vs log of the dose

[6] In the presence of S9, cells were incubated with diethanolamine or solvent for 2 hours at 37° C The cells were then washed, and medium containing BrdU was added Cells were incubated for a further 25 5 hours, with Colcemid present for the final 2 to 3 hours S9 was from the livers of Aroclor 1254 induced male Sprague Dawley rats

TABLE D4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Diethanolamine[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 Harvest time 10 5 hours					Trial 1 Harvest time 12 hours				
Summary Negative					Summary Negative				
Medium					Medium				
	100	1	0 01	1 0		100	3	0 03	2 0
Mitomycin C					Cyclophosphamide				
5	100	33	0 33	24 0	50	100	55	0 55	34 0
Diethanolamine					Diethanolamine				
101	100	1	0 01	1 0	303	100	1	0 01	1 0
505	100	0	0 00	0 0	1 010[4]	100	2	0 02	2 0
2 010[4]	100	2	0 02	2 0	3 010	100	8	0 08	7 0
				P=0 340[5]					P=0 019

[1] Study performed at Bioassay Systems Corporation A detailed description of these data is presented by Loveday *et al*, 1989 Briefly, Chinese hamster ovary cells were incubated with diethanolamine or solvent (medium) as indicated in [2] and [3] Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa

[2] In the absence of S9 cells were incubated with diethanolamine or solvent for 8 hours at 37 C Cells were then washed and fresh medium containing Colcemid was added for an additional 2 5 hours followed by harvest

[3] In the presence of S9 cells were incubated with diethanolamine or solvent for 2 hours at 37 C Cells were then washed, medium was added, and incubation was continued for 10 hours Colcemid was added for the last 2 to 3 hours of incubation before harvest S9 was from the livers of Aroclor 1254 induced male Sprague Dawley rats

[4] Alkaline pH at this concentration during chemical exposure

[5] Significance of percent cells with aberrations tested by linear regression trend test vs log of the dose

TABLE D5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Exposed Dermally for 13 Weeks to Diethanolamine[1]

Treatment (mg/kg)	% Normochromatic Erythocytes with Micronuclei[2]	Number of mice
MALE		
Control	0 14 ± 0 02	10
80	0 14 ± 0 02	10
160	0 14 ± 0 01	9
320	0 11 ± 0 01	9
630	0 11 ± 0 02	10
1,250	0 08 ± 0 01	8
FEMALE		
Control	0 08 ± 0 01	10
80	0 08 ± 0 01	10
160	0 08 ± 0 01	10
320	0 07 ± 0 01	10
630	0 07 ± 0 01	10
1,250	0 07 ± 0 01	5
Urethane[3]		
0 2%	1 87 ± 0 24	3

[1] Smears were prepared from peripheral blood samples obtained by cardiac puncture of dosed and control animals at the termination of the 13-week study Slides were stained with Hoechst 33258/pyronin Y (MacGregor *et al*, 1983) 10,000 normochromatic erythocytes from each animal were scored for micronuclei No significant elevation in the frequency of micronucleated erythrocytes was observed in either male or female mice following dermal administration of diethanolamine

[2] Values are mean ± standard error of the mean

[3] Positive control Three male mice were treated with urethane in drinking water, these animals were not part of the main 13 week study