FINAL

Report on Carcinogens Background Document for

Diethanolamine

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Prepared for the:
U.S. Department of Health and Human Services
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National Toxicology Program
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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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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.

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

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.

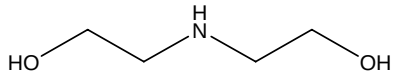


Figure 1-1. Structure of DEA

1.2 Physical-chemical properties

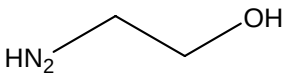
DEA melts at 28°C and is soluble in water, alcohol, ethanol, and benzene but insoluble in most other organic solvents. It is hygroscopic and reacts with the carbon dioxide in air. DEA is corrosive to copper, zinc, and galvanized iron. It reacts violently with oxidizers, strong acids, acid anhydrides, 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

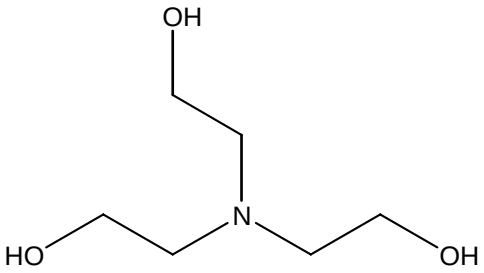
Property	Information	Reference
Molecular weight	105.14	ChemFinder 2001
Color	colorless to faintly colored	NTP 1999a
Odor	mild, ammonia-like	Budavari *et al.* 1996
Physical state	crystalline solid	Budavari *et al.* 1996
Melting point (ºC)	28	Lide 1999
Boiling point (ºC)	268.8	Lide 1999
Flash point (°C)	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 x 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.



Ethanolamine



Triethanolamine

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.]

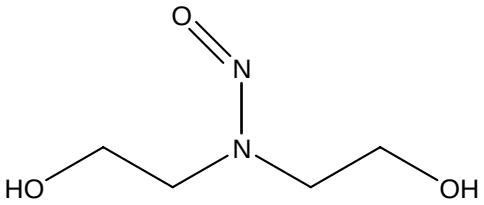


Figure 1-3. Structure of *N*-nitrosodiethanolamine

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) 0 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 *uvr*A)	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.

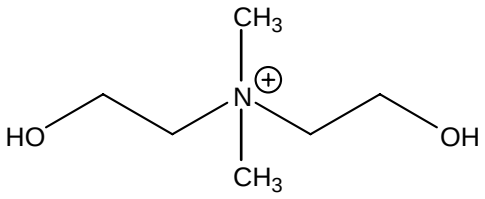
6 Other Relevant Data

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.

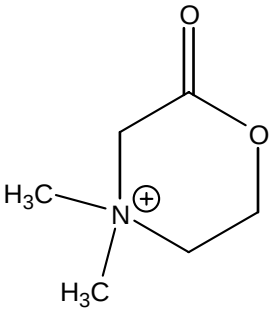
The IARC Working Group (2000) reviewed the literature on absorption, distribution, metabolism, and excretion of DEA and reported that dermal absorption occurred in both rats and mice. However, *in vitro* studies with full-thickness skin preparations from rats, mice, rabbits, and humans demonstrated that 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.

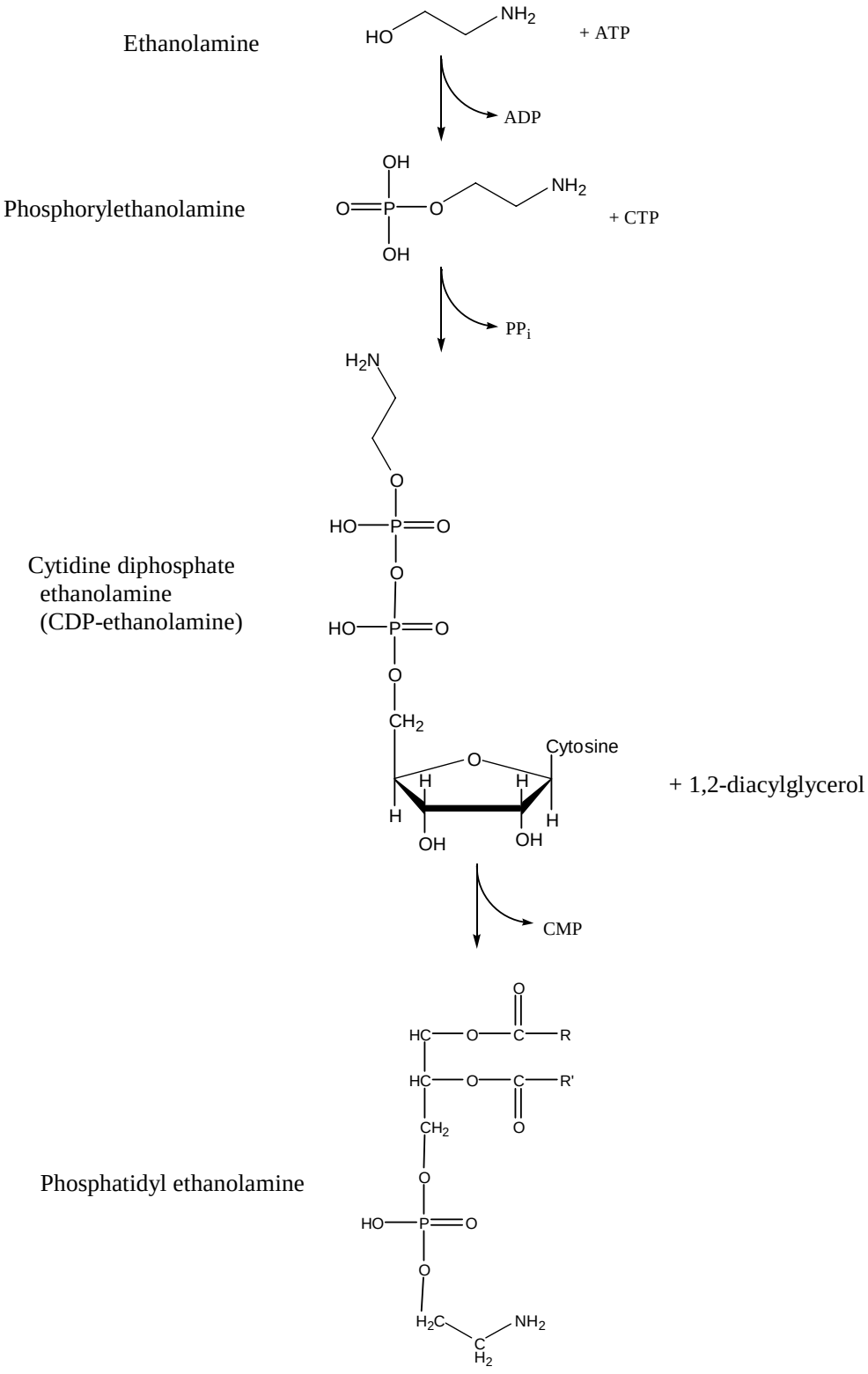


Figure 6-2. Phosphatidyl ethanolamine biosynthesis

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).]

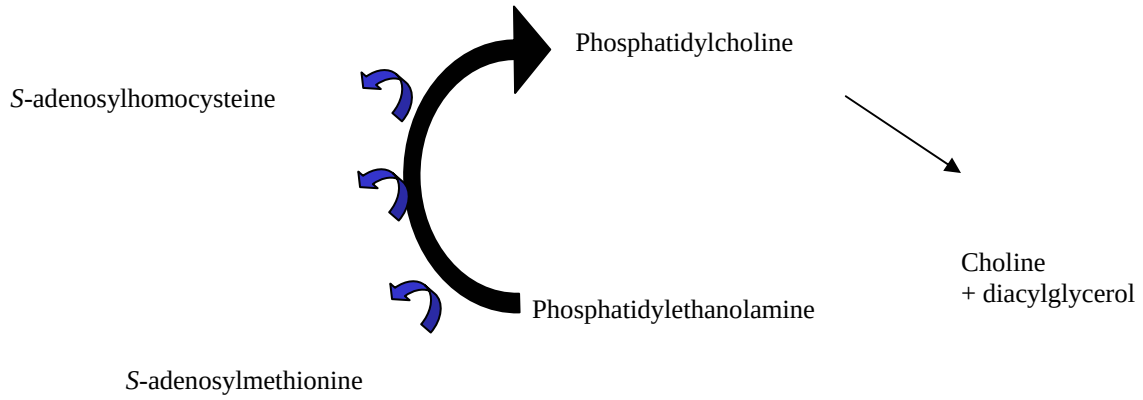


Figure 6-3. Synthesis of phosphatidyl choline from phosphatidylethanolamine and hydrolysis of phosphatidyl choline to choline and diacylglcyerol

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.

7 References

1. Balbaa, M., N. Abdel-Hady, F. El-Rashidy, L. Awad, E.-S.H. El-Ashry, and R.R. Schmidt. 1999. Inhibition of some hepatic lysosomal glycosidases by ethanolamines and phenyl 6-deoxy-6-(morpholin-4-yl)-ß-D-glucopyranoside. *Carbohydr Res* 317:100-109.

2. Barak, A.J., D.J. Tuma, and M.F. Sorrell. 1973. Relationship of ethanol to choline metabolism in the liver: a review. *Am J Clin Nutr* 26:1234-1241.

3. Barak, A.J., D.J. Tuma, and H.C. Beckenhauer. 1985. Ethanol, the choline requirement, methylation and liver injury. *Life Sci* 37:789-791.

4. Barbee, S.J. and R. Hartung. 1979a. Diethanolamine-induced alteration of hepatic mitochondrial function and structure. *Toxicol Appl Pharmacol* 47:431-440.

5. Barbee, S.J. and R. Hartung. 1979b. The effect of diethanolamine on hepatic and renal phospholipid metabolism in the rat. *Toxicol Appl Pharmacol* 47:421-430.

6. Bardin, J.A., E.A. Eisen, P.E. Tolbert, M.F. Hallock, S.K. Hammond, S.R. Woskie, T.J. Smith, and R.R. Monson. 1997. Mortality studies of machining fluid exposure in the automobile industry V: a case-control study of pancreatic cancer. *Am J Ind Med* 32:240-247.

7. Beyer, K.H., W.F. Bergfeld, W.O. Berndt, R.K. Boutwell, W.W. Carlton, D.K. Hoffmann, and A.L. Schroeter. 1983. Final report on safety assessment of triethanolamine, diethanolamine, and monoethanolamine. *J Am Coll Toxicol* 2:183-235.

8. Buchman, A.L., M.E. Ament, M. Sohel, M. Dubin, D.J. Jenden, M. Roch, H. Pownall, W. Farley, M. Awal, and C. Ahn. 2001. Choline deficiency causes reversible hepatic abnormalities in patients receiving parenteral nutrition: proof of a human choline requirement: a placebo-controlled trial. *J Parenter Enteral Nutr* 25:260-268.

9. Budavari, S., M.J. O'Neil, A. Smith, P.E. Heckelman, and J.F. Kinneary. 1996. The Merck Index. Merck & Co., Inc., Whitehouse Station, NJ.

10. ChemFinder. 2001. Cambridge Soft Corporation. Diethanolamine. Available at: (http://www.chemfinder.camsoft.com and search 111-42-2).

11. da Costa, K.A., E.F. Cochary, J.K. Blusztajn, S.C. Garner, and S.H. Zeisel. 1993. Accumulation of 1,2-*sn*-diradylglycerol with increased membrane-associated protein kinase C may be the mechanism for spontaneous hepatocarcinogenesis in choline-deficient rats. *J Biol Chem* 268:2100-2105.

12. da Costa, K.A., S.C. Garner, J. Chang, and S.H. Zeisel. 1995. Effects of prolonged (1 year) choline deficiency and subsequent re-feeding of choline on 1,2-*sn*-diradylglycerol, fatty acids and protein kinase C in rat liver. *Carcinogenesis* 16:327-334.

13. Dean, B.J., T.M. Brooks, G. Hodson-Walker, and D.H. Hutson. 1985. Genetic toxicology testing of 41 industrial chemicals. *Mutat Res* 153:57-77.

14. Driver, H.E. and A.E. McLean. 1986. Dose-response relationships for initiation of rat liver tumours by diethylnitrosamine and promotion by phenobarbitone or alcohol. *Food Chem Toxicol* 24:241-245.

15. Edens, M.R. and J.F. Lochary. 2000. Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc. Alkanolamines from olefin oxides and ammonia. Available at: (http://jws-edck.wiley.com:8095/articles/alkaeden.a01/sect15.html registered users only).

16. Eisen, E.A., P.E. Tolbert, R.R. Monson, and T.J. Smith. 1992. Mortality studies of machining fluid exposure in the automobile industry I: a standardized mortality ratio analysis. *Am J Ind Med* 22:809-824.

17. Eisen, E.A., P.E. Tolbert, M.F. Hallock, R.R. Monson, T.J. Smith, and S.R. Woskie. 1994. Mortality studies of machining fluid exposure in the automobile industry III: a case-control study of larynx cancer. *Am J Ind Med* 26:185-202.

18. Eisen, E.A., J. Bardin, R. Gore, S.R. Woskie, M.F. Hallock, and R.R. Monson. 2001a. Exposure-response models based on extended follow-up of a cohort mortality study in the automobile industry. *Scand J Work Environ Health* 27:240-249.

19. Eisen, E.A., T.J. Smith, D. Kriebel, S.R. Woskie, D.J. Myers, S.M. Kennedy, S. Shalat, and R.R. Monson. 2001b. Respiratory health of automobile workers and exposures to metal-working fluid aerosols: lung spirometry. *Am J Ind Med* 39:443-453.

20. Eustis, S.L., J.R. Hailey, G.A. Boorman, and J.K. Haseman. 1994. The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol Pathol* 22:457-472.

21. Fernandez, M., J. L'Haridon, L. Gauthier, and C. Zoll-Moreux. 1993. Amphibian micronucleus test(s): a simple and reliable method for evaluating in vivo genotoxic effects of freshwater pollutants and radiations. Initial assessment. *Mutat Res* 292:83-99.

22. Ghoshal, A.K. and E. Farber. 1984. The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. *Carcinogenesis* 5:1367-1370.

23. Ghoshal, A.K. and E. Farber. 1993. Choline deficiency, lipotrope deficiency and the development of liver disease including liver cancer: a new perspective. *Lab Invest* 68:255-260.

24. Goodridge, A.G. and H.S. Sul. 2000. Lipid metabolism-synthesis and oxidation. In Biochemical and Physiological Aspects of Human Nutrition. Stipanuk, M.H., ed. W.B. Saunders Company, Philadelphia, Pennsylvania. pp. 305-350.

25. Haseman, J.K., J.R. Hailey, and R.W. Morris. 1998. Spontaneous neoplasm incidences in Fischer 344 rats and $B6C3F_1$ mice in two-year carcinogenicity studies: a National Toxicology Program update. *Toxicol Pathol* 26:428-441.

26. Haworth, S., T. Lawlor, K. Mortelmans, W. Speck, and E. Zeiger. 1983. Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen* 5:1-142.

27. HSDB. 2000. Hazardous Substance Data Bank, National Library of Medicine. Revised February 8, 2000. Diethanolamine. Last reviewed November 7, 1991. Available at: (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search 111-42-2).

28. IARC. 1988. Alcohol Drinking. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 44. World Health Organization, Lyon, France.

29. IARC. 2000. Some Industrial Chemicals. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 77. World Health Organization, Lyon, France.

30. Inoue, K., T. Sunakawa, K. Okamoto, and Y. Tanaka. 1982. Mutagenicity tests and in vitro transformation assays on triethanolamine. *Mutat Res* 101:305-313.

31. Järvholm, B., B. Lavenius, and G. Sällsten. 1986. Cancer morbidity in workers exposed to cutting fluids containing nitrites and amines. *Br J Ind Med* 43:563-565.

32. Järvholm, B. and B. Lavenius. 1987. Mortality and cancer morbidity in workers exposed to cutting fluids. *Arch Environ Health* 42:361-366.

33. Kenyon, E.M., S.K. Hammond, J. Shatkin, S.R. Woskie, M.F. Hallock, and T.J. Smith. 1993. Ethanolamine exposures of workers using machining fluids in the automotive parts manufacturing industry. *Appl Occup Environ Hyg* 8:655-661.

34. Kerchaert, G.A., R. Brauninger, R.A. LeBoeuf, and R.J. Isfort. 1996. Use of the Syrian hamster embryo cell transformation assay for carcinogenicity prediction of chemicals currently being tested by the National Toxicology Program in rodent bioassays. *Environ Health Perspect* 104 Suppl 5:1075-1084.

35. Knaak, J.B., H.W. Leung, W.T. Stott, J. Busch, and J. Bilsky. 1997. Toxicology of mono-, di-, and triethanolamine. *Rev Environ Contam Toxicol* 149:1-86.

36. Kreiss, K. and J. Cox-Ganser. 1997. Metalworking fluid-associated hypersensitivity pneumonitis: a workshop summary. *Am J Ind Med* 32:423-432.

37. Lehman-McKeeman, L.D. and E.A. Gamsky. 1999. Diethanolamine inhibits choline uptake and phosphatidylcholine synthesis in Chinese hamster ovary cells. *Biochem Biophys Res Commun* 262:600-604.

38. Lehman-McKeeman, L.D. and E.A. Gamsky. 2000. Choline supplementation inhibits diethanolamine-induced morphological transformation in Syrian hamster embryo cells: evidence for a carcinogenic mechanism. *Toxicol Sci* 55:303-310.

39. L'Haridon, J., M. Fernandez, V. Ferrier, and J. Bellan. 1993. Evaluation of the genotoxicity of *N*-nitrosoatrazine, *N*-nitrosodiethanolamine and their precursors *in vivo* using the newt micronucleus test. *Water Res* 27:855-862.

40. Lide, D.R. 1999. CRC Handbook of Chemistry and Physics, 1999-2000. Lide, D.R. ed. CRC Press, Boca Raton.

41. Lombardi, B. and M.L. Smith. 1994. Tumorigenesis, protooncogene activation, and other gene abnormalities in methyl deficiency. *J Nutr Biochem* 5:2-9.

42. Loveday, K.S., M.H. Lugo, M.A. Resnick, B.E. Anderson, and E. Zeiger. 1989. Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. II. Results with 20 chemicals. *Environ Mol Mutagen* 13:60-94.

43. Mathews, J.M., C.E. Garner, and H.B. Matthews. 1995. Metabolism, bioaccumulation, and incorporation of diethanolamine into phospholipids. *Chem Res Toxicol* 8:625-633.

44. Mathews, J.M., C.E. Garner, S.L. Black, and H.B. Matthews. 1997. Diethanolamine absorption, metabolism and disposition in rat and mouse following oral, intravenous and dermal administration. *Xenobiotica* 27:733-746.

45. Melnick, R.L. and R.E. Tomaszewski. 1990. Diethanolamine. In Ethel Browning's Toxicity and Metabolism of Industrial Solvents, Vol II, Nitrogen and Phosphorus Solvents. Buhler, D.R. and D.J. Reed, eds., Elsevier, Amsterdam. pp. 401-410.

46. Melnick, R.L., J. Mahler, J.R. Bucher, M. Hejtmancik, A. Singer, and R.L. Persing. 1994a. Toxicity of diethanolamine. 2. Drinking water and topical application exposures in B6C3F_1 mice. *J Appl Toxicol* 14:11-19.

47. Melnick, R.L., J. Mahler, J.R. Bucher, M. Thompson, M. Hejtmancik, M.J. Ryan, and L.E. Mezza. 1994b. Toxicity of diethanolamine. 1. Drinking water and topical application exposures in F344 rats. *J Appl Toxicol* 14:1-9.

48. Mendrala, A.L., J.M. Waechter, G.A. Bormett, M.J. Bartels, and W.T. Stott. 2001. The pharmacokinetics of diethanolamine in Sprague-Dawley rats following intravenous administration. Food Chem Toxicol 39:931-939.

49. Mikol, Y.B., K.L. Hoover, D. Creasia, and L.A. Poirier. 1983. Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. *Carcinogenesis* 4:1619-1629.

50. Newberne, P.M. and A.E. Rogers. 1986. Labile methyl groups and the promotion of cancer. *Annu Rev Nutr* 6:407-432.

51. NIOSH. 1976. National Institute for Occupational Safety and Health. Revised 1997. Nitrosamines in industrial hygiene practices to minimize dermal and respiratory exposure to cutting fluids. Available at: (http://www.cdc.gov/niosh/78127_15.html).

52. NTP. 1992. NTP Technical Report on Toxicity Studies of Diethanolamine (CAS No. 111-42-2) Administered Topically and in Drinking Water to F344/N Rats and B6C3F1 Mice. National Toxicology Program TR No 20.

53. NTP. 1999a. Toxicology and Carcinogenesis Studies of Diethanolamine in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). National Toxicology Program Technical Report Series No 478.

54. NTP. 1999b. Toxicology and Carcinogenesis Studies of Coconut Oil Acid Diethanolamine Condensate (CAS No. 68603-42-9) in F344/N Rats and $B6C3F_1$ Mice (Dermal Studies). National Toxicology Program Technical Report Series No 479.

55. NTP. 1999c. Toxicology and Carcinogenesis Studies of Lauric Acid Diethanolamine Condensate (CAS No.120-40-1) in F344 Rats and $B6C3F_1$ Mice (Dermal Studies). National Toxicology Program Technical Report Series No 480.

56. NTP. 1999d. Toxicology and Carcinogensis Studies of Oleic Acid Diethanolamine Condensate in F344/N rats and $B6C3F_1$ mice. National Toxicology Program Technical Report Series No 481.

57. NTP. 2001. National Toxicology Program. NTP Chemical Repository. Revised August 13, 2001. Diethanolamine. Available at: (http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html, search 111-42-2, & select H&S:DIETHANOLAMINE 111-42-2).

58. OSHA. 1987. Occupational Safety & Health Administration, U.S. Department of Labor. Revised November 1987. Diethanolamine. Available at: (http://www.osha-slc.gov/dts/sltc/methods/partial/pv2018/2018.html).

59. Park, R.M., D.H. Wegman, M.A. Silverstein, N.A. Maizlish, and F.E. Mirer. 1988. Causes of death among workers in a bearing manufacturing plant. *Am J Ind Med* 13:569-580.

60. Park, R.M. and F.E. Mirer. 1996. A survey of mortality at two automotive engine manufacturing plants. *Am J Ind Med* 30:664-673.

61. Park, R.M. 2001. Mortality at an automotive engine foundry and machining complex. *J Occup Environ Med* 43:483-493.

62. Piipari, R., M. Tuppurainen, T. Tuomi, L. Mäntylä, M.L. Henriks-Eckerman, H. Keskinen, and H. Nordman. 1998. Diethanolamine-induced occupational asthma, a case report. *Clin Exp Allergy* 28:358-362.

63. Preussmann, R., B. Spiegelhalder, G. Eisenbrand, G. Würtele, and I. Hofmann. 1981. Urinary excretion of *N*-nitrosodiethanolamine in rats following its epicutaneous and intratracheal administration and its formation in vivo following skin application of diethanolamine. *Cancer Lett* 13:227-231.

64. Radike, M.J., K.L. Stemmer, and E. Bingham. 1981. Effect of ethanol on vinyl chloride carcinogenesis. *Environ Health Perspect* 41:59-62.

65. Rosenman, K.D., M.J. Reilly, and D. Kalinowski. 1997. Work-related asthma and respiratory symptoms among workers exposed to metal-working fluids. *Am J Ind Med* 32:325-331.

66. Rotimi, C., H. Austin, E. Delzell, C. Day, M. Macaluso, and Y. Honda. 1993. Retrospective follow-up study of foundry and engine plant workers. *Am J Ind Med* 24:485-498.

67. Schroeder, J.C., P.E. Tolbert, E.A. Eisen, R.R. Monson, M.F. Hallock, T.J. Smith, S.R. Woskie, S.K. Hammond, and D.K. Milton. 1997. Mortality studies of machining fluid exposure in the automobile industry IV: a case-control study of lung cancer. *Am J Ind Med* 31:525-533.

68. Shenkin, A. 2001. Adult micronutrient requirements. In Artificial Nutrition Support in Clinical Practice Second Edition. Payne-James, J., G.K. Grimble and D.B.A. Silk, eds. Greenwich Medical Media Limited, London. pp. 193-212.

69. Sidransky, H. and E. Farber. 1960. Liver choline oxidase activity in man and in several species of animals. *Arch Biochem Biophys* 87:120-133.

70. Silverstein, M., R. Park, M. Marmor, N. Maizlish, and F. Mirer. 1988. Mortality among bearing plant workers exposed to metalworking fluids and abrasives. *J Occup Med* 30:706-714.

71. Sorsa, M., L. Pyy, S. Salomaa, L. Nylund, and J.W. Yager. 1988. Biological and environmental monitoring of occupational exposure to cyclophosphamide in industry and hospitals. *Mutat Res* 204:465-479.

72. Spalding, J.W., J.E. French, S. Stasiewicz, M. Furedi-Machacek, F. Conner, R.R. Tice, and R.W. Tennant. 2000. Responses of transgenic mouse lines $p53^{+/-}$ and Tg·AC to agents tested in conventional carcinogenicity bioassays. *Toxicol Sci* 53:213-223.

73. Sprince, N.L., J.A. Palmer, W. Popendorf, P.S. Thorne, M.I. Selim, C. Zwerling, and E.R. Miller. 1996. Dermatitis among automobile production machine operators exposed to metal-working fluids. *Am J Ind Med* 30:421-429.

74. SRC. 2001. Revised August 23, 2001. Syracuse Research Corporation. Available at: (http://esc.syrres.com/interkow/webprop.exe?CAS=111-42-2).

75. Stott, W.T., M.J. Bartels, K.A. Brzak, M. Mar, D.A. Markham, C.M. Thornton, and S.H. Zeisel. 2000. Potential mechanisms of tumorigenic action of diethanolamine in mice. *Toxicol Lett* 114:67-75.

76. Sullivan, P.A., E.A. Eisen, S.R. Woskie, D. Kriebel, D.H. Wegman, M.F. Hallock, S.K. Hammond, P.E. Tolbert, T.J. Smith, and R.R. Monson. 1998. Mortality studies of metalworking fluid exposure in the automobile industry: VI. A case-control study of esophageal cancer. *Am J Ind Med* 34:36-48.

77. Sun, J.D., J.L. Beskitt, and M.J. Tallant. 1996. In vitro skin penetration of monoethanolamine and diethanolamine using excised skin from rats, mice, rabbits, and humans. *J Toxicol Cut Ocular Toxicol* 15:131-146.

78. Takada, A., J. Nei, S. Takase, and Y. Matsuda. 1986. Effects of ethanol on experimental hepatocarcinogenesis. *Hepatology* 6:65-72.

79. Tolbert, P.E., E.A. Eisen, L.J. Pothier, R.R. Monson, M.F. Hallock, and T.J. Smith. 1992. Mortality studies of machining-fluid exposure in the automobile industry. II. Risks associated with specific fluid types. *Scand J Work Environ Health* 18:351-360.

80. Zeisel, S.H. 1996. Choline. A nutrient that is involved in the regulation of cell proliferation, cell death and cell transformation. In Dietary Fats, Lipids, Hormones and Tumorigenesis. Hebver, D. and D. Kritchevsky, eds. Plenum Pres, New York. pp. 131-141.

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

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.

Appendix C: NTP (1992). NTP Technical Report on Toxicity Studies of Diethanolamine (CAS No. 111-42-2) Administered Topically and in Drinking Water to F344/N Rats and B6C3F$_1$ Mice. TR 20. pp 7 - 53 and D1 - D9.