DIETHANOLAMINE

Diethanolamine was considered by a previous IARC Working Group in 2000 (IARC, 2000). Since that time new data have become available, which have been incorporated to this *Monograph*, and taken into consideration in the present evaluation.

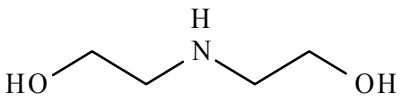
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

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

1.1.2 Structural and molecular formulae and relative molecular mass



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

1.1.3 Chemical and physical properties of the pure substance

Description: Deliquescent prisms; viscous liquid with a mild odour of ammonia (O'Neil *et al.*, 2006)
Boiling-point: 268.8 °C (O'Neil *et al.*, 2006)
Melting-point: 28 °C (O'Neil *et al.*, 2006)
Density: 1.0940 at 25 °C (O'Neil *et al.*, 2006)
Spectroscopy data: Infrared (proton [5830]; grating [33038]), nuclear magnetic resonance (proton [6575]; C-13 [2936]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide, 2000)
Solubility: Miscible with water, methanol, acetone, ethanol, chloroform and glycerine; soluble at 25 °C in benzene (4.2%), ether (0.8%), carbon tetrachloride (< 0.1%) and *n*-heptane (< 0.1%); slightly soluble to insoluble in petroleum ether (O'Neil *et al.*, 2006)
Vapour pressure: 0.00037 hPa at 25 °C (IUCLID, 2000)
Stability and reactivity: Stable at usual use temperatures; incompatible with some metals, halogenated organics, nitrites,

strong acids and strong oxidizers (Dow Chemical Company, 1999)
Octanol/water partition coefficient (P): log P, –1.43 (Sangster, 2006)
Conversion factor: $mg/m^3 = 4.30 \times ppm$ (calculated from: mg/m^3 = (relative molecular mass/24.45) ppm, assuming a temperature of 25 °C and a pressure of 101 kPa)

1.1.4 Technical products and impurities

Diethanolamine is commercially available with the following specifications: purity, 99% min.; monoethanolamine, 0.5% max.; triethanolamine 0.5% max.; and water content, 0.15% max. (Huntsman Corporation, 2008). A lower grade of diethanolamine is commercially available with the following specifications: purity, 55% min.; monoethanolamine, 5% max.; triethanolamine 40% max.; and water content, 1% max. (Elarum, 2010). In Europe, diethanolamine is typically marketed with the following specifications: purity, > 99%; triethanolamine, 1% max.; monoethanolamine, 0.5% max.; and water content, 0.2% max. (OECD, 2007).

Diethanolamine is also available as a blend of 83–87% diethanolamine and 13–17% deionized water with monoethanolamine and triethanolamine present as impurities at a maximum concentration of 1% (Huntsman Corporation, 2007).

1.1.5 Analysis

Diethanolamine can be determined in workplace air by drawing the air sample through aqueous hexanesulfonic acid and analysing with ion chromatography. The range for this method is 0.30–19.5 mg for a 100-L air sample (NIOSH, 2003).

Diethanolamine can be determined in air by drawing the air sample through sampling tubes containing XAD-2 resin coated with 10% 1-naphthylisothiocyanate. Samples are analysed by desorbing the adsorbent with dimethylformamide and quantitating the amine derivative by high performance liquid chromatography using ultraviolet detection (OSHA, 2010).

Exposure to diethanolamine from metal working fluids has been determined by high performance liquid chromatography/mass spectrometry analysis of aqueous hand-washing solutions and personal air samples collected on acid-treated glass fibre filters (Henriks-Eckerman *et al.*, 2007).

Levels of diethanolamine in shampoo products can be determined by liquid chromatography/thermal energy analysis after conversion to *N*-nitrosodiethanolamine with acetic acid and sodium nitrite (Chou, 2005).

1.2 Production and use

1.2.1 Production

Diethanolamine is produced by reacting ethylene oxide with ammonia. In most production facilities, ethylene oxide and ammonia are reacted in a batch process that yields a crude mixture of ethanolamine, diethanolamine and triethanolamine. The mixture is then distilled to separate and purify the individual compounds (Edens & Lochary, 2004).

Ethanolamines became available commercially in the early 1930s; they assumed steadily growing commercial importance as intermediates after 1945, because of the large-scale production of ethylene oxide. Since the mid-1970s, economical production of very pure, colourless ethanolamines has been possible (IARC, 2000).

It has been estimated that 45 900 and 75 400 tonnes of diethanolamine were produced in the USA in 1972 and 1983, respectively (HSDB, 2010). Estimated annual production of diethanolamine in the USA over three decades is presented in Table 1.1.

Worldwide production of ethanolamines in 1985 was approximately (thousand tonnes per

Table 1.1 Estimated annual production of diethanolamine in the USA (thousand tonnes)

Year	1960	1965	1970	1975	1980	1985	1989	1995
Production	24	35	42	39	56	76	92	149

From Edens & Lochary (2004)

year): USA, 220; western Europe, 145; south-eastern Asia, 40; South America, 18; and eastern Europe, 4. Of the world production of ethanolamines in 1985, approximately 50% was monoethanolamine, 30–35% diethanolamine and 15–20% triethanolamine (Hammer *et al.*, 1987).

The annual world capacity for the ethanolamines in 2005 was estimated at 1 510 000 tonnes, subdivided into 400 000 tonnes for Europe (eight production sites), 780 000 tonnes for North and South America (seven production sites), 30 000 tonnes for the Middle East (one production site) and 300 000 tonnes for the Asia/Pacific region (11 production sites). No data on individual capacities for diethanolamine were available (OECD, 2007).

Information available in 2010 indicated that diethanolamine was manufactured by 29 companies in the USA, seven companies in Mexico, three companies each in the People's Republic of China and the United Kingdom, two companies each in Canada, Germany, China (Hong Kong SAR) and India, and one company each in Belgium, Slovak Republic and Switzerland (Chemical Sources International, 2010). Other sources indicated that diethanolamine was produced by five companies in the USA (HSDB, 2010), five companies in Germany, three companies in the United Kingdom, three companies in the Netherlands and one company each in Austria, Belgium, Denmark and Sweden (IUCLID, 2000).

1.2.2 Use

Diethanolamine is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos and hair conditioners. Diethanolamine is also used in the production of lubricants in the textile industry, in industrial gas purification to remove acid gases and as an emulsifier and dispersing agent in preparations of agricultural chemicals. Diethanolamine is used in metalworking fluids for cutting, stamping and die-casting operations as a corrosion inhibitor. In the production of detergents, cleaners, fabric solvents and metalworking fluids, diethanolamine is used for acid neutralization and soil deposition. Aqueous diethanolamine solutions are used as solvents for numerous drugs that are administered intravenously. Shampoos and hair dyes may contain free diethanolamine as a component and/or as a contaminant of fatty acid alkanolamides, generally in the range of 0.2–10% (Bailey, 2007). Diethanolamine is used with sulfolane in the sulfinol process to absorb carbon dioxide and hydrogen sulfide gases (NTP, 1999a; Edens & Lochary, 2004; OECD, 2007, 2008).

Table 1.2 presents estimates of uses in major applications in the USA (Knaak *et al.*, 1997).

The database for substances in preparations in Nordic countries lists a wide variety of uses of diethanolamine registered in Denmark, Norway, Sweden and Finland. In 2004, 520 preparations containing diethanolamine, accounting for a total volume of 19 865.8 tonnes, were registered in Denmark. In Norway, Sweden, and Finland, 103 (856.8 tonnes), 307 (459.0 tonnes), and 75 (132.7 tonnes) products were registered, respectively. Use categories included intermediates, cleaning/washing agents, paints, lacquers and varnishes, surface treatments, cutting fluids, pH-regulation agents, impregnation materials, surface-active agents, corrosion inhibitors, process regulators, colouring agents, reprographic agents, lubricants and additives. Its use in consumer preparations was indicated for products registered in Norway and Sweden (SPIN, 2006; OECD, 2008).

Table 1.2 Major uses of diethanolamine in the USA

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

From Knaak *et al.* (1997)

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Diethanolamine is not known to occur as a natural product.

1.3.2 Occupational exposure

Diethanolamine is present in water-based machining and grinding fluids (soluble oils, semi-synthetic and synthetic metalworking fluids) and has been detected in workplace air in the metal manufacturing industry. It was detected in bulk metalworking fluids at levels ranging from 4 to 5% (Kenyon *et al.*, 1993). Recent exposure to diethanolamine can be inferred from studies showed dermal sensitivity among workers exposed to metalworking fluids (Geier *et al.*, 2004a, b). Moreover, the presence of *N*-nitrosodiethanolamine in bulk fluids and in the urine of exposed workers may provide indirect evidence for the exposure to diethanolamine from these fluids (Ducos & Gaudin, 2003).

According to the 1981–83 National Occupational Exposure Survey (NIOSH, 1999), 800 000 workers (many of whom were metalworkers) in the USA were potentially exposed to diethanolamine.

The median air concentration of diethanolamine in nine machine shops in Finland was found to be 64 μg/m^3 (Henriks-Eckerman *et al.*, 2007).

The presence of diethanolamine has also been reported in wetting fluids used in road paving. A level of 0.05 mg/m^3 was detected in a stationary sample at a slurry machine discharging a bitumen emulsion containing 0.2% of the amine. All personal exposures were below the limit of detection (0.02 mg/m^3) (Levin *et al.*, 1994). In a study in Germany (1992–94), diethanolamine was detected in samples of metalworking fluids at a range of 0–44% (n = 69). The proportion of samples in which diethanolamine was present steadily declined from 90 to 60% over the study period (Pfeiffer *et al.*, 1996).

In 1996, 51 samples of cooling lubricant concentrates from the German market were analysed. Of these, six (12%) showed diethanolamine concentrations of more than 0.2%, with a maximum concentration reaching 0.85%. The occurrence of diethanolamine levels above 0.2% in these concentrates declined from 80% (1991–92), to 53% (1993), 25% (1994), 21% (1995), and 12% (1996). The reduction was due to a change in the composition of the coolant fluids that followed regulatory requirements in Germany (see Section 1.4). The detected residues above 0.2% were not due to the direct addition of diethanolamine as an ingredient, but to contamination by other components in the coolant fluids (Kaup *et al.*, 1997).

At a site in Germany, diethanolamine is produced in one production plant and is processed further within eight other operations and

plants. Between January 2001 and December 2006, data on 53 workplace exposures covering all operations were collected by means of personal air sampling. The reported data are 8-hour time-weighted average (TWA) values for shifts. In the production plant, the highest value recorded was 0.026 mg/m^3; at the filling stations, the maximum value recorded was 0.062 mg/m^3; and the overall range of the measurements (53) was < 0.019–0.062 mg/m^3 (OECD, 2008).

1.3.3 Environmental occurrence

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

(a) Air

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

(b) Water

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

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

Diethanolamine was not detected in a study carried out in 1978 in any of the 21 samples taken from surface water in Japan (Japanese Department of Environmental Health, 1985). Diethanolamine was detected in German surface waters of the Rivers Elbe at 0.34–0.58 µg/L, Mulde at 2.54–4.6 µg/L, Neibe at 0.72–1.8 µg/L and Rhine at 0.30–0.59 µg/L (Pietsch *et al.*, 2001; OECD, 2008).

(c) Soil

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

1.3.4 Occurrence in personal care products

Free diethanolamine is reported to be a contaminant in fatty acid-diethanolamine condensates (amides of coconut oil acid, oleic acid and lauric acid) at levels ranging from < 1% to nearly 19%. Diethanolamine also occurs as a contaminant in triethanolamine products (see Table 1.3).

Potential exposure to diethanolamine in personal care products arises from the use of alkanolamides of diethanolamine, which are condensation products of diethanolamine and fatty acids (e.g. cocamide diethanolamine, a reaction product of diethanolamine and coconut oil-derived fatty acids). Cocamide diethanolamine, lauramide diethanolamine, linoleamide diethanolamine and oleamide diethanolamine are fatty acid diethanolamides that may contain 4–33% diethanolamine, and are present in cosmetics at concentrations of < 0.1–50% (Dea, 1986).

Table 1.3 Diethanolamine content of several condensates

Product	Diethanolamine content	Reference
Coconut oil acid diethanolamine condensate	18.2%	NTP (2001)
Lauric acid diethanolamine condensate	0.83%	NTP (1999a)
Oleic acid diethanolamine condensate	0.19%	NTP (1999c)
Triethanolamine	0.49%	NTP (1999d)

Twenty shampoo products were analysed and 19 were found to contain diethanolamine at levels ranging from 140 to 15 200 ppm (Chou, 2005). In a substudy to assess skin absorption, a commercially available body lotion was found to contain 1.8 mg/g diethanolamine (Craciunescu *et al.*, 2009). In a study of skin penetration, two representative shampoo formulations containing coconut diethanolamide at a concentration of 4% were found to contain 0.98% diethanolamine; two shampoos and a bubble bath containing 4.75% lauramide diethanolamine contained 0.25% diethanolamine; a leave-on emulsion containing 2% triethanolamine contained 0.008% diethanolamine; and an oxidative hair dye containing 4.7% lauramide diethanolamine contained 0.25% diethanolamine, while two other hair dye products containing 1.4% lauramide diethanolamine contained 0.075% diethanolamine (Brain *et al.*, 2005). In a study of the penetration of cosmetic products through intact human skin, a shampoo containing cocamide diethanolamine was found to include 0.092% free diethanolamine, and a second shampoo containing lauramide diethanolamine included 0.28% free diethanolamine (Kraeling *et al.*, 2004).

1.3.5 Detection in body fluids and daily exposure estimates

After about 3 or 4 weeks of using a body lotion containing 1.8 mg/g diethanolamine, plasma concentrations of the compound in three volunteer subjects ranged from 3 to 7 nmol/mL (Craciunescu *et al.*, 2009) [data were read from a graph].

Craciunescu *et al.* (2006) provided exposure estimates of 8–200 mg/kg per day from daily use of shampoo. An alternative calculation using a lower diethanolamine content in shampoo and lower skin penetration rates suggested that the exposure to diethanolamine for a 60-kg adult would be in the range of 0.2–2 µg/kg per day (Bailey, 2007). [The Working Group noted the large discrepancy in the estimated values between the two studies.]

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for diethanolamine are presented in Table 1.4.

The Food and Drug Administration (FDA) permits the use of diethanolamine as a component of adhesives in food packaging, as an indirect food additive, as a component of uncoated or coated food contact surfaces of paper and paperboard for use with dry solid foods with no free fat or oil on the surface, and for use only as an adjuvant to control pulp absorbance and pitch content in the manufacture of paper and paperboard or for use only in paper mill boilers in the USA (FDA, 2010).

A technical standard in Germany limits the level of diethanolamine in water-mixable cooling lubricants to 0.2% (Kaup *et al.*, 1997).

Table 1.4 Occupational exposure limits and guidelines for diethanolamine

Country	Year	Concentration (mg/m^3)	Interpretation
Argentina[a]	2007	11	TWA
Australia	2008	13	TWA
Belgium	2002	2 (sk)	TWA
Bulgaria[a]	2007	11	TWA
Columbia[a]	2007	11	TWA
Denmark	2002	2	TWA
France	2006	15	VME
Jordan[a]	2007	11	TWA
Netherlands	2003	2	TWA
New Zealand	2002	13 (sk)	TWA
Norway	1999	15	TWA
Republic of Korea	2006	15	TWA
Russian Federation	2003	5 (sk)	STEL
Singapore[a]	2007	11	TWA
Sweden	2005	15	TWA
		30 (sk)	STEL
Switzerland	2006	1	MAK-week
		1	KZG-week
USA			
ACGIH[a]	2007	2	TWA
NIOSH	1995	15	TWA
Viet Nam[a]	2007	11	TWA

[a] These countries follow the recommendations of the ACGIH threshold limit values.
ACGIH, American Conference of Governmental and Industrial Hygienists; KZG, Kurz Zeit Gedächtnis; MAK, Maximale Arbeitsplatz-Konzentration; NIOSH, National Institute of Occupational Safety and Health; sk, skin notation; STEL, short-term exposure limit; TWA, time-weighted average; VME, Valeur Moyenne d'Exposition
From RTECS (2009) and ACGIH (2010)

2. Cancer in Humans

The Working Group was not aware of any study that specifically examined the risk of cancer among persons exposed to diethanolamine. While diethanolamine is found in personal care products, no epidemiological studies evaluating human cancer in association with diethanolamine were identified. However, ethanolamines have been used as additives in metalworking fluids since the 1950s and are present in wetting fluids used in asphalt paving. Exposures to these agents occur as complex mixtures and there is a large database of studies on workers exposed in these occupational settings. In light of the complex mixtures, and concomitant occupational exposures, any observed elevations in risk cannot be specifically attributed to diethanolamine or to any other constituent of the complex mixtures. The Working Group, therefore, did not make a detailed evaluation of these studies. The data on metalworking fluids are reviewed below, although a formal evaluation by the Working Group is not provided.

There are four major types of metalworking fluid: straight (generally mineral oils), soluble and semi-synthetic (straight oils diluted with water and additives) and synthetic (water and additives with no oil). [Exposure assessments for soluble and synthetic are often combined for analysis.] Ethanolamines — either diethanolamine or triethanolamine — are common additives to

soluble, semi-synthetic and synthetic metalworking fluids (see Section 1). Diethanolamine may also be present as an unintended impurity of intended triethanolamine or fatty acid diethanolamide additives. Metalworking fluids are complex mixtures that may vary considerably, depending on the type of fluid and the additives used. These mixtures may contain many potential carcinogens and, in particular, potential exposure to *N*-nitrosodiethanolamine occurred in all of the studies considered. The use of diethanolamine and nitrites together as additives to metalworking fluids can lead to the formation of *N*-nitrosodiethanolamine. Therefore, workers in any study that noted exposure to *N*-nitrosodiethanolamine would also have been exposed to the diethanolamine from which the nitroso derivative was formed. In this review, only studies that included workers exposed to water-based (soluble, synthetic and semi-synthetic) metalworking fluids were included.

IARC (2000) previously reviewed several studies that examined the risk of cancer among workers potentially exposed to diethanolamine and *N*-nitrosodiethanolamine through metalworking fluids. Virtually all the cohorts described included workers with exposure to soluble, semi-synthetic or synthetic fluids. Only studies with potential exposure to ethanolamines (no studies indicated diethanolamine alone) were considered by the Working Group. The previous *IARC Monograph* (IARC, 2000) concluded that small excesses were observed for cancers at various sites, in particular the stomach, oesophagus and larynx. In those studies, only associations with the use of soluble oils or synthetic fluids were presented and no results were given specifically in relation to exposure to diethanolamine.

Studies reviewed previously included two proportionate mortality studies, two cohort studies and two nested case–control studies. The proportionate mortality studies included a study of workers employed at a bearing-manufacturing plant (Park *et al.*, 1988) and a study of workers employed at two large automotive engine manufacturing plants (Park & Mirer, 1996) in the USA. The cohort studies analysed the mortality of Swedish men employed in the grinding or turning departments of a company producing bearing rings (Järvholm *et al.*, 1986; Järvholm & Lavenius, 1987), and that of a large cohort of 46 384 workers employed in three facilities manufacturing automotive parts in the USA (Eisen *et al.*, 1992).

The later cohort (Eisen *et al.*, 1992) represents the most extensive database and the findings were reported in a series of publications. Exposure was assessed for all three types of metalworking fluid (straight, soluble and synthetic) (Eisen *et al.*, 1992; Tolbert *et al.*, 1992). Findings for the follow-up from 1940 to 1984 were reported by Eisen *et al.* (1992). Tolbert *et al.* (1992) reported the results of a cohort study of 33 619 persons who had worked for at least 3 years before 1985 in two of the three facilities studied by Eisen *et al.* (1992). Case–control studies nested among the members of the cohort studied by Eisen *et al.* (1992) were reported in Eisen *et al.* (1994) for laryngeal cancer and Sullivan *et al.* (1998) for oesophageal cancer.

The present Working Group examined results of studies of workers exposed to water-based metalworking fluids published since the previous review. These included an update of the major cohort of automobile workers in the USA (Eisen *et al.*, 1992) and a series of publications related to that cohort, an independent cohort study of workers in an engine plant (Kazerouni *et al.*, 2000) and a population-based case–control study of urinary bladder cancer (Colt *et al.*, 2011).

Eisen *et al.* (2001) extended the follow-up of the Eisen *et al.* (1992) cohort for an additional 10 years for 46 399 automobile manufacturing workers with potential exposure to metalworking fluids. In external analyses, significant excesses in risks were found for leukaemia and cancers of the liver, lung and stomach among white workers and pancreatic cancer in black

workers. In some circumstances, the risks were higher in the extended follow-up period than in the entire period of observation. In internal analyses using the unexposed workers as a reference group, workers with the highest cumulative exposure (mg/m^3-years) to grinding with soluble metalworking fluids (modelled as a categorical variable) had a significant increased risk for skin cancer and indications for an increased risk of cancer of the larynx, with some evidence of a trend (P = 0.065). When exposure to different types of metalworking fluids was modelled as a continuous variable, significant increases were observed for an exposure–response to synthetic metalworking fluids and liver cancer and exposure to soluble metalworking fluids and cancers of the skin and prostate. Excess risks of borderline significance were observed for other sites.

A series of analyses and subanalyses stemmed from the Eisen *et al.* (2001) cohort (Zeka *et al.*, 2004; Agalliu *et al.*, 2005a, b; Bardin *et al.*, 2005; Thompson *et al.*, 2005; Malloy *et al.*, 2007; Friesen *et al.*, 2009; Mehta *et al.*, 2010; Costello *et al.*, 2011). Each study assessed cumulative exposure to the three types of metalworking fluid (straight, soluble and synthetic) using a common quantitative exposure matrix. These studies used a variety of analytical methods (such as analysis of relevant biological time windows of exposure and latency) to evaluate risks at specific cancer sites. [An advantage of many of these studies is that they examined cancer incidence rather than mortality.] A few studies have also calculated risk estimates for exposure to specific components of metalworking fluids (i.e. ethanolalamines and nitrosamines).

Exposure to soluble metalworking fluids was associated with an elevated risk for cancers of the breast (Thompson *et al.*, 2005), prostate (Agalliu *et al.*, 2005a, b) and skin (melanoma) (Costello *et al.*, 2011) but not with cancers of the urinary bladder (Friesen *et al.*, 2009), lung (Friesen *et al.*, 2009), larynx, oesophagus, stomach (Zeka *et al.*, 2010), rectum (Malloy *et al.*, 2007) or hepatobiliary tract (Bardin *et al.*, 2005).

None of the studies found a statistically significant association with exposure to synthetic metalworking fluids. A marginally significant inverse relationship for lung cancer was observed for exposure to synthetic fluids (Mehta *et al.*, 2010). Significant elevated risks for several cancer sites were associated with exposure to straight metalworking fluids. [The Working Group noted the complexity of the exposure assessment because workers were often exposed to multiple types of metalworking fluid, the types of fluids had changed over time (the use of water-based fluids has increased) and workers often changed jobs.]

Kazerouni *et al.* (2000) updated the mortality experience of 11 838 workers (Decoufle, 1976, 1978) exposed to a variety of metalworking fluids, including cutting-oil mist. [The Working Group inferred that the description of metalworking fluid defined by the authors included straight, soluble, synthetic and semi-synthetic types.] Exposures were qualitatively categorized as 'heavy, moderate or low' based on job history. Among workers exposed to oil mist, significantly (or of borderline significance) elevated standardized mortality ratios were observed for cancers of the liver and biliary tract, testis and lung and Hodgkin disease; the magnitude of effect was higher among heavily exposed workers. Among workers with heavy exposure to oil mists, mortality from lung cancer was higher for those with ≥ 5 years of exposure compared with those with < 5 years of exposure [although the confidence intervals overlapped].

Colt *et al.* (2011) conducted a large, population-based case–control study in the USA of 1158 incident urinary bladder cancer cases and 1402 population controls. Men reporting use of metalworking fluids at a personal interview had a significantly elevated risk for urinary bladder cancer after adjusting for demographic factors, tobacco smoking and employment in

other high-risk occupations. [The Working Group noted that the type of metalworking fluid was not specified and exposure was based on self-reporting.]

[The Working Group noted that the mixed and varied exposures may explain the variability of the results of the different studies and also make the attribution of excesses of cancer observed to any single agent very difficult. It is probable that most of the cohorts studied included workers exposed to water-reduced metalworking fluids, who were probably exposed to diethanolamine by skin penetration and inhalation. Because the exposures reviewed here occur were to complex mixtures, the metalworking fluid environment might be evaluated better as an exposure circumstance.]

3. Cancer in Experimental Animals

3.1 Skin application

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F$_1$ mice, 6 weeks of age, received dermal applications of 0, 40, 80 or 160 mg/kg body weight (bw) diethanolamine (purity, > 99%) in 95% ethanol on 5 days per week for 103 weeks. Survival of treated males was similar to that of vehicle controls, but that of treated females was significantly reduced (44/50, 33/50, 33/50 and 23/50 for the control, low-, mid- and high-dose groups, respectively). The mean body weights of the mid- and high-dose males were lower than those of the vehicle controls after weeks 88 and 77, respectively. The mean body weights of the low- and mid-dose females were lower than those of the vehicle controls from week 73, but those of the high-dose females were reduced compared with the vehicle controls from week 53. In male mice, the incidence of hepatocellular adenoma, hepatocellular carcinoma and of hepatocellular adenoma and carcinoma (combined) in all dosed groups was significantly greater than that in the vehicle-control group (hepatocellular adenoma: 31/50, 42/50, 49/50 and 45/50 ($P < 0.001$, poly-3 trend test); hepatocellular carcinoma: 12/50, 17/50, 33/50 and 34/50 ($P < 0.001$, poly-3 trend test), for the control, low-, mid- and high-dose groups, respectively). In addition, the incidence of hepatoblastoma in the mid- and high-dose groups was significantly increased compared with vehicle controls (0/50, 2/50, 8/50 ($P = 0.004$, pairwise comparison) and 5/50 ($P = 0.028$, pairwise comparison) in the control, low-, mid- and high-dose groups, respectively). In female mice, the incidence of hepatocellular adenoma and carcinoma was significantly higher than that in the vehicle controls (hepatocellular adenoma: 32/50, 50/50, 48/50 and 48/50 ($P < 0.001$, poly-3 trend test); hepatocellular carcinoma: 5/50, 19/50, 38/50 and 42/50 ($P < 0.001$, poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). The incidence of renal tubule adenoma in males showed an increase after standard single-section examination (1/50, 4/50, 6/50 and 6/50 ($P = 0.05$, poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). When single sectioning and extended-step sectioning were combined, the incidence was: 1/50, 6/50, 8/50 and 7/50 ($P = 0.046$, poly-3 trend test) for the control, low-, mid- and high-dose groups, respectively (NTP, 1999b; Table 3.1).

[The Working Group noted that tumours of the kidney and hepatoblastomas are rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats, 6 weeks of age, received dermal applications of diethanolamine (purity, > 99%) in 95% ethanol on 5 days per week for 103 weeks. Males received 0, 16, 32 or 64 and females received 0, 8, 16 or 32 mg/kg bw. Survival rates for treated males and females were similar to those of corresponding

Table 3.1 Carcinogenicity studies of exposure to diethanolamine in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 105 wk NTP (1999b)	Dermal application 0, 40, 80 or 160 mg/kg bw (M, F), 5x/wk, 103 wk 50/group rats	Liver (hepatocellular adenoma): M–31/50 (62%), 42/50 (84%), 49/50 (98%), 45/50 (90%) F–32/50 (64%), 50/50 (100%), 48/50 (96%), 48/50 (96%)	$P > 0.001$ (trend in M, F) (poly-3 test)	> 99% pure
		Liver (hepatocellular carcinoma): M–12/50 (24%), 17/50 (34%), 33/50 (66%), 34/50 (68%) F–5/50 (10%), 19/50 (38%), 38/50 (76%), 42/50 (84%)	$P > 0.001$ (trend in M, F) (poly-3 test)	
		Liver (hepatoblastoma): M–0/50, 2/50 (4%), 8/50 (16%), 5/50 (10%)	$P = 0.004$ (mid-dose M) $P = 0.028$ (high-dose M)	
		Kidney (renal tubule adenoma, standard evaluation): M–1/50 (2%), 4/50 (8%), 6/50 (12%), 6/50 (12%)	$P = 0.049$ (trend in M) (poly-3 test)	
		Kidney (renal tubule adenoma, standard + extended evaluations): M–1/50 (2%), 6/50 (12%), 8/50 (16%), 7/50 (14%)	$P = 0.046$ (trend in M) (poly-3 test)	

bw, body weight; F, female; M, male; wk, week or weeks

vehicle-control groups. The mean body weight of the high-dose males was lower than that of the vehicle controls from week 8 and that of the high-dose females was lower than that of the vehicle controls from week 97. There was no increase in tumour incidence in treated groups compared with the vehicle controls (NTP, 1999b).

3.2 Genetically modified mouse

Groups of 15–20 female Tg.AC mice, which carry a zeta-globin promoted v-Ha-*ras* gene on an FVB background, 14 weeks of age, received dermal applications of diethanolamine in 95% ethanol. The diethanolamine used was from the same chemical batch as that used in the study in $B6C3F_1$ mice (NTP, 1999b). The diethanolamine was administered in 200-µL volumes, five times a week for 20 weeks. The concurrent negative-control groups were treated with 200 µL 95% ethanol. The positive-control group was treated with 1.25 µg 12-*O*-tetradecanoylphorbol 13-acetate (approximately 99% pure) twice a week for 20 weeks. The doses of diethanolamine selected were based on the maximum tolerated dose used earlier (NTP, 1999b) and were 5, 10 or 20 mg/mouse per application (higher than the maximum tolerated dose). Survival was high in both the control (90%) and treated groups (80–95%). Lesions were diagnosed as papillomas when they reached at least 1 mm in diameter and persisted for 3 weeks. Animals that did not survive to the end of week 10 were not included in the data summaries or calculations. Six weeks after the last application, all surviving mice were killed. There was no evidence of chronic irritation or ulceration at the site of application. In contrast to the positive controls, 18/20 of which developed multiple papillomas, there was no increase in the incidence of skin tumours in diethanolamine-treated animals in this model (Spalding *et al.*, 2000).

[The Working Group was aware of three carcinogenicity bioassays (by dermal application studies) in $B6C3F_1$ mice and F344/N rats of fatty acid-diethanolamine (coconut oil acid, lauric acid and oleic acid diethanolamine) condensates conducted by the National Toxicology Program (NTP, 1999c, d, 2001). The same three condensates were also tested in the transgenic Tg.AC and *Tp53*$^{+/-}$ mouse models (Spalding *et al.*, 2000). The Working Group concluded that these studies could not be used in an evaluation of the carcinogenicity of diethanolamine per se. This judgement was based on the fact that the substances tested were complex mixtures of imprecise composition, that the actual diethanolamine content had not been measured in any of the three studies and therefore the precise levels of exposure were indeterminable, and that these studies were not designed as, and did not represent, conventional or adequate carcinogenesis bioassays of diethanolamine.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

Studies of the penetration of [^{14}C]diethanolamine from cosmetic formulations (shampoos hair dyes and body lotions) through human skin samples indicated that approximately 0.1% of the applied dose of shampoo and hair dye formulations was absorbed into the receptor fluid after 5–30 minutes; in a 72-hour repeated-dose study with a body lotion formulation, nearly 30% of applied diethanolamine accumulated in the skin and approximately 1% was absorbed into the receptor fluid (Kraeling *et al.*, 2004). A subsequent study found similar levels of permeation of diethanolamine from cosmetic formulations through human skin (Brain *et al.*, 2005). [These studies did not address the permeability of diethanolamine through the skin of young

children, the effects of elevated temperatures associated with bathing or showering, or the effect of abrasions that alter skin integrity on the dermal absorption of diethanolamine.]

Diethanolamine was absorbed by human liver slices and incorporated into phospholipids (Mathews *et al.*, 1995).

4.1.2 Experimental systems

The absorption and distribution of dermally applied [^{14}C]diethanolamine in 95% ethanol was characterized in F344 rats and B6C3F$_1$ mice (Mathews *et al.*, 1997; IARC, 2000). In both species, the percentage absorbed 48 hours after a single administration increased with increasing dose (rats, 2.1–27.5 mg/kg bw; mice, 8–81 mg/kg bw). At comparable dose levels, the percentage absorbed in mice (30–40%) was about 2.5 times higher than that in rats (10–20%). In contrast to carcinogenicity studies, the studies of disposition of diethanolamine prevented oral exposure through grooming activities by gluing a wire mesh over the area of skin application. Tissue/blood ratios of [^{14}C] were substantially greater than 1 for all tissues examined (adipose, brain, heart, kidney, liver, lung, muscle, skin and spleen), with the greatest accumulation (tissue/blood > 100) in the liver and kidney of rats and mice. [The 2.5-fold increase in absorption of diethanolamine in mice compared with rats does not appear to account for the differential liver tumour response in these species: 100% incidence in mice exposed to 40 mg/kg or higher and the lack of a liver tumour response in rats exposed to up to 64 mg/kg bw.]

Diethanolamine was well absorbed in rats after oral exposure. Forty-eight hours after a single oral dose of 7 mg/kg bw [^{14}C]diethanolamine, 22% of the recovered dose was excreted in the urine and 60% of the dose remained in the tissues (Mathews *et al.*, 1997). The tissue distribution of [^{14}C] was similar after oral, intravenous and dermal administration. After daily oral administration of 7 mg/kg bw [^{14}C]diethanolamine to rats, diethanolamine equivalents accumulated in the tissues at high concentrations during 4–8 weeks of repeated treatment, and reached a level of 0.3 mg/g tissue in the liver. In a comparative study of the penetration of [^{14}C] diethanolamine through excised skin samples obtained from multiple species, the permeability rate constant for an aqueous solution of diethanolamine (37% w/w) through mouse skin was approximately 10 times higher than that through rat skin and about 20 times higher than that through human skin (Sun *et al.*, 1996).

After a single intravenous or oral administration of diethanolamine, rats predominantly excreted the parent compound in the urine (Mathews *et al.*, 1997); after repeated oral administration, the parent compound was still the major product excreted in the urine but *N*-methylated metabolites were also detected. The parent compound also accounted for the majority of radioactivity extracted from the liver and brain of rats administered [^{14}C]diethanolamine; two minor metabolites identified in tissues were *N*-methyldiethanolamine and *N,N*-dimethyldiethanolamine (Mathews *et al.*, 1995).

Diethanolamine is known to be incorporated into membrane phospholipids (Artom *et al.*, 1949; Barbee & Hartung, 1979; IARC, 2000). It can be *O*-phosphorylated and *N*-methylated to metabolites that are incorporated into polar head groups as aberrant membrane phospholipids (phosphoglyceride and sphingomyelin analogues) via the ethanolamine metabolic pathway (Mathews *et al.*, 1995). Following repeated exposure of rats to diethanolamine, the extent of methylation and accumulation of aberrant sphingomylenoid lipids in tissues increased. *N*-Nitrosodiethanolamine, a hepatocarcinogen, was not detected in the urine, blood or gastric contents of B6C3F$_1$ mice that were administered 160 mg/kg per day diethanolamine by dermal application or oral gavage with or without 140

ppm (≈40 mg/kg per day) sodium nitrite in their drinking-water for 2 weeks (Stott *et al.*, 2000). Thus, liver tumour induction in mice is unlikely to be due to nitrosation of diethanolamine to this mutagenic nitrosamine.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Toxicology studies of diethanolamine were conducted in male and female F344 rats and B6C3F$_1$ mice for 13 weeks by administration in the drinking-water (160–5000 ppm, ≈equivalent to daily doses of 15–440 mg/kg bw for rats; and 630–10 000 ppm, ≈equivalent to daily doses of 100–1700 mg/kg bw for mice) and by topical application (five times a week at daily doses of 32–500 mg/kg bw in rats and 80–1250 mg/kg bw in mice) (Melnick *et al.*, 1994a, b). In both species, diethanolamine induced dose-dependent toxic effects at multiple organ sites. In rats, induced toxic responses were observed in the bone marrow (poorly regenerative microcytic anaemia), kidney (increased weight, tubular necrosis, decreased renal function, and tubular mineralization), brain and spinal cord (demyelination), testis (seminiferous tubule degeneration) and skin (ulceration, inflammation, hyperkeratosis and acanthosis at site of application). In mice, diethanolamine induced toxicity in the liver (hepatocellular cytological alterations and single-cell necrosis), kidney (nephropathy and tubular epithelial necrosis in males), heart (cardiac myocyte degeneration) and skin (ulceration, inflammation, hyperkeratosis and acanthosis at site of application). Minimal to mild cytological alterations in the liver of mice comprised multiple hepatocyte changes, including enlarged cells that were frequently multinucleated, increased nuclear pleomorphism, and increased eosinophilia; these changes were observed in all treated groups of male and female mice, except for females that received topical applications of 80 mg/kg bw. No liver lesions were observed in rats exposed to diethanolamine by either route. The mechanism of diethanolamine toxicity at multiple organ sites in rats and mice is unknown, but may be related to its high tissue accumulation and effects on phospholipid metabolism resulting in alterations in membrane structure and function (Melnick *et al.*, 1994a, b).

4.3 Genetic and related effects

4.3.1 Experimental systems

The genetic toxicology of diethanolamine has been reviewed (IARC, 2000). Diethanolamine was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538, or TA98, in *Escherichia coli* WP2 uvrA, or in L5178Y mouse lymphoma cells incubated in the presence or absence of metabolic activation systems. It did not induce sister chromatid exchange or chromosomal aberrations in cultured Chinese hamster ovary cells or mitotic gene conversion in *Saccharomyces cerevisiae*. Diethanolamine did not increase the frequency of micronuclei in peripheral erythrocytes of B6C3F$_1$ mice exposed to 80–1250 mg/kg by dermal application for 13 weeks (NTP, 1999b).

Diethanolamine (10–500 μg/mL, 0.1–5.0 mM) induced morphological transformation in Syrian hamster embryo (SHE) cells cultured for 7 days in media containing 28 μM [3 μg/mL] choline; however, this effect was prevented by supplementation of the medium with excess choline (30 mM [3.125 mg/mL]) (Lehman-McKeeman & Gamsky, 2000). Diethanolamine also inhibited choline uptake and decreased phosphatidylcholine synthesis by these cells. The latter changes were also prevented by supplementation of the medium with 30 mM choline.

Based on these findings, the authors suggested that diethanolamine-induced morphological transformation of SHE cells was due to inhibition of choline uptake. [Because the high level of choline supplementation may have blocked diethanolamine uptake by the cells and any subsequent cellular effects of this agent, a study of morphological transformation in SHE cells by choline deficiency would help to determine whether reduced choline uptake alone was sufficient to induce cell transformation.]

Primary cultures of hepatocytes isolated from B6C3F$_1$ mice were grown in the presence of diethanolamine (4.5 mM [473 mg/mL]) or in choline-deficient medium (0.86 µM, 0.09 mg/L) for 48 hours and evaluated for DNA methylation status in GC-rich regions (Bachman *et al.*, 2006). Both diethanolamine and choline-deficient treatments resulted in 54 regions of altered methylation, of which 43 and 49 regions were hypomethylations, respectively, and only one hypermethylation with each treatment. Based on the size of polymerase chain reaction products of methylation-sensitive restriction digests, the authors concluded that 39 of the 54 regions of altered methylation (72%) were similar after both diethanolamine and choline-deficient treatments. Thus, the authors suggested that, by inhibiting choline uptake into cells, diethanolamine may decrease the supply of *S*-adenosyl methionine (SAM), the main methyl donor for many methylation reactions, leading to hypomethylations in promoter regions of genes and consequent alterations in gene expression.

Four-day-old *Drosophila melanogaster* females were given 4% sucrose solutions containing 5, 10, 20, 40 or 80% diethanolamine for 24 hours; after a 2-hour recovery period, they were mated with 7-day-old males (Muñoz & Barnett, 2003). The frequencies of meiotic non-disjunction in *Drosophila* oocytes were analysed in the progeny from three successive 24-hour broods. Diethanolamine induced similar increases in the frequencies of female non-disjunction (chromosome malsegregation) at all concentrations. The authors suggested that the induction of aneupolidy may be a genotoxic effect of diethanolamine.

DNA was isolated from sections of liver tumours obtained in the 2-year dermal application study of diethanolamine in B6C3F$_1$ mice (NTP, 1999b) and analysed for genetic alterations in β-catenin *Catnb* and H-*ras* genes (Hayashi *et al.*, 2003). *Catnb* encodes β-catenin protein, which is involved in cell–cell adhesion and Wnt signal transduction. Genetic alterations in exon 2 of the latter gene included deletion mutations and point mutations that occurred at much higher frequencies in liver neoplasms from diethanolamine-exposed mice compared with controls. The frequency of *Catnb* mutations was 100% in diethanolamine-induced hepatoblastomas, 32% in hepatocellular neoplasms from mice exposed to diethanolamine, and 10% in control hepatocellular neoplasms. These findings indicate the occurrence of in-vivo mutagenesis in diethanolamine-induced tumours. Hepatocellular neoplasms obtained from mice exposed to diethanolamine lacked H-*ras* codon 61 mutations; this is in sharp contrast to spontaneous liver tumours in this strain of mice, in which the frequency of H-*ras* mutations is slightly higher than 50%.

4.4 Mechanistic data

4.4.1 Hepatic choline deficiency

Diethanolamine induces hepatic choline deficiency in mice (Lehman-McKeeman *et al.*, 2002), probably due to the inhibition of choline uptake (Lehman-McKeeman & Gamsky, 2000). Application of diethanolamine in 95% ethanol to the skin of B6C3F$_1$ mice at doses of 0, 20, 40, 80 or 160 mg/kg bw on 5 days a week for 4 weeks caused decreases in liver concentrations of choline, phosphocholine, phosphatidylcholine, glycerophosphocholine and SAM, while levels of *S*-adenosyl homocysteine were increased.

Phosphocholine, which is the intracellular storage form of choline, was most sensitive to treatment with diethanolamine. These metabolic changes were qualitatively similar to those caused by feeding diets deficient in choline to mice (Lehman-McKeeman *et al.*, 2002) or rats (Pomfret *et al.*, 1990) for 2 weeks. Based on these changes and earlier studies that observed the induction of liver neoplasms in rats and mice fed choline-deficient diets (Newberne *et al.*, 1982), the authors suggested that diethanolamine-induced hepatocarcinogenicity is due to the induction of choline deficiency and the consequent reduction in hepatic levels of SAM (a methyl donor for methyltransferases); the latter change can lead to DNA hypomethylation and altered gene expression. However, in the study by Lehman-McKeeman *et al.* (2002), there was no effect on hepatic levels of SAM in mice treated with 40 mg/kg bw, a dose that produced a significant increase in the incidence of hepatocellular neoplasms in male and female mice in a carcinogenicity study (NTP, 1999b). Liver choline levels have not been measured in mouse kidney or in rat liver.

4.4.2 Cell proliferation

Dermal exposure of B6C3F$_1$ mice to diethanolamine in 95% ethanol at daily doses of 0, 20, 40, 80 or 160 mg/kg bw was reported to increase hepatocyte proliferation (assessed by the 7-day 5-bromo-2′-deoxyuridine (BrdU) labelling index) over 13 weeks of treatment without affecting the number of apoptotic cells (Mellert *et al.*, 2004), but there was no clear dose–response in the liver. Because diethanolamine is known to cause syncytia formation (polyploidization; enlarged, multinucleated hepatocytes) in mice (Melnick *et al.*, 1994a), an increase in the hepatocyte labelling index, which is a measure of DNA synthesis, may not reflect a true increase in hepatocyte number.

The BrdU labelling index was increased in primary cultures of mouse or rat hepatocytes incubated with 5–750 µg/mL diethanolamine for 24 hours; supplementation of the culture medium with choline reduced the level of diethanolamine-induced DNA synthesis, and reducing the concentration of choline in the culture medium (in the absence of diethanolamine) also caused increases in DNA synthesis. In contrast, DNA synthesis was not increased in cryopreserved human hepatocytes incubated with diethanolamine or in culture medium depleted of choline (Kamendulis & Klaunig, 2005).

Interestingly, the effects of reduced choline uptake in mouse neural precursor cells appear to be converse to those reported in mouse hepatocytes. In neural precursor cells, treatment with 3 mM [312 µg/mL] diethanolamine reduced choline uptake and decreased intracellular levels of phosphocholine; however, these changes resulted in a reduced BrdU labelling index and increased DNA fragmentation (apoptosis) (Niculescu *et al.*, 2007). The effects of diethanolamine on DNA synthesis and apoptosis were prevented by choline supplementation of the growth medium. Based on these findings, the authors concluded that prenatal exposure to diethanolamine may adversely affect brain development.

4.5 Mechanisms of carcinogenesis

Induction of choline deficiency has been proposed as the means by which diethanolamine induces liver neoplasms in mice (IARC, 2000; Lehman-McKeeman *et al.*, 2002; Kamendulis & Klaunig, 2005; Leung *et al.*, 2005). Choline is an essential nutrient that can be acquired from the diet or by de-novo synthesis via the sequential methylation of phosphatidylethanolamine, catalysed by phosphatidylethanolamine *N*-methyltransferase using SAM as the methyl donor (Zeisel, 2008). This pathway is activated when cellular levels of choline are low, resulting

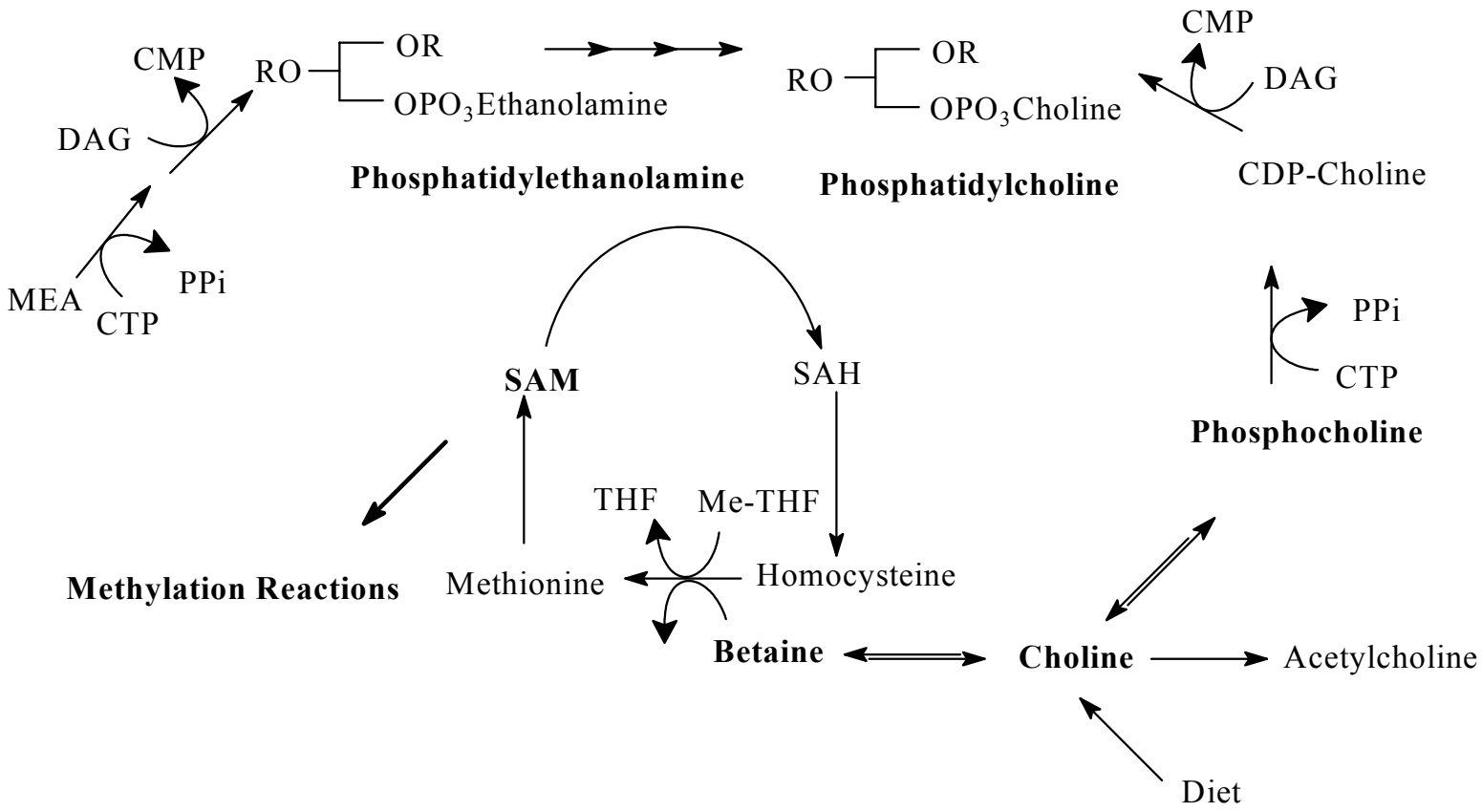
in greater utilization of SAM. Pathways of choline formation and utilization are shown in Fig. 4.1. Choline is involved in numerous physiological functions; the major fate of choline is conversion to phosphatidylcholine after its phosphorylation to phosphocholine. Acetylation of choline produces the neurotransmitter acetylcholine. Dietary choline is also the major source of methyl groups via its oxidation to betaine and subsequent conversion to SAM. Depletion of intracellular choline leads to a reduced availability of SAM and can result in hypomethylation of DNA, altered expression of genes that regulate growth and possible tumour development.

The basis for the proposed mechanism of diethanolamine-induced choline deficiency in the induction of liver tumours includes the following: (a) dermal exposure of mice to maximum tolerated doses of diethanolamine resulted in reductions in levels of choline, choline metabolites and SAM in the liver (Lehman-McKeeman *et al.*, 2002); (b) the induction of morphological transformation in SHE cells by diethanolamine was prevented by the addition of excess choline (Lehman-McKeeman & Gamsky, 2000); (c) the inhibition of choline uptake by diethanolamine in SHE and Chinese hamster ovary cells was prevented by the addition of excess choline to the culture medium (Lehman-McKeeman & Gamsky, 1999; Lehman-McKeeman & Gamsky, 2000); (d) DNA methylation status was similarly altered (mainly hypomethylations) in isolated mouse hepatocytes grown in the presence of diethanolamine or in choline-deficient medium (Bachman *et al.*, 2006); (e) increases in DNA synthesis in primary cultures of mouse or rat hepatocytes incubated with diethanolamine were prevented by the addition of excess choline (Kamendulis & Klaunig, 2005); (f) *N*-nitrosodiethanolamine was not detected in mice that were administered diethanolamine by dermal application with or without sodium nitrite in their drinking-water (Stott *et al.*, 2000).

Several issues raise uncertainties about the reliability of the proposed choline deficiency mechanism: (a) no effect on hepatic levels of SAM was observed in mice administered a dose of diethanolamine (40 mg/kg bw) that produced a significant increase in hepatocellular neoplasms (Lehman-McKeeman & Gamsky, 2000); (b) studies of induced choline deficiency have not been evaluated in mouse kidney, the second site of tumour induction by diethanolamine in mice; (c) although rats are highly sensitive to choline deficiency, the 2-year carcinogenicity study of diethanolamine found no evidence of a liver tumour response in this species (NTP, 1999b). The lack of published studies on the effects of maximum tolerated doses on liver levels of choline and choline metabolites in rats creates a critical gap in the data on the proposed mechanism of choline-deficiency in the induction of liver tumours by this chemical; (d) the hallmark of dietary choline deficiency is a fatty liver; however, a fatty liver was not diagnosed in rats or mice exposed to diethanolamine; (e) the detection of mutations in the β-catenin gene in liver tumours from diethanolamine-exposed mice indicates that in-vivo mutagenesis may be involved (Hayashi *et al.*, 2003). No studies have been reported on the mutational profile in liver tumours induced in mice fed a choline-deficient diet.

Several factors relate to the applicability of the proposed mechanism of diethanolamine-induced choline deficiency in mice for the evaluation of carcinogenic risks to humans. Similar to studies in rats and mice, diethanolamine was absorbed by human liver slices and incorporated into phospholipids. The finding that healthy adults deprived of dietary choline develop signs of organ dysfunction (e.g. fatty liver), which was reversed by consumption of a high-choline diet, contributed to the recognition of choline as an essential nutrient; the current recommended adequate intake of choline is 425 mg/day for women and 550 mg/day for men (Zeisel & da

Fig. 4.1 Interrelationship between the intracelular pathways of choline and methionine



CDP-choline, cytidyl diphosphate-choline; CMP, cytidyl monophosphate; CTP, cytidyl triphosphate; DAG, diacylglycerol; MEA, monoethanolamine; PC, phosphatidylcholine; PPi, pyrophosphate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate

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Costa, 2009). Variations in dietary requirements for choline have been attributed in part to polymorphisms in genes involved in choline metabolism. The rodent choline-deficient model has been widely used to understand the progression of non-alcoholic steatohepatitis (Larter & Yeh, 2008), a risk factor for liver cancer in humans (Severi *et al.*, 2010). Thus, the choline-deficient mechanism is relevant to humans; however, the relationship between exposure to diethanolamine, a reduction in liver choline levels and the development of liver cancer in humans is not known.

[Sufficient data that would allow reliable comparisons of the sensitivity of humans and rodents to diethanolamine-induced choline deficiency are not available. Data are also lacking on tissue levels of diethanolamine in rodents and humans following repeated exposures to this chemical and the effects on intracellular concentrations of choline and choline metabolites. Tissue levels of diethanolamine are dependent on the frequency of exposure and factors that affect dermal absorption, e.g. skin temperature. The level of dietary intake of choline may also affect sensitivity to agents such as diethanolamine that competitively inhibit its intracellular uptake. In this regard, it is worth noting that the ingestion of choline from diets fed to rodents in the carcinogenicity study (NTP, 1999b) or to mice in the study that demonstrated the induction of hepatic choline deficiency (Lehman-McKeeman *et al.*, 2002) (0.05–0.2%, equivalent to 300–320 mg/kg bw) provided approximately 35–40 times higher intakes of choline on a per kilogram basis than the current adequate intake amount recommended for humans.]

The induction of liver tumours in mice by diethanolamine was suggested to be a consequence of choline deficiency. This mechanism is applicable to human health, especially for subgroups that are highly susceptible to dietary choline deficiency. Other possible mechanisms include diacylglycerol accumulation and activation of protein kinase C (Leung *et al.*, 2005), incorporation of diethanolamine into membrane phospholipids and generation of lipid second messengers, e.g. aberrant ceramides (Mathews *et al.*, 1995; NTP, 1999b), and induction of aneuploidy (Muñoz & Barnett, 2003).

5. Summary of Data Reported

5.1 Exposure data

Diethanolamine has been produced since the 1930s by the reaction of ethylene oxide with ammonia. Occupational exposure can occur through its production and use in metalworking fluids and coolant fluids, textile processing, and industrial gas purification. Exposure of the general population to diethanolamine occurs through dermal contact with cosmetics, soaps and detergents where it is present as a contaminant in fatty acid-diethanolamide surfactants.

5.2 Human carcinogenicity data

Numerous studies have investigated exposure to metalworking fluids and the risk of cancer in workers who were probably exposed to diethanolamine and other agents. Excess risks of cancer were observed among workers exposed to metalworking fluids that probably contained diethanolamine. However, these studies cannot distinguish the carcinogenic effect of diethanolamine alone from that of the complex mixture. No studies were identified that evaluated human cancer associated with the use of personal care products that contain diethanolamine.

5.3 Animal carcinogenicity data

In male and female mice, dermal application of diethanolamine increased the incidence of hepatocellular carcinoma and hepatocellular adenoma in males and females, and of hepatoblastoma in males. The incidence of renal tubule adenoma was also increased in males. Dermal application of diethanolamine did not induce tumours in rats.

Tumours of the kidney and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

Diethanolamine is absorbed only weakly through human skin. No data were available on the absorption of diethanolamine after other routes of exposure in humans.

A genotoxic mechanism is supported by the induction of aneuploidy in Drosophilia and the elevated frequency of mutations in β-catenin *Catnb* genes in liver tumours induced by diethanolamine. However, diethanolamine was not genotoxic in most in-vitro systems and did not increase the frequency of micronuclei in exposed mice.

A mechanism for liver tumour induction of diethanolamine in mice that involves the inhibition of choline uptake in the liver has been proposed based on the reduced levels of choline metabolites observed in the liver of mice exposed to diethanolamine, the fact that supplementation of incubation medium with choline prevented diethanolamine-induced morphological transformation in Syrian hamster embryo cells and prevented diethanolamine-induced increases in DNA synthesis in rat hepatocytes, and the similarity in DNA hypomethylation in mouse hepatocytes grown in either the presence of diethanolamine or choline-deficient medium. This hypothesis is challenged by the finding that reductions in hepatic levels of *S*-adenosyl methionine do not occur at all doses of diethanolamine that induced liver tumours in mice, and the fact that the hallmark liver phenotype for choline deficiency, i.e. fatty liver, was not observed in rats or mice exposed to diethanolamine.

There is weak evidence that a genotoxic mechanism is involved in the induction of liver tumours by diethanolamine. There is moderate experimental support for choline deficiency as a mechanism for diethanolamine-induced liver cancer in rodents. The human relevance of this mechanism to humans cannot be excluded, especially for subgroups that are highly susceptible to dietary choline deficiency. No mechanistic data are available on the induction of kidney tumours by diethanolamine.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Diethanolamine is *possibly carcinogenic to humans (Group 2B).*

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