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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

last updated: 10/2005

Diethanol-amine

No. 158

CAS No. 111-42-2

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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Diethanolamine

1 Summary and assessment

Following oral administration, diethanolamine is rapidly absorbed via the gastrointestinal tract. Skin absorption diethanolamine is slow and incomplete; rats only absorb 16% of the applied amount of substance within 48 hours, while mice absorb 34% of a comparable dose and 58% of a three times higher dose in the same period of time. Application several times a day increases the absorption ability of the rat skin at the site of application. In-vitro studies using skin preparations have demonstrated that there are considerable interspecies differences in the rate of transdermal penetration of diethanolamine and that when the penetration rate is determined for a 37-percent aqueous solution of the chemical, a very marked decrease is noted from the mouse (294 µg/cm²/hour) via the rabbit and rat to humans (12.7 µg/cm²/hour). The respiratory tract provides a further route for the absorption of diethanolamine into the body, as demonstrated in a number of subacute and subchronic studies which were conducted with aerosols due to the chemical's very low vapour pressure. Once absorbed into the body, diethanolamine is excreted slowly, regardless of the route of administration. Multiple administration results in marked accumulation. The major route of elimination is via the urine. Only small quantities undergo faecal excretion and only traces are eliminated via the expired air. Upon single oral or dermal administration of diethanolamine, 20 to 30% of the absorbed dose is excreted in the urine, 2 to 3% in the faeces and approx. 0.2% in the expired air within 48 hours. Of the absorbed diethanolamine, 40 to 60% accumulates in the tissues, with the major fractions being recovered in the liver and kidneys, but also in numerous other organs and in the muscles. Following daily oral administration of diethanolamine on 5 consecutive days only about 40% of the total administered dose is excreted and the tissue concentrations of diethanolamine are 3 to 5 times higher than after single-dose administration. When daily diethanolamine treatment is continued for 8 weeks, tissues become saturated after about 4 weeks and excretion in terms of percentage of the dose administered increases to 92% at week 8. When diethanolamine treatment is discontinued after 4 weeks, elimination of the accumulated compound takes place with a half-life of roughly one week, with more than 90% disappearing from the tissues by 4 weeks later.

Following single intravenous administration to rats, 25% of a diethanolamine dose of 10 mg/kg body weight undergoes urinary excretion within 96 hours, elimination being biphasic with half-lives of 9.2 minutes and 258 hours. The corresponding percentage at a dose level of 100 mg/kg body weight is 36%, the half-lives being 16.3 minutes and 206 hours. Portions of 64.1 and 52.5% of the administered doses of diethanolamine remain in the tissues. Faeces and breath hardly contribute to excretion. Similar results are obtained in studies with rats and mice treated intravenously with diethanolamine to determine their elimination kinetics over a period of 48 hours. Only a small portion of the diethanolamine is metabolised in the body. Analysis of the compounds excreted in urine after administration of diethanolamine reveals that a single dose yields almost exclusively unchanged diethanolamine and hardly any metabolites. Following 8 weeks' treatment, N-methylation products of diethanolamine also appear in significant amounts in urine. The major portion of the diethanolamine retained in tissue also remains structurally unchanged. The aqueous extract from liver and brain homogenates, which represents 80 to 90% of the diethanolamine retained in tissue after a single administration, primarily contains unchanged diethanolamine as the phosphate. Liver homogenate also contains small quantities of N-methyldiethanolamine and N,N-dimethyldiethanolamine as phosphates (each accounting for 2% of the diethanolamine peak). These compounds are not detected in brain homogenate. Smaller portions of all diethanolamine retained in liver and brain are bound to lipids and can be extracted with chloroform/methanol. After a single dose of diethanolamine, this applies to 9% and 6% in liver and brain, respectively, relative to the total amount of diethanolamine present, whereas after 8 weeks of treatment the respective recoveries are 2 and 21%. Analysis of the chloroform/methanol extract reveals that brain contains diethanolamine almost exclusively as unchanged compound, which occurs in a phosphatidylethanolamine fraction. Fifteen percent of this is bound to ceramides and 85% to phosphoglycerides. This distribution is found after a single dose of diethanolamine and remains essentially unchanged after 8 weeks' treatment. After single-dose administration, the chloroform/methanol extractable, bound diethanolamine in liver is distributed in equal parts to a phosphatidylethanolamine fraction and a phosphatidylcholine fraction. The diethanolamine present the phosphatidylethanolamine fraction is structurally unchanged, with roughly 30% and 70% of the compound bound to ceramides and phosphoglycerides, respectively. The phosphatidylcholine fraction

contains no unchanged diethanolamine, but rather contains N-methyldiethanolamine (15%) and N,N-dimethyldiethanolamine (85%), with about 30 and about 70% of the metabolites being bound to ceramides and phosphoglycerides, respectively. Following 8 weeks' treatment, the chloroform/methanol-extractable portion is markedly reduced and the chemical is no longer detectable in the phosphatidylethanolamine fraction. The phosphatidylcholine fraction contains only N,N-dimethylethanolamine, almost all of which is bound to ceramides. In the blood of rats receiving single-dose or long-term treatment with diethanolamine, 16 to 20% of the chemical is also bound to phospholipids as N-methyl derivatives. In-vitro studies in human liver slices have shown that diethanolamine is absorbed by the tissue (29% of the administered dose within 12 hours), remains largely metabolically unchanged and is present to a minor extent in phospholipids (ceramide derivatives), again largely as the unchanged molecule. It may therefore be presumed that a small portion of the diethanolamine that is absorbed into the body is methylated at the nitrogen atom and/or phosphorylated at the oxygen atom and that these metabolites are then incorporated into phospholipids instead of the structurally related compounds ethanolamine and choline.

Diethanolamine is of low toxicity following single administration even at high doses. The LD_{50} data reported for the rat vary greatly from 1410 to 3540 mg/kg body weight, with one value of 710 mg/kg body weight lying completely outside the range. Whereas the LD_{50} values for acute oral toxicity are found to be in a similar dose range in rabbits and guinea pigs, mice appear to be less sensitive than rats, judging by the values of 3300 and 4570 mg/kg body weight reported for mice. Dermal toxicity is very low in rabbits, with LD_{50} values being given as 12200 and 13000 mg/kg body weight. Due to its very low vapour pressure, diethanolamine can cause respiratory toxicity only when administered as an aerosol. Inhalation of atmosphere saturated with diethanolamine gas is survived by rats without signs of toxicity in the inhalation hazard test. High aerosol concentrations result in mortality after only a few hours. The toxicity data for single intraperitoneal administration of diethanolamine to rats and mice vary greatly but show that there is marked toxicity even in the range of 200 mg/kg body weight. The subcutaneous and intramuscular LD_{50} values are in the order of several thousand mg/kg body weight. Data on clinical signs of intoxication are very sparse. They are rather unspecific. The liver and the kidneys are reported as the target organs, and in one case there is report of

changes in serum electrolytes occurring at a very high intraperitoneal dose of diethanolamine.

Diethanolamine has an irritant effect on the skin following single exposure for several hours. Short-term exposure and aqueous dilutions of the substance do not cause skin irritation. Longer-term treatment (2 to 13 weeks) of rats and mice with a 95-percent ethanol solution of diethanolamine results in damage to the skin including chronic-active inflammation, hyperkeratosis and acanthosis. Following direct contact, diethanolamine has a severely irritating to corrosive effect and can cause serious eye injury.

Diethanolamine has shown no skin-sensitising effect in several studies in the guinea pig. A modified local lymph node assay carried out in mice has detected an effect of diethanolamine on the proliferation of lymph node cells and the production of interleukin-4 and interferon-gamma in these cells, but only after pretreatment of the application site with a 10-percent solution of sodium dodecyl sulphate. However, as sodium dodecyl sulphate itself has a marked effect on the parameters measured, the diethanolamine effect observed results from a synergism between sodium dodecyl sulphate and diethanolamine.

Numerous studies have been carried out to investigate the subacute, subchronic and chronic toxicity of diethanolamine as well as its toxic actions on individual organs. For better clarity, the results are summarised in Table 1.

Beginning of Table 1

Table 1. Summary of the toxic effects of diethanolamine on the internal organs of rats and mice, excluding carcinogenicity and reproductive toxicity

Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Acute						
Rat, male	oral	100–3200	single dose (sacrifice after 18 hours)	none	increased liver and kidney weights; increased urea concentration in serum, decreased arginine concentration; increases in all serum enzyme activities studied; degenerative changes in the hepatic parenchyma and renal tissue, tubular necrosis, swollen endoplasmic reticulum, loss of ribosomes; *no observed effect level* (NOEL) not given	Korsrud et al., 1973

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Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Rat, male	oral	1000	single dose (sacrifice after 2, 5 or 24 hours)	none	liver and pancreas changes, swelling of mitochondria, endoplasmic reticulum and Golgi complex; focal degeneration of the pancreatic acinar cells	Hruban et al., 1965
Rat, male	intraperitoneal	100 or 500	single dose (sacrifice after 24 hours)	none	cytoplasmic vacuolisation seen in liver and kidney tissues, swollen mitochondria; elevated serum LDH and AST levels	Grice et al., 1971
Subacute						
Rat, male	oral	1000	4 days	none	effects slightly intensified as compared with single-dose administration (see above)	Hruban et al., 1965
Rat, male	oral	3.3, 33 or 330 (≙ 0.01, 0.1 or 1% in feed, study with an independent repeat)	32 days	9/10 and 8/10 at 330 mg/kg body weight	increased liver weight; decreased haemoglobin and haematocrit, decreased serum protein; no effects at 3.3 mg/kg body weight	Eastman Kodak, 1967 a, 1968
Rat, male (findings not broken down according to mode of administration)	oral or intraperitoneal	26, 557 or 1000	3 to 5 days	no data	hypocalcaemia with signs of tetany, 3-fold increase in urinary calcium excretion after short-term treatment at high dose levels, adrenocortical hyperfunction with hyperglycaemia and increased corticosterone, increased blood urea nitrogen, thymus involution and anaemia after prolonged treatment	Foster, 1972
		26–557	2 to 49 days			
Rat, male and female	oral	males: ca. 77, 162, 319, 622 or 1016; females: ca. 79, 158, 371, 670 or 1041	14 days	2/5 males and 5/5 females at the highest dose; 5/5 females at 670 mg/kg body weight	normochromic, microcytic anaemia with decreases in erythrocyte and reticulocyte counts, haemoglobin and haematocrit; elevated serum levels of creatinine, total protein, albumin, and bile acids; increased kidney weight; renal tubular epithelial necrosis; increased urine concentrations of urea nitrogen, glucose, protein and LDH activity; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992

Table 1. Summary of the toxic effects of diethanolamine on the internal organs of rats and mice, excluding carcinogenicity and reproductive toxicity						
Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Rat, sex unspecified	oral	105, 210 or 315	11 days (postnatal days 5 to 15)	none	increased liver and kidney weights; increases in succinic dehydrogenase activity in the nuclear and mitochondrial fractions of the liver cells and in the mitochondrial fractions of the kidney cells, respectively; increased cholinesterase activity in the mitochondrial fraction	Burdock and Masten, 1979
Rat, sex unspecified	oral	105, 210 or 315	11 days (postnatal days 5 to 15)	none	histopathological liver changes (periportal swelling and vacuolisation, swollen mitochondria); sequestration of diethanolamine by hepatic and renal mitochondrial fractions; decreased aniline hydroxylase activity in hepatic microsomes, increased succinic dehydrogenase in liver and kidney	Burdock, 1981
Rat, male	oral	0.25, 1.3 or 5 mg/ml drinking water	7, 14 or 21 days	none	decline of respiratory control and increase in oxygen consumption in the hepatic mitochondria	Barbee and Hartung, 1976
Rat, male	oral	42, 160 or 490	7 to 35 days	none	increases in state 4 oxygen consumption in hepatic mitochondria and ATPase activity after 3 weeks' treatment; alterations of size and shape of hepatic mitochondria after 2 weeks' treatment	Barbee and Hartung, 1979 a
Mouse, male and female,	oral	110–2169	14 days	none	dose-dependent increase in liver weight; minimal tissue alterations in the liver	NTP, 1992
Rat, male and female	dermal	125, 250, 500, 1000 or 2000	16 days	3/5 males and 5/5 females at the highest dose level, 1/5 females at 1000 mg/kg body weight	dose-dependent skin changes at the site of application; normochromic, microcytic anaemia with decreases in erythrocyte and reticulocyte counts, haemoglobin and haematocrit; increased kidney weight; renal tubular epithelial necrosis; increased urine concentrations of urea nitrogen, glucose, protein and LDH activity; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992

Table 1. Summary of the toxic effects of diethanolamine on the internal organs of rats and mice, excluding carcinogenicity and reproductive toxicity						
Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Mouse, male and female,	dermal	160, 320, 630, 1250 or 2500	16 days	5/5 males and 3/5 females at the highest dose level	dose-dependent skin changes at the site of application; increased liver weight; minimal histopathological alterations of the liver	NTP, 1992
Rat, sex unspecified	inhalation	109 mg/m³	9 days	none	increased liver weight; elevated AST activity, increased kidney weight and elevated blood urea nitrogen	Hartung et al., 1970
Rat, male and female	inhalation	100, 200 or 400 mg/m³, 6 hours per day	14 days	none	increased liver weights in females from the highest dose group	BASF, 1993 a
Rat, sex unspecified	intraperitoneal	250	repeated (no further details)	none	increased liver weight, decreased total lipid content of the liver	Hartung et al., 1970
Mouse, sex unspecified	intraperitoneal	6, 8 or 12 mg/animal	2 to 48 days	none	increased liver weight; increased glycogen and water content of the liver, decreased total lipid content of the liver	Annau et al., 1950
Subchronic and chronic						
Rat, sex unspecified	oral	4 mg/ml drinking water	7 weeks	high, no further data	Liver and kidney damage, pronounced normocytic anaemia without bone-marrow depletion and without increase in the number of reticulocytes	Hartung et al., 1970
Rat, male and female	oral	171, 350, 680, 560 or 580 (amount ingested)	90 days	10/10 from 350 mg/kg body weight; 1/10 at 171 mg/kg body weight	increased liver and kidney weights; histopathological changes in liver and kidney tissue, lung infections	Mellon Institute, 1950; Smyth et al., 1951
	oral	5.1, 20, 90 or 390	90 days	7/10 in the highest dose group; 2/10 or 3/10 in the other dose groups	increased liver and kidney weights, extensive lung infections; the *no effect level* (NOEL) derived from the two studies (see above) was between 90 and 171 mg/kg body weight	
Both studies are of limited significance due to deaths unrelated to the test substance and because of inadequate documentation.						

Table 1. Summary of the toxic effects of diethanolamine on the internal organs of rats and mice, excluding carcinogenicity and reproductive toxicity						
Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Rat, male and female	oral	25, 50, 100, 200 or 400	13 weeks	4/20 at 400 mg/kg body weight, 4/20 at 200 mg/kg body weight, 1/20 at 100 mg/kg body weight	sever dose-dependent depressions of body weight gain from 50 (males) and 100 (females) mg/kg body weight; no other findings; *no observed effect levels* (NOELs): 25 (males) and 50 (females) mg/kg body weight	GSRI, 1980
Rat, male	oral	25, 48, 97, 202 or 436	13 weeks	2/10 at the highest dose level	depressed body weight gains; normochromic, microcytic anaemia with decreases in erythrocyte and reticulocyte counts, haemoglobin and haematocrit; increased blood levels of urea nitrogen, total protein, albumin and bile acids; increased liver and kidney weights; decreased testis and epididymis weights; increased urinary protein; histopathological changes in the kidneys and testes and the spinal cord and medulla oblongata; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992; Melnick et al., 1994 a
Rat, female	oral	14, 32, 57, 124 or 242	13 weeks	1/10 at the lowest dose level		
Mouse, male and female,	oral	50, 100, 200, 400 or 800	13 weeks	1/20 at 800 mg/kg body weight, 1/20 at 100 mg/kg body weight (deaths not treatment-related)	no clear treatment-related effects	GSRI, 1980
Mouse, male	oral	104, 178, 422, 807 or 1674	13 weeks	10/10 at the two highest dose levels	depressed body weight gains; increased liver weight, elevated serum ALT and SDH activities, hepatocytic lesions; nephropathies; increased heart weight, myocardial degeneration at high dose levels; cytological alterations of the submandibular salivary gland; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992; Melnick et al., 1994 b
Mouse, female	oral	142, 347, 884, 1154 or 1128	13 weeks	10/10 at the two highest dose levels; 3/10 at 884 mg/kg body weight		

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Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Rat, male and female	dermal	32, 63, 125, 250 or 500	13 weeks	3/20 at the highest dose level	skin changes at the site of application; depressed body weight gains; normochromic, microcytic anaemia with decreases in erythrocyte and reticulocyte counts, haemoglobin and haematocrit; increased blood levels of albumin, urea nitrogen, protein, bile acids and serum ALT; increased liver and kidney weights; decreased testis weight; nephropathies, histopathological changes in the medulla oblongata; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992; Melnick et al., 1994 a
Rat, male and female	dermal	16, 32 or 64 (males); 8, 16 or 32 (females)	2 years	not different from the control	skin changes at the site of application; increase in incidence and severity of nephropathy, dose-related from the lowest dose in females, seen only at the highest dose in males	NTP, 1999 a
Mouse, male and female,	dermal	80, 160, 320, 630 or 1250	13 weeks	6/20 at the highest dose level	skin changes at the site of application; increased liver weight; hepatocytic lesions; elevated serum ALT and SDH activities, increased heart and kidney weights, myocardial lesions; cytological alterations of the submandibular salivary gland; *no observed adverse effect level* (NOAEL) not achieved	NTP, 1992; Melnick et al., 1994 b
Mouse, male and female,	dermal	40, 80 or 160	2 years	shorter survival at the highest dose level	skin changes at the site of application; decreased body weights; cytoplasmic alterations in the liver and thyroid gland, primarily in males	NTP, 1999 a

Table 1. Summary of the toxic effects of diethanolamine on the internal organs of rats and mice, excluding carcinogenicity and reproductive toxicity

Species studied, sex	Dosage form	Dose (mg/kg body weight)	Duration of exposure	Mortality	Toxicologically significant effects of treatment	Reference
Rat, male and female	inhalation	15, 150 or 400 mg/m³, 6 hours per day, 5 days per week	13 weeks	none	mild normochromic, microcytic anaemia, decreased red blood cells, mean corpuscular volume, haemoglobin and haematocrit at the highest concentration; increased liver weight; elevated ALT and ALP activities; increase kidney weight, nephropathies; histopathological changes in the larynx and trachea; systemic *no observed adverse effect level* (NOAEL) between 15 and 150 mg/m³; no local NOAEL due to squamous metaplasia of the larynx even at 15 mg/m³	BASF, 1996
Rat, male	inhalation	26 mg/m³	13 weeks	several deaths	depression of growth rate; increased lung and kidney weights	Hartung et al., 1970
Rat, dog, guinea pig, male and female	inhalation	1.14 mg/m³ whole day	13 weeks	none	increased liver weights in female rats	Hazleton, 1967

ALP	alkaline phosphatase	ATPase	adenosine triphosphatase
ALT	alanine aminotransferase	LDH	lactate dehydrogenase
AST	aspartate aminotransferase	SDH	sorbitol dehydrogenase

End of Table 1

Irrespective of the route of administration and the duration of treatment, the liver and kidney are the target organs for diethanolamine toxicity. Apart from this, rats are observed to develop normochromic, microcytic anaemia after only 14 days of treatment with diethanolamine. Acute pancreatic lesions, hypocalcaemia and adrenocortical hyperfunction, decreased testis and epididymis weights and degenerative changes in the generative cells are seen after high doses of diethanolamine. Prolonged treatment with high doses of diethanolamine results in myocardial degeneration with increased heart weight, histopathological changes in the spinal cord and medulla oblongata and cytological alterations of the submandibular salivary gland. In all cases however, the most prominent toxic effects of diethanolamine consist in liver and kidney lesions. They occur after only a single 10 mg/kg dose of diethanolamine and increase in a dose-related manner. In correlation, these organs show dysfunctions and histopathological changes to-

gether with corresponding clinical chemistry results for the blood and urine obtained from treated animals. Various studies have demonstrated that diethanolamine treatment affects the cell membranes and organelles. It is suspected that diethanolamine toxicity can be attributed, at least partially, to the incorporation of the chemical into phospholipids in place of its close structural congeners ethanolamine and choline, which serve as constituents of cell membranes. The non-natural phospholipids thus formed interfere with the natural functions of membranes, affecting mitochondria and microsomes in particular. This is indicated not only by light and electron microscopy studies, which have demonstrated morphological changes in the cell organelles even after treatment with very low doses of diethanolamine, but also by studies showing the functional impairment of the organelles after diethanolamine treatment of animals from which they were obtained. For instance, hepatic mitochondria from rats given 2 weeks' oral treatment with diethanolamine show an increase in oxygen consumption and a decline of respiratory control. After 5 days of intraperitoneal treatment of rats with diethanolamine, there is dose-related inhibition of the drug-metabolising enzymes involved in the hydroxylation of acetanilide and N-demethylation of aminopyrine. Hexobarbitone sleeping time is markedly prolonged in a dose-related manner in these animals. This correlates with a decrease in hepatic microsomal cytochrome P-450 and b_5 content in treated animals. Moreover, phenobarbitone induction of liver enzymes is blocked by diethanolamine when the two compounds are administered simultaneously. The incorporation of diethanolamine into the phospholipids of the liver and kidney has been demonstrated in vivo and in vitro. Diethanolamine then inhibits the synthesis of the natural phospholipids from ethanolamine and choline and the diethanolamine-containing phospholipids that form instead are metabolised at a markedly lower rate, thus shifting the ratio of natural to non-natural phospholipids in favour of the latter as treatment progresses. The toxicokinetic studies investigating the distribution and excretion of diethanolamine yield the same results.

Based on the available findings, diethanolamine is devoid of genotoxic potential. All in-vitro and in-vivo tests carried out thus far have been negative. Diethanolamine has proven to be cytotoxic neither in the standard tests on micro-organisms (*Salmonella typhimurium*, *Escherichia coli*, *Saccharomyces cerevisiae*), the in-vitro mammalian cell tests for chromosome aberrations and sister chromatid change nor in the in-vivo tests for single strand DNA breaks or micronucleus formation.

A comprehensive carcinogenicity study of diethanolamine is available in which rats and mice were treated dermally with the chemical for 2 years. Whereas no carcinogenic activity was detected in rats, the investigators and scientific reviewers for the US National Toxicology Program (NTP) concluded that, under the conditions of the study, there was clear evidence of carcinogenic activity of diethanolamine in male and female mice. This statement is based upon the great, dose-dependent increases relative to the control group noted in the incidences of hepatocellular adenoma and carcinoma in both sexes at the end of the treatment period. Significant, dose-related increases in the incidences of adenoma and renal tubule hyperplasia were additionally observed in male mice. In order to evaluate the carcinogenic potential of diethanolamine in mice, the NTP also utilised three studies of the diethanolamine condensates of coconut oil acid, lauric acid and oleic acid, which were carried out in parallel using the same methodology. These products contain free diethanolamine as an impurity, with concentrations varying from 0.19% in oleic acid diethanolamine condensate up to 18% in coconut oil acid diethanolamine condensate. Increased incidences of hepatocellular adenoma and carcinoma corresponded with the diethanolamine content in these studies, with activities ranging from no effect with oleic acid diethanolamine condensate up to very pronounced activity noted for coconut oil diethanolamine condensate. The above-mentioned carcinogenicity studies were criticised because exposure was accomplished by open dermal application without cover, for which reason partial ingestion of the chemical could not be excluded. Moreover, the chemicals under investigation were dissolved in 95-percent ethanol, which in itself has been ascribed properties representing a risk factor for liver carcinogenicity. A study in transgenic mice was negative. Two cell transformation assays were performed on Syrian hamster embryo cells, one giving a positive, the other a negative result. The latter, however, was questionable because the concurrent positive controls did not provide a clear positive result. As diethanolamine was devoid of genotoxic potential according to the available data, a study was conducted to investigate a possible non-genotoxic mechanism causing the tumorigenesis observed in mice. To this end, B6C3F1 mice were treated with the same dose of diethanolamine under the same conditions for up to 13 weeks. It was found that the chemical increased cell proliferation in the liver and kidneys of the animals and, in addition, increased the mitotic index in the kidneys, a finding in support of the assumption that the mechanism of tumorigenesis is of

a non-genotoxic nature. It must also be taken into account, however, that diethanolamine, upon entering the body, is converted in the stomach in the presence of nitrate or nitrite to yield N-nitrosodiethanolamine, a known carcinogen. Rats given diethanolamine dermally and sodium nitrite simultaneously in their drinking water excrete N-nitrosodiethanolamine in their urine. However, N-nitrosodiethanolamine is not detected in the blood or urine of male mice given diethanolamine daily by the dermal or oral route and co-administered sodium nitrite in their drinking water for 2 weeks, nor is N-nitrosodiethanolamine found in the gastric contents following dermal treatment. The potential carcinogenicity of diethanolamine is more likely related to dramatic decreases in choline-containing lipids in the liver. Diethanolamine has long since been known to act as a “pseudo” ethanolamine when administered to rats or mice in that it is incorporated, irrespective of the route of administration, into hepatic phospholipids, thereby interfering with phospholipid biosynthesis. Non-natural phospholipids containing diethanolamine accumulate in liver tissue. Moreover, diethanolamine has been demonstrated to produce marked inhibition of phospholipid biosynthesis, particularly the synthesis of choline-containing phospholipids. Recent in-vitro data show that diethanolamine also has a strong inhibitory effect on hepatocellular choline uptake and hence greatly reduced hepatocellular content of choline-containing phospholipids. Supplementation of excess choline cancelled the effects. Since induction of cell transformation by diethanolamine in Syrian hamster embryo cells is also cancelled by supplementation of excess choline, the decrease in intracellular choline levels and related effects on phospholipid metabolism can be considered a potential mode of carcinogenic action of diethanolamine. This assumption is supported by a further study using the same strain of mouse and dose levels as in the NTP dermal carcinogenicity study. Dietary choline deprivation, known to produce hepatocarcinogenic or tumour-promoting effects in mice in the absence of diethanolamine treatment, results in the same disruption of hepatocellular choline metabolism with marked decreases in phosphocholine levels, as is seen in diethanolamine-treated mice, in which the effect is dose-dependent with a *no observed effect level* (NOEL) of 10 mg/kg body weight. Hepatic S-adenosylmethionine and S-adenosylhomocysteine levels in mice fed choline-devoid diet and in mice treated with diethanolamine are also altered in the same manner and to the same extent, thus allowing the conclusion that, in connection with the decreases in phosphocholine levels, there is a similar disturbance of methyl

group metabolism in the differently treated mice. Therefore, it seems reasonable to assume that the liver tumours observed in mice treated with diethanolamine are induced via a non-genotoxic mode of action involving the disruption of choline and methyl group metabolism. Conclusive evaluation of the carcinogenic potential of diethanolamine is not possible on the basis of the body of data currently available, which includes one clearly positive result from a long-term study, though only in one species, the absence of any indication that diethanolamine has a genotoxic potential, data on the aberrant incorporation of the chemical into the body's own phospholipids and the available evidence supporting a non-genotoxic mechanism.

No teratogenicity was observed for diethanolamine in any of the studies conducted. No teratogenicity or embryotoxicity related to diethanolamine was found in pregnant rats given oral treatment at dose levels of 50 or 200 mg/kg body weight from days 6 to 15 post coitum. A further comprehensive study in pregnant rats treated with diethanolamine on days 6 to 19 of gestation and subsequently observed together with their offspring until postpartum day 21 showed increased postnatal toxicity from a dose level of 125 mg/kg body weight. The same dose also resulted in maternal toxicity (increased kidney weights) so that the *no observed adverse effect levels* (NOAELs) for reproductive toxicity and maternal toxicity are both given as 50 mg/kg body weight. A Chernoff/Kavlock screening study in mice treated orally with diethanolamine at 450 mg/kg body weight showed mortality among the delivered pups to be markedly increased, and body weight gain decreased, relative to the controls on post partum day 3, a finding that can be evaluated as representing developmental toxicity due to diethanolamine. Dermal treatment of pregnant female rats did not produce any embryotoxicity or teratogenicity; only the highest dose, 1500 mg/kg body weight, resulted in slight retardation of foetal development following daily administration on days 6 to 15 of gestation, as indicated by reduced ossification in the skull, axial skeleton and distal limbs observed in conjunction with marked maternal toxicity. The *no observed effect level* (NOEL) for embryotoxicity/teratogenicity established by the investigators was 500 mg/kg body weight, whereas a NOEL for maternal toxicity was not established. Similar investigations in the rabbit demonstrated no embryotoxic or teratogenic activity of diethanolamine up to the highest test dose of 350 mg/kg body weight. The maternal NOEL found in that study was 100 mg/kg body weight. Inhalation exposure of pregnant rats to diethanolamine from day 6

to day 15 of gestation in accordance with OECD guideline No. 414 resulted in a statistically significant increase in the number of cervical ribs at the highest test concentration of 200 mg/m³, a finding that can be considered to be an embryotoxic effect. The NOAELs for maternal and foetal toxicity and for teratogenicity were 50 mg/m³ and 200 mg/m³, respectively. While no effects were observed on the female reproductive organs in several subchronic studies, administration of high doses to male rats resulted in marked testicular damage. Fourteen days' oral treatment at 1016 mg diethanolamine/kg body weight, for instance, resulted in seminiferous tubule degeneration, characterised by a reduction in tubule size and in the number of male germ cells. Oral treatment of male rats with diethanolamine at dose levels ≥ 97 mg/kg for 13 weeks has been found to result in significant, dose-related decrease in testis and epididymis weights, sperm count and epididymal sperm count and motility. Testicular degeneration was diagnosed in all males at the highest dose level of 436 mg/kg body weight and in 3 at the second highest dose level of 202 mg/kg body weight. Sixteen days of dermal administration of 2000 mg/kg body weight, a very high dose which was lethal to several treated male rats, was observed to produce the same effects in the testes and epididymides. Dermal treatment of male rats for 13 weeks at dose levels of up to 500 mg/kg body weight resulted only in decreased testicular weight in the absence of any detectable effects on sperm cells or histopathological findings. Male mice treated with diethanolamine orally or dermally in parallel with the rats for 2 or 13 weeks exhibited no testicular or epididymal damage. In rats, 13 weeks of inhalation treatment with 400 mg diethanolamine/m³ also gave rise to testicular atrophy and mild atrophy of prostate in some of the animals. No multi-generation reproductive toxicity study is available. Therefore the significance of the testicular damage observed in rats at high dose levels of diethanolamine can not be evaluated at this time.

The effect of diethanolamine on the immune system has also been investigated in young female mice. Daily oral administration of 100, 300 or 600 mg diethanolamine/kg body weight for 14 days results in a dose-dependent increase in the number of Ig^{+} lymphocytes and a decrease in the number of $CD^{4+}CD^{8-}$-lymphocytes in these animals. The spleen antibody-forming cell response to sheep erythrocytes is statistically significantly decreased in all dose groups in a dose-dependent manner. Diethanolamine treatment does not affect the proliferative actions of the mitogens concanavalin A and lipo-

polysaccharide on spleen B cells or of the allogenic spleen cells from DBA/2 mice on the T cells of normal, diethanolamine-treated mice. Natural killer cell activity in the spleen is decreased only in animals in the highest dose group. The ability to form killer cells after incubation with allogenic P815 mastocytoma cells is similarly decreased by 10 to 14% in all dose groups. In the highest dose group, there is decreased cytotoxicity of macrophages isolated by peritoneal lavage towards melanoma cells. Host resistance of diethanolamine-treated mice to *Listeria monocytogenes* is unaffected whereas decreased host resistance is observed towards *Streptococcus pneumoniae* as well as in the B16F10 melanoma model. Treatment of mice at diethanolamine levels of up to and including 600 mg/kg body weight has no significant effect on body weight, and necropsy also reveals no pathological changes. All organ weights are comparable with those of the controls, with the exception of liver weight, which is increased in a dose-dependent, statistically significant manner. The clinical chemistry results are comparable for treated animals and controls. Dose-dependent decreases in erythrocyte and reticulocyte counts and haemoglobin and haematocrit values are found in treated animals, whilst leukocytes remain unaffected. In a preceding range-finding study using dose levels of up to and including 900 mg/kg body weight, diethanolamine treatment also leads to decreases in spleen antibody-forming cell response to sheep erythrocytes and in the formation of cytotoxic T lymphocytes in spleen cells. Hence, diethanolamine has a marked effect on the immune system of mice and thus could have immunotoxic potential. A *no effect level* has not been established.

Indications of a possible neurotoxic effect are reported only in two 13-week studies in which rats received diethanolamine in their drinking water or as applications of a 95-percent ethanol solution to the shaved dorsal skin in order to investigate subchronic toxicity. In both cases, findings included toxic effects on the brain, and in the case of oral administration, effects on the spinal cord were also seen and described as demyelination. In both cases, however, the effects were observed only at the two highest dose levels ($\geq$ 124 and $\geq$ 250 mg/kg body weight) and in the complete absence of any corresponding clinical signs. There were no indications of neurotoxic effects in a subsequent neurotoxicity study, in which rats received diethanolamine by inhalation treatment for 13 weeks and were examined very carefully both clinically and histopathologically for neurotoxicological effects

in accordance with the guidelines. On the other hand, there is a report describing a total of 39 cases in dogs and 12 in cats affected by a neuroparalytic syndrome following oral administration of a 54-percent aqueous solution of diethanolamine given as a flea repellent for an average of 5 months. In the case of one dog, gross pathology and histopathology revealed lesions in the brain and cervical spinal cord with spongiosis of the corona radiata of the brain and spongiosis of the spinal cord. The corresponding daily dose of diethanolamine was 23 mg/kg body weight.

Reports of experience with diethanolamine in humans are, with the exception of skin sensitisation studies, very rare and of very limited value. In comparative studies with different patch-test systems, for example, diethanolamine has been found to cause only mild irritation to the human skin. Skin sensitisation has been described in various studies and case reports. For instance, a patch test carried out in 32 patients who were sensitive to ethylenediamine gave a positive response to diethanolamine in one of the patients. On the other hand, the case is reported of a metalworker who handled cutting fluids and developed contact allergy of the hands and forearms. The man showed a positive response to diethanolamine as well as a number of other constituents. A further case was reported of a worker who came into frequent contact with a diethanolamine-containing coolant in connection with metal grinding and developed an allergy to the substance. Out of 17 metalworkers with contact allergic eczema, 2 showed a clear positive reaction to diethanolamine in the patch test. By contrast, the patch test with diethanolamine was negative in 3 metalworkers with contact allergy to alkanolamineborates as ingredients in cutting oils, and also there was no case of contact allergy to diethanolamine in a sample of approx. 200 eczematous patients in whom the focus was not on metalworking occupations. Comprehensive surveys which were conducted by the Informationsverbund Dermatologischer Kliniken (IVDK; “Information Network of Dermatological Clinics”) over the past 10 years and recorded all patients tested for contact allergy to diethanolamine at the participating centres (> 4000) found positive reactions in 1.7% of patients. When analysis was limited to a subgroup of patients with jobs in metalworking occupations (> 2000), the frequency of positive reactions to diethanolamine rose to 2.9%, and further restriction to those who were metalworkers with a metal-cutting occupation (353) increased the frequency of positive reactions to diethanolamine in the patch test to > 10%. These data show a clear trend

suggesting that an increase in potential exposure to diethanolamine is associated with an increase in the incidence of allergies to the chemical. Based on these data, diethanolamine definitely is a relevant potential contact allergen in humans under appropriate conditions of exposure. There is one other reported case of occupational asthma, the induction of which was attributed to the handling of cutting fluids containing diethanolamine. It was possible to induce the signs of asthma in a chamber provocation test at very low concentration levels of diethanolamine. The odour thresholds for diethanolamine dilutions in air and water are given as 0.27 ppm and 22000 ppm respectively.

When addressing the question of the carcinogenic potential of diethanolamine in humans, it is important to consider that the chemical's N-nitroso derivative, N-nitrosodiethanolamine, has been demonstrated to be carcinogenic in several studies in rats. It must be taken into account that diethanolamine, upon entering the body, is converted to N-nitrosodiethanolamine in the stomach in the presence of nitrate or nitrite. Mixtures of sodium nitrite and triethanolamine contained in cooling lubricants have reportedly caused colon carcinoma, via partial formation of N-nitrosodiethanolamine, in the case of one exposed human in whose urine N-nitrosodiethanolamine was detected. The German TRGS 615 "Verwendungsbeschränkungen für Korrosionsschutzmittel, bei deren Einsatz N-Nitrosamine auftreten können", i.e. the Technical Rules for Hazardous Substances, No. 615, which governs the restrictions on the use of corrosion inhibitors involving potential exposure to N-nitrosamines, covers *inter alia* the use of diethanolamine in order to prevent the formation of N-nitrosodiethanolamine.

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has assigned diethanolamine to category 3A of carcinogenic substances in the List of MAK and BAT values. Category 3A comprises "Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional. Substances for which the criteria for classification in Category 4 or 5 are fulfilled but for which the database is insufficient for the establishment of a MAK or BAT value". The Commission has designated the substance with "Sh" and "H" on account of its skin-sensitising potential and the risk of percutaneous absorption, respectively.

2 Name of substance

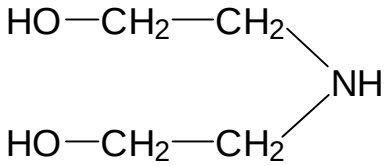
2.1	Usual name	Diethanolamine
2.2	IUPAC name	Bis-(2-hydroxyethyl)amine
2.3	CAS No.	111-42-2
2.4	EINECS No.	203-868-0

3 Synonyms, common and trade names

2,2'-Aminodiethanol
Bis(hydroxyethyl)amin
Bis(hydroxyethyl)amine
N,N-Bis(2-hydroxyethyl)amin
N,N-Bis(2-hydroxyethyl)amine
DEA
DELA
N,N-Diethanolamin
N,N-Diethanolamine
Diethylolamin
β,β'-Dihydroxydiethylamin
2,2'-Dihydroxydiethylamine
β,β'-Di(hydroxyethyl)amin
Dihydroxyethylamine
Di(2-hydroxyethyl)amine
Diolamin
Ethanol, 2,2'-iminobis-
Ethanol, 2,2'-iminodi-
2-[(Hydroxyethyl)amino]ethanol
2-[(2-Hydroxyethyl)amino]ethanol
2,2'-Iminobisethanol
2,2'-Iminobis(ethanol)
Iminodiethanol
2,2'-Iminodiethanol
2,2'-Iminodi-1-ethanol

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_4H_{11}NO_2$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	105.14	
5.2	Melting point, °C	27.8 (solidification point)	(Condea, 1998)
		28	(INRS, 1991; Edens and Lochary, 1991; Lide and Frederikse, 1997; BASF, 2000; O'Neil et al., 2001)
5.3	Boiling point, °C	268	(Edens and Lochary, 1991; BASF, 2000)
		268.8	(Lide and Frederikse, 1997; O'Neil et al., 2001)
		269 (decomposition)	(INRS, 1991)
		270.3 (decomposition)	(Condea, 1998)
5.4	Vapour pressure, hPa	< 0.01 (at 20 °C)	(INRS, 1991)
		0.0037 (at 25 °C)	(BASF, 2000)
		5 (at 126.7 °C)	(Condea, 1998)
		6.5 (at 138 °C)	(INRS, 1991)
5.5	Density, g/cm³	1.1004 (at 15 °C)	(BASF, 2000)
		1.0966 (at 20 °C)	(Lide and Frederikse, 1997)
		1.0881 (at 30 °C)	(O'Neil et al., 2001)
		1.0919 (at 30 °C)	(INRS, 1991)
		1.0838 (at 40 °C)	(Condea, 1998)
5.6	Solubility in water	Miscible	(INRS, 1991; O'Neil et al., 2001)
		Completely soluble (at 20 hPa and 25 °C)	(Edens and Lochary, 1991; BASF, 2000)
		Very soluble	(Lide and Frederikse, 1997)

5.7	Solubility in organic solvents	Miscible with acetone and ethanol, low solubility in hydrocarbons and diethyl ether (INRS, 1991) 0.01 g/100 g n-heptane (at 25 °C) (Edens and Lochary, 1991) High solubility in ethanol, low solubility in diethyl ether and benzene (Lide and Frederikse, 1997) Miscible with methanol and acetone; solubility at 25 °C: 4.2% in benzene, 0.8% in diethyl ether, < 0.1% in carbon tetrachloride and < 0.1% in n-heptane (O'Neil et al., 2001)
5.8	Solubility in fat	No information available
5.9	pH value	11.28 (at 53 g/l, 20 °C) (BASF, 2000)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 4.370 mg/m³ 1 mg/m³ ≙ 0.229 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Diethanolamine and the amides derived from it are used for a vast number of household and industrial applications. The 1990 estimate for the various areas of application shows the following distribution (BUA, 1994):

Raw materials for detergents	50%
Gas purification	15%
Drilling and cutting oils (cooling lubricants)	15%
Cosmetics/pharmaceuticals	< 10%
Cement additives	1%
Agrochemicals	1%
Others	10%

The main areas of application are summarised as follows: gas purification; starting material in the manufacture of surface-active agents, soaps, emulsifiers, lubricating oils and cosmetics; intermediate in the synthesis of pharmaceuticals and pesticides, plastics, anti-corrosive agents and vulcanisation accelerators; and lastly, solvent in pesticide formulations (INRS, 1991).

A detailed survey of the uses of diethanolamine is also presented by Edens and Lochary (1991).

7 Experimental results

7.1 Toxicokinetics and metabolism

The distribution of orally administered diethanolamine in the body and its excretion were investigated in male rats (F344-M, weighing approx. 160 to 190 g). A group of 4 animals received a single dose of diethanolamine, dissolved in water, by oral gavage at 7.9 mg/kg body weight, containing an admixture of approx. 3 µCi ^{14}C-diethanolamine (7.3 mCi/mmol, radiochemical purity 95 to 96%). Urine and faeces were collected for two 24-hour periods, and radiolabelled components in breath were trapped. At study termination after 2 days, the animals were sacrificed and samples were obtained of the blood, adipose tissue, muscle, skin, kidney, liver, spleen, heart, lung and brain. All samples were analysed for radioactivity. Of the administered diethanolamine, determined as radioactivity, 22% and 2% was excreted in the urine and faeces, respectively, within 48 hours. By contrast, the tissues, and particularly the liver and kidney, were found to contain considerable portions (57% in total) of the administered radioactivity. In breath, a mere 0.2% and 0.01% of the administered radioactivity was recovered as CO_2 and other volatiles, respectively. Analysis by high performance liquid chromatography (HPLC) of the radioactive compounds excreted in urine revealed that the very predominant compound was unchanged diethanolamine, accompanied by small quantities of metabolites which were not identified further. This demonstrated that diethanolamine is absorbed well by the body after oral administration, accumulates in various organs and undergoes slow excretion predominantly via the urine. There was little metabolism. Very similar values were obtained when animals were treated with diethanolamine at 200 instead of 7.9 mg/kg body weight using the same experimental design. The chemical was absorbed rapidly and completely by the body. Total urinary excretion within 48 hours was only 20 to 30% of the administered radioactivity. Less than 3% was excreted in the faeces. The distribution pattern of diethanolamine storage in the organs was comparable at both dose levels. Hence, absorption, distribution and excretion rate were not dose-dependent for diethanolamine (RTI, 1991; Mathews et al., 1997).

The form in which diethanolamine accumulates in the tissues was investigated by analysing the radiolabelled compounds in the blood, liver and

brain of rats treated as described above. One gram of minced tissue or 1 ml of whole blood was homogenised with 10 ml phosphate-buffered saline (pH 7.1), vortexed with 20 ml chloroform/methanol 2 : 1 and centrifuged. This procedure extracted 87 to 89% of the radioactive compounds into the aqueous phase, while 6 to 9% distributed into the chloroform phase. Analysis of the aqueous phase by HPLC showed 70 to 80% of the radioactivity accumulated in the liver and brain to represent unchanged diethanolamine. Additionally, small quantities of N-methyldiethanolamine and N,N-dimethyldiethanolamine were detected in liver along with one fraction containing phosphates of diethanolamine and its methyl and dimethyl derivatives (95, 2 and 2%, respectively), as demonstrated by incubation of that fraction with alkaline phosphatase and subsequent analysis by HPLC. Brain was found to contain neither the methyl nor the dimethyl compound, but instead contained only unchanged diethanolamine and diethanolamine phosphate. It was possible to separate the chloroform extract of liver into two fractions which proved to be phosphatidylethanolamine and phosphatidylcholine and each contained 50% of the bound diethanolamine that was extractable from this phase. Following incubation with phospholipase D, the phosphatidylethanolamine fraction was detected to contain unchanged diethanolamine while the phosphatidylcholine fraction yielded N-methyldiethanolamine (15%) and N,N-dimethyldiethanolamine (85%). For brain, 97% of the radioactivity accumulating in the chloroform extract was analysed as unchanged diethanolamine in the phosphatidylethanolamine fraction. By first incubating the chloroform-extractable material with sphingomyelinase, an enzyme which specifically cleaves the phosphate-ceramide bond, it was demonstrated that about 30% and 70% of the radioactivity in the liver extract was bound to ceramides and phosphoglycerides, respectively. About one third of the radioactivity associated with ceramide and about 60% of that associated with phosphoglycerides represented N-methyl derivatives of diethanolamine. Fifteen percent and 85% of the radiolabelled phospholipids isolated from brain tissue were ceramide derivatives and phosphoglycerides, respectively. N-methyl derivatives of diethanolamine were found only in small quantities in brain. About 20% of blood radioactivity was also present in the form of phospholipids, of which 80% contained N-methylated diethanolamine. Protein binding was largely excluded for diethanolamine and its metabolites. Upon ultracentrifugation of liver homogenate, only 6 to 7% of the radioactivity was bound to proteins. The study demonstrated that by far the largest portion of diethanolamine

retained in tissue was present in the form of unchanged compound but that part of the diethanolamine was methylated at the nitrogen atom and/or phosphorylated at the oxygen atom and that these metabolites were incorporated into phospholipids instead of choline. The investigators concluded that diethanolamine and its N-methyl derivatives were, like choline, transported into the cell, where they accumulated and were to some extent incorporated into phospholipids. The latter were then incorporated into the cell membranes. In the investigators' opinion, these reactions could be responsible for the longer-term effects of diethanolamine in the body (Mathews et al., 1995, 1997).

Based on the same experimental design, diethanolamine was administered to a further group of 4 male rats (F344-M, weighing approx. 160 to 190 g) by oral gavage for 5 days. The daily dose was 7.8 mg/kg body weight and radioactive diethanolamine was admixed at approx. 3 µCi/administration. Urine, faeces, blood and organs were obtained and examined as described above. Urine and faeces were additionally collected for all 24-hour periods and analysed for radioactivity. On none of the days did urinary and faecal excretion of diethanolamine keep pace with the rate of administration, indicating steady accumulation of diethanolamine. Only about 40% of the total dose absorbed was excreted. The detectable levels of radioactivity in the tissues after 5 days were 3 to 5 times higher than levels noted after single dosing without there being any marked changes in the patterns of distribution to specific tissues. Analysis of the radioactive substances present in the urine and the liver extract revealed no qualitative differences between single-dose administration and 5 repeated doses. When additional rats, instead of receiving diethanolamine at 7.8 mg/kg body weight, were given daily oral doses of 0.7 or 200 mg/kg body weight with the same amount of radioactivity per administration, excretion observed for the 0.7 and 7.8 mg/kg doses was proportional to the dose administered. At 200 mg/kg body weight there was saturation. Excretion was increased proportionally, but there were also very high diethanolamine concentrations in the liver and kidney that reached 2 mg/g tissues (RTI, 1991; Mathews et al., 1997).

In order to investigate the bioaccumulation of diethanolamine in greater detail, a further study was conducted in additional groups of 4 to 6 male rats (F344-M, weighing approx. 160 to 190 g) given daily oral treatment with diethanolamine 5 times per week for 2, 4 or 8 weeks. Examinations were performed in the same manner as described above. The daily dose

was 7 to 8 mg/kg body weight and the amount of radioactivity administered per dose was about 1 to 2 µCi. At the end of each treatment period, the animals were sacrificed and blood and organ and tissue samples were collected and analysed as described above. During the last week of each dosing schedule, the urine and faeces were collected for all 24-hour periods and analysed for radioactivity. It was found that the percentage of diethanolamine excreted increased with the duration of treatment and that, hence, accumulation ceased to increase once steady state was attained. Thus the percentage of the radioactivity administered in the final week that was excreted in that same period was 68.9%, 79.1% and 92.1% for 2, 4 and 8 weeks' treatment, respectively. Analysis of the organs for residual radioactivity revealed a steady increase up to the end of week 4 and attainment of plateau levels between 4 and 8 weeks. It was concluded from this that tissue levels reached steady state somewhere between 4 and 8 weeks. Any additionally absorbed diethanolamine was excreted. As with single-dose administration, diethanolamine was again essentially excreted in urine as unchanged compound. In addition, however, significant amounts of N-methyldiethanolamine and a metabolite identified as N,N-dimethyl-2-oxomorpholinium were detected after 8 weeks' exposure. One group of rats was not terminated after 4 weeks of diethanolamine administration but kept on without treatment for another 4 weeks. Their urine and faeces were collected on selected days and indicated an elimination half-life of roughly one week. This value was confirmed by the levels of radioactivity measured in the tissues at the end of the study at 8 weeks, showing that > 90% of the administered diethanolamine had been excreted within 4 weeks (RTI, 1991; Mathews et al., 1997).

As studied for single oral administration of diethanolamine to rats, tissues from rats receiving 8 weeks' treatment with radiolabelled diethanolamine as described above were examined with regard to the form in which the chemical accumulated there. The same methods were employed as in tissues from animals given a single dose. Of the radioactivity present in the liver and brain, 97 and 77%, respectively, was found in the aqueous extract and almost exclusively represented unchanged diethanolamine. There were no detectable N-methyl derivatives of diethanolamine. Of the radioactivity present in the liver and brain, 2 and 21%, respectively, was extractable with chloroform/methanol 2 : 1. In blood, about 20% of radioactivity was found to be bound to phospholipids, as noted after single-dose ad-

ministration. All diethanolamine was present as the N-methyl derivative. The radioactivity recovered in the extract of liver was present as a constituent of the phosphatidylcholine fraction and represented N,N-dimethyldiethanolamine, whereas the brain extract was found to contain only compounds of the phosphatidylethanolamine fraction, 97% of which represented unchanged diethanolamine as the carrier of radioactivity. Incubation of the phosphatidylcholine fraction of liver extract with sphingomyelinase demonstrated that almost all N,N-dimethyldiethanolamine present was bound to ceramides. In brain, 65 and 35% of diethanolamine was bound to ceramides and phosphoglycerides, respectively. Compared with the data obtained after single-dose treatment, the picture was essentially the same after a treatment period of 8 weeks. By far the largest portion of diethanolamine retained in tissue was aqueous-extractable, although in brain markedly less was extractable than after single-dose administration. Correspondingly, 21% of the radioactivity present in brain was extractable with chloroform/methanol. The distribution of the N-Methyl derivatives of diethanolamine was now clearer. They were found only in liver and blood. In brain, they were no longer detectable. Unmetabolised diethanolamine was no longer detectable as a phospholipid constituent in liver (Mathews et al., 1995, 1997).

A further study was carried out to investigate the absorption, distribution and excretion of diethanolamine in male rats (F344-M, weighing approx. 170 to 190 g) after dermal administration. Groups of 4 or 5 animals were dermally exposed to diethanolamine at dose levels of 2.1, 7.6 or 27.5 mg/kg body weight, the dorsal skin having been clipped 24 hours in advance, and degreased. A special metal “micro tissue capsule” was glued onto the skin with cyanoacrylate adhesive to cover the application site (2 cm^2) and left in place for 48 hours. ^{14}C-Diethanolamine (7.3 mCi/mmol, 95 to 96% radiochemically pure) was used in quantities of approx. 6 to 20 µCi/dose. The dosing volume was 25 µl of a solution of labelled and unlabelled diethanolamine in ethanol. Urine and faeces were collected for two 24-hour periods, and radiolabelled components in breath were trapped. At the end of the study, the animals were killed and the excess diethanolamine that was not dermally absorbed was carefully recovered from the skin. The skin washes and blood, adipose tissue, muscle, skin, kidney, liver, spleen, heart, lungs and brain were analysed for radioactivity. When administered according to the experimental procedure described above,

diethanolamine was only moderately well absorbed but absorption occurred in a dose-related manner (maximum 16.2% at the highest dose). A significant portion (approx. 30%) thereof remained in the tissue at the dose site. Another significant portion (approx. 40%) was retained in various organs of the body. No more than 26 and 1.9% of the absorbed radioactivity was excreted in urine and faeces, respectively. Less than 0.1% of the absorbed radioactivity was recovered in the expired air. Distribution of absorbed diethanolamine in the body followed the same pattern as that observed after oral administration. The liver and kidney were most predominantly involved, and apart from them the lung and heart. No further analytical determinations were performed (RTI, 1991; Mathews et al., 1997).

Another dermal study investigated the absorption and distribution of diethanolamine in 10 female rats (Sprague-Dawley, weighing 230 to 300 g). A single dermal dose of diethanolamine, containing ^{14}C-diethanolamine (21 µCi/rat, 15 mCi/mmol, 98% radiochemically pure), was applied to 19.5 cm² of clipped dorsal skin at 1500 mg/kg body weight. The skin was covered with nonabsorbent gauze and Saran® film, the cover being left in place for 6 hours. The cover was subsequently removed and the skin was washed in half of the animals. Urine and faeces were collected for 48 hours after dosing, after which period the animals were killed and the organs analysed to determine the distribution of radioactivity. Blood was withdrawn at scheduled intervals via an indwelling cannula implanted in the jugular vein and the samples were also analysed for radioactivity. Absorption was low, with respective recovery rates for administered radioactivity being 1.4% and 0.64% in animals with unwashed skin and animals which had the diethanolamine washed off after 6 hours. The resulting absorption rates were calculated as 45 and 21 g/cm²/hour, respectively. Very little of the administered radioactivity was excreted in urine (0.11%) and faeces. The blood contained detectable but non-quantifiable amounts of diethanolamine as radioactivity. The absorbed diethanolamine was recovered mainly in liver and kidney and in the eviscerated carcass (Dow, 1995).

The effect of multiple dosing on the dermal absorption rate of diethanolamine was also investigated in female rats (Sprague-Dawley, weighing 230 to 300 g). The same methods were used as described above. Groups of 4 animals had diethanolamine doses applied to 25 cm² of the clipped dorsal skin at 1500 mg/kg body weight once daily for 2 or 5 days. The skin was covered with nonabsorbent gauze and Saran® film, the cover being left

in place for 6 hours. Subsequently, the gauze was removed and the skin washed. One day after the treatment with non-radiolabelled diethanolamine, the rats were exposed to a single dose of the same strength which contained ^{14}C-diethanolamine (21 µCi/rat, 15 mCi/mmol, 98% radiochemically pure) and was left in place at the dose site for 48 hours. Animals that were treated three times absorbed a total of 21% of the administered radioactivity, while those treated six times absorbed 42%. Of the absorbed radioactivity, 4.3 and 13% underwent urinary excretion in animals that were treated three and six times, respectively. Faecal excretion accounted for 0.06 and 0.3% after 3 and 6 treatments, respectively. The majority of the absorbed radioactivity was recovered in liver and kidney and in the eviscerated carcass. Less than 0.3% was detected in the brain, fat or heart. In total, 16% (2-day pretreatment) and 28% (5-day pretreatment) of the administered radioactivity was found in the tissues. Diethanolamine was also detected in blood at a concentration level of approx. 10 µg/g during dosing days. Pretreatment caused a very marked increase in the absorption of diethanolamine via the skin (Dow, 1995).

Mice (B6C3F1 strain, weighing 23 to 25 g) were also studied in order to investigate the absorption, distribution in the body, and elimination of diethanolamine following dermal administration. Four males had diethanolamine applied to 1 cm² of clipped and degreased dorsal skin as a single dose of 8, 23 or 81 mg/kg body weight containing ^{14}C-labelled diethanolamine (6 to 20 µCi, 7.3 mCi/mmol, 95 to 96% radiochemically pure) in a dosing volume of 15 µl/animal. The dose site was covered by means of a special “micro tissue capsule” which was glued to the skin using cyanoacrylate adhesive and left in place for 48 hours. Urine and faeces were collected for two 24-hour periods, and radiolabelled components in breath were trapped. At the end of the study, the animals were killed and the excess diethanolamine that was not dermally absorbed was carefully recovered from the skin. The skin washes and blood, adipose tissue, muscle, skin, kidney, liver, spleen, heart, lungs and brain were analysed for radioactivity. The respective amounts of the radioactivity absorbed within 48 hours were 27, 34 and 58% after application together with diethanolamine doses of 8, 23 and 81 mg/kg body weight. At the highest dose level, 19% of the absorbed radioactivity was excreted in urine and faeces (16.4 and 2.6%, respectively), whilst 39.2% remained in the body, 2.2% of which remained at the dose site. Of the radioactivity administered, 24.8% was recovered as unabsorbed radio-

activity. Thus, the overall recovery rate in this study was 82.9%. Determination of the radioactivity present in the tissues revealed high accumulation in liver (16.7% of the administered radioactivity), muscle (9.5%) and kidney (4.2%). All other values were below 3%. Less than 1% was found in breath (RTI, 1991; Mathews et al., 1997).

In a further, detailed study, groups of 11-week-old female Sprague-Dawley rats (weighing 247 to 271 g) were implanted with a jugular vein cannula via which they were treated intravenously with ^{14}C-labelled diethanolamine (97.4% pure, specific activity 15 mCi/mmol). Groups of 5 animals were administered diethanolamine (99.3% pure) at 10 or 100 mg/kg body weight as a single dose containing radiolabelled diethanolamine (4.2 µCi/animal). Subsequently, the urine and faeces were collected at 12-hour intervals for a total of 96 hours, and blood was withdrawn via the jugular vein cannula at 5, 10, 15 and 30 minutes and 1, 2, 4, 6, 12, 24, 36, 48, 60, 72 and 84 hours after administration. The animals were sacrificed by exsanguination via cardiac puncture at 96 hours after dosing. Blood, liver, kidney, heart, brain, stomach and fat as well as skin and skinned carcass were taken and the tissues and faeces processed to yield aqueous homogenates. All homogenates and the blood and urine were analysed for radioactivity. The levels of radioactivity in the blood, blood cells and urine were also measured as a function of time after dosing. In total, 96% and 94.8% of the administered radioactivity was recovered in the 10 mg/kg and 100 mg/kg groups, respectively. By the time of sacrifice, 64.1% and 52.5% of the administered radioactivity had been distributed to the tissues in animals from the 10 mg/kg and 100 mg/kg groups, respectively. Respective portions of 34.6% and 28.2% were accounted for by the eviscerated and skinned carcass, 20.9% and 17.1% by the liver, 7.2% and 4.9% by the kidneys and 5% by the two dermal dosages. Each of the other organs examined contained less than 1% of the administered radioactivity. The highest tissue concentrations were 26 and 199 µg equivalents of diethanolamine/g tissue, which were found in the kidneys. Excretion of the absorbed diethanolamine took place mainly via the urine. In total, 25% (at 10 mg/kg body weight) and 36% (at 100 mg/kg body weight) were excreted via this route during the observation period of 96 hours, whereas faecal excretion accounted for as little as 1.2% and 1.5%, respectively. A significant portion (23%) of the total amount excreted was recovered in urine within the first 12 hours after administration at the 100 mg/kg dose level, whereas only 8.5% was excreted

during that period at the 10 mg/kg level. Peak concentrations of plasma radioactivity were measured 5 minutes after administration. Elimination subsequently took place according to a biphasic process with half-lives of 9.2 minutes and 258 hours at the 10 mg/kg dose level and 16.3 minutes and 206 hours at the 100 mg/kg level. The latter value was corrected to 113 hours in a later publication (Dow, 1995; Mendrala et al., 2001).

Three male rats (F344-M, weighing approx. 169 to 190 g) were also intravenously injected with ^{14}C-labelled diethanolamine (7.3 mCi/mmol, 95 to 96% radiochemically pure), dissolved in phosphate-buffered physiological saline. A single dose of the chemical was injected into the lateral tail vein at 7.5 mg/kg body weight (together with approx. 3 µCi). Urine and faeces were collected during two 24-hour periods and analysed for radioactivity. The radiolabelled components in breath were collected in traps and determined. At the end of the study, the animals were sacrificed and the levels of radioactivity determined in the blood, adipose tissue, brain, heart, kidney, liver, lung, muscle, skin and spleen. By 48 hours after administration, 28.3% of the administered radioactivity was excreted in urine whereas 0.6% was recovered in faeces and 0.2% as CO_2 in breath. A fraction of 0.01% of the radioactivity found in the expired air was not CO_2. By far the largest portion of radioactivity (53.7%) was retained in the organs and in muscle (15.3%). Primarily involved were the liver (27.1%), skin (4.5%) and kidney (4%). Other tissues and blood retained only minor quantities of radioactivity. Analysis of urine and liver homogenate by HPLC revealed that the very predominant radioactive compound present was unchanged diethanolamine, accompanied by small quantities of metabolites which were not identified further. The same study, when conducted in mice (B6C3F1, weighing 23 to 25 g) given diethanolamine at 14.9 mg/kg body weight, yielded very similar results, which differed only marginally from those obtained in rats (RTI, 1991; Mathews et al., 1997).

An in-vitro study on the skin penetration and dermal absorption of diethanolamine employed skin preparations from female rats (CD, about 10 weeks old), female mice (CD1, about 6 weeks old) and female New Zealand White rabbits (11 to 12 weeks old) as well as fresh human skin samples from mammoplasty patients. The animal preparations originated from the clipped dorsal trunk in the thoracic region. All skin preparations were maintained in minimum essential medium (MEM), mounted in a skin penetration apparatus and bathed with minimum essential medium at a flow rate

of 2.5 ml/hour. After 30 minutes, diethanolamine (99% pure) was applied at 20 mg/cm² to the surface of the skin (1.77 cm² total exposed surface area) mounted in the apparatus. Per skin sample, 5 to 10 µCi ^{14}C-diethanolamine (150 mCi/mmol, approx. 96.5% radiochemically pure) was admixed. Diethanolamine was applied either undiluted or as a 37-percent aqueous solution and the skin preparations were covered with two-ply gauze after dose application in order to simulate the conditions of in-vivo dosing. The medium passing under the skin preparations was collected over a period of 6 hours and analysed for radioactivity. ^{14}C-Ethanol was used as a control for which the rates of penetration through the various types of skin were known. The skin samples studied showed values in the normal range, thus demonstrating the integrity of the skin preparations. At the termination of each experiment, determinations were carried out for unabsorbed radioactivity, radioactivity which had penetrated through the skin, radioactivity absorbed into the skin and residual radioactivity rinsed out of the apparatus. Additionally, the recovery rate was calculated together with the penetrating radioactivity found in the effluent medium. It was approx. 80% in all cases, with the exception of the rat skin experiments with undiluted diethanolamine, where it was 95%. By far the largest portion of radioactivity remained on the skin unabsorbed, with only small amounts having penetrated into or through the skin. Dilution of diethanolamine with water was found to enhance these processes. A steady-state penetration rate was determined by plotting versus time the cumulative amount of radioactivity that penetrated the skin. The data are summarised in Table 2.

Table 2. Skin penetration and absorption of diethanolamine in vitro after treatment with undiluted diethanolamine as compared with a 37-percent solution*

	Mouse	Rabbit	Rat	Human
Dose absorbed in 6 hours (% of radioactivity administered)	1.30/6.68	0.02/2.81	0.04/0.56	0.08/0.23
Skin absorption in 6 hours (% of radioactivity administered)	0.68/1.28	0.44/2.06	0.08/0.46	0.95/1.88
Penetration rate (µg/cm²/hour)	46.3/294.40	0.9/132.20	1.8/23.00	5.7/12.70

* The data obtained with undiluted diethanolamine are separated by a slash from those obtained with the 37-percent aqueous solution.

Absorption into and penetration through the skin were markedly higher in the experiments using the 37-percent solution. Moreover, the investigators clearly pointed out that there were very marked interspecies differences. There was a marked decrease in the skin permeability of diethanolamine from the mouse to the rabbit, rat and humans (Sun et al., 1996).

The absorption and distribution of diethanolamine in human tissue was investigated in liver slices, which were maintained in modified Krebs-Henseleit buffer at pH 7.4. The specimens were 250 µm thick and were obtained from a medical research institute in Baltimore. They were incubated at 37 °C in buffer containing ^{14}C-diethanolamine at a concentration of 1.05 mM (equivalent to 110 µg/ml). The slices were removed after 4 and 12 hours and homogenised in water by ultrasonication for one minute. Further processing was carried out as described above for the same investigators' work on liver from animal sources. The liver slices absorbed 11% and 29% of the administered radioactivity within 4 and 12 hours, respectively. Of the radioactivity remaining in the buffer medium, 85 to 97% were recovered from the medium as unchanged diethanolamine, accompanied by small quantities of 4 metabolites which were not further characterised. The aqueous extract from the homogenate contained 74 and 92% of the radioactivity absorbed by the liver slices after incubation for 4 and 12 hours, respectively. By far the largest portion of this radioactivity was accounted for by unchanged diethanolamine. Of the 4 metabolites found in total, two compounds each represented 1.5% of the radioactivity recovered after 4 hours' incubation. After 12 hours, there were 3 detectable metabolites, which together accounted for 14% of the radioactivity. One of the metabolites detected at 4 hours no longer appeared. The chemical structures of the metabolites were not given. More than 90% of the radioactivity present in the chloroform/methanol extract was attributed to phospholipids which contained diethanolamine in the unchanged form. After 12 hours' incubation, small amounts (0.3%) of N-methylated diethanolamine were also detected in the phospholipids. More than 80% and 95% of phospholipids containing radiolabelled diethanolamine after 4 and 12 hours, respectively, consisted of ceramide derivatives known as sphingomyelins, in which choline had been replaced by diethanolamine. This evidence demonstrated that the absorption, metabolism and incorporation of diethanolamine take place in same manner in human tissue as in the rat (Mathews et al., 1995).

7.2 Acute and subacute toxicity

Acute toxicity

The acute toxicity data for diethanolamine following single-dose administration are summarised in Table 3.

Beginning of Table 3

Table 3. Acute toxicity of diethanolamine following single-dose administration

Species, strain, sex[1]	Route	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Rat, Sherman, male	oral	1820, 2830	LD_{50} (with batches of various origins)	14 days	Mellon Institute, 1950; Smyth et al., 1951
Rat, Sherman, female	oral	1410, 1650, 1660	LD_{50} (with batches from different years of production)	14 days	Mellon Institute, 1950; Smyth et al., 1951
Rat	oral	3540	LD_{50}	n. d.	Anonymous, 1968
Rat	oral	710	LD_{50}	n. d.	Smyth et al., 1970
Rat	oral	ca. 2300	LD_{50}	n. d.	Eastman Kodak, 1982
Rat	oral	3460	LD_{50}	n. d.	Izmerov et al., 1982
Rat	oral	ca. 1600 (males: 2500, females: 1100)	LD_{50}, reeling, forced respiration; gastrointestinal irritation, hydrothorax	7 days	BASF, 1966
Mouse	oral	4570	LD_{50}	n. d.	Eastman Kodak, 1982
Mouse	oral	3300	LD_{50}	n. d.	Izmerov et al., 1982
Rabbit	oral	2200	LD_{50}	n. d.	Izmerov et al., 1982
Guinea pig	oral	2200	LD_{50}	n. d.	Izmerov et al., 1982
Rabbit	dermal	13000	LD_{50}	n. d.	INRS, 1991
Rabbit	dermal	12200	LD_{50}	n. d.	National Association of Printing Ink Research Institute, 1974
Rat	inhalation	inhalation hazard test, enriched atmosphere at 20 °C, 8-hour exposure	all 12 animals survived; no toxic effects	7 days	BASF, 1966

Table 3. Acute toxicity of diethanolamine following single-dose administration					
Species, strain, sex[1]	Route	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Rat	inhalation	inhalation hazard test, saturated vapours, 8-hour exposure	all 6 animals survived	n. d.	Union Carbide, 1966
Rat, male	inhalation	875 (vapours, duration of exposure unspecified)	deaths and respiratory difficulties	n. d.	Hartung et al., 1970
Rat, male	inhalation	6122 (aerosol, duration of exposure unspecified)	deaths and respiratory difficulties	n. d.	Hartung et al., 1970
Rat, Sprague-Dawley, male	inhalation	6433 (vapour and aerosol, duration of exposure unspecified)	deaths within less than 2 hours; pulmonary oedema, liver and kidney lesions	n. d.	Foster, 1972
Rat	intraperitoneal	1500	changes in serum electrolytes (decreased Na^+ and PO_4^{3-}; increased K^+, Ca^{2+} and Mg^{2+}); depressed heart rate and EEG abnormalities; ascites, increased kidney and liver water content; degenerative changes of hepatocytes and renal tubular epithelia, vacuolisation of cytoplasm and mitochondrial swelling in liver cells	n. d.	Foster, 1972
Rat	intraperitoneal	160	LD_{50}	n. d.	Eastman Kodak, 1982
Mouse	intraperitoneal	ca. 400	LD_{50}; lying on the side, dyspnoea, convulsions; adhesions between intestinal loops	7 days	BASF, 1966
Mouse	intraperitoneal	210	LD_{50}	n. d.	Eastman Kodak, 1982
Mouse, Swiss-Webster	intraperitoneal	2300	LD_{50}	24 hours	Blum et al., 1972
Mouse, Swiss-Webster	intraperitoneal	1700	sedation, ataxia, loss of righting reflex followed by death after 15 minutes to 24 hours; microscopy revealed lesions in the liver tissue and electron microscopy revealed swollen mitochondria in the hepatocytes	24 hours	Blum et al., 1972
Rat	subcutaneous	2200	LD_{50}	n. d.	Izmerov et al., 1982
Mouse	subcutaneous	3553	LD_{50}	n. d.	Koch, 1954
Rat	intramuscular	1500	LD_{50}	n. d.	Izmerov et al., 1982

Table 3. Acute toxicity of diethanolamine following single-dose administration					
Species, strain, sex[1]	Route	Dose (mg/kg body weight or mg/m³)	Effects	Observation period	Reference
Dog	intrave-nous	400	all animals died; sympatho-mimetic and parasympatho-lytic effects; blood pressure increase, vasoconstriction, in-crease in respiratory volume, depression of the central nervous system, increased diuresis, cardiotoxicity, intes-tinal spasms; signs preceding death: fall in blood pressure, increased diuresis and defe-cation, mydriasis	–	Mellon Institute, 1954
[1] where specified			n. d. no data		

End of Table 3

In most cases no signs of toxicity were reported and only a numerical value was given for the LD_{50} so that the methodology by which it was obtained remains unknown. With oral LD_{50} values for rats, mice, guinea pigs and rabbits ranging from 1410 to 4570 mg/kg body weight (with the exception of one value, which was reported as 710 mg/kg body weight), diethanolamine was of low toxicity after single-dose treatment. Based on the experimentally determined, markedly lower absorption via the skin as compared with oral administration (see Section 7.1), the chemical's dermal toxicity in rabbits was found to be even lower, with LD_{50} values being ascertained as 12200 and 13000 mg/kg body weight. Single inhalation exposure of rats to diethanolamine resulted in deaths only at very high concentration levels. However, the validity of the inhalation toxicity data for diethanolamine is unclear due to the complete absence of experimental details. Intraperitoneal administration of diethanolamine to rats and mice at dose levels between 160 and approx. 400 mg/kg body weight was lethal in 50% of animals. An LD_{50} value of 2300 mg/kg body weight following intraperitoneal administration and an observation period of only 24 hours does not fit into the overall picture, but it was not possible to compare the value with the other data because of the lack of experimental details (Blum et al., 1972). Subcutaneous LD_{50} values for rats and mice and an intramuscular LD_{50} value for rats were found to be between 1500 and 3553 mg/kg body weight and thus were in the oral LD_{50} range. Acute toxicity was not clearly related to sex or species in the available data. However, the impression is that mice were somewhat less sensitive to diethanolamine than rats.

Little has been reported on the target organs for, and signs of, acute diethanolamine intoxication. Apart from rather nonspecific effects, the liver and kidney have been under discussion as target organs and changes in serum electrolytes have been reported. The liver as a target organ for diethanolamine toxicity has been the subject of several rat studies in which light and electron microscopy examinations were carried out in addition to determinations of serum enzymes specific to liver damage.

Groups of 7 to 9 male Sprague-Dawley rats (mean body weight 380 g) were treated once with diethanolamine, dissolved in water and neutralised with HCl, at dose levels of 0 (controls), 100, 200, 400, 800, 1600 or 3200 mg/kg body weight by oral gavage. The volume administered was 5 ml/kg body weight in each case. The animals were exsanguinated and necropsied 18 hours after the administration of diethanolamine. Blood, liver and kidneys were obtained and the tissues examined by light and electron microscopy. Serum enzyme activities were measured for 10 enzymes, and serum concentrations of urea, ornithine and arginine were determined. All dose groups exhibited increases in liver and kidney weights in a dose-related manner. However, the kidney showed a marked increase only at dose levels of 1600 mg/kg body weight and above. From 800 mg/kg body weight, serum concentrations of urea increased greatly in a dose-related manner whereas arginine concentrations dropped. Ornithine concentrations were unchanged at all dose levels. The activities of all 10 enzymes in serum were markedly increased by administration of diethanolamine at dose levels of 800 and 1600 mg/kg body weight. The increases occurred in a dose-related manner, with the most pronounced increases being seen at 3200 mg/kg body weight (see Table 4).

Table 4. Serum enzyme activities in rats after a single oral dose of diethanolamine at various dose levels (nmol substrate converted/minute/ml serum)

Enzyme	Diethanolamine dose (mg/kg body weight)			
	0 (controls)	800	1600	3200
Ornithine carbamoyl transferase	0.007	0.008	0.031	0.032
Glutamate dehydrogenase	0.51	0.51	0.69	0.98
Isocitrate dehydrogenase	0.45	0.78	1.14	2.14
Sorbitol dehydrogenase	0.47	1.09	1.65	1.95
Fructose-1-phosphate aldolase	0.24	0.89	1.36	1.56
Fructose-1,6-diphosphate aldolase	1.39	1.48	1.94	2.03
Glutamic-pyruvic transaminase	1.11	1.42	1.63	2.14
Glutamic-oxaloacetic transaminase	1.62	1.98	2.33	2.97
Malate dehydrogenase	1.87	2.16	2.50	3.75
Lactate dehydrogenase	2.57	2.68	2.84	3.16

Light microscopy revealed degenerative changes of the parenchymal cells of the liver from 200 mg/kg body weight. These were more extensive at 400 mg/kg body weight, at which dose level degenerative kidney changes with tubular necrosis also occurred. Higher dose levels were associated with even more pronounced degenerative changes; in particular tubular necrosis was noted in the kidneys at 800 and 1600 mg/kg body weight. Electron microscopy revealed widespread changes in the liver cells from rats given diethanolamine at 1600 mg/kg body weight. The cells contained numerous large lipid droplets. The rough and the smooth endoplasmic reticulum were both swollen, and this was accompanied by loss of ribosomes. Diethanolamine did not affect the morphology of the mitochondria, lysosomes, nuclei and nucleoli or the glycogen distribution in any of the dose groups. The increased serum enzyme activities led the investigators to conclude, none the less, that the mitochondria were affected by diethanolamine (Korsrud et al., 1973).

In a further study, male Sprague-Dawley rats (weighing 300 g) were force fed orally (no further details) with diethanolamine at a dose level of 1000 mg/kg body weight in a casein hydrolysate-containing diet lacking choline. Three animals received the dose as a single administration and were sacrificed at 2, 5 or 24 hours after dosing. Another three animals were treated with one-third of the dose three times daily at 6-hour intervals for 4 consecutive days and sacrificed at 24 hours after the last administration. Two of the animals were fed with a diet containing choline and cholesterol during the last 24 hours. Biopsies of pancreas were obtained at 0, 2 and 5 hours after feeding from one animal and at 0 and 1 hours from the other. Tissue samples of pancreas and liver were taken from all animals upon sacrifice and examined by light and electron microscopy with particular attention to serous acini of pancreas. Light microscopy revealed large hepatocellular vacuoles in the livers from rats given repeated doses, while the basophilic granules in the cytoplasm were very small. The same animals were found to have only very few granules (zymogen granules) in their pancreatic acinar cells. Feeding of choline and cholesterol in the diet reduced the number of hepatocellular vacuoles after only 5 hours and completely reversed the effect within 24 hours, but did not influence the changes in the cytoplasm. Following a single dose of diethanolamine, swelling of the mitochondria was observed with a maximum at 5 hours, a finding which was reversible after 24 hours. Upon repeated treatment, the rough endoplasmic reticulum was markedly changed, the shape of the

Golgi complex showed minor alterations and the smooth endoplasmic reticulum was slightly enlarged. When the animals received diet containing choline and cholesterol the changes in rough endoplasmic reticulum returned to normal within 5 hours. After single administration of diethanolamine, the pancreatic acinar cells exhibited slight to moderate focal degeneration, disappearance of zymogen granules and vacuolisation of the Golgi complex. Furthermore, there was damage to the rough endoplasmic reticulum (disruption, vacuoles). Repeated treatment intensified the effects. Feeding of diet containing choline and cholesterol resulted in a rapid recovery of the rough endoplasmic reticulum and a slower normalisation of the quantity of zymogen granules. The investigators concluded from their findings that, in the cells studied, diethanolamine prevented formation of intracellular membranes with subsequent depletion of the endoplasmic reticulum. They suspected that diethanolamine inhibited lipid metabolism in the cells (Hruban et al., 1965).

Groups of 6 male Sprague-Dawley rats (weighing 225 to 300 g) were treated once with diethanolamine at dose levels of 0 (water control), 100 or 500 mg/kg body weight, fasted for 4 or 24 hours after dosing and then sacrificed. Diethanolamine was dissolved in water, neutralised with HCl and administered to the animals intraperitoneally. Serum lactate dehydrogenase and aspartate aminotransferase determinations and light and electron microscopy studies of liver and kidney tissues were carried out for all animals. At 4 hours after administration of diethanolamine at 100 or 500 mg/kg body weight, vacuolisation of the cytoplasm and cytoplasmic basophilia were observed in the liver tissue of animals from either dose group. Electron microscopic findings were also characterised by cytoplasmic vacuolisation. The mitochondria appeared swollen and less dense. The effects were even more marked at 24 hours after administration. No necrotic liver cells were observed. The renal tubules exhibited the same effects as seen in liver tissue (vacuolisation and rarefaction of the cytoplasm). The renal effects were observed to a markedly lesser degree at 100 mg/kg body weight. Elevation in serum lactate dehydrogenase to approximately twice the control level was seen only at the 500 mg/kg dose level at both 4 and 24 hours after administration. Aspartate aminotransferase activity levels were only slightly elevated at 100 mg/kg body weight whereas a three-fold increase was seen at 500 mg/kg body weight. This increase also did not depend on the post-dosing interval (Grice et al., 1971).

Subacute toxicity

Groups of 10 male albino rats (average body weight 319 g) were fed diethanolamine at levels of 0.01, 0.1 or 1% in their respective diets for a period of 32 days. A control group of 10 animals received food without diethanolamine. The animals' body weights and food consumption data were recorded throughout the entire dosing period. Blood samples were collected on treatment day 28 for haematology studies in the controls and animals treated with diethanolamine at 1 or 0.1%. All surviving animals were sacrificed at the end of treatment. The liver, kidneys, spleen, heart, lungs, brain and testes were excised, weighed and comprehensively examined by histopathology. Of the 10 animals in the group given 1% dietary diethanolamine (which, according to the investigators, corresponded to 330 mg/kg body weight when food consumption was taken into account), 9 died between treatment days 13 and 21 and therefore most of this group was lost to assessment. All animals in the group exhibited a steady decrease in food intake between days 0 and 14, accompanied by a marked loss of weight. The one survivor showed some recovery after treatment day 21. The animals fed dietary diethanolamine levels of 0.1 or 0.01% showed no marked differences relative to controls with regard to food consumption and body weight gain, apart from decreases in both parameters seen in the lowest dose group between treatment days 4 and 8, a finding for which there was no ready explanation. Organ weights, including testes weights, were unchanged in both dose groups relative to the control group, except for the liver weights, which were increased. The one survivor of the 1% group exhibited a severe decrease in blood haemoglobin concentration, a low haematocrit and a very high white blood cell count. Animals fed dietary diethanolamine at 0.1% showed statistically significant decreases in haemoglobin concentration and haematocrit relative to concurrent study controls, although the values were in the normal range. The white blood cell count did not differ from the control. No substance-related histopathological changes were found (Eastman Kodak, 1967 a).

The study discussed above was repeated using exactly the same experimental design, only that determinations of alkaline phosphatase, aspartate aminotransferase and serum protein and urinalyses were additionally carried out in all animals. The repeat study confirmed the findings of the original study, except for the decreases in body weight and food intake previously noted from treatment days 4 to 8 in animals fed 0.01% diethano-

lamine in their diet. Of the 10 animals in the 1% group, 7 died between days 14 and 21 of treatment, and another, eighth animal had to be euthanised during this period. No organ weights were determined for this group. Liver, kidney and testes weights did not differ from the controls. The increased liver weights seen previously were not confirmed. Confirmation was obtained for the haematology results obtained earlier for one animal of the 1% group (4 animals were examined on day 21 of dosing). The same applied to the other two dietary concentrations. Serum protein was markedly decreased at the highest dietary concentration, but was normal at the other two concentrations. Serum enzyme activities were in the normal range, with a high variability being observed at the highest diethanolamine concentration. Histopathological examinations were carried out on the liver, kidney and testes of the controls and the animals of the 1% group. They showed no organ toxicity for diethanolamine (Eastman Kodak, 1968).

Repeated oral or intraperitoneal administration to male Sprague-Dawley rats of diethanolamine, dissolved in water and neutralised with HCl, at a daily dose level of 9.5 mmol/kg body weight (equivalent to 1000 mg/kg body weight), depending on the duration of treatment, resulted in hypocalcaemia, accompanied by the classical signs of tetany and very greatly reduced blood clotting within 3 to 5 days. A three-fold increase in urinary calcium excretion was partially responsible for development of calcium deficiency. The effect was less pronounced at a daily dose of 5.3 mmol/kg body weight (equivalent to 557 mg/kg body weight). Treatment of rats at daily doses of 0.25 to 5.3 mmol/kg body weight (equivalent to 26 to 557 mg/kg body weight) for 2 to 49 days resulted in adrenocortical hyperfunction, manifested by increased plasma corticosterone, increased liver glycogen, hyperglycaemia, thymus involution and increased blood urea nitrogen. Prolonged administration of diethanolamine resulted in a dose-related normochromic-normocytic anaemia (no further details; Foster, 1972).

Neonatal Sprague-Dawley rats received daily oral treatment with diethanolamine at dose levels of 1, 2 or 3 mmol/kg body weight (equivalent to 105, 210 or 315 mg/kg body weight) from 5 to 15 days post-partum. The liver, heart, kidney and brain were obtained at the end of the treatment period for determination of organ weights, moisture content and enzyme activity of the membrane-bound enzymes cholinesterase and succinic dehydrogenase in various cell fractions. Significant increases in relative organ weights were noted for the liver and kidney at the intermediate and highest

dose levels and for the other examined organs at the highest dose level. No increases in moisture content were observed for any organs. The succinic dehydrogenase activity in the nuclear and mitochondrial fractions was elevated for the liver and in the mitochondrial fractions for the kidneys, respectively, following the highest dose. Significant increases in cholinesterase activity were also noted in the mitochondrial fraction of these organs (no further details; Burdock and Masten, 1979).

The neutralised hydrochloride of diethanolamine, dissolved in water, was administered orally to neonatal Sprague-Dawley rats at daily dose levels of 1, 2 or 3 mmol/kg body weight (equivalent to 105, 210 or 315 mg/kg body weight) between the 5th and 15th day of life. Examination of the livers by microscopy and electron microscopy revealed periportal swelling and vacuolisation. The mitochondria were swollen and exhibited numerous fat droplets, glycogen accumulation and fingerprint degeneration (no further details). The animals' livers and kidneys were homogenised and nuclear, mitochondrial and microsomal fractions isolated. The fractions were assayed for ^{14}C-diethanolamine uptake (presumably following co-administration with unlabelled diethanolamine). Additional assays were carried out for protein, lipid and phosphorus content and succinic dehydrogenase, acetylcholinesterase, aminopyrine N-demethylase and aniline hydroxylase activity. Lipids were extracted from the fractions and separated by thin-layer chromatography. Uptake of radiolabelled diethanolamine was found to occur primarily in the renal and hepatic mitochondrial fractions. Thin-layer chromatography revealed that the phospholipid radioactivity was heterogeneously distributed; the microsomal fraction contained various phospholipid intermediates whereas radioactivity was more directly associated with the phospholipids themselves in the nuclear and mitochondrial fractions. Protein and lipid content was increased in nuclear and mitochondrial fractions of liver with no concomitant increase in phosphorus, indicating the formation of atypical phospholipids. All three parameters in the hepatic microsomal fraction remained unchanged by diethanolamine treatment. Succinic dehydrogenase activity was increased in both nuclear and mitochondrial fractions of liver and mitochondrial fraction of kidney, indicating an increase in the friability of the membranes. Acetylcholinesterase activity was unchanged, as was aminopyrine N-demethylase activity. Aniline hydroxylase activity was depressed in the microsomal fraction of liver. The findings led the investigator to conclude that diethanolamine was incorporated into mi-

corporated into mitochondrial phospholipid membranes, resulting in enzymatic and morphological changes (Burdock, 1981).

Male Sprague-Dawley rats were given as their drinking water as solution of diethanolamine in water, neutralised to pH 7.4. Concentration levels of 0.25, 1.3 or 5 mg/ml were administered for 1, 2 or 3 weeks. The animals were then sacrificed and the hepatic and renal mitochondria isolated and assayed for proper function. The hepatic mitochondria showed a decline of respiratory control and an increase in oxygen consumption during state 4 respiration. The mitochondria in the kidney cells were not affected by the administration of diethanolamine. Effects on hepatic mitochondria occurred only after several days. Drinking water with 3 mg/ml showed no effect when given for 24 hours. The permeability of the inner and outer mitochondrial membranes was unchanged after 3 weeks, even at the highest concentration. Electron micrographs revealed no structural abnormalities in the mitochondria (no further details; Barbee and Hartung, 1976).

In a further study, male Sprague-Dawley rats were administered drinking water with the following diethanolamine concentrations (calculated from the actual water consumption data) for varying periods of time: 0.25 mg/ml (42 mg/kg body weight) for 2 or 5 weeks, 1 mg/ml (160 mg/kg body weight) for 1, 3 or 5 weeks or 3 mg/ml (490 mg/kg body weight) for 1 or 3 days or 1, 2, 3 or 5 weeks. The diethanolamine-containing water was neutralised to pH 7.4 with hydrochloric acid in all cases. The animals were sacrificed by exsanguination via cardiac puncture under anaesthesia at the end of treatment. The hepatic mitochondria were isolated and assayed in suspension for oxygen consumption by means of an oxygen electrode on addition of adenosine diphosphate. Measurements were carried out for the rapid consumption of oxygen occurring until completion of adenosine diphosphate phosphorylation (state 3) and the subsequent slower consumption of oxygen (state 4). The values thus obtained were used to calculate the acceptor control ratio (state 3 : state 4) and the adenosine diphosphate/oxygen ratio. In addition, assays were carried out to measure mitochondrial adenosine triphosphatase activity after 3 weeks' administration of diethanolamine at 3 mg/ml drinking water and to determine the permeability of the inner and outer mitochondrial membranes by means of permeation-dependent biochemical reactions. Mitochondria were examined by electron microscopy after administration of 3 mg/ml drinking water for 2 weeks. No effect was noted on the measured parameters when diethanolamine was admin-

istered for only one day. Diethanolamine was also ineffective at 5 mM in a suspension of mitochondria in vitro. When diethanolamine was administered for up to 5 weeks there was a marked time and dose related increase in state 4 oxygen consumption from a concentration level of 1 mg/ml. After 3 weeks, the same degree of saturation was attained at the two high concentrations (plus 45 to 40%) and was not exceeded after 5 weeks. State 3 oxygen consumption and the adenosine diphosphate/oxygen ratio remained constant for all treatment periods and all diethanolamine concentrations so that there was a decline in acceptor control ratio corresponding to the increased oxygen consumption at state 4 (to approx. 75% of control). The activity of magnesium-dependent adenosine triphosphatase was significantly increased in the mitochondria, which also exhibited increased oxygen consumption at state 4, there being a correlation between these effects. Hepatic mitochondria from animals treated with diethanolamine at 3 mg/ml for 2 weeks showed no effect in the membrane permeability test employed. Electron microscopy revealed slight changes in these mitochondria as compared with controls, particularly with regard to size and shape. The investigators suspected none the less that the increase in state 4 oxygen consumption and the elevation in adenosine triphosphatase activity were due to damage to the mitochondrial membrane (Barbee and Hartung, 1979 a).

In a range-finding study for a 13-week study, groups of 5 male and 5 female 6-week-old F344/N rats (males and females weighing 72 to 74 g and 79 to 81 g, respectively) were administered diethanolamine (> 99% pure) in the drinking water for 14 days. The diethanolamine concentrations used were 0 (controls), 630, 1250, 2500, 5000 and 10000 ppm, with the solutions being adjusted to pH 7.4 with hydrochloric acid. Based on the recorded body weight and water consumption data, the actual dose levels for males/females were 0 (controls), 77/79, 162/158, 319/371, 622/670 or 1016/1041 mg/kg body weight per day. The animals were housed individually for 16 hours on study day 12 during which time urine was collected from each animal. At the end of the 14-day treatment period, blood samples were collected from the retro-orbital sinus and necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis and thymus were weighed. Histopathological examinations of the organs were performed for animals in the control group and the highest surviving dose group as well as all early-death animals and, where necessary, also for lower dose groups in order to determine a *no observed effect level*

(NOEL). Comprehensive haematology and clinical chemistry analyses were performed on blood samples from all survivors, and the concentrations of glucose, protein, urea nitrogen and creatinine were measured in urine, as were the activities of alkaline phosphatase and lactate dehydrogenase. All females in the group given 10000 ppm diethanolamine in the drinking water died on days 4 or 6 of treatment, and 2 males in that group died on day 14. All females in the 5000 ppm group also died on days 5 or 8 of treatment whereas the males survived. There were no further deaths. Body weight gains were dose-dependently reduced in males and females from 5000 and 1250 ppm, respectively. Water consumption was reduced by 10 to 15% in all treated animals. Treatment of rats with diethanolamine resulted in moderate normochromic, microcytic anaemia in both sexes, as indicated by dose-dependent decreases in erythrocyte and reticulocyte counts, haemoglobin concentration and haematocrit. Serum concentrations of creatinine, total protein, albumin and bile acids were increased by treatment with diethanolamine (no further details). Kidney damage occurred dose-dependently in both sexes after administration of diethanolamine. Absolute and relative kidney weights were increased, and renal tubular epithelial necrosis was observed at higher diethanolamine concentrations (from 10000 and 2500 ppm in males and females, respectively). Urine concentrations of urea nitrogen, glucose and protein and lactate dehydrogenase activity were dose-dependently increased. All males treated at 10000 ppm diethanolamine in their drinking water exhibited mild to marked seminiferous tubule degeneration, characterised by a reduction in tubule size and in the number of spermatogenic cells. Large numbers of degenerated cells appeared in the lumen of epididymal tubules. As even the lowest drinking water concentration tested caused nephropathy and haematological changes, the study did not achieve a *no observed adverse effect level* (NOAEL) for diethanolamine (NTP, 1992).

Mice were also investigated in a range-finding study for a 13-week oral study. Groups of 5 male and 5 female 6-week-old B6C3F1 mice (weighing 19 and 21 g, respectively) were offered, as their only drinking water, solutions of diethanolamine (> 99% pure) in water, adjusted to pH 7.4 with hydrochloric acid, for 14 days. The various concentration levels of diethanolamine were 0 (controls), 630, 1250, 2500, 5000 and 10000 ppm. The recorded water consumption and body weight data were used to calculate the actual mean dose levels for males/females as 110/197, 205/326,

415/793, 909/1399 or 1362/2169 mg/kg body weight. All animals were housed individually. After 14 days of treatment, blood samples were collected and necropsies performed on all animals. The brain, heart, right kidney, liver, lung, right testis and thymus were weighed. Histopathological examinations of the organs were performed for animals in the control group and the highest dose group and, where necessary, also for lower dose groups in order to determine a *no observed effect level* (NOEL). Comprehensive haematology and clinical chemistry studies were carried out on the blood samples. All mice survived until the end of treatment. Clinical signs of toxicity were observed only at the highest concentration of 10000 ppm. Animals exhibited rough haircoat and abnormal posture at this level. In addition, body weights were reduced in this group and water consumption was depressed. Necropsy revealed no treatment-related macroscopic findings. Relative and absolute liver weights were dose-dependently increased. These findings correlated with minimal hepatic changes (cellular enlargement, increased cytoplasmic eosinophilia, necrosis of single cells). No marked differences were observed between males and females. All other data for the treated animals did not differ from the findings in the controls (NTP, 1992).

A range-finding study for a 13-week dermal study was conducted in groups of 5 male and 5 female F344/N rats. They were exposed to diethanolamine (> 99% pure, dissolved in 95% ethanol) at dose levels of 0 (controls), 125, 250, 500, 1000 or 2000 mg/kg body weight, applied without cover to the shaved dorsal skin once daily, excluding weekends, over a 16-day period (12 applications). The animals were housed individually, their body weights were recorded, and urine from each animal was collected for 16 hours on study day 12. At the end of the treatment period, blood samples were collected from the retro-orbital sinus and gross pathology was performed on all animals. Weights were recorded for the brain, heart, right kidney, liver, lungs, right testis and thymus. Histopathological examinations of the organs, including the skin from the application site, were performed for animals in the control group and the highest surviving dose group (1000 mg/kg body weight) as well as all early-death animals and, where necessary, also for lower dose groups in order to determine a *no observed effect level* (NOEL). Comprehensive haematology and clinical chemistry analyses were performed on blood samples from all survivors, and the concentrations of glucose, protein, urea nitrogen and creatinine were measured in

urine, as were the activities of alkaline phosphatase and lactate dehydrogenase. Administration of the highest dose of 2000 mg/kg body weight resulted in the death of all females (4 deaths on day 5 of treatment and one on day 15) and 3 males (on days 6, 8 and 14 of treatment). One female in the 1000 mg/kg group died on day 5 of treatment. All other animals survived. Body weight gains were markedly reduced in males and females of the two highest dose groups. As in the oral study, diethanolamine treatment of rats produced moderate normochromic, microcytic anaemia in both sexes. There were dose-dependent decreases in erythrocyte and reticulocyte counts, haemoglobin concentration and haematocrit. The observed increases in white blood cell counts were attributed to inflammatory processes at the site of application. Kidney lesions similar to those seen after oral administration of diethanolamine were noted in both sexes. Absolute and relative kidney weights were dose-dependently increased and renal tubular epithelial necrosis was found in early-death animals. There were dose-dependent increases in the urine concentrations of urea nitrogen, glucose and protein in both sexes and increases in the lactate dehydrogenase activity in the two highest dose groups. Dose-dependent skin lesions ranging up to necrosis were seen at the application site in all treated animals. Severe inflammation and crusting of the skin occurred at 500 mg/kg body weight and above. Hyperkeratosis was observed at the lower dose levels. Of the 5 male rats treated at the highest dose, 2000 mg/kg body weight, 4 exhibited mild to moderate seminiferous tubule degeneration, morphologically similar to that found after administration of diethanolamine in the drinking water. Similarly to the drinking water study, the dermal study did not yield a NOAEL due to nephropathy and haematological changes occurring at the lowest dose (NTP, 1992).

A range-finding study for a 13-week dermal study was conducted in groups of 5 male and 5 female B6C3F1 mice. They were exposed to diethanolamine (> 99% pure, dissolved in 95% ethanol) at dose levels of 0 (controls), 160, 320, 630, 1250 or 2500 mg/kg body weight, applied without cover to the shaved dorsal skin once daily, excluding weekends, over a 16-day period (12 applications). The animals were housed individually and their body weights recorded. At the end of the treatment period, blood samples were collected from the retro-orbital sinus and gross pathology was performed on all animals, including early-death animals. The brain, heart, right kidney, liver, lung, right testis and thymus were weighed. Histo-

pathological examinations of the organs, including the skin from the application site, were performed for animals in the control group and the highest surviving dose group as well as all early-death animals and, where necessary, also for low dose groups in order to determine a *no observed effect level* (NOEL). Comprehensive haematology and clinical chemistry studies were carried out on the blood samples from all survivors. In the highest dose group, all males and 3 females died between study days 11 and 14. In the other dose groups, body weight development corresponded to that observed in the controls, and there were no signs of intoxication. Moderate to marked inflammation, ulceration and crusting of the skin were observed at the site of application from 1250 and 2500 mg/kg body weight in males and females, respectively. Mild acanthosis occurred in all dose groups down to the lowest dose of 160 mg/kg body weight and was more pronounced in the two highest dose groups. Absolute and relative liver weights were dose-dependently increased at dose levels of 320 mg/kg body weight and above. Histopathological findings, however, were limited to minimal hepatocellular alterations (cellular enlargement, increased eosinophilia) in the mice from the highest dose group. All other data obtained for the treated animals corresponded to the findings in the control (NTP, 1992).

Short-term inhalation of high concentrations of diethanolamine vapours (200 ppm, equivalent to 874 mg/m³) or aerosols (1400 ppm, equivalent to 6118 mg/m³) resulted in respiratory difficulties and some deaths in rats. The continuous inhalation of 25 ppm diethanolamine (equivalent to 109 mg/m³) for 216 hours (9 days) caused increased liver weight, elevated serum aspartate aminotransferase activity, increased kidney weights and elevated blood urea nitrogen (no further details; Hartung et al., 1970).

Groups of 10 male and 10 female Wistar rats (Chbb:THOM) in a range-finding study for a 90-day study received daily inhalation treatment with diethanolamine (99.5% pure) in the form of an aerosol on 5 days/week for 14 days in accordance with OECD guideline No. 412. The animals were 7 weeks old at the beginning of the study and had mean body weights of 218 g (males) and 166 g (females). Concentrations of 100, 200 or 400 mg/m³ were administered to the animals for 6 hours per day by means of a head-nose exposure system. A group comprising the same number of animals was treated with air as a control. All animals in the study were housed individually. Body weight determinations and observations of the animals for clinical signs were performed in accordance with the OECD guideline.

Comprehensive examination for neurotoxicity was performed in all treated animals and the controls according to the EPA “Functional Observational Battery” (see also Section 7.10). At the end of the treatment period, blood was taken from the retro-orbital venous plexus of 5 males and 5 females from each group for comprehensive haematology and clinical chemistry determinations in compliance with the guidelines. Additionally, clotting analyses were carried out using Hepato Quick’s test. Three males and 3 females from each group were sacrificed by perfusion fixation with Soerensen’s phosphate buffer and Karnovsky fixative at the end of the treatment period and examined for neurotoxic effects in accordance with the EPA “Neuropathology” guidelines. Another 5 animals per sex and group were examined by gross pathology and histopathology in accordance with OECD guideline No. 412. All animals survived until the end of treatment and showed no signs of intoxication. The highest concentration of diethanolamine, 400 mg/m³, resulted in slightly decreased body weight and slightly retarded body weight gain in male rats. The same concentration also caused slightly decreased serum cholesterol levels in both sexes as well as a statistically significant increase in relative liver weight and a tendency towards increased absolute liver weight in females. All other examinations, including the neurotoxicological assessments, yielded no diethanolamine inhalation-related differences from the control data (see also Section 7.10; BASF, 1993 a).

Repeated intraperitoneal administration to rats of diethanolamine doses of 250 mg/kg body weight resulted in increased liver weight accompanied by decreased total liver lipids (no further details; Hartung et al., 1970).

The daily intraperitoneal injection of diethanolamine into white mice for 2 to 48 days led to an increase in glycogen content of the liver and to a decrease in total lipid content. The weight, size and water content of the liver were increased. As a rule, a treatment period of 3 days was sufficient to produce the effects. Longer periods of treatment produced no enhanced effect. Diethanolamine was administered in the form of its hydrochloride at dose levels of 6, 8 or 12 mg/animal in maximal quantities of 0.4 ml of water. A dose-dependent effect on the liver was found only with regard to liver weight. Experiments were performed on the last day of treatment after a starvation period of 5 hours (no further details; Annau et al., 1950).

7.3 Skin and mucous membrane effects

In an exploratory skin irritation study, diethanolamine was applied to the backs of rabbits either undiluted or as a 20-percent solution in water (pH > 10) for 1, 5 or 15 minutes. The treated areas of skin were subsequently washed with a 25-percent aqueous Lutrol solution to which 5% acetic acid had been added. No visible signs of irritation were elicited using the experimental procedure described above (no further details; BASF, 1956).

In a further skin irritation study, undiluted diethanolamine was applied to the dorsal skin of rabbits and left there for 1, 5 or 15 minutes or for 20 hours. Twenty-four hours after the end of exposure, there were no signs of irritation for exposure periods of up to 15 minutes. When the dorsal skin of rabbits was exposed to undiluted diethanolamine for 20 hours, reactions seen after 24 hours included mild redness, severe oedema formation and slight necrosis with mottled skin. After 8 days, slight necrosis was still seen and there was severe scaling. When applied to the skin of the rabbit ear for 20 hours, undiluted diethanolamine produced no signs of irritation (no further details; BASF, 1966). Based on these data, diethanolamine is to be considered a skin irritant.

A further acute skin irritation study involved applying diethanolamine to the clipped dorsal skin of white rabbits for 1, 5 or 15 minutes (patch test) and 20-hour tests on the dorsal skin and the skin of the rabbit ear. Following the short exposure, the treated skin area was washed first with undiluted Lutrol 9 and subsequently with a 50-percent aqueous Lutrol solution. Following the 20-hour exposure, the residual chemical was not washed off. Skin reactions were assessed on removal of the patches as well as after 1, 3 and 8 days. Diethanolamine caused barely perceptible reddening of the skin following exposure for 1 to 15 minutes. The 20-hour exposure caused marked reddening of the dorsal skin and slight swelling with partly mottled, anaemic superficial necrotic lesions, which were shed in the form of coarse, parchment-like scales. Following 20-hour exposure, the skin of the rabbit ear developed mild redness with circumscribed superficial, up to lentil-sized crusts (no further details; BASF, 1967). Diethanolamine proved to be irritating to the skin also in this study.

A skin irritation study in New Zealand white rabbits (weighing 2.5 to 3.8 kg) was carried out to investigate the effect of diethanolamine (98% pure) on

the clipped and unclipped skin. The French guidelines for skin and eye irritation studies were employed. Diethanolamine proved to produce moderate irritation (with a score of 2.6 out of a maximum score of 8) in all animals, both those with clipped and those with unclipped skin, but the effect on the clipped skin was clearly more pronounced. After 72 hours, there was a tendency towards improvement of the oedema but increase in erythema (no further details; Dutertre-Catella et al., 1982). Diethanolamine was irritating to the skin also in this study.

There is also report of skin irritation studies of diethanolamine in rabbits involving semiocclusive application of 50- and 30-percent solutions of the chemical to the intact and abraded shaved dorsal skin, followed by assessment of the reactions at 24 and 72 hours. Respective average Draize scores of 0.29 and 0.17 were found using groups of 6 animals/concentration. The diethanolamine solutions tested were evaluated as nonirritating (no further details; Anonymous, 1983).

In addition, rabbits were treated with 0.1 ml pure diethanolamine or with a 10-percent solution of diethanolamine in water. The animals received 10 applications of pure diethanolamine or aqueous solution to the ear over a period of 14 days or 10 consecutive semiocclusive 24-hour applications to the shaved abdomen. Pure diethanolamine showed some skin denaturation on the ear and belly following 10 and 3 doses, respectively, and was therefore considered by the authors of the report to be “moderately irritating” (no further details; Anonymous, 1983).

A group of 6 rabbits had a single dose of 0.5 ml undiluted diethanolamine applied to the skin of the flank under occlusive cover for 4 hours. The application site was then gently cleansed and the animals were examined after 1, 24, 42 and 69 hours. All animals exhibited mild to moderate erythema and oedema after 1 and 24 hours. These reactions persisted for up to 69 hours in some animals, but cleared up completely after 5 or 6 days. The investigators referred to their findings as “signs of mild skin irritation” (ICI, 1985).

The skin irritancy of diethanolamine and 20 other compounds was also investigated in a comparative study conducted in guinea pigs. Diethanolamine was found not to be irritating (no further details; Kharchenko and Ivanova, 1980).

A large-scale study was conducted to investigate eye irritation and injury due to diethanolamine, *inter alia*. Five rabbits with healthy eyes each had 0.005 or 0.02 ml undiluted diethanolamine applied directly onto the cornea. The eyelids were then kept retracted for one minute. Eighteen to 24 hours later the eyes were examined before and after fluorescein staining. The results thus obtained were classified according to a system in which the various types of eye injury were assigned separate scores. The individual scores were then added together to yield a total score of maximum 20. The chemicals under investigation were then allocated one of 10 grades based on the amount of substance causing eye injury and their dangerousness to the eye. When tested according to the study design described above, 0.005 ml diethanolamine caused eye injury with a total score of less than 5, i.e. the area of the cornea showing necrosis was < 63%, whilst 0.02 ml resulted in a score of more than 5. It was therefore placed in grade 5 of the system (no further details; Carpenter and Smyth, 1946).

In a mucous membrane irritation study of diethanolamine in the rabbit eye, a single instillation of 0.05 ml undiluted chemical into the conjunctival sac of the eye resulted in severe reddening, oedema formation, clouding of the cornea and mucosal bleeding after an observation period of one hour. The same effects, undiminished in severity, were seen after an observation period of 24 hours. Severe reddening of the eyes and slight clouding of the cornea were still seen after 8 days, and scarring and mucosal bleeding were noted in addition (no further details, BASF, 1966). Diethanolamine thus was corrosive to the eye with the risk of serious eye injury.

In a further acute mucous membrane irritation study, one drop of undiluted diethanolamine was instilled into the conjunctival sac of the rabbit eye and spread evenly over the surface of the eye. The findings were scored after 10 minutes, 1 and 24 hours and 3 and 8 days. Diethanolamine caused greyish-red, superficial corrosion of the conjunctivae together with mucosal bleeding, reddening, swelling and hazy clouding, which progressed to milky clouding. In addition, scarification of the eyelids occurred. The authors evaluated the substance as “corrosive” (BASF, 1967).

Without specifying the method used for testing, the irritant effect on the rabbit eye was referred to as “severe”. A 40-percent solution still had severe effects on the animals’ eyes (no further details; Eastman Kodak, 1982).

In an eye irritation study in white New Zealand rabbits (weighing 2.5 to 2.8 kg), which was conducted in accordance with the French guidelines for skin and eye irritation studies, diethanolamine (98% pure) produced severe irritation of the iris, cornea and conjunctiva. After observation periods of 24 and 48 hours, the respective scores were found to be > 50 and 56 (out of a maximum score of 110). The irritation subsided very slowly; the score was still 41 after 7 days (no further details; Dutertre-Catella et al., 1982). Based on these findings, diethanolamine is severely irritating to the eye with the risk of serious eye injury.

Furthermore, one study is reported to have been conducted in groups of 6 rabbits which had 0.2 ml of either a 30-percent or a 50-percent solution of diethanolamine (> 99% pure) instilled into the conjunctival sac of the eye. The eyes were rinsed after 15 seconds. Whereas the 30-percent solution of diethanolamine was nonirritation to the eye under the conditions of the study, the 50-percent solution produced moderate to severe conjunctival irritation and corneal injury with slight reddening of the iris. The effects cleared up within 7 days. The investigators therefore considered the chemical to be a “severe irritant” (no further details; Anonymous, 1983).

7.4 Sensitisation

The sensitising potential of diethanolamine and 20 other compounds was investigated in a comparative study conducted in guinea pigs. An epicutaneous test was employed. Diethanolamine was devoid of skin-sensitising properties in this test (no further details; Kharchenko and Ivanova, 1980).

A Magnusson and Kligman maximisation test was carried out in 8-week-old guinea pigs (Himalayan spotted, weighing 296 to 369 g) in accordance with OECD guideline No. 406. The test group comprised 20 animals, while 10 animals each were used as control groups for the first challenge and the second challenge; the latter, however, proved not to be necessary. Intradermal induction was performed with a 5-percent solution of “Diethanolamin, rein” (diethanolamine, pure) in physiological saline whilst epidermal induction was accomplished with a 75-percent solution. Both solutions, in their respective mode of administration, were mildly irritating to the skin. Challenge was carried out with a nonirritating 25-percent solution of diethanolamine. Following a post-challenge observation period of 24 hours, 2 out

of 20 test group animals exhibited mild erythema (score 1 out of 4), which was still seen in one animal after 48 hours. No effects were observed in the control animals. Therefore, the chemical was non-sensitising (RCC, 1990).

A further maximisation test on white guinea pigs (Dunkin Hartley strain) was performed with monoethanolamine, diethanolamine and triethanolamine in order to assess the chemicals with regard to skin sensitisation and potential cross-reactivity. Groups of 15 animals were treated with one of the three amines per group. At challenge each group received all three amines. Pure diethanolamine containing 0.1% monoethanolamine and less than 0.3% triethanolamine was given as an aqueous solution. A 1-percent solution was administered intradermally and a 17.6-percent solution (pH 9.8) epidermally after pretreatment of the application area with a 10-percent sodium dodecyl sulphate solution. Challenge was accomplished with 7, 3.5 or 0.7% aqueous diethanolamine solutions and equimolar solutions of monoethanolamine and triethanolamine. The readings at 48 and 72 hours after challenge showed no significant difference in reactivity between the actively induced animals and the control animals. Therefore, the investigators felt certain that diethanolamine was devoid of skin-sensitising potential and that there were no cross-reactions with the other two amines in the study as conducted (Wahlberg and Boman, 1996).

Diethanolamine was tested for sensitisation in a modified local lymph node assay (LLNA), together with three moderate human sensitisers (tetramethylthiuram disulphide, 2-mercaptobenzothiazole and zinc dimethyldithiocarbamate). Groups of 3 or 4 male and female mice (BALB/c, 6 to 8 weeks old) had each ear treated daily with 25 µl of acetone/olive oil (4 : 1) solutions containing different concentrations of diethanolamine for 3 consecutive days. The animals were killed 5 days after initiation of treatment, their auricular lymph nodes excised and single-cell suspensions of lymph node cells prepared. Proliferation of the cells was determined by incubating them with ^{3}H-methylthymidine for 24 hours at 37 °C. Subsequently, the amount of radioactivity incorporated into the DNA was determined. The proliferation index was calculated versus the vehicle control data. In addition, the lymph node cell suspensions obtained from diethanolamine-treated mice were incubated with concanavalin A at 37 °C and the cell suspension supernatants subsequently assayed for the cytokine levels of interleukin-4 and interferon gamma they contained. The other three chemicals were tested in the same manner. Whereas tetramethylthiuram disul-

phide and zinc dimethyldithiocarbamate displayed marked and dose-related effects on the proliferation of lymph node cells at concentration levels of 1 to 5% (proliferation index values of up to 30), 2-mercaptobenzothiazole and diethanolamine yielded contradictory results at concentration levels of 25% and 30%, respectively. The proliferation index for diethanolamine as determined in 4 independent assays carried out at a concentration level of 20% was found to be in the range between 1.2 and 3.7, with values ≥ 3 being considered positive. Pretreatment of the application sites with a 10-percent solution of sodium dodecyl sulphate in acetone/olive oil (4 : 1) one hour before application of test chemical clearly enhanced the proliferative responses of the lymph node cells. A 20-percent solution of diethanolamine resulted in a proliferation index of 20.1 under these conditions. Sodium dodecyl sulphate, however, also produced a marked effect as evidenced by a proliferation index of up to 14.8, so that the result could not be used in the evaluation of diethanolamine. A 1-percent solution of sodium dodecyl sulphate without further treatment was not observed to cause proliferation of lymph node cells. However, pretreatment of the application site at that level also did not lead to any relevant increase in proliferation index for diethanolamine or 2-mercaptobenzothiazole. The cytokine production studies carried out in suspensions of treated lymph node cells, for which groups of 4 to 10 mice were used, yielded a result comparable to that obtained for cell proliferation. In the absence of pretreatment, only tetramethylthiuram disulphide and zinc dimethyldithiocarbamate enhanced the interleukin and interferon concentrations, whilst diethanolamine and 2-mercaptobenzothiazole were devoid of any effect. Only after pretreatment with 10-percent sodium dodecyl sulphate was there a marked increase in cytokines in 6 out of 8 animals, whereas sodium dodecyl sulphate alone enhanced cytokine production in only one out of 8 animals. The investigators interpreted their results as being indicative of a synergistic effect of diethanolamine and sodium dodecyl sulphate. In their opinion, diethanolamine exhibited a sensitising effect following pretreatment of the skin with sodium dodecyl sulphate in the local lymph node assay (De Jong et al., 2002).

7.5 Subchronic and chronic toxicity

Neutralised diethanolamine, when administered in the drinking water at a concentration of 4 mg/ml for 7 weeks, caused a high mortality among rats.

Liver and kidney damage was observed. The most obvious finding was a pronounced normocytic anaemia without bone-marrow depletion and without marked increase in the number of reticulocytes. The investigators considered their findings as clear indications of the appreciable chronic toxicity of diethanolamine (no further details; Hartung et al., 1970).

Groups of 5 male and 5 female rats received diethanolamine at concentration levels of 0 (controls), 0.25, 0.5, 1, 2 or 4% in their food (respective ingested amounts: 171, 350, 680, 560 and 580 mg/kg body weight). An untreated group of 10 male and 10 female rats served as a control. Initial body weights for males and females were about 100 g and 90 g, respectively. Animals treated at the two highest concentration levels died between days 4 and 6 of treatment. At dietary diethanolamine levels of 1% and 0.5% (equivalent to 680 and 350 mg/kg body weight), the animals survived for average periods of 23 and 30 treatment days, respectively. All animals treated at 0.25% (equivalent to 171 mg/kg body weight) survived to the end of the 90-day study period, except for one animal from that group, which died on day 31 of treatment. The most frequent cause of death was lung infection, which occurred in 7 out of 10 animals in the group given 350 mg/kg body weight and in 5 out of 10 animals in the 171 mg/kg group. Two animals from the control group also died of lung infection. The survivors in the lowest concentration group had increased absolute and relative liver and kidney weights. Histopathologically, 25 out of 26 animals that survived longer-term treatment exhibited cloudy swelling and degeneration of the renal tubules and 7 out of 26 showed early fatty degeneration of the liver. Because of the numerous deaths, the study was repeated with lower dietary concentrations of diethanolamine. The levels selected were 0.0075, 0.030, 0.125 and 0.5% (equivalent to 5.1, 20, 90 and 390 mg/kg body weight, as calculated on the basis of the animals' actual food consumption and body weights). A number of animals also died of lung infection in the repeat study: 3 out of 10 in control group, 7 out of 10 in the highest concentration group and 2 out of 10 or 3 out of 10 in the other treatment groups. Animals surviving for 90 days exhibited increased liver and kidney weights in the two highest dose groups at the end of treatment. However, no other marked effects were observed. The investigators reported the *no effect level* (NOEL) to be between 0.125 and 0.25% (equivalent to 90 and 171 mg/kg body weight; Mellon Institute, 1950; Smyth et al., 1951). The study is of limited significance due to the numerous deaths that were un-

related to the test substance and also occurred amongst the controls, and due to very inadequate reporting of the study conduct and assessments.

A subchronic toxicity study of diethanolamine (97.2% pure) was conducted in groups of 10 male and 10 female rats (Fischer 344, weighing approx. 150 and 120 g, respectively). The rats received the chemical as a solution in distilled water at dose levels of 0 (controls), 25, 50, 100, 200 or 400 mg/kg body weight by oral gavage on 5 working days per week for 13 weeks. At the end of treatment, the animals were sacrificed and necropsied. Histopathological examinations were performed on all animals that died prematurely or were killed in a moribund condition, the controls and the animals in the highest dose group. The kidneys and adrenal glands of all animals were histopathologically examined. Chemical-related mortality occurred at dose levels of 100 (one male), 200 (3 males, one female) and 400 mg/kg body weight (3 males, one female). A dose-related negative effect on body weight gains was seen in males and females from dose levels of 50 and 100 mg/kg body weight, respectively, with respective weight gains in males and females of the highest dose group being reduced by 51 and 75% relative to their controls at the end of the treatment period. Gross pathology and histopathology failed to reveal any changes that could clearly be related to diethanolamine treatment. No clinical signs of intoxication were observed (GSRI, 1980). Based on body weight gain depressions, *no observed effect levels* (NOELs) were 25 and 50 mg/kg body weight (for males and females, respectively) in this study.

Groups of 10 male and 10 female rats (F344/N strain, 6 weeks old, respective initial weights from 117 to 123 g and from 102 to 105 g) were given 13 weeks of treatment with diethanolamine (> 99% pure) in their drinking water, adjusted to pH 7.4 with hydrochloric acid. Males received concentrations of 0 (controls), 320, 630, 1250, 2500 or 5000 ppm, equivalent to mean daily dose levels of 25, 48, 97, 202 and 436 mg/kg body weight as determined from water consumption and body weight data. Females were treated with concentrations of 0 (controls), 160, 320, 630, 1250 or 2500 ppm, equivalent to mean daily dose levels of 14, 32, 57, 124 and 242 mg/kg body weight. The number of animals housed per cage was 5. At treatment week 12, animals were separated into individual cages for urine collection over a period of 16 hours. Each urine sample was analysed for concentrations of glucose, protein, urea and creatinine and for activities of alkaline phosphatase and lactate dehydrogenase. At the end of treatment,

for the 7 days before necropsy, females were subjected to vaginal lavage for cytology and determination of oestrous cycle length. Finally, after 13 weeks, all animals were sacrificed and necropsied after blood samples had been taken by venipuncture of the orbital sinus. The brain, heart, right kidney, liver, lung, right testis and thymus were weighed. Complete histopathological examinations were performed for all control animals and for all animals in the highest surviving dose group with at least 60 % of surviving animals as well as for all early-death animals and, where necessary, also for lower dose groups in order to determine a *no observed effect level* (NOEL). Blood samples from all animals were comprehensively examined for haematology and analysed for concentrations of total protein, albumin, urea nitrogen, creatinine, glucose and total bile acids and activities of alanine aminotransferase and sorbitol dehydrogenase. In addition, males were examined for the motility, viability and number of sperm obtained from the epididymis. All animals survived to the end of treatment, except for 2 males in the highest dose group and one female in the lowest dose group. Body weight gain was dose-dependently depressed in both sexes, the effect being more pronounced in males. At the end of the study, male body weights at and above 630 ppm (48 mg/kg body weight) were more than 10% lower than controls, and at 5000 ppm they were down to as little as 56% of control weights (436 mg/kg body weight). Females exhibited marked depression of body weight gain from 1250 ppm (124 mg/kg body weight), at which level terminal weight was 84% of the control, up to the highest dose level of 2500 ppm (242 mg/kg body weight), where it was 75% of the control. Water consumption was also decreased in both sexes in a dose-related fashion with marked effects among the higher dose groups. This may have contributed to the decreased body weight gain. Clinical signs of toxicity included tremor, emaciation, abnormal posture and rough haircoat in the two highest dose groups of both sexes. Administration of diethanolamine produced a poorly regenerative, normochromic, microcytic anaemia in rats. Statistically significant effects (on mean corpuscular volume and mean corpuscular haemoglobin) were observed in males and females even at the lowest dose tested. There were dose-dependent decreases in erythrocyte and reticulocyte counts, haemoglobin concentration and haematocrit in both sexes. Blood levels of urea nitrogen, total protein, albumin and bile acids were increased in a dose-related fashion from the lowest dose level in all treated animals. Enzyme activities determined in the blood of treated animals were not different from the control.

Necropsy of all treated and untreated animals revealed no significant lesions. Dose-related increases in relative liver weights were observed in male and female rats. Absolute liver weights were decreased in males, particularly in the high dose groups, whereas they were increased in females. Histopathology revealed no hepatocellular lesions; the only indication of impaired hepatic function was provided by increased concentrations of bile acids in the blood. In addition, marked, dose-dependent increases were seen in the relative kidney weights of all treated males and females. Females also exhibited increased absolute kidney weights, whereas males showed no such change during treatment. Urinary protein content was increased in all treated males. Histopathology revealed only minimal nephropathy in various animals from all groups, including controls, which increased in severity and incidence with increasing dose levels. While 6 out of 10 males in the control group exhibited minimal nephropathy, all 10 animals were observed to have slight to moderate nephropathies at a daily dose level of 436 mg/kg body weight. At this dose level, all males showed tubular epithelial necrosis and mild renal tubular mineralisation, effects which did not occur at the low dose levels and in the control group. Females were found to have minimal nephropathy in 2 out 10 controls and in 9 out of 10 or 10 out of 10 animals in all treatment groups. Renal tubular epithelial necrosis which was minimal in severity was present in 3 out of 10 females in the highest dose group. All female controls showed minimal renal tubular mineralisation, a lesion which was also observed in treated females in a non-dose-dependent manner and was of up to moderate severity. In addition to the kidney, the brain and spinal cord were identified as further targets of diethanolamine toxicity. Mild vacuolisation and demyelination in the medulla oblongata and the spinal cord were noted for all males and females in the two highest dose groups. No corresponding clinical signs of toxicity were observed. In treated males, testis weights were dose-dependently decreased at and above a concentration of 1250 ppm in the drinking water, reaching 36% of the control at the highest concentration, 5000 ppm. Likewise, epididymis weights dropped to 30% of the control. Morphologically, there were reductions in the numbers of spermatogenic cells and the size of seminiferous tubules. Epididymal sperm count was greatly reduced, as was percent motility of the epididymal sperm. Testicular degeneration was diagnosed in all males in the highest dose group and in 3 out of 10 in the second highest dose group. Females showed no effect of diethanolamine treatment on oestrous cycle length. Due to the

haematological changes and nephropathy seen even at the lowest dose levels of 25 (males) and 14 mg/kg body weight (females) a *no observed adverse effect level* (NOAEL) was not achieved in this study (see also Section 7.8; NTP, 1992; Melnick et al., 1994 a).

In basically the same manner as described for rats (see above; GSRI, 1980), a subchronic toxicity study of diethanolamine (97.2% pure) was conducted in male and female mice (B6C3F1, weighing approx. 25 and 19 g, respectively). Groups of 10 mice per sex were treated at dose levels of 0 (controls), 50, 100, 200, 400 or 800 mg/kg body weight on 5 working days per week for 13 weeks. No mortality or depression of body weight gain occurred that could clearly be related to treatment with diethanolamine. Gross pathology and histopathology failed to reveal any changes and the animals exhibited no clinical signs of toxicity. However, the study is problematic insofar as male mice of the 200, 400 and 800 mg/kg dose groups had considerably higher initial weights than did the controls and, based upon these weights, actually showed less but not dose-dependently reduced weight gain in the course of treatment, when compared with the controls. Female mice did not show this effect but rather clearly gained weight in all dose groups. Also, one death among the males in the 800 mg/kg group and another death among the females in the 100 mg/kg group were not considered to result from diethanolamine treatment (GSRI, 1980). Up to the highest tested dose of 800 mg/kg body weight, there was no finding that was certain to be related to treatment.

Using basically the same experimental design as in the 13-week drinking water study in rats (see above; NTP, 1992; Melnick et al., 1994 a), the effect of diethanolamine (> 99% pure) was also investigated in mice (B6C3F1, 6 weeks old, mean weights of males and females 23.6 and 20 g, respectively) after administration in their drinking water for 13 weeks. Groups of 10 males and 10 females received diethanolamine at concentration levels of 0 (controls), 630, 1250, 2500, 5000 or 10000 ppm in their drinking water, which was adjusted to pH 7.4 with hydrochloric acid. Based on water consumption and body weight data, mean daily dose levels for males and females were calculated as being 104, 179, 422, 807 or 1674 mg/kg body weight and 142, 347, 884, 1154 or 1128 mg/kg body weight, respectively. The animals were housed individually in cages. All animals surviving treatment to the end of week 13 were examined in the same manner as described for rats, except that no urinalyses or haematology

tests were performed in this case. Similarly to the rats, animals that died prematurely were autopsied and histopathologically examined. In the 10000 ppm group, all males died between treatment days 24 and 32 and all females between treatment days 15 and 27. All animals treated at 5000 ppm in their drinking water succumbed, with the males dying between days 19 and 70 and the females between days 14 and 31. Three females in the 2500 ppm group died on days 30, 78 and 82. All other mice survived the treatment. All animals in the 2500 ppm group and the females of the 1250 ppm group exhibited retardation of body weight gain. Drinking water consumption in the surviving animals was not affected by addition of diethanolamine. No gross findings were observed at necropsy in animals that died early or survived to sacrifice at the end of the study. The absolute and relative liver weights for all examined animals from all dose groups were significantly greater than controls. This effect was associated with increased serum alanine aminotransferase and sorbitol dehydrogenase activities at a diethanolamine concentration of 2500 ppm in the drinking water, and with histopathological findings in the liver tissue. Multiple hepatocytic changes were observed, including hypertrophy, increased eosinophilia, nuclear pleomorphism and/or multinucleated cells in all concentration groups, and small areas of necrosis from 2500 ppm. In males, absolute and relative kidney weights were increased from 1250 ppm, whereas in females only relative kidney weight was increased at 2500 ppm. Minimal nephropathy, which was dose-related in incidence, was observed in various males in all dose groups, but only in one female survivor in the 2500 ppm group. Renal tubular epithelial necrosis was observed only in a few early-death animals in the highest dose group. In addition, absolute heart weight was increased in females treated with 2500 ppm diethanolamine, while relative heart weight was increased in males and females at 2500 ppm and in females at 1250 ppm. The increase seen at 2500 ppm was associated with minimal to mild myocardial degeneration and was more marked in severity among all early-death animals in the 5000 and 10000 ppm groups. The investigators considered early death possibly to be linked to the myocardial changes. The 2500 ppm animals and the early-death animals in the two higher dose groups also exhibited dose-dependently increased cytological alterations of the submandibular salivary gland. No effect of diethanolamine treatment was observed with regard to testis weight, sperm count and sperm motility in male mice and oestrous cycle length in female mice. Due to the liver alterations seen even at the lowest dose levels

of 104 (males) and 142 mg/kg body weight (females) a *no observed adverse effect level* (NOAEL) was not achieved (NTP, 1992; Melnick et al., 1994 b).

In a dermal study, groups of 10 male and 10 female rats (F344/N, 7 weeks old, initial weights ranging from 120 to 124 g and from 107 to 114 g for males and females, respectively) were dermally treated with diethanolamine (> 99% pure) daily for 13 weeks (except on weekends and public holidays). The chemical was applied to the dorsal skin as a solution in 95% ethanol at dose levels of 0 (controls), 32, 63, 125, 250 or 500 mg/kg body weight without any cover. The animals were housed individually. At treatment week 12, the urine was collected for each animal over a period of 16 hours. Concentrations of glucose, protein, urea nitrogen and creatinine and activities of alkaline phosphatase and lactate dehydrogenase were determined for each urine sample. For the 7 days before the end of treatment, females were subjected to vaginal lavage for cytology and determination of oestrous cycle length. Finally, after 13 weeks of treatment, all animals were sacrificed after blood samples had been taken by venipuncture of the orbital sinus. All animals were necropsied and examined by gross pathology. Weights were recorded for the brain, heart, right kidney, liver, lungs, right testis and thymus. Complete histopathological examinations were performed for all control animals and for all animals in the highest surviving dose group with at least 60 % of surviving animals as well as for the early-death animals and, where necessary, also for lower dose groups in order to determine a *no observed effect level* (NOEL). Blood samples from all animals were comprehensively examined for haematology and analysed for concentrations of total protein, albumin, urea nitrogen, creatinine, glucose and total bile acids and activities of alanine aminotransferase and sorbitol dehydrogenase. In addition, males were examined for the motility of sperm obtained from the epididymis, and the number and viability of caudal sperm was determined. In the highest dose group, given 500 mg/kg body weight, one male died during week 9 and 2 females were killed in a moribund condition during week 10. Body weight gains were dose-dependently retarded in treated males and females, starting at dose levels of 250 and 125 mg/kg body weight, respectively. All animals in the three highest dose groups showed signs of irritation and crusting at the site of dermal application. Dermal treatment of rats with diethanolamine additionally produced a poorly regenerative, normochromic, microcytic anaemia. In females, erythrocytes and reticulocytes, haemoglobin and haematocrit were

significantly lower than the control even at the lowest dose, and from 63 mg/kg body weight, mean corpuscular volume and mean corpuscular haemoglobin were also reduced. All effects were dose-related and increased in severity up to the highest dose. In males, no marked decreases in reticulocyte count were observed up to the highest dose; however, all other haematological effects seen in females were also present in males, but in some cases only at higher dose levels. There were no histopathological changes in the femoral bone marrow. A serum finding for animals in all treatment groups, except males in the lowest dose group, was a dose-related increase in albumin concentration. In females, serum concentrations of urea nitrogen were increased in a dose-related manner at all dose levels, whilst in males increases were noted only at and above doses of 250 mg/kg body weight. Females additionally exhibited dose-dependent increases in serum protein and bile acids from dose levels of 63 mg/kg body weight and 125 mg/kg body weight, respectively. Males exhibited significant increases in serum alanine aminotransferase activity from the 125 mg/kg dose level. There were dose-dependent increases in relative liver weights at all dose levels in both males and females, and in absolute liver weights in females. Males showed slight but not dose-related increases in absolute liver weights from a dose level of 125 mg/kg body weight. There were no histopathological changes of the liver. In this study the kidney was the main target organ of diethanolamine action, as identified by the investigators. Absolute and relative kidney weights were significantly increased even at the lowest dose of 32 mg/kg body weight, an effect which was hardly intensified by higher doses. The kidney weight increases at and above the lowest dose were associated with increases in incidence and severity of nephropathy, renal tubular cell necrosis or tubular mineralisation. Instances of minimal nephropathy were also observed in the controls (9 out of 10 males, 3 out of 10 females). In treated females, they increased in incidence and severity up to a dose of 125 mg/kg body weight, but then returned to control values at the higher dose levels. Males showed no differences from the controls. Minimal tubular epithelial necrosis was observed in the two highest dose groups (2 out of 10 and 10 out of 10 animals) in females but not in males. Mineralisation was evident in minimal form in 4 out of 10 control females. In treated females, mineralisation followed the same dose-response pattern as that found for nephropathy, whilst in males it occurred only in a mild form in 9 out of 10 animals of the highest dose group. Occasional, non-dose-related urinalysis findings which

differed from the controls and were present only at the highest dose in some instances were considered to be unrelated to treatment. Minimal demyelination with vacuolisation was evident in the medulla oblongata of all males and females at the highest dose level and was also noted in 7 out of 10 females at 250 mg/kg body weight. There were no spinal cord lesions. Testis weight was decreased for males in the highest dose group. No effects on sperm were observed. Treatment with diethanolamine did not affect the oestrous cycle of female rats. Dermal treatment with diethanolamine resulted in dose-related minimal to moderate lesions of the skin at the application site which were described as ulceration (from 250 mg/kg body weight), chronic-active inflammation (from 250 and 125 mg/kg body weight in males and females, respectively), acanthosis (from 63 mg/kg body weight) and hyperkeratosis (in all treated females and in males treated at and above 63 mg/kg body weight). Due to the haematological changes, nephropathy and skin lesions seen even at the lowest dose levels of 32 mg/kg body weight (females) a *no observed adverse effect level* (NOAEL) was not achieved in this study (NTP, 1992; Melnick et al., 1994 a).

In a study conducted to assess the carcinogenic potential of diethanolamine (see also Section 7.7), groups of 50 male and 50 female rats (F344/N, 6 weeks old, mean initial weights of males and females: 130 and 105 g, respectively) received daily dermal treatment with diethanolamine (> 99% pure, dissolved in 95% ethanol) on 5 working days/week for 2 years. Males and females were treated at dose levels of 0 (controls), 16, 32 or 64 mg/kg body weight and 0 (controls), 8, 16 or 32 mg/kg body weight, respectively. The animals were housed individually throughout the entire treatment period. Animals that died early, those sacrificed in a moribund condition and all animals that survived until the end of treatment were necropsied and comprehensively examined both macroscopically and histopathologically. No clinical chemistry or haematology studies were carried out. Survival of dosed male and female groups was similar to that of the controls. Body weight gains also showed no marked differences relative to the control data. The only treatment-related clinical sign of toxicity observed during the treatment period consisted in minimal to mild skin lesions at the application site, which were dose-related and more common in females than in males. Lesions reported included acanthosis, hyperkeratosis, focal accumulations of exudate on the epidermal surface and occasional ulceration. The incidences and severity of observed nephropathy were sig-

nificantly and dose-dependently greater in dosed females than in the control group. Treatment caused interstitial fibrosis and loss of nephrons in severe cases. Renal effects observed male rats were minimal in severity and restricted to the highest dose group but were significant when compared with the control group. No marked treatment-related toxicity was found in any other organ (see also Section 7.7; NTP, 1999 a).

Using basically the same experimental design as in the 13-week dermal study in rats (NTP, 1992; Melnick et al., 1994 a), groups of 10 male and 10 female mice (B6C3F1, 6 weeks old, 6 weeks old, body weights of males and females ranging from 22.5 to 23.2 g and 18.9 to 19.5 g, respectively) received dermal treatment with diethanolamine (> 99% pure) for 13 weeks. A solution of the chemical in 95% ethanol was applied to the dorsal skin at dose levels of 0 (controls), 80, 160, 320, 630 or 1250 mg/kg body weight once daily except on weekends and public holidays. The animals were housed individually in cages. All animals surviving treatment to the end of week 13 were examined in the same manner as described for rats, except that no haematology tests or urinalyses were performed in this case. Clinical chemistry determinations were carried out. Similarly to the rats, animals that died prematurely were autopsied and histopathologically examined. There were 4 deaths among the females in the highest dose group during treatment weeks 2 and 3, and among the males there was one death each during treatment weeks 2 and 9 (a few of these animals were sacrificed in a moribund condition). All other animals survived the treatment. Body weight gain was slightly depressed by 8%, on average, but only in males in the highest dose group. The only evident clinical signs of toxicity were inflammation and crusting of the skin at the application site in the two highest dose groups. All animals in all dose groups showed dose-related increases in absolute and relative liver weights. Multiple hepatocytic changes, including hypertrophy, increased eosinophilia, nuclear pleomorphism or multinucleated cells, were observed in all animals treated at dose levels of and above 160 mg/kg body weight and in 4 out of 10 males given the lowest dose. Males treated at dose levels from 320 mg/kg body weight and 2 males given the lowest dose additionally exhibited hepatocellular necrosis, a lesion which did not occur in females. Alanine aminotransferase activity was statistically significantly elevated in a dose-related fashion from dose levels of 320 mg/kg body weight in males and at 1250 mg/kg body weight in females. A significant and dose-dependent increase in sorbitol dehydro-

genase activity was seen in males only, starting at a dose level of 320 mg/kg body weight. Absolute and relative kidney weights were increased in animals in all dose groups (with the exception of relative kidney weights in females in the lower dose groups). However, mild tubular epithelial necrosis was observed only in occasional animals from the highest dose group. The highest dose group showed increased absolute and relative heart weights in association with cardiac myocyte degeneration, and cytological alteration of the submandibular salivary gland. Testis weight, sperm count and sperm motility in male mice and oestrous cycle length in female mice were not affected by treatment with diethanolamine. Dermal treatment with diethanolamine resulted in dose-related minimal to moderate lesions of the skin at the application site which manifested themselves as acanthosis in all animals from all dose groups, as ulceration from 640 mg/kg body weight, chronic-active inflammation from 630 and 320 mg/kg body weight in males and females, respectively, and hyperkeratosis in males treated at and above 63 mg/kg body weight and females given the highest dose. Due to the liver alterations and skin lesions seen at the lowest dose level tested, 80 mg/kg body weight, a *no observed adverse effect level* (NOAEL) was not achieved in this study (examination of the blood for micronucleus formation is discussed in Section 7.6.2; NTP, 1992; Melnick et al., 1994 b).

Groups of 50 male and 50 female mice (B6C3F1, 6 weeks old, mean initial weights of males and females were 23.5 and 19.2 g, respectively) participating in a 2-year carcinogenicity study (see also Section 7.7) received daily dermal treatment with diethanolamine (> 99% pure, dissolved in 95% ethanol) on 5 working days/week for 103 weeks. The animals received doses of 0 (controls), 40, 80 or 160 mg/kg body weight. They were housed individually throughout the entire treatment period. Animals that died early, those sacrificed in a moribund condition and all animals that survived until the end of treatment were necropsied and examined comprehensively both macroscopically and histopathologically. No clinical chemistry or haematology studies were carried out. Survival was similar in dosed male groups and controls, although there was a tendency towards shorter survival for the highest dose group. Survival of females was lower than that of the controls and this was particularly evident in the highest dose group. Males exhibited decreased body weights at the highest dose and at 80 mg/kg body weight after treatment weeks 77 and 88, respectively. Females showed decreased body weights at the highest dose level and at the two lower dose

levels after treatment weeks 53 and 73, respectively. Histopathological examination of the liver revealed cytoplasmic alteration in about one third of males from each treatment group in addition to the instances of adenoma and carcinoma discussed in Section 7.7. Cytoplasmic alteration was characterised as mild to moderate enlargement of centrilobular hepatocytes, and syncytial alteration was characterised as scattered hepatocytes with 3 or more small nuclei. In females, these effects occurred only at the two highest dose levels. The kidneys of dosed animals exhibited no changes of histopathological significance other than the instances of adenoma and hyperplasia discussed in Section 7.7. The thyroid gland exhibited significantly increased incidences of follicular cell hyperplasia in all dose groups as compared with the control. Only the two highest male dose groups were found to have cytoplasmic alterations of the cells lining the secretory duct. The application site skin of dosed mice was only slightly damaged. Minimal to mild hyperkeratosis occurred in a dose-related manner in up to one-third of the animals in the highest dose group. Acanthosis and exudate formation were observed only occasionally (see also Section 7.7; NTP, 1999 a).

A subchronic inhalation toxicity and neurotoxicity study (see also Section 7.10) of diethanolamine was carried out in Wistar rats (Chbb:THOM, 7 weeks old, mean initial weights of males and females 233 and 184 g, respectively) in accordance with OECD guideline for testing No. 413, the EPA guidelines for subchronic toxicity studies and the EPA guidelines for neurotoxicity testing. Groups of 13 male and 13 female rats were housed individually and treated for 6 hours/day on 5 working days/week for 13 weeks with liquid aerosols of diethanolamine (99.4% pure), produced in a generator system at approx. 40 °C and administered by means of a head-nose exposure system. Aerosol particle size analysis revealed the mass median aerodynamic diameter to be between 0.6 and 1.9 µm, with the content of respirable particles ranging from 92 to 95%. The aerosol concentration levels used were 0 (controls), 15, 150 and 400 mg/m³. They were analytically monitored throughout the entire treatment period and found to deviate only slightly from target values. In accordance with the guideline, all animals were weighed and checked for signs of toxicity at regular intervals. Comprehensive examinations for neurotoxic effects were carried out in 10 males and 10 females from each group, including the controls, in compliance with EPA “Functional Observational Battery” requirements (see Section 7.10). Before and at the end of treatment, the

eyes of the animals in the highest dose group and the control group were examined with an ophthalmoscope. At the end of the treatment period, 10 animals per sex per group had their urine collected overnight and blood taken from the retro-orbital venous plexus before sacrifice. Comprehensive clinical chemistry and haematology studies and urinalyses were carried out according to the guidelines and all animals underwent necropsy and complete gross pathology and histopathology examinations. The additional 3 males and 3 females from each group were sacrificed by perfusion fixation with Soerensen's phosphate buffer and Karnovsky fixative at the end of the treatment period and subsequently necropsied and neurohistopathologically examined in accordance with the EPA guideline. All animals survived to the end of treatment without exhibiting any treatment-related clinical signs. A statistically significant substance-related decrease in body weight gain was found only for the male rats treated at the highest concentration. Animals treated at the intermediate and highest concentrations were observed to have squamous metaplasia and hyperplasia of the laryngeal and tracheal epithelium, which were concentration-dependent in incidence and severity. A few animals in the lowest concentration group showed squamous metaplasia in the larynx only, associated with some submucosal infiltration of inflammatory cells. There was no functional or morphological evidence of diethanolamine neurotoxicity. A mild normochromic, microcytic anaemia was present in male and female rats at the highest concentration. There were statistically significant decreases in red blood cells, haemoglobin, haematocrit and mean corpuscular volume, whereas the white blood cell count and differential blood count were unchanged. Females in the two highest concentration groups exhibited statistically significant, dose-related increases in absolute and relative liver weights relative to the control, whereas males showed an increase only in relative liver weight at the highest concentration. Serum alanine aminotransferase and alkaline phosphatase activities were elevated in a statistically significant and dose-related manner in the two highest concentration groups of male rats, whilst only alkaline phosphatase activity was similarly affected in females. No corresponding histopathological changes were found in liver. Absolute and relative kidney weights showed statistically significant and dose-related increases in the two highest female concentration groups. Males also exhibited statistically significant and dose-related increases in relative kidney weights at the intermediate and highest concentration levels. Concentration-dependently increased incidence of mild haematuria was diagnosed

for both sexes in the intermediate and high concentration groups, with males also exhibiting increases in epithelial cells and casts from the renal tubules. Histopathology revealed minimal to slight tubular hyperplasia in some females from the intermediate and high concentration groups, while some males from these groups were observed to have slight intratubular lithiasis (mineralisation). Dose-dependent erosion of the glandular stomach was seen in some females in the two highest concentration groups. Three males treated at the highest concentration exhibited diffuse testicular atrophy and 4 of the males showed minimal to mild atrophy of prostate, findings which were interpreted as treatment-related effects. All other examinations revealed no diethanolamine treatment-related changes relative to the concurrent controls. The *no observed adverse effect level* for systemic toxicity was between 15 and 150 mg/m³. A *no observed adverse effect level* (NOAEL) for local irritation was not established due to the occurrence of squamous metaplasia of the larynx even in the lowest concentration group (15 mg/m³; see also Section 7.10; BASF, 1996).

An abstract reported that inhalation of 6 ppm diethanolamine (equivalent to 26 mg/m³) on a workday schedule for 13 weeks resulted in depression of growth rate, increased lung and kidney weights and some deaths among male rats (no further details; Hartung et al., 1970).

Three beagle dogs, 10 Sprague-Dawley rats and 6 guinea pigs (Hartley strain) were placed in an inhalation chamber together and exposed to a diethanolamine concentration of 0.6 ppm (equivalent to 2.6 mg/m³ air) for 6 hours per day on 5 working days per week. (No details were given as to the animals' sex, age or weight). Air was passed through the diethanolamine (99% pure) present in liquid form in a heated vapour generating system, thus yielding the above-mentioned vapour concentration. Another inhalation chamber was used to expose to air, as a control, the same numbers of animals of the same species. The treatment lasted 9 weeks (45 exposures). Assuming respiratory minute volumes of 5.2 l/minute for dogs and 0.1 l/minute for rats, the investigators calculated the respective daily diethanolamine doses for dogs and rats as being 0.5 mg/kg and 0.2 mg/kg body weight. Dermal absorption due to whole-body vapour exposure was not taken into account. All animals were placed under careful clinical observation. Blood was obtained from all animals at the end of the treatment period. The animals were sacrificed and necropsied 18 hours after the last treatment. Differential blood counts were obtained, haematocrit and hae-

moglobin determined and red and white blood cell counts performed. The lungs, livers and kidneys of all animals were weighed and all important tissues, including the central nervous system, were comprehensively examined by histopathology. All animals survived the study without showing any clinical signs of toxicity related to treatment with diethanolamine. Body weight gains were comparable to controls, as were the haematology data. The guinea pigs exhibited increases in absolute and relative kidney weights. The dogs showed a slight increase in relative liver weight. Necropsy revealed no treatment-related effects and the histopathology studies yielded no findings attributable to treatment with diethanolamine. No signs of eye or respiratory tract irritation were observed (Eastman Kodak, 1967 b).

A further inhalation toxicity study of diethanolamine was conducted over a treatment period of 13 weeks. Placed in an inhalation chamber of 3.85 m^3 volume, 2 male and 2 female (beagle) dogs, 10 weanling and 10 adult male rats, 10 weanling and 10 adult female rats and 5 male and 5 female guinea pigs (weighing 484 to 580 g, no further details regarding the experimental animals) were exposed 24 hours/day to a stream of diethanolamine-saturated air for 90 days. In order to generate atmosphere saturated with diethanolamine, air was passed through heated liquid diethanolamine in a vapour generator and subsequently filtered through a fibre-glass filter to remove any particles that were carried over. The concentration of diethanolamine measured in the atmosphere that was fed into the chamber was 0.26 ppm (equivalent to 1.14 mg/m^3). Animals in a control group of identical composition were exposed to clean atmosphere in a second exposure chamber. Inhalation exposure was interrupted only for 30 minutes in the mornings for feeding and cage cleaning and for further feeding in the evenings. During treatment, all signs of toxicity were carefully recorded and body weights were determined twice weekly. Before the initiation of treatment and within the last 24 hours of treatment, blood samples were collected for haematology (determination of red and white blood cell counts, differential blood count, haemoglobin and haematocrit) from all dogs and guinea pigs and from 50% of the rats. In cases where deviations from the control were found the same groups of animals was re-examined after an observation period of 14 days. Urinary albumin and glucose concentrations were determined in the same animals and by the same procedure. The dogs were given an ophthalmological examination of the cornea, including a fluorescein staining test, at the beginning of treatment, after 5 and 10 days and at the

end of treatment. All animals that died intercurrently, those sacrificed at the end of treatment and those sacrificed at the end of the observation period were necropsied and examined by gross pathology. Histopathological examinations of the lungs, liver, kidneys, spleen, reproductive organs, bone marrow and brain were performed in one female and one male dog, 2 male and 2 female adult rats, all weanling rats and 50% of the surviving male and female guinea pigs. Many of the guinea pigs died in the first two weeks of treatment, a finding which the investigators attributed to poor adaptation to the exposure chamber and above all to the feeding times. Because of this mortality, the original guinea pigs were replaced by another group of 5 males and 5 females (weighing 326 to 399 g), which were given food and water ad libitum during vapour exposure but otherwise received exactly the same treatment as the main group. The diethanolamine concentration in the air was 0.31 ppm (equivalent to 1.32 mg/m³). All dogs survived the entire treatment period. There were 2 early deaths among the adult males, but the investigators did not attribute the deaths to diethanolamine treatment. Many guinea pigs among those in the original exposure group died during the first two weeks of treatment, before the inhalation exposure device was changed and the animals had uninterrupted access to food and water during exposure. Several animals died of pneumonia during weeks 9 and 10. Several animals in the replacement group also died of pneumonia. The number of deaths among the controls being comparable, the high mortality was not considered to have been caused by diethanolamine. Body weight gains were comparable to those of the controls except in dogs and male weanling rats. Dogs lost weight at the beginning of treatment, but later their weights normalised, whereas body weight gains in male weanling rats were significantly lower than control from week 8 of treatment. All rats had bloody noses for several weeks after the beginning of treatment, but normalised again later on. No further clinical signs of toxicity were observed. Haematology and urinalysis findings did not differ from the controls. The relative organ weights for all treated animals were unchanged relative to controls, with the exception of female rats, where statistically significant increases in relative liver weights were seen in the adults and weanlings at the end of the observation period and immediately upon treatment, respectively. Histopathological examination of the treated animals revealed no changes attributable to treatment with diethanolamine (Hazleton, 1967).

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of diethanolamine are summarised in Tables 5 and 6.

Table 5. In-vitro genotoxicity tests with diethanolamine on micro-organisms

Test system	Concentration range tested (µg/plate)[1]	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Salmonella/microsome test, *Salmonella typhimurium* TA 100, TA 1535	no data	S-9 mix from Clophen A50-induced rat liver	negative	negative	Hedenstedt, 1978
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, preincubation test	33–3333	S-9 mix from Aroclor-induced rat and hamster liver	negative	negative	Haworth et al., 1983; NTP, 1993
Salmonella/microsome test, *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538, standard plate incorporation test	0.2–2000 (99.7% pure)	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985
Escherichia coli WP2, WP2uvrA, standard plate incorporation test	0.2–2000 (99.7% pure)	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985
Mitotic gene conversion, *Saccharomyces cerevisiae* JD1	no information (99.7% pure)	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985

[1] Unless stated otherwise, publications give no details of cytotoxic effects or of the purity of the diethanolamine used and/or any impurities it may have contained.

As Table 5 shows, no positive effect of diethanolamine was demonstrated in any of the studies on micro-organisms. In three Salmonella/microsome assays, performed on various strains of *Salmonella typhimurium* in the presence and absence of metabolic activation, the chemical proved not to be mutagenic both in the standard plate incorporation test and in the preincubation test. A further study, in which *Escherichia coli* was used as the test organism, also gave negative results, both with and without metabolic activation. A test for mitotic gene conversion in *Saccharomyces cerevisiae* also produced a negative result. Therefore, diethanolamine has no genotoxic potential in bacterial test systems.

Table 6. In-vitro genotoxicity tests with diethanolamine on mammalian cells					
Test system	Concentration range tested (µg/ml)[1]	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Chromosome aberration test, rat liver cells, RL1 and RL4, 100 metaphases examined per concentration	0.125 to 0.5 times the concentration that inhibited growth by 50%	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985
Chromosome aberration test, Chinese hamster ovary (CHO) cells, 100 metaphases examined per concentration	303–3010 with S9 mix, 101–2010 without S-9 mix	S-9 mix from Aroclor-induced rat liver	slight increase in the number of aberrations with increasing concentration	negative	Loveday et al., 1989
Sister-chromatid exchange test (SCE) test, Chinese hamster ovary (CHO) cells, 50 metaphases examined per concentration	4352, 2176 or 1088 (toxic at the highest level	S-9 mix of unspecified origin	negative	negative	Sorsa et al., 1988
Sister-chromatid exchange test (SCE) test, Chinese hamster ovary (CHO) cells, 50 metaphases examined per concentration	150–1500	S-9 mix from Aroclor-induced rat liver	negative	negative	Loveday et al., 1989
L5178Y/TK assay, trifluorothymidine resistance, mouse lymphoma cells (L5178Y/TK$^{+/-}$)	no data	S-9 mix from Aroclor-induced rat liver	negative	negative	Myhr et al., 1986

[1] Unless stated otherwise, publications give no details of cytotoxic effects or of the purity of the diethanolamine used and/or any impurities it may have contained.

All studies conducted in mammalian cells also gave negative results. Neither rat liver cells nor Chinese hamster ovary cells showed chromosome aberrations following treatment with diethanolamine. A slight increase in the number of chromosome aberrations noted upon treatment of ovary cells in the presence of S-9 mix was considered by the investigators themselves not to be of sufficient magnitude to be relevant (Loveday et al., 1989). A test for sister chromatid exchanges in Chinese hamster ovary cells and a trifluorothymidine resistance assay in mouse lymphoma cells also yielded negative results. In-vitro studies in mammalian cells thus also provided no indication of a genotoxic potential of diethanolamine.

7.6.2 In vivo

The question as to whether diethanolamine can cause single-strand breaks in rat liver DNA in vivo was addressed by a study in male and female

Wistar rats. The 6 to 8-week-old animals underwent partial hepatectomy. During the phase of liver regeneration they were treated with thymidine that was tritium-labelled at the methyl group and incorporated into the DNA. Following a recovery period of two weeks, 2 males and 2 females were each treated with a single oral dose of diethanolamine (40% solution in water; 99.7% pure) at 910 mg/kg body weight. The same numbers of animals were used for a negative control group (given plain water) and a positive control, which received methyl methanesulphonate (20% solution in water) at dose levels of 350 mg/kg body weight (males) or 300 mg/kg body weight (females). The animals were sacrificed 6 hours after dosing, and their livers were removed and homogenised. The alkaline elution method was employed to separate the DNA fragments, and the radioactivity present in each fraction was determined. Diethanolamine produced a clearly negative result, as did the negative control, whilst methyl methanesulphonate as the positive control caused marked single-strand DNA breakage (Shell, 1982).

In the context of a subchronic study (see also Section 7.5), peripheral blood from mice was also studied for micronucleus formation. Groups of 10 male and 10 female mice (B6C3F1) received dermal applications of diethanolamine to the dorsal skin at dose levels of 0 (controls), 80, 160, 326, 630 or 1250 mg/kg body weight. Treatment was administered daily, except weekends and public holidays, for 13 weeks. Blood was obtained from the animals at the end of treatment, spread onto slides, fixed with absolute methanol, stained and scored for the number of micronuclei per 10000 normochromatic erythrocytes. Diethanolamine caused no increase in the number of micronuclei at any dose level (NTP, 1992).

7.7 Carcinogenicity

In a 2-year carcinogenicity study conducted in the context of the US National Toxicology Program, groups of 50 male and 50 female rats (F344/N, 6 weeks old, mean initial weights of males and females 130 and 105 g, respectively) received daily dermal treatment with diethanolamine (> 99% pure, dissolved in 95% ethanol) on 5 working days/week. Males and females had doses applied to the dorsal skin at levels of 0 (controls), 16, 32 or 64 mg/kg body weight and 0 (controls), 8, 16 or 32 mg/kg body weight, respectively. Additionally, groups of 50 male and 50 female mice (B6C3F1,

6 weeks old, mean initial weights of males and females 23.5 and 19.2 g, respectively) were treated dermally in the same manner. Males and females received dermal doses of 0 (controls), 40, 80 or 160 mg/kg body weight. All rats and mice housed individually throughout the entire treatment period. Animals that died early, those sacrificed in a moribund condition and all animals that survived until the end of treatment were necropsied and examined comprehensively both macroscopically and histopathologically. Mean survival of treated rats was similar to that of the controls. Body weight gains also showed no marked deviations from the control data. In male mice, survival was the same as in the controls, whereas survival of females was reduced in a dose-related fashion, the reduction attaining statistical significance at the highest dose. Males and females exhibited dose-dependent inhibition of body weight gain. Findings unrelated to neoplastic lesions as observed during necropsy and histopathological examination are discussed in Section 7.5 above. Neoplasms occurred to the same extent in male and female rats as they did in the controls. Incidences of hepatocellular carcinoma and hepatocellular adenoma observed in all dose groups of male and female mice were markedly increased over controls (see Table 7). Furthermore, the size and number of neoplasms/animal were significantly greater in treated animals than in the controls.

Table 7. Number of mice/group with neoplasms and non-neoplastic lesions of the liver after 2-year dermal treatment with diethanolamine

	Dose (mg/kg body weight per day)			
	0 (controls)	40	80	160
Males				
Hepatocellular adenoma	31/50 (62%)	42/50 (84%)	49/50 (98%)	45/50 (90%)
	12/50 (24%)*	36/50 (72%)*	47/50 (94%)*	41/50 (82%)*
Hepatocellular carcinoma	12/50 (24%)	17/50 (34%)	33/50 (66%)	34/50 (68%)
	2/50 (4%)*	5/50 (10%)*	14/50 (28%)*	17/50 (34%)*
Hepatoblastoma	0/50 (0%)	2/50 (4%)	8/50 (16%)	5/50 (10%)
Lung metastases	3/50	4/50	9/50	7/50
Females				
Hepatocellular adenoma	32/50 (64%)	50/50 (100%)	48/50 (96%)	48/50 (96%)
	16/50 (32%)*	43/50 (86%)*	46/50 (92%)*	45/50 (90%)*
Hepatocellular carcinoma	5/50 (10%)	19/50 (38%)	38/50 (76%)	42/50 (84%)
	0/50 (0%)*	6/50 (12%)*	21/50 (42%)*	26/50 (52%)*
Lung metastases	0/50	3/50	6/50	1/50

* animals with multiple adenomas or carcinomas

The histopathological appearance of liver neoplasms was typical of that usually observed in B6C3F1 mice in that there was a morphological continuum from adenoma to carcinoma. The latter lesions were frequently 1 cm or more in diameter, whereas adenomas were generally smaller and more discrete. Lung metastases were observed in various animals with carcinomas, including some male controls. No other type of tissue exhibited any increase over control in the incidence of malignant tumours following treatment with diethanolamine. Male mice showed a trend towards an increased incidence of renal tubule adenoma with increasing dose levels. Step sectioning of the entire kidney revealed a dose-related, statistically significant increase in the incidences of adenoma and hyperplasia of the renal tubules in male mice. The incidence of renal carcinoma was not increased relative to the control. Comparison with the controls showed female and male mice to have increased follicular cell hyperplasia in the thyroid gland. The effect was significant and dose-related. There were no tumours. Upon completion of the peer review process, the National Toxicology Program summarised the outcome of the 2-year study as follows. Under the conditions of the study, there was “no evidence of carcinogenic activity” of diethanolamine for male and female F344/N rats, but “clear evidence of carcinogenic activity” of diethanolamine for male and female B6C3F1 mice. Concerns with the study, which were voiced by the manufacturers and pertained to the choice of route of administration and the interpretation of the chemical-related increase in liver carcinoma incidence observed in mice, remained and were addressed during the review meeting of the peer review committee. It was argued that the possibility of mixed oral/dermal exposure could not be excluded for the administration of diethanolamine as an ethanol solution without cover. Moreover, it was pointed out that ethanol itself has potential tumour promotional/carcinogenic effects. It was also said that oral ingestion of diethanolamine could result in the formation of the known carcinogen N-nitrosodiethanolamine in the stomach, a process which could have been enhanced by the high bacterial count in the feed used and the very high dose levels of diethanolamine administered. The high incidence of tumours in control mice was also criticised. Despite these concerns almost all reviewers confirmed the conclusion that the study presented clear evidence of the carcinogenic activity of diethanolamine following dermal administration to mice (NTP, 1999 a).

A study was conducted in mice to elucidate the mechanism of liver tumour induction by diethanolamine. Groups of 5 to 6 male B6C3F1 mice (weighing about 28 to 30 g) received different diethanolamine (99.7% pure) treatments, some including supplemental sodium nitrite. In the first trial, two groups of animals had a diethanolamine dose of 160 mg/kg body weight per day applied to the clipped and depilated dorsal skin for 2 weeks, one group of animals being allowed access to the site of application during grooming, whilst the other group was prevented from such exposure by means of an appropriate device. A third group received diethanolamine, as a solution in water, at 160 mg/kg body weight in parallel by daily oral gavage. A control group of animals only had their backs clipped and depilated but otherwise remained untreated. All animals were singly housed, and urine was collected on test days 5 to 7 and 12 to 14. The animals were sacrificed 1 to 2 hours after the last dose and exsanguinated by cardiac puncture. In the second trial, the same groups, dose levels and routes of administration were chosen, except that animals were administered supplemental sodium nitrite at about 40 mg/kg body weight via their drinking water. Again, animals were sacrificed 1 to 2 hours after the last dose and exsanguinated by cardiac puncture, but in addition, their livers and gastric contents were also removed. Blood and urine from all animals in both trials were analysed for N-nitrosodiethanolamine concentrations, as were the gastric contents from all animals in the second trial. Blood from all animals was also assayed for alanine aminotransferase and aspartate aminotransferase activities as well as for diethanolamine levels. Various phospholipids, choline and choline metabolites were determined in the livers from animals in the second trial. No N-nitrosodiethanolamine was identified in the blood or urine of animals from any of the treatment groups in the first and second trial. The gastric contents of the animals in the second trial also contained no N-nitrosodiethanolamine. The concentration levels of diethanolamine attained at the end of treatment were dependent upon the route/method of administration. Respective blood levels found after oral administration, dermal application with access during grooming and dermal application without access during grooming were 7.7, 6.6 and 5 µg/g, the latter corresponding to 65% of the level for oral administration. Treated animals showed no clinical signs of toxicity and their alanine aminotransferase and aspartate aminotransferase activities did not differ from the controls. Analysis of the livers from animals in the second trial revealed statistically significant decreases in choline, phosphocholine and glycero-

phosphocholine levels that were dependent upon the route/method of administration. Oral administration of diethanolamine produced the most pronounced effect, with levels being reduced by 64, 84 and 70%, respectively. Dermal dosing without access to the application site during grooming was associated with decreases by approx. 50, 70 and 42%, respectively. Dermal dosing with access to the application site during grooming was only slightly less effective than oral administration. Levels of phosphatidylcholine and phosphatidylethanolamine in the liver were only slightly and not significantly reduced (with the exception of phosphatidylethanolamine, which was decreased by 20% after oral administration of diethanolamine). In contrast, sphingomyelin levels found in the livers of animals treated by oral administration or by dermal exposure with possible oral ingestion were more than twice as high as in the control. In the absence of possible ingestion during grooming, dermal application resulted in an approx. 1.5-fold rise in liver sphingomyelin levels. The investigators therefore took this as conclusive evidence that diethanolamine exposure was markedly higher in mice receiving uncovered dermal applications with additional oral exposure, the method used in the NTP carcinogenicity study (NTP, 1999 a), than in mice dosed by covered dermal application. In the opinion of Stott et al., N-nitrosodiethanolamine played no part in tumorigenesis in the NTP study. The investigators did assume, however, that dramatic decreases in choline-containing lipids in the liver were causally related to the occurrence of liver tumours (Stott et al., 2000).

In order to evaluate the carcinogenic potential of diethanolamine following lifetime dermal administration to mice, the NTP utilised three studies which investigated the diethanolamine condensates of coconut oil acid, lauric acid or oleic acid in exactly the same manner as pure diethanolamine (see Table 8). All experimental conditions, except for the dose levels of fatty acid condensates tested, were the same as those described by the NTP (NTP, 1999 a) as the procedure used for testing diethanolamine as the free amine.

Table 8. Results from 2-year dermal carcinogenicity studies in mice exposed to fatty acid diethanolamine condensates as compared with pure diethanolamine

	Diethanolamine	Coconut oil acid condensate	Lauric acid condensate	Oleic acid condensate
Purity	> 99%	no data	90%	95%
Diethanolamine content	> 99%	18.2%	0.83%	0.19%
N-nitrosodiethanolamine content	not detectable	219 ppb	3600 ppb	68 ppb
Diethanolamine dose tested (mg/kg body weight)	40, 80, 160	100, 200	100, 200	15, 30
Findings in male mice	increased incidences of hepatocellular adenoma and carcinoma, increased incidence of lung metastases and a trend towards increased incidence of renal tubule adenoma from the lowest dose	increased incidences of hepatocellular adenoma and carcinoma and increased incidence of renal tubule adenoma at the high dose	no effects	no effects
Findings in female mice	increased incidences of hepatocellular adenoma and carcinoma and increased incidence of lung metastases from the lowest dose	increased incidences of hepatocellular adenoma and carcinoma at both dose levels	increased incidences of hepatocellular adenoma and carcinoma (combined) significant at both dose levels	no effects

Coconut oil acid diethanolamine condensate and lauric acid diethanolamine condensate were found to be associated with significant increases in incidences of hepatocellular adenoma and carcinoma, but not to the extent seen after administration of pure diethanolamine. The investigators attributed the effect to free diethanolamine present in both fatty acid condensates and pointed to the fact that the coconut oil condensate, which contained the highest percentage of free diethanolamine, exhibited the most marked effect, whereas the lauric acid condensate, with a much lower content of free diethanolamine, was less active and the oleic acid condensate, which was free of unreacted diethanolamine, caused no increase in tumour incidence (NTP, 1999 a, b, c, 2001). The concerns discussed for the study with pure diethanolamine also apply in the case of these studies. In particular, the possibility of oral ingestion of part of the dermally applied fatty acid condensates should be taken into consideration as the condensates can be cleaved in the stomach to yield the free fatty acid and diethanolamine.

As diethanolamine had no detectable genotoxic activity, it seemed reasonable to assume that the carcinogenicity observed in mice was more likely to have been caused via a non-genotoxic mechanism. In order to corroborate this hypothesis, a study was conducted in mice to investigate the extent to which treatment with diethanolamine affected cell proliferation in hepatic and renal tissues, when the same strain of mouse and the same dose regimen were used as in the carcinogenicity study described above (NTP, 1999 a). To this end, groups of 10 male mice (B6C3F1, 11 to 12 weeks old, initial body weights between 25.1 and 28.9 g) had diethanolamine (99.6% pure), dissolved in 96-percent ethanol, applied to the clipped dorsal skin at 160 mg/kg body weight once daily on 5 days/week for 1, 4 or 13 weeks. Each group of treated animals was matched with a concurrent control group which received only the vehicle but otherwise identical treatment. All animals were implanted with subcutaneous osmotic minipumps containing approx. 200 µl of a solution of 5-bromo-2'-deoxyuridine (BrdU) one week before the end of treatment. These animals received treatment with diethanolamine on all 7 days during the final week. Body weight and food consumption were determined on a weekly basis for all animals. Liver and kidney weights were determined following necropsy at the end of the study. Haematoxylin and eosin stained slides were prepared for the liver (lobus dexter lateralis and lobus dexter medialis) kidney and jejunum, the latter serving as a positive control tissue in the immunohistochemical determination of cell proliferation. The liver and kidney tissue was used in order to determine the mitotic index (relative number of cells at mitosis). Furthermore, cell proliferation (S phase) and biologically programmed cell death (apoptosis) were measured in the same tissues by means of immunohistological methods, a monoclonal antibody (anti-aBrdU) on the one hand and the streptavidin method on the other. More than 1000 cells were assessed in every structural compartment of liver and kidney to establish the percentage of cells which had incorporated bromodeoxyuridine (cell proliferation) or streptavidin (apoptosis). Apart from one spontaneous death during treatment week 13, all animals survived until the end of treatment without any clinical signs of toxicity except for mild erythema at the site of application, which was noted in a few animals. Body weight changes, food consumption and terminal body weights also showed no treatment-related differences as compared with the controls. Absolute and relative kidney weights were significantly increased for all exposure periods. Absolute and relative liver weights were significantly increased after 1 and 4 weeks'

treatment, whereas after 13 weeks' treatment only relative liver weight was increased. Histopathological alterations were not observed in the kidney after 1 and 4 weeks' treatment and those found in the liver were limited to mild cytoplasmic eosinophilia in the periportal zone in half of the mice studied. There were also no treatment-related histopathological changes in the kidneys after 13 weeks' treatment. The livers of all diethanolamine-treated mice exhibited mild cytoplasmic eosinophilia in the periportal zone, and multinucleated cells were present in the centrilobular zone in 4 out of 10 animals treated for 13 weeks. A small focus of liver cell necrosis was seen in one animal from that treatment group. For all treatment periods, the number of mitoses was increased relative to control in the kidney, but not so in the liver. The number of cells incorporating bromodeoxyuridine during the last treatment week of each treatment period, thus representing a measure of cell proliferation, was significantly increased in the liver and kidneys relative to controls for all treatment periods. In the liver, this effect was concentrated in the centrilobular region, whereas in the kidney it was concentrated in the proximal tubules of the renal cortex and the outer stripe of the outer medulla. Measurement of apoptosis yielded no indication that diethanolamine treatment, regardless of duration, had any effect on the liver or kidneys. The sustained increase in cell proliferation in the liver and kidneys and the increased mitotic index in the kidney after administration of diethanolamine support the assumption that tumour formation in the liver and kidney of male mice treated with the chemical is induced via a non-genotoxic mechanism (BASF, 2001, Mellert et al., 2004).

A further study in mice investigated the non-genotoxic mechanism of diethanolamine-induced carcinogenicity. As shown in earlier studies, diethanolamine is incorporated into liver phospholipids instead of choline, thus interfering with the biosynthesis of phosphatidylethanolamine and phosphatidylcholine (Barbee and Hartung, 1979 b; Mathews et al., 1995), it inhibits choline uptake into cultured Syrian hamster embryo cells as well as phosphatidylcholine synthesis and it is incorporated into phospholipids in the absence of a great excess of choline in the culture medium (Lehman-McKeeman and Gamsky, 1999, 2000). A number of studies by various investigators have shown that dietary choline deprivation causes hepatocarcinogenesis or, in combination with known carcinogens, promotes the formation of liver tumours in rodents. Therefore it seemed reasonable to assume that there was a relation between the carcinogenicity observed with

diethanolamine and the chemical's impact on choline metabolism. Hence, groups of 8 male mice (B6C3F1, 6 weeks old) were fed either a diet containing normal choline levels (0.25%) or a choline-devoid diet for a period of 2 weeks. Subsequently, the livers were removed, flash-frozen and analysed for phosphocholine, glycerophosphocholine, choline, phosphatidylcholine, S-adenosylmethionine and S-adenosylhomocysteine. The animals' body weights and liver weights were determined at the end of the study. Concurrent separate groups of 4 animals were included for the collection of blood for clinical chemistry analyses, and the livers were obtained for histopathological assessment. In parallel, groups of 6 male B6C3F1 mice were treated with diethanolamine at daily dose levels of 10, 20, 40, 80 or 160 mg/kg body weight on 5 days/week for a period of 4 weeks. The animals had a 95-percent ethanol solution of diethanolamine (99% pure) applied to the shaved dorsal skin. Concurrent controls included mice treated with ethanol alone and mice which remained completely untreated. Two further groups of mice of the same strain were also treated either with diethanolamine at 160 mg/kg body weight or with ethanol alone for 4 weeks and placed under observation for 2 weeks after treatment. All these animals were fed a normal diet containing choline. At the end of the dosing or recovery period the animals were sacrificed, the livers removed and the same liver analyses performed as in the animals fed a choline-devoid diet. Concurrent groups of treated animals were included for the collection of blood for clinical chemistry analyses, and the livers were obtained for histopathological assessment. Finally, the investigations as described above were also conducted in another strain of mouse (C57BL/6) in order to detect any possible differences between the individual mouse strains. Again, males aged 6 weeks at the beginning of the study were treated in the same manner as described above either with diethanolamine at 160 mg/kg body weight or with ethanol for a period of 4 weeks. An untreated control group was included. Subsequently, the livers were also removed and analyses were carried out in the same fashion as in the other mice. Feeding B6C3F1 mice a choline-devoid diet as compared with a normal diet resulted in a statistically significant decrease in the levels of choline and its metabolites in the liver, with the greatest decrease, representing a 75% reduction, being noted for phosphocholine, the intracellular storage form of choline. The smallest decrease was seen in the phosphatidylcholine levels. Hepatic S-adenosylmethionine concentrations were reduced by 20%, whereas S-adenosylhomocysteine levels increased. Body weight and

relative liver weight were unaffected by choline-devoid diet and there were no histopathological changes in terms of fatty liver. Most notably affected were serum triglyceride levels, which were decreased by about 50%. The livers of diethanolamine-treated mice displayed the same effects as when mice were fed a choline-devoid diet. Phosphocholine levels were dose-dependently and significantly decreased from the 20 mg/kg dose level, reaching a 50% reduction at the highest dose level. Glycerophosphocholine and choline levels were dose-dependently decreased from 40 and 80 mg/kg body weight, respectively, whereas reduced phosphatidylcholine levels were observed only at the highest dose level. From 80 mg/kg body weight, mice fed choline-devoid diet had significantly decreased S-adenosylmethionine levels but significantly increased S-adenosylhomocysteine levels. The effects were no longer detectable after a treatment-free 14-day observation period. There was no evidence of fatty liver in diethanolamine-treated mice and, in contrast with mice fed choline-devoid diet, serum triglyceride levels were not decreased. The C57BL/6 mice, which received the same treatment, are distinguished from B6C3F1 mice by, *inter alia*, higher hepatic S-adenosylmethionine levels. They reacted to diethanolamine treatment with significant decreases in hepatic phosphocholine levels but not in hepatic S-adenosylmethionine levels. No differences were observed between ethanol-treated mice and untreated controls with regard to the study parameters, except for a reduction in betaine concentrations by about 20%. Treatment of mice with diethanolamine in ethanol did not further enhance this effect. The investigators concluded that treatment with diethanolamine at the same dose levels as those used in the NTP carcinogenicity study (NTP, 1999 a) resulted in hepatic biochemical changes in mice that were consistent with those observed after choline-devoid diet, supporting the hypothesis that choline deficiency is the mode of action by which diethanolamine is hepatocarcinogenic following dietary administration to mice. This effect of diethanolamine was dose-dependent with a *no effect level* (NOEL) of 10 mg/kg body weight and supports the high likelihood of a non-genotoxic mechanism of liver tumorigenesis observed in the carcinogenicity study. Given that treatment with ethanol reduces hepatic betaine levels, which interferes with methyl group metabolism as much as do changes in the levels of choline, choline metabolites, S-adenosylmethionine and S-adenosylhomocysteine, ethanol may have exacerbated the effects of diethanolamine (Lehman-McKeeman et al., 2002).

Diethanolamine was tested in a cell transformation assay using Syrian hamster embryo cells. X-irradiated “feeder cells” were seeded at 6 x 10^4 cells per 60-mm plastic dish. One day later about 500 “target cells” were seeded into the same dish. A total of 9 dishes were treated with each concentration. Diethanolamine concentrations of 0 (controls), 25, 50, 100, 200 or 500 µg/ml were added 24 hours later and the dishes were maintained under culture conditions for 8 days. The dishes were subsequently fixed with methanol and stained by the Giemsa method. The total number of colonies and the number of transformed colonies per dish were counted. 3-Methylcholanthrene served as a positive control at a concentration of 0.1 µg/ml. Diethanolamine did not cause transformation in any of the cells. At 100 µg/ml and above, markedly increased cytotoxicity occurred in a dose-dependent manner up to 500 µg/ml, with only approx. 40% of colonies being found at the highest concentration relative to solvent control (0,2 % DMSO), as compared with 100% at the lowest concentration (Inoue et al., 1982). The relevance of the result appears questionable, however, because only one transformed colony per 1000 normal colonies was observed at the intermediate and highest concentrations of the positive control substance.

The ability of diethanolamine to induce cell transformation was also tested in a further study using Syrian hamster embryo cells. Per concentration, 20 culture dishes with 25 to 45 colonies of the embryo cells were incubated with diethanolamine concentrations of 0 (controls), 2500, 3000, 3500, 4000 or 4500 µg/ml medium for 24 hours. After another 6 to 7 days without diethanolamine exposure, the grown colonies were fixed with methanol, stained with Giemsa and scored for morphological transformation with a stereo microscope. Two such trials were carried out and the data pooled for analysis in order to examine 1000 or more colonies per concentration. A further study was conducted in parallel, in which the dishes were exposed to diethanolamine concentrations of 0 (controls), 250, 500, 1000, 1500 or 2500 µg/ml medium throughout the 7-day period of colony growth. The numbers of transformed colonies observed at two concentration levels (3000 and 4500 µg/ml) after 24 hours’ exposure were significantly increased relative to the control, and two other levels (3500 and 4000 µg/ml) showed a clear tendency. When diethanolamine was left to act on the cells and growing colonies for 7 days, the numbers of transformed colonies were significantly increased (13 to 18 transformed colonies) at all concentrations except the highest level, which exerted a strong cytotoxic effect (Kerckaert et al., 1996).

Based on the work of Kerckaert et al. (1996), studies were conducted to elucidate the molecular/biochemical mode of action by which diethanolamine induces cell transformation. The starting point and rationale for the study was the close structural relationship between diethanolamine and its congeners choline and ethanolamine and the fact that diethanolamine has been shown to be incorporated into phospholipids (see also Section 7.1). Therefore, Syrian hamster embryo (SHE) cells were used to ascertain the extent to which diethanolamine affects phospholipid biosynthesis, whether diethanolamine is incorporated into phospholipids and how these processes could be responsible for the transformation of SHE cells. When the cells were incubated in petri dishes for 48 hours following addition of 10 µCi ^{33}P-phosphoric acid or 10 µCi ^{14}C-choline to incubation medium containing 0 (controls) or 500 µg diethanolamine (99% pure), ^{33}P incorporation into phosphatidylcholine and ^{14}C incorporation were found to be inhibited by 86 and 70%, respectively, in the presence of diethanolamine. When SHE cells were incubated in medium containing ^{3}H-choline (5 µCi/ml) and diethanolamine at 0 (controls), 10, 50, 100, 250 or 500 µg/ml, choline uptake (measured as the amount of radioactivity incorporated after 10 minutes) showed marked inhibition at diethanolamine concentrations from 50 µg/ml, with inhibition by as much as 85% being reached at the two highest concentration levels. Further studies used exactly the same conditions as those in the transformation assays reported by Kerckaert et al. (1996). SHE cells were exposed to 10 µCi ^{33}P-phosphoric acid and diethanolamine at 0 (controls), 10, 50, 100, 250 or 500 µg/ml, and in some instances to choline (30 mM as the chloride) in great excess. At the end of the 7-day incubation, the ^{33}P incorporation into cellular phosphatidylcholine, the incorporation of diethanolamine into the phospholipid fraction and the effect of choline supplementation were determined. In these studies, incorporation of ^{33}P into phosphatidylcholine was inhibited by 60% at the highest diethanolamine concentration, and 12% of the ^{14}C-diethanolamine was found in the phospholipid fraction from SHE cells after 7 days. Supplementation of excess choline completely cancelled the effects. Significant concentration dependence of the inhibition of phosphatidylcholine synthesis (measured as ^{33}P incorporation) was demonstrated for diethanolamine concentrations of 100 µg/ml and higher. Finally, the SHE cell transformation assay as performed by Kerckaert et al. (1996) was repeated in the absence and presence of 30 mM choline. The result from 1996 was confirmed and again it was demonstrated that excess choline blocked the effects of diethanolamine. The in-

vestigators concluded from their findings that diethanolamine inhibited the uptake of choline into the cell, thus reducing the availability of intracellular choline. Moreover, diethanolamine was incorporated into phospholipids as a non-natural component. The processes were competitive as the effects were cancelled by excess choline. The investigators considered this a plausible mechanism explaining cell transformation by diethanolamine and suspected intracellular choline deficiency to be the likely cause of diethanolamine carcinogenicity (Lehman-McKeeman and Gamsky, 2000).

Syrian hamster ovary (CHO-K1) cells were also studied to investigate the effect of diethanolamine on choline uptake and intracellular phospholipid synthesis. Cells seeded at 3 x 10^5 cells/dish were incubated for 48 hours with 3 ml medium containing diethanolamine (99% pure) at levels of 0 (controls), 20, 50, 100, 200, 500 or 1000 µg/ml. For determination of phospholipid synthesis, 10 µCi of ^{33}P-phosphoric acid were added per dish, and in some instances excess choline (30 mM as the chloride) was supplemented. Diethanolamine incorporation into cellular phospholipids was measured by adding ^{14}C-diethanolamine at 500 µg/ml (10 µCi/dish) to an otherwise untreated culture and incubating it for 48 hours. The phospholipids were isolated at the end of the incubation period and separated by thin-layer chromatography. The radioactivity was measured in various fractions as well as in total. In order to study the effect of diethanolamine on choline uptake, CHO cells were incubated in medium overnight. Fresh medium containing diethanolamine levels from 0 to 500 µg/ml and 100 µM ^{3}H-choline (5 µCi/dish) was added for 10 minutes. Choline uptake was then stopped by the addition of ice-cold saline solution and the radioactivity content of the cells measured. Diethanolamine was shown to inhibit the incorporation of ^{33}P into phosphatidylcholine by up to 76% in a dose-dependent manner. The effect was clearly discernible even at the 50 µg/ml dose. Even more marked was the effect of diethanolamine on cellular choline uptake, which was greatly decreased at all diethanolamine concentrations studied, with inhibition reaching a maximum of 95% at diethanolamine levels of 250 and 500 µg/ml. Simultaneous supplementation of excess choline completely prevented the inhibition by diethanolamine of the incorporation of ^{33}P into phosphatidylcholine. About 20% of ^{14}C-diethanolamine was incorporated into cellular phospholipids during the 48-hour incubation period and appeared predominantly in the phosphatidylethanolamine fraction upon analysis. These findings show that diethanolamine inhibited cellular

phosphatidylcholine synthesis in a reversible and competitive manner by blocking cellular choline uptake (Lehman-McKeeman and Gamsky, 1999).

Diethanolamine was additionally assessed for its carcinogenic potential in a short-term bioassay using transgenic mice. The TG.AC strain carrying a mutant v-Ha-ras gene was employed. Within just a few weeks after dermal application of tumour promoters or initiators or deep skin injury the strain readily develops benign skin papillomas, which may progress to malignancies of the skin. Spontaneous incidence of papillomas is very low to zero without treatment. Diethanolamine was tested in groups of 10 to 15 female TG.AC mice aged 10 to 12 weeks. Each animal received a daily application of 20 mg diethanolamine, 200 µl ethanol (solvent control) or 1.25 µg 12-O-tetradecanoylphorbol-13-acetate (positive control) to the clipped dorsal skin 5 days/week for 20 weeks. Animals treated with diethanolamine or ethanol exhibited no papillomas at the end of treatment (no data provided for the positive control; Tennant et al., 1995).

When addressing the question of the carcinogenic potential of diethanolamine, it is important to consider that the chemical's N-nitroso derivative, N-nitrosodiethanolamine, has been demonstrated to be carcinogenic in several studies in rats (Preussmann et al., 1982; Berger et al., 1990). In addition, genotoxic properties of N-nitrosodiethanolamine have also been demonstrated in vitro in hepatocytes derived from rats, hamsters and pigs as marked increases in single-strand DNA breaks following treatment with the chemical (Pool et al., 1990). N-Nitrosodiethanolamine forms in the stomach from diethanolamine and nitrite. The latter can be ingested with the food or be formed from nitrate by reduction. N-Nitrosodiethanolamine was excreted in the urine after dermal administration of diethanolamine to rats simultaneously given sodium nitrite in their drinking water. Male Sprague-Dawley rats (weighing 300 ± 50 g) were offered a solution of 2000 ppm sodium nitrite (equivalent to 2 g/l) in water as their only drinking water for 6 days. Undiluted diethanolamine was applied as a single dose of 100, 200, 300 or 400 mg/animal (9, 9, 26 and 16 animals, respectively) to the shaved dorsal skin on day 6. Administration of sodium nitrite was continued for another 5 days while the individual 24-hour urines were collected and analysed for N-nitrosodiethanolamine. Concurrent control groups comprised 21 animals given no diethanolamine and 16 animals treated with 200 or 300 mg diethanolamine but no sodium nitrite in the drinking water. N-Nitrosodiethanolamine was not detected in the urines from the control groups. Uri-

nary N-nitrosodiethanolamine was detected in all other animals, with amounts varying greatly up to a total of 150 µg in one animal treated at 300 mg diethanolamine. The investigators considered this finding as evidence indicating that N-nitrosodiethanolamine forms from diethanolamine after absorption into the body and excretion into saliva or gastric juice (Preussmann et al., 1981).

7.8 Reproductive toxicity

An embryotoxicity/teratogenicity study was conducted in rats (CD Sprague-Dawley). Groups of 7 to 11 pregnant female rats aged 12 to 14 weeks were treated daily by oral gavage with an aqueous solution of diethanolamine (99.8% pure), neutralised with hydrochloric acid, at dose levels of 0 (controls), 50, 200, 500, 800 or 1200 mg/kg body weight on days 6 to 15 of gestation. All survivors were sacrificed and subjected to caesarean section on day 20 of gestation. The body weights, numbers of implantation sites, numbers of resorptions, numbers of live and dead foetuses and gravid uterine weights were recorded. All animals in the 3 higher dose groups (500, 800 and 1200 mg/kg body weight) died within a few days of treatment initiation or had to be killed in a moribund condition. Maternal body weights in the diethanolamine group treated at 200 mg/kg body weight were lower than in the controls, the entire litter having been resorbed in one animal. All other dams in that dose group and the lowest dose group showed no effects related to diethanolamine treatment. Embryotoxic or teratogenic activity was also not detected (EHRT, 1990).

A further extensive study investigated the reproductive toxicity of diethanolamine in groups of 12 pregnant female rats (Sprague-Dawley, weighing approx. 250 g) administered diethanolamine (98% pure), dissolved in water and neutralised with hydrochloric acid, once daily by oral gavage on days 6 to 19 of gestation. The dose levels tested were 0 (controls), 50, 125, 200, 250 and 300 mg/kg body weight. Maternal food and water consumption and body weight data were recorded at intervals throughout gestation and lactation. The dams were sacrificed on day 21 after delivery of pups, liver and kidney weights were recorded and the number of uterine implantation sites was determined in order to calculate postimplantation loss. The numbers of live and dead pups/dam were determined and any detectable external anomalies recorded. Live pups were weighed immediately after birth

and on postnatal days 4, 7, 14 and 21. Litters were reduced to a maximum size of 8 pups (optimally 4 males and 4 females) at postnatal day 7, with the remaining pups being subjected to gross necropsy. All surviving pups were also necropsied and examined by gross pathology on postnatal day 21. All dams in the highest dose group exhibited marked body weight loss after the beginning of treatment, two died on treatment day 6 and the remaining dams were sacrificed on treatment day 9. Therefore they were excluded from the study. Two animals in the 250 mg/kg diethanolamine group died, one on day 15 and the other on day 21 of gestation. Body weights and body weight gains were markedly decreased in that group, both during treatment and thereafter. Similar but less severe effects were observed in the group given 200 mg/kg body weight. One female died on day 22 of gestation, and body weights and body weight gains were decreased, albeit to a lesser extent. The two lowest dose groups (50 and 125 mg/kg body weight) exhibited no maternal mortality or body weight changes as compared with the controls. During treatment, maternal relative feed intake was transiently but not dose-dependently decreased at the two high dose levels, and relative water intake was reduced for several days in the 250 and 125 mg/kg groups. For all groups, necropsy revealed no treatment-related findings in any of the dams. The relative and absolute liver weights were unaffected. Absolute kidney weights were significantly and dose-dependently elevated starting at the 125 mg/kg dose level. Postimplantation losses occurred more frequently at and above 200 mg/kg body weight, and pup mortality was increased for postnatal days 0 to 4 from a dose level of 125 mg/kg body weight. Pup body weight was decreased at and above the 200 mg/kg dose level. No further mortality occurred among the pups from day 5 after delivery. Pups sacrificed on postnatal days 7 or 21 exhibited no treatment-related morphological anomalies or organ defects. The study discussed above established the *no observed adverse effect level* (NOAEL) for both maternal toxicity and developmental toxicity of diethanolamine in rats as 50 mg/kg body weight (RTI, 1999)

A Chernoff/Kavlock screening study for reproductive toxicity was conducted in a group of 50 6 to 8-week-old *primigravida* CD-1 mice. They were treated daily by oral gavage with a solution of diethanolamine in water on days 6 to 15 of gestation. The selected exposure level was the lethal dose 10 (LD_{10}) of 450 mg/kg body weight, as determined in preliminary studies, which was administered in 10 ml water/kg body weight. A control group of

50 pregnant mice received parallel treatment with water alone. Observations included maternal body weights from initiation of diethanolamine treatment until post partum day 3, clinical signs of maternal toxicity occurring during treatment, the number of live and dead neonatal pups, pup weights post partum and at day 3 and the number of pups which did not survive the first three days of life. Maternal mortality and signs of intoxication did not occur, neither in the control group nor following treatment with diethanolamine. Throughout the treatment period and up to the day of delivery, maternal body weights recorded for the treated dams were similar to those for the controls. Their weight was increased relative to controls when determined post partum (from 30.3 to 32.2 g) but it was decreased at post partum day 3 (from 34.5 g to 32.4 g). There were no differences between the treated group and the controls in respect of the number of dams delivering live pups under the conditions of the study, the number of dams delivering dead litters or litter size. On postnatal day 3, there was a marked increase in mortality amongst the pups from the treated group as compared with the controls. Survival rate was 77% in the diethanolamine group, while it was 95% in the control group. Body weights of surviving neonates in the treated group and the control group were the same on the day of birth. Body weights of surviving neonates in the treated group and the control group were the same on the day of birth. The decreases in pup survival and body weight gain were considered by the investigators as being indicative of the reproductive toxicity of diethanolamine (EHRT, 1986).

In an embryotoxicity/teratogenicity study, groups of 25 timed-pregnant female rats (Sprague-Dawley, CD) received daily dermal treatment with diethanolamine (99.5 to 99.9% pure) from days 6 to 15 of gestation. The animals had a solution of the chemical in water applied to the clipped dorsal skin under occlusive cover for 6 hours per exposure. Doses of 0 (controls), 150, 500 or 1500 mg/kg body weight were administered, each in 4 ml water/kg body weight. Body weights, food consumption and skin reactions to diethanolamine at the site of application were monitored regularly. On day 21 of gestation, blood for haematology determinations was obtained from the animals by orbital sinus puncture. They were subsequently necropsied and examined by gross pathology. Maternal livers, uteri and kidneys were weighed and the sex and reproductive organs examined macroscopically. Live and dead foetuses and resorptions were counted. All live foetuses were weighed and examined for external variations and malfor-

mations. About one-half of the live foetuses were examined for visceral abnormalities and craniofacial malformations. The other half was stained with alizarin red S and examined for skeletal malformations and variations. No deaths were recorded in any of the groups during the study. There was dose-dependent damage to the application-site skin of treated animals. Crusting, necrosis and ecchymosis were observed at the highest dose but the crusting decreased in severity at lower dose levels. All females had a normal pregnancy, except for a small number of females that failed to become pregnant (2 in the control group, one in the 150 mg/kg group and 3 in the highest dose group). Maternal body weight gains and food consumption were not affected by diethanolamine treatment in the lower dose groups. At the highest dose level, there was a significant decrease in body weight gain during and after treatment. All dose groups exhibited treatment-related anaemia, with statistically significant findings comprising reduced erythrocyte numbers, decreased haematocrit, decreased haemoglobin and decreased mean corpuscular volume with decreased mean corpuscular haemoglobin in the highest dose group, though these changes were observed as a trend down to the lowest dose level tested. A similar trend was also observed with respect to increased leukocytes, a finding which, again, attained statistical significance only in the top dose group. Maternal necroscopy revealed no treatment-related changes compared with the control. Uterine and liver weights were unaffected. Kidney weights showed statistically significant increases at the two highest dose levels and a trend towards an increase at the lowest dose level. No effect was observed on the number of live or dead foetuses, the foetal sex ratio or the number of resorptions. Examination of the foetuses gave no indications that diethanolamine was embryotoxic or caused malformations. Diethanolamine had no effect on foetal body weights and caused no external or visceral malformations or variations. The highest dose group exhibited slight delay in foetal development, as indicated by such changes as reduced ossification in the skull, axial skeleton and distal limb areas. The investigators concluded on the basis of the available findings that a *no observable effect level* for the maternal toxicity of diethanolamine could not be established and that the *no observable effect level* (NOEL) for developmental toxicity was 500 mg/kg body weight (Union Carbide, 1992; Marty et al., 1999).

A developmental toxicity range-finding study of cutaneous diethanolamine administration to rabbits was conducted in groups of 8 timed-pregnant New

Zealand White rabbits (5.5 to 6 months old, weighing 2.9 to 3.9 kg). The animals had diethanolamine (99.7% pure) applied to the clipped dorsal skin at dose levels of 0 (control), 250, 500, 1000 or 2000 mg/kg body weight under occlusive cover for 6 hours per day on days 6 to 18 of gestation. The highest dose was administered as the undiluted substance, while the other doses were obtained by appropriate dilution with water to enable a constant dosing volume of 2 ml/kg body weight. After 6 hours' exposure, the dressing was removed and the application site wiped gently. On the day after the last application (day 19 of gestation), blood for haematology determinations was obtained from the animals' ear veins. They were subsequently necropsied and examined by gross pathology. The liver, kidneys and uterus were weighed and, where necessary, examined by histopathology. Sections of treated skin and healthy skin were also prepared for histopathological examination. All live and dead foetuses and resorptions and their locations were recorded. Uteri from females that appeared nongravid were stained with ammonium sulphide and examined for early resorptions. All 8 animals from the highest dose group were sacrificed on days 3 to 5 of treatment due to development of widespread necrosis in the treated skin area. In addition, 6 and 2 animals from the 1000 and 500 mg/kg groups, respectively, were sacrificed on treatment days 5 to 7 due to severe skin necrosis. At the end of the study, 8 animals from the control group, 8 animals from the lowest dose group, 6 animals from the 500 mg/kg group and 2 animals from the 1000 mg/kg group were examined. Among those animals, one female each from the lowest and highest dose groups and 2 from the intermediate dose group were not pregnant. All other females bore litters with viable foetuses. Clinical signs of toxicity only involved the skin at the dosing site and were observed in a dose-related manner in all dose groups except the lowest dose group. The primary findings were necrosis, eczema and skin vesicles, which reached a considerable extent in several animals even at 500 mg/kg body weight. No effects of treatment were noted on body weight, food consumption or haematological parameters. Necropsy also revealed no treatment-related effects. Relative and absolute liver weights were dose-dependently increased in all treated animals at the end of treatment. Relative kidney weights were increased in animals from the highest and intermediate dose groups. There was no effect of diethanolamine treatment on uterine weight relative to the control group. There was also no effect of treatment on all reproductive parameters investigated.

The severe skin effects led the investigators to recommend dose levels below 500 mg/kg body weight for the main study (Union Carbide, 1993 a).

The main embryotoxicity/teratogenicity study of cutaneous diethanolamine administration to New Zealand White rabbits was conducted in groups of 8 timed-pregnant females (5.5 to 6 months old, weighing 2.7 to 4.3 kg). The animals had diethanolamine (99.7% pure, dissolved in water) applied to the clipped dorsal skin at dose levels of 0 (water control), 35, 100 or 350 mg/kg body weight under occlusive cover for 6 hours per day on days 6 to 18 of gestation. A constant dose volume of 2 ml/kg body weight was administered. After each 6-hour exposure, the dressing was removed and the application site wiped gently. On day 29 of gestation, the surviving pregnant females had blood drawn from the ear vein for haematology determinations. They were subsequently necropsied and examined by gross pathology. The uterus, liver and kidneys were weighed. All live and dead foetuses and resorptions and the corpora lutea were counted. Uteri from females that appeared nongravid were stained with ammonium sulphide and examined for early resorptions. Upon removal from the uterus, foetuses were weighed, sexed and examined externally for variations and malformations. All live foetuses were necropsied and examined by gross pathology. One-half of the live foetuses was decapitated and examined for craniofacial deformations subsequent to fixation in Bouin's solution. All foetuses were stained with alizarin red S and examined for skeletal variations and malformations. No maternal deaths were recorded during the study. Three females in the control group and one female in each dose group were not pregnant, and one female in the 100 mg/kg group bore only one early resorption. One female in the highest dose group aborted her litter on day 27 of gestation. Clinical signs of toxicity related to diethanolamine treatment were observed only in the highest dose group at the site of application. The skin in that area exhibited barely perceptible to severe erythema from treatment day 5 and eczema, crusting and necrosis from treatment day 6, which were not reversible by the end of the study. There were no effects of treatment on gestational body weights, body weight gains, food consumption or haematological parameters. Maternal necropsy also revealed no treatment-related effects apart from discoloration of the kidneys in some of the animals from the highest dose group. That group also exhibited slight increases in absolute and relative liver weights and relative kidney weight as compared with the controls. Uterine weights did not differ from the con-

trols in any of the treated pregnant females. There were no differences between animals from all treatment groups and animals in the control group with regard to the numbers of corpora lutea, live or dead foetuses or implantations/litter, the percentage of preimplantation loss or the foetal sex ratio. Foetal examination revealed neither skeletal nor visceral effects of treatment. The *no observed effect level* (NOEL) for maternal toxicity established in this study was 100 mg/kg body weight/day, while the NOEL for developmental toxicity coincided with the highest test dose, i.e. 350 mg/kg body weight/day (Union Carbide, 1993 b; Marty et al., 1999).

In a range-finding study for a prenatal inhalation toxicity study conducted in accordance with OECD guideline for testing No. 414, groups of 10 pregnant rats (Wistar/Chbb:THOM, 10 to 15 weeks old, mean initial weight approx. 200 g) underwent daily 6-hour inhalation exposure to diethanolamine (> 98% pure) on days 6 to 15 of gestation. Diethanolamine concentrations present in the head-nose inhalation exposure system were 0 (controls), 100, 200 or 400 mg/m³ and were generated by atomisation of the chemical with compressed air and controlled dilution with air. The mean aerosol particle size was in the range of 0.6 to 1.2 µm. The dams were observed once daily before the treatment period and three times per day on treatment days. Body weights were recorded every 3 days. On day 16 of gestation, blood for haematology and clinical chemistry measurements and determination of clotting time was obtained from all animals by orbital sinus puncture. The animals were then necropsied and examined by gross pathology and their lung, liver and kidney weights determined. Each uterus was weighed, and the numbers of corpora lutea, implants and early resorptions were determined. The foetuses were removed and examined grossly. Foetal and placental weights were recorded. Treated dams showed no clinical signs of toxicity during the entire study. There were no deaths. Body weights of the treated animals did not differ from the controls. The absolute and relative liver weights were significantly increased in animals from the top dose group. All other organ weights, including uterine weight, recorded for treated animals did not differ from those of the controls. The treated dams showed no treatment-related effects on the reproductive parameters under investigation. Examination of the foetuses revealed no indication of any effect of diethanolamine treatment. The clinical chemistry findings exhibited significant increases in sodium and creatinine levels as well a concentration-dependent decrease in triglyceride levels in the high-

est and intermediate concentration groups. All other examinations revealed no indication of any effect of diethanolamine in this range-finding study (BASF, 1991).

The main prenatal inhalation toxicity study of diethanolamine in rats was conducted in groups of 25 female Wistar rats (Chbb:THOM, aged 68 to 70 days and weighing about 214 g at the beginning of the study) in accordance with OECD guideline for testing No. 414. All animals were mated separately after an acclimatisation and adaptation period, and fertilisation was considered confirmed by a sperm-positive vaginal smear. On days 6 to 15 of gestation, females underwent daily 6-hour exposure in a head-nose inhalation system to atmosphere containing concentration levels of 0 (controls), 10, 50 or 200 mg/m³, which were generated by atomisation of the chemical with compressed air and controlled dilution with air. The mean aerosol particle size was less than 1.2 µm. The dams were observed for clinical signs once daily before the treatment period and three times per day on treatment days. Body weights were recorded every 3 days. On day 20 of gestation, the dams were sacrificed, necropsied and examined grossly. Uterus and ovaries were removed and the uterine weight and the number of corpora lutea, number and distribution of implantations and number of live and dead foetuses determined. The foetuses were removed, weighed, examined externally for treatment-related effects and sexed. One-half of the foetuses were then fixed in alcohol while the other half was fixed in Bouin's solution. The latter was examined for any effects on the organs. Foetuses fixed in alcohol were stained for skeletal analysis and examined for malformations, variations and retardations. No maternal deaths were recorded during the entire study. Body weights of the treated groups did not differ from the controls. Clinical signs of toxicity were seen only at the highest concentration in the form of bloody discharge from the vagina noted in 8 of the 21 pregnant animals on postcoital day 14. Maternal necropsy revealed no gross pathological findings that were related to diethanolamine treatment. There were no statistically significant differences from concurrent study controls or historical control data with regard to conception rate, mean number of corpora lutea and implantation sites, pre- and postimplantation losses, resorptions or viable foetuses. Foetuses were not affected by diethanolamine treatment with respect to sex ratio, body weight or external findings. Examination of the foetal organs revealed no treatment-related malformations. Occasional findings which occurred across all

treatment and control groups were considered incidental by the investigators. Examination of the foetal skeletons revealed several malformations and retardations at all concentration levels and among the controls, but they were considered to be of no biological relevance on account of their small number and distribution across the groups. Only the significant increase in the number of cervical ribs noted in the highest concentration group was considered to be treatment-related. The investigators interpreted this finding as an embryotoxic effect rather than a manifestation of diethanolamine teratogenicity. Therefore, the *no observed adverse effect level* (NOAEL) for maternal and foetal toxicity was 50 mg/m³ while the NOAEL for teratogenicity was 200 mg/m³, the highest concentration tested (BASF, 1993 b).

A number of subacute and subchronic studies in rats and mice have also investigated the effect of diethanolamine on the male and female reproductive organs (see also Sections 7.2 and 7.5). In this context, females exhibited no effects on the reproductive organs or oestrus cycle.

When male rats were fed diets containing admixtures of 0.1 or 0.01% diethanolamine for 32 days, no differences were observed with regard to testicular weight or histopathological examination of the testes at the end of treatment (Eastman Kodak, 1967 a). The 10-fold concentration, 1% diethanolamine, was also devoid of any effect (see also Section 7.2; Eastman Kodak, 1968).

Groups of 5 6-week-old male F344/N rats that received diethanolamine in their drinking water at dose levels of 0 (controls), 77, 162, 319, 622 or 1016 mg/kg body weight per day for 14 days were examined for, *inter alia*, possible damage to the reproductive organs at the end of treatment. All animals in the highest dose group exhibited mild to marked seminiferous tubule degeneration, characterised by a reduction in tubule size and in the number of male germ cells. In addition, large numbers of degenerated cells appeared in the lumen of epididymal tubules. Parallel studies conducted in the same manner in B6C3F1 mice, which received diethanolamine in their drinking water at 0 (controls), 110, 205, 415, 909 or 1362 mg/kg body weight per day, did not reveal any chemical-related effects on the male reproductive organs (see also Section 7.2; NTP, 1992).

A further study, in which groups of 10 6-week-old male F344/N rats were treated with diethanolamine in their drinking water at dose levels of 0 (con-

trols), 25, 48, 97, 202 or 436 mg/kg body weight per day for 13 weeks, confirmed the above-discussed findings of the 14-day range-finding study. At doses of 97 mg/kg and above, there was a statistically significant and dose-dependent decrease in testis weights down to 36% of control in the highest dose group at the end of treatment. Likewise, epididymis weights dropped to 30% of the control. Morphologically, the numbers of male germ cells and the size of seminiferous tubules were reduced. Epididymal sperm count and percent motility were greatly reduced. Testicular degeneration was diagnosed in all males at the highest dose level and in 3 at the second highest dose level. Parallel studies conducted in the same manner in male B6C3F1 mice, which were administered diethanolamine orally in their drinking water at daily dose levels of 0 (controls), 104, 178, 422, 807 or 1674 mg/kg body weight for 13 weeks, did not reveal any effects on testis weight, sperm count or sperm motility (see also Section 7.5; NTP, 1992).

When groups of 5 6-week-old male F344/N rats were treated dermally with diethanolamine in 95% ethanol daily except on weekends, application of the highest dose of 2000 mg/kg body weight to the shaved dorsal skin without cover was found to produce the same degenerative changes in the testes and epididymides after 16 days as those seen in rats given oral treatment for 14 days. However, this very high dose also resulted in several deaths. Lower dose levels of 1000, 500, 250 or 125 mg/kg body weight had no effect on the testes or epididymides. Again, parallel studies conducted in the same manner in male B6C3F1 mice treated dermally with up to 2500 mg diethanolamine/kg body weight revealed no chemical-related effects on the reproductive organs (see also Section 7.2; NTP, 1992).

Additionally, groups of 10 7-week-old male F344/N rats were treated dermally with diethanolamine in 95% ethanol daily except on weekends for 13 weeks. Doses of 0 (controls), 32, 63, 125, 250 or 500 mg/kg body weight were applied to the dorsal skin without any cover. Testis weight was decreased for males in the highest dose group at the end of treatment. No effects on sperm were observed. The animals from the other dose groups had no lesions in the reproductive organs. Again, parallel studies conducted in the same manner in male B6C3F1 mice, which had up to 1250 mg diethanolamine/kg body weight applied to the dorsal skin daily except on weekends for 13 weeks, revealed no chemical-related effects on the reproductive organs (see also Section 7.5; NTP, 1992).

A subchronic inhalation toxicity and neurotoxicity study of diethanolamine in Wistar rats was conducted in groups of 13 7-week-old males which were exposed to liquid aerosols for 6 hours/day on 5 working days/week. The aerosol concentration levels used were 0 (controls), 15, 150 and 400 mg/m³. At the end of the 13-week treatment period, 3 males in the group treated at the highest concentration exhibited diffuse testicular atrophy while 4 males in that group had minimal to mild atrophy of the prostate gland, findings which were considered treatment-related (see also Sections 7.5 and 7.10; BASF, 1996).

7.9 Effects on the immune system

A range-finding study was conducted in young female mice (B6C3F1, 4 to 6 weeks old, weighing approx. 20 g) to determine the effects of diethanolamine on the immune system. Groups of 4 animals were treated by oral gavage with aqueous solutions of diethanolamine (99.8% pure) at daily dose levels of 0 (controls), 100, 300, 400, 500, 600, 700, 800, 900, 1000, 1250 or 1500 mg/kg body weight for 14 consecutive days. The animals' body weights were recorded on days 1 and 8 of treatment and 24 hours after the last administration, before they were sacrificed, necropsied and examined by gross pathology. Organ weights were recorded for the thymus, lungs, liver, spleen and kidneys, including adrenal glands, and routine haematology parameters, including differential leukocyte count and reticulocyte number, were determined in blood samples collected by retro-orbital puncture. The indicator employed to measure the effect on the immune system was the spleen IgM (immunoglobulin M) antibody-forming cell (AFC) response to the T cell-dependent antigen of sheep erythrocytes. Mice received 2 x 10^8 sheep erythrocytes by intravenous injection on day 11 of treatment. Upon sacrifice, spleen cell suspensions were prepared and mixed with guinea pig complement, sheep erythrocytes and warm agar. After 3 hours' incubation at 37 °C, plaques generated by the lysis of sheep erythrocytes indicated the number of antibody-forming cells, because each plaque was generated from a single ACF. All animals receiving diethanolamine in doses of up to 800 mg/kg body survived the experimental period and showed no overt signs of toxicity. Fifty percent of animals treated with diethanolamine at 900 mg/kg body weight died by the end of the experimental period, and all mice in the dose groups above that level

died. Therefore, analysis of the above-mentioned parameters only included animals treated at dose levels from 100 up to and including 800 mg/kg body weight. No changes in body weight or body weight gain were observed in those animals as compared with water-treated controls up to the end of the study. Gross pathology revealed no remarkable findings. Liver weight was dose-dependently increased up to 78.2% in the treatment group given 900 mg/kg body weight but thymus weight was dose-dependently decreased by up to 54.8% relative to the controls. The haematology determinations revealed dose-dependent, statistically significant decreases in erythrocyte count and haemoglobin and haematocrit values from a dose level of 300 mg/kg body weight, and very marked, dose-dependent decreases in reticulocyte count were noted at dose levels of and above 700 mg/kg body weight, with a 73.8% decrease being seen in the highest dose group. No effects were observed on leukocyte numbers relative to the controls. Diethanolamine caused statistically significant decreases in IgM antibody-forming cell response at and above the 300 mg/kg dose level without any further intensification up to 900 mg/kg body weight, with decreases ranging between 38.2 and 74% at the various dose levels. A subsequent study in mice treated with diethanolamine at lower dose levels of 0 (controls), 50, 100, 125, 150, 200, 250 or 300 mg/kg body weight found no suppression of the immune response even at the highest dose level. This led the investigators to conclude that the *no effect level* was within that dose range. The range-finding study additionally encompassed in-vitro assays of murine spleen cells to investigate the antibody response to sheep erythrocytes. Single-cell suspensions of splenocytes were exposed to diethanolamine at concentrations ranging from 10^{-10} to 10^{-4} M, or water as the control, and incubated at 37 °C on addition of sheep erythrocytes. On day 5, enumeration of IgM antibody-forming cells was performed and spleen cell viability determined. Diethanolamine had no effect on spleen cell viability but did reduce the IgM antibody-forming response at 10^{-8} M and higher concentrations in a statistically significant and dose-dependent manner. At concentrations of 10^{-8} M and 10^{-4} M, the immune response was suppressed by 17.7 and 60.4%, respectively. In addition, a third substudy conducted in the context of the range-finding study employed the cytotoxic T lymphocyte (CTL) assay, which measures the final differentiation of the T cell to the cytotoxic effector (killer) cell. This again involved treating groups of 4 young female mice with diethanolamine in the same manner at the same dose levels as for the AFC assay, but without

sheep erythrocytes. The animals were also sacrificed on day 15, 24 hours after the last treatment. The results obtained for the general toxicity parameters were comparable with those found in the first investigation. For immunological testing, single-cell splenocyte suspensions were prepared from treated and control mice and incubated for 5 days at 37 °C on addition of mitomycin-treated P815 mastocytoma cells. The responder : sensitiser ratio between spleen cells and allogenic P815 cells was 50 : 1. Splenocytes were subsequently isolated and incubated with ^{51}Cr-labelled P815 cells at ratios between 25 : 1 and 0.75 : 1 for 4 hours in order to determine the number of cytotoxic T lymphocytes formed. Treatment of mice with diethanolamine resulted in a dose-dependently decreased ability of splenocytes to generate toxic T lymphocytes, a finding which, however, attained statistical significance only at dose levels of and above 900 mg/kg body weight. In summary, diethanolamine was found to have a marked effect on the immune system of mice but at dose levels that were also associated with considerable general toxicity (Immunotoxicology Program, 1992 a).

A comprehensive immunotoxicity study of diethanolamine was also conducted in female mice (B6C3F1, 4 to 6 weeks old, mean initial weight 21.7 g). Groups of 8 mice/dose and scheduled examination were treated by oral gavage with diethanolamine dissolved in water at dose levels of 0 (control), 100, 300 or 600 mg /kg body weight per day for 14 days. The animals were sacrificed 24 hours after the last treatment. Numerous separate substudies were conducted to determine a larger number of immunological parameters and to collect general toxicity data. Body weights and organ weights were recorded for the liver, spleen, lungs, thymus, kidneys and one representative mesenterial lymph node per animal, and in addition, haematology and clinical chemistry determinations were performed. Additional groups of 11 or 12 mice were treated with diethanolamine in the same manner in order to investigate their host resistance to *Listeria monocytogenes* or *Streptococcus pneumoniae* and their immune defence against B16F10 melanoma cells. To this end, mice were treated with one of the bacterial strains or the melanoma cells 24 hours after the last exposure to diethanolamine. *Listeria monocytogenes* cells were administered intravenously at three different challenge levels, and morbidity among the treated animals was recorded for 14 days. *Streptococcus pneumoniae* cells were also administered at three different challenge levels by intraperitoneal injection, and morbidity was recorded for 7 days. Mice treated with 3 x 10^5 melanoma cells were

given ^{125}UdR 12 days after cell administration and sacrificed 24 hours thereafter. The nodules in the lungs were counted and radioassayed. The methods used in the immunology studies and the results obtained with them are summarised in Table 9.

Beginning of Table 9

Table 9. Results of immunological studies in female mice given 14 days' oral treatment with diethanolamine at 100, 300 or 600 mg/kg body weight (Immunotoxicology Program, 1992 b)

Study methods	Results
One day after the last exposure to diethanolamine, the animals were anaesthetised and splenectomised; a splenocyte cell suspension was prepared and the B(Ig^+) lymphocytes and T lymphocytes counted, including Ty1.2$^+$, $CD^{4+}CD^{8-}$, $CD^{4-}CD^{8+}$ and $CD^{4+}CD^{8+}$ (no further details provided as to the method of determination)	Ig^+ lymphocytes were dose-dependently increased, with the 30% increase seen at the highest dose attaining statistical significance; $CD^{4+}CD^{8-}$ lymphocytes was dose-dependently decreased, with the 18% decrease seen at the highest dose attaining statistical significance; $CD^{4-}CD^{8+}$ and $CD^{4+}CD^{8+}$ lymphocytes were not affected in number by exposure to diethanolamine
Animals were treated intravenously with 2 x 10^8 sheep erythrocytes on day 11 of diethanolamine treatment (as an exception, doses administered in this case were 300, 600 or 800 mg/kg body weight) and 4 days later the effect was determined on the spleen IgM antibody-forming cell (AFC) response to the T-dependent sheep erythrocyte antigen (further details are provided in the above-mentioned study by the same investigators)	Spleen weight was statistically significantly decreased by 14% in the lowest, 300 mg/kg dose group, whereas it remained unaffected in the higher dose groups; spleen cellularity was decreased by 15 to 19% in all dose groups in a dose-independent manner; the immunoglobulin (Ig) M antibody-forming cell response was dose-dependently and statistically significantly decreased in all dose groups when the data were expressed as total spleen activity (40, 58 and 64%) and also in the intermediate and highest dose group when the data were evaluated as specific activity (48 and 58%, respectively)
One day after the last exposure to diethanolamine, the animals' spleens were removed and processed into splenocyte cell suspensions; the effect of the mitogens concanavalin A and lipopolysaccharide from *Salmonella typhosa* 901 on the proliferation of the spleen cells was determined by 3 days' incubation with mitogen followed by addition of ^{3}H-thymidine 2 days later and, finally, measurement of the incorporated radioactivity (no further details of the methodology)	Treatment with diethanolamine showed no effect in this study when compared with the concurrent controls
One day after the last exposure to diethanolamine, the animals' spleens were removed and processed into splenocyte cell suspensions; these were then co-cultured for 5 days with mitomycin C-treated (40 µg/ml) spleen cells from DBA/2 mice; the endpoint measured was the reaction of the allogenic DBA/2 mouse spleen cells (4 x 10^5 cells/suspension) on the ability of the T cells among the spleen cells from diethanolamine-treated mice (1 x 10^5 cells/suspension) to proliferate, this ability being determined by labelling the proliferating cells with ^{3}H-thymidine 18 to 24 hours before the end of the assay (no further details of the methodology)	No significant effects were observed in comparison with the concurrent controls; a nonsignificant 33% decrease in proliferation occurred at the highest diethanolamine dose

Table 9. Results of immunological studies in female mice given 14 days' oral treatment with diethanolamine at 100, 300 or 600 mg/kg body weight (Immunotoxicology Program, 1992 b)	
Study methods	Results
One day after the last exposure to diethanolamine, the animals' spleens were removed and processed into splenocyte cell suspensions; these were then co-cultured for 5 days with mitomycin C-treated allogenic P815 mastocytoma cells at a 50 : 1 ratio; the spleen cells were isolated for the subsequent cytotoxic T lymphocyte (CLT) assay and incubated with ^{51}Cr-labelled P815 cells at a 25 : 1 ratio for 4 hours before the enumeration of cytotoxic T lymphocytes	All dose groups showed a similar diethanolamine treatment-related decrease by 10 to 14% in the ability to form cytotoxic T lymphocytes (killer cells), as compared with the controls
One day after the last exposure to diethanolamine, the animals' spleens were removed and processed into splenocyte cell suspensions; these were assayed for natural killer cell activity 24 hours later by using ^{51}Cr-labelled YAC-1 tumour cells as target cells and determining ^{51}Cr release (no further details of the methodology)	Only the highest dose group exhibited a significant decrease in natural killer cell activity by 20% at a spleen cell/tumour cell ratio of 25 : 1, as compared with the concurrent control
At the end of the diethanolamine treatment, the animals' macrophages were isolated by peritoneal lavage and adhered in culture plate wells for 2 hours; the adherent cells were subsequently treated with gamma interferon and lipopolysaccharides or incubated without admixture for another 4 hours at 37 °C; after a change of medium, the macrophages were further incubated upon addition of B16F10 target cells at a 10 : 1 ratio; the plates were then rinsed, stained and read on an ELISA plate reader (no further details of methodology)	Only in the highest dose group and in the absence of gamma interferon and lipopolysaccharide as stimulants of macrophage activity was a decrease noted in macrophage cytotoxicity to B16F10 melanoma cells as compared with the controls treated in the same manner; the other dose groups without macrophage stimulation and all dose groups with such stimulation showed no significant effects of diethanolamine treatment
One day after the last treatment with diethanolamine, the animals were sacrificed and the cells of the peritoneal cavity harvested by lavage with heparin-containing buffer; absolute cell counts and relative counts by type of cell were determined	The animals in the intermediate dose group exhibited a significant decrease in the absolute count of harvested cells; for lymphocytes, neutrophils, monocytes, eosinophils and mast cells, no significant differences relative to the controls were observed in the absolute numbers or the percentages

End of Table 9

Diethanolamine was not found to affect body weight or body weight changes in treated animals. Only the animals in the highest dose group gained weight faster than the controls in the first week of treatment, something which normalised, however, in the second week of treatment. Liver weight was dose-dependently increased in the two high dose groups by a maximum of 43%. All other organ weights, including thymus and spleen weights, showed no treatment-related alterations. Necropsy of the mice revealed no pathological findings. The erythrocyte count was dose-dependently decreased in treated mice, with the highest dose reaching statistical significance. Haemoglobin and haematocrit values and the reticulocyte count were also dose-dependently decreased, with the de-

creases attaining statistical significance in the two upper dose groups. All other haematological parameters and the serum chemistry determinations were unaffected by diethanolamine treatment. Pretreatment with diethanolamine had no effect on *Listeria monocytogenes*-related mortality but did affect mortality seen after administration of *Streptococcus pneumoniae*, which showed a marked 73% increase in the highest dose group, a finding suggestive of decreased host resistance to the latter bacterium. The resistance of the mice to the melanoma cells was also decreased. A 161% increase in the levels of radioactivity was found in the lungs after pretreatment with diethanolamine at 300 mg/kg body weight, as compared with the controls. The investigators concluded that diethanolamine could be an immunotoxicant. A *no effect level* could not be established (Immunotoxicology Program, 1992 b).

7.10 Neurotoxicity

In the context of a subchronic study, groups of 10 male and 10 female rats were treated for 13 weeks with diethanolamine at daily dose levels of 0 (controls), 25, 48, 97, 202 or 436 mg/kg body weight (males) or 0 (controls), 14, 32, 57, 124 or 242 mg/kg body weight (females) in their drinking water (as calculated from the animals' actual water consumption, their body weights and the diethanolamine levels in the drinking water). During the study, death occurred in 2 males in the highest dose group and one female in the lowest dose group. Body weight gains in males were greatly depressed in a dose-related fashion at and above a dose of 48 mg/kg body weight to the extent that body weight in the highest male dose group was reduced by 44% relative to the controls at the end of treatment. The females exhibited retarded body weight development at and above a dose of 124 mg/kg body weight, with final body weight also showing a marked 16% decrease at that level, relative to controls, and a 25% decrease at the highest dose level, though decreases were not as pronounced as in males. Clinical signs of toxicity included tremor, emaciation, abnormal posture and rough haircoat in the two highest dose groups of both sexes. In addition to other findings, the investigators also identified the brain and spinal cord as targets of diethanolamine toxicity. Histopathological examination revealed dose-related demyelination in the medulla oblongata and the spinal cord of all male and female rats in the two highest dose groups. This finding was

classed as minimal to mild and found to be slightly more pronounced in males than in females. There were no clinical signs that could be clearly attributed to these lesions (see also Section 7.5; NTP, 1992).

Similar results were obtained from a 13-week study in which groups of 10 male and 10 female rats received daily applications of diethanolamine to the shaved dorsal skin, without cover, at dose levels of 0 (controls), 32, 63, 125, 250 or 500 mg/kg body weight (except on weekends and public holidays). In the highest dose group, one male died during treatment week 9 and 2 females were killed in a moribund condition during treatment week 10. Male body weights in were depressed in a clearly dose-related manner in the two highest dose groups, reaching 86% and 69% of control body weights at the end of treatment. Females showed the same effect in a more moderate form. Their body weights in the two highest dose groups were 90% and 79% of control weights. Clinical signs of toxicity were observed only in the 3 highest dose groups and consisted in irritation and crusting of the skin at the site of application. Histopathological examination revealed minimal demyelination in the medulla oblongata in all males and females in the highest dose group and in 7 out of 10 females in the 250 mg/kg group, a lesion which the investigators considered to be clearly treatment-related. There were no lesions in spinal cord. No clinical signs indicative of neurotoxicity were observed (see also Section 7.5; NTP, 1992).

Cases of a neuroparalytic syndrome affecting dogs and cats treated with the flea repellent, FLEE, were reported by American veterinarians from Florida during 1980 and 1981. The neuromuscular deficits were characterised by tremor, stiffness of the hind limbs, ataxia, paresis and paralysis. A total of 39 cases in dogs and 12 cases in cats were reported, with a mortality rate of 41% in both species. The flea repellent was given with food at a daily dose level of 44 mg/kg body weight. Analysis revealed that it was a 53-percent aqueous solution of diethanolamine and the corresponding daily dose of diethanolamine was 23 mg/kg body weight. Exposure resulted in the onset of neurological signs after 2 days to 28 months (mean value 5 months). Discontinuation of treatment was followed by recovery in most cases. A detailed case report was presented of one dog that was treated with FLEE for 2 to 3 months. It died 9 days after referral to hospital and underwent necropsy. In addition to an enlarged liver with periportal hydropic degeneration of hepatocytes, macroscopic and histopathological examination revealed lesions in the brain and cervical spinal cord with spongiosis of

the corona radiata of the brain and spongiosis of the spinal cord. No evidence of any tissue necrosis was detected (Sundlof and Mayhew, 1983).

A range-finding study for a 90-day study of diethanolamine included comprehensive assessment of the treated animals for neurotoxic effects. Groups of 10 male and 10 female Wistar rats were treated daily with an aerosol of liquid diethanolamine by means of a head-nose inhalation exposure system for 6 hours/day on 5 days/week for 14 days. The administered aerosol concentration levels of diethanolamine were 100, 200 and 400 mg/m³, and a control group was treated with clean air. In addition to other clinical, macroscopic and histopathological examinations, a comprehensive assessment of the neurotoxicological effects of diethanolamine treatment was carried out in all animals in accordance with the EPA “Functional Observational Battery” and the EPA “Neuropathology” guideline. Apart from the clinical observations, this also included that 3 males and 3 females from each group were killed by perfusion with Soerensen’s phosphate buffer and treated with Karnovsky fixative at the end of the treatment period. Epoxy resin embedding and histopathology of cross and longitudinal sections were performed on the sciatic nerve (nervus ischiadicus), the tibial nerve (nervus tibialis) and the sural nerve (nervus suralis). Paraffin embedding and histopathology were performed on various parts of the brain as cross sections and on parts of the spinal cord as cross and longitudinal sections. None of the studies showed any treatment-related changes indicative of neurotoxicity from diethanolamine when compared with the control animals (see also Section 7.2; BASF, 1993 a).

The 90-day main study was conducted using identical methodology, except that groups comprised 13 male and 13 female Wistar rats, concentration levels of 0 (controls), 15, 150 and 400 mg/m³ were selected and treatment was administered for 13 weeks. This study also showed no neurotoxic effects for diethanolamine (see also Section 7.5; BASF, 1996).

7.11 Other effects

Diethanolamine as a close congener of the natural cell constituent ethanolamine was studied for its effects on the formation of phospholipids. Male rats (Sherman or Wistar strains, initially weighing between 100 and 110 g) were fed low-protein diets (5% casein) containing either 5 or 32% fat for 7

or 12 days. At the end of treatment the animals were injected intraperitoneally with a solution of disodium hydrogen phosphate which was radioactively labelled with ^{32}P (2 to 4 µCi). The animals were sacrificed 6 hours later, their livers removed and the lipids extracted from the livers. Part of the extract was analysed for radioactivity and part for phosphorus content. A third portion was treated with magnesium oxide to separate choline-containing from non-choline-containing phospholipids and similarly analysed for radioactivity and phosphorus content. Groups of 2 to 7 animals were studied. This model was used in a number of studies comparing diethanolamine-treated with ethanolamine-treated or untreated animals. The chemicals were administered by gavage (50 mg ethanolamine or 100 mg diethanolamine/animal in 1 ml water) 5 minutes before the injection of disodium hydrogen phosphate, or the diet was supplemented with 0.5% diethanolamine or ethanolamine. In some instances, animals maintained on diet containing diethanolamine each additionally received 100 mg diethanolamine in 1 ml water by oral gavage 5 minutes before the injection of disodium hydrogen phosphate. It was found that total radioactivity and specific radioactivity in liver phospholipids from rats given a single massive dose of either diethanolamine or ethanolamine increased considerably relative to the controls, indicating that an increase in phospholipid synthesis was also induced by diethanolamine. In contrast, however, when diethanolamine was added to the diet for 7 or 12 days, a marked decrease by up to 50% occurred in specific radioactivity of phospholipids, indicating inhibition of phospholipid biosynthesis. Separation and analysis of total phospholipids in these studies showed that in the first case, synthesis of choline-containing and non-choline-containing phospholipids was stimulated to the same extent, whereas in the second case the inhibition of synthesis essentially affected the choline-containing phospholipids, while radioactivity from non-choline-containing phospholipids was practically unchanged and their specific radioactivity was only slightly decreased. Chemical analysis of liver phospholipids from treated animals demonstrated that animals maintained on a diethanolamine-containing diet exhibited markedly increased total phospholipids and non-choline-containing phospholipids in conjunction with an unchanged content of choline-containing phospholipids in their livers. Ethanolamine showed none of the effects described above. The investigators concluded from their findings that diethanolamine was incorporated into phospholipids instead of ethanolamine and that the atypical phospholipids formed in the process were

metabolised less easily than their natural analogues, and therefore accumulated in the liver (Artom et al., 1949).

In a further study, young male rats (40 g initial weight) were fed a low-protein diet (8% casein) for 7 to 10 days and only the diet was supplemented with 0.5% diethanolamine neutralised with hydrochloric acid. The animals were then maintained on the diethanolamine-containing diet for another 5, 7 or 9 days. Animals fed diet without diethanolamine served as controls. Some of the controls received diet supplemented with 0.5% choline chloride or 0.5% ethanolamine neutralised with hydrochloric acid. At the end of the treatment period, the animals were sacrificed and their livers removed. From these, the lipids were extracted, quantified and analysed for content of phosphorus and choline, and the phospholipids were isolated and chromatographically fractionated. Additionally, male rats maintained on a normal diet were injected intramuscularly with ^{14}C-labelled diethanolamine or ethanolamine (8 µCi, 8 µmol in 1 ml) upon having reached a body weight of 100 to 150 g, and groups of 3 to 6 rats were sacrificed after 45 minutes, 1.5, 3, 6 or 16 hours. The animals' livers were removed and the lipids extracted, and the content of radioactivity was measured both with and without further fractionation. In agreement with the findings described above (Artom et al., 1949), the content of non-choline-containing phospholipids was markedly increased in the livers of diethanolamine-treated animals relative to the controls (124 mg : 67 mg, per whole liver) while the content of choline-containing phospholipids was markedly reduced (46 mg : 57 mg, per whole liver). The effect was inverse when diethanolamine was replaced by choline as the dietary supplement; the amounts of non-choline-containing phospholipids found in the liver were lower than in the controls (36 mg : 67 mg, per whole liver) whereas the amounts of choline-containing phospholipids were higher (88 mg : 57 mg, per whole liver). In both cases, the amounts of neutral fat in the livers of treated animals were significantly decreased. In the short-term experiments in which animals received ^{14}C-diethanolamine or ^{14}C-ethanolamine by intramuscular injection shortly before sacrifice, the peak of radioactivity in the liver lipids was reached between 3 and 6 hours following administration. However, in each instance, diethanolamine-derived radioactivity was exceeded by ethanolamine-derived radioactivity by more than one order of magnitude. This demonstrated that when a single dose of the chemical was administered, incorporation of the chemical into phospholipids was consid-

erably less extensive than in longer-term treatment, a finding for which the explanation provided was that diethanolamine-containing phospholipids were metabolised more slowly than natural phospholipids and therefore accumulated in the liver (Artom et al., 1958).

In a further study, white mice were treated by daily intraperitoneal injection of 12.4 mg diethanolamine (presumably per animal) for 3 or 6 days. The homogenates prepared from animals' livers were extracted in the cold and subsequently assayed for alkaline phosphatase activity, using sodium glycerophosphate as the substrate. The amount of free phosphoric acid formed during the one-hour incubation at pH 8.8 was determined. Several analyses revealed a significant increase in alkaline phosphatase activity, independently of the duration of treatment with diethanolamine. Histopathological examination of the livers from mice treated with diethanolamine for 3 days confirmed the finding. Again, there was a marked increase in alkaline phosphatase activity. The observations seemed to suggest that phosphatase was formed primarily in the cell nuclei. Hepatocyte nuclei grew larger, small and medium-sized nuclei disappeared and large nuclei appeared in greater quantity. The investigators considered their findings as confirmation that diethanolamine acted on the liver of mice essentially by altering the phospholipid pattern of the cell nuclei (Annau and Manginelli, 1950).

The effect of diethanolamine on lipid metabolism in liver and kidney cells of rats was studied in vivo and in vitro using radiolabelled ethanolamine, choline and diethanolamine. Adult male Sprague-Dawley rats were used. The in-vitro studies were carried out by processing the animals' livers into homogenates, from which supernatants were obtained by refrigerated centrifugation at 1000 x g. Incorporation of ^{3}H-ethanolamine or ^{3}H-choline into phosphatidylethanolamine or phosphatidylcholine was used as a measure of phospholipid synthesis. Incorporation of ^{14}C-diethanolamine as a non-natural component was also determined. The substrate used was diolein, which was incubated in an incubation medium for one hour at 37 °C together with one of the radiolabelled chemicals and the homogenate supernatant under addition of increasing amounts of unlabelled diethanolamine. The reaction was then stopped by the addition of chloroform/methanol (2 : 1), and the phospholipids were extracted from the reaction mixture and analysed for their content of radioactivity. Incorporation of labelled chemical occurred in a linear manner within one hour. The concentrations of labelled ethanolamine and choline were varied between 25 and 200 µM with a spe-

cific radioactivity of 1 µCi/50 nmol. Unlabelled diethanolamine was added at concentrations of 0 (controls), 100, 300, 1000 or 3000 µM, while labelled diethanolamine was added at concentrations from 5 to 20 mM with a specific radioactivity of 0.05 µCi/µmol. The results were presented as Lineweaver-Burk plots. It was found that phosphatidylcholine synthesis was inhibited by diethanolamine in a dose-dependent and competitive fashion. Phosphatidylethanolamine synthesis was also dose-dependently inhibited by diethanolamine, but in a not solely competitive manner and not to the extent observed for the choline derivative. Incorporation of diethanolamine itself into phospholipids was also demonstrated. The Lineweaver-Burk plot yielded a K_m (substrate concentration at half-maximal reaction velocity) value of 11.6 mM and a V_{max} (maximum velocity of the enzyme reaction) value of 21.0 nmol/mg protein per 60 minutes. Diethanolamine thus appeared to be markedly less effective as a substrate for the studied enzyme reaction than were choline and ethanolamine, for which the respective determined K_m values of 75.5 and 53.5 µM were smaller by two orders of magnitude. In-vivo investigations included a first study consisting of two trials in each of which 3 groups of 4 animals were dosed with diethanolamine at 2 mg/ml (equivalent to a daily dose of 320 mg/kg body weight) for 1, 2 or 3 weeks in the drinking water. The same number of untreated animals served as the controls. At the completion of each dosing regimen, each animal was injected intraperitoneally with 5 µCi ethanolamine (specific radioactivity 3.8 Ci/nmol) or 5 µCi choline (specific radioactivity 4.2 Ci/nmol) containing no added carrier. The animals were sacrificed 6 hours later, their livers and kidneys removed, the phospholipids extracted and the extracts analysed for radioactivity. It was found that diethanolamine, when administered for periods of several weeks, very markedly inhibited ^{3}H-ethanolamine and ^{3}H-choline incorporation into hepatic and renal phospholipids. The most significant inhibition of ethanolamine incorporation was observed in liver, where there was a 73% decrease after only one week. The inhibition of hepatic choline incorporation exhibited a more gradual decline, but also showed decreases by 53 and 59% after 2 and 3 weeks, respectively. In the kidney, ethanolamine and choline incorporation into the phospholipids was also inhibited by multiple administration of diethanolamine, although not to the degree observed in liver. After 3 weeks of diethanolamine treatment, incorporation of ethanolamine and choline was found to be inhibited by 53 and 29%, respectively. A second trial carried out in the context of the in-vivo studies investigated the elimination kinetics of the

phospholipid derivatives of ethanolamine, choline and diethanolamine. Two groups of 32 rats received a single intraperitoneal injection of 250 mg diethanolamine/kg body weight containing 10 µCi ^{14}C-diethanolamine. One group additionally received 6 µCi ^{3}H-ethanolamine, the other 5 µCi ^{3}H-choline by intraperitoneal injection. Four animals from each of the two groups were sacrificed 6 hours, 1, 2, 4, 5, 6, 7 or 9 days after dosing, and their livers and kidneys were removed and assayed for residual radioactivity as described above, with ^{14}C and ^{3}H being measured separately. The data recorded 6 hours after dosing were compared with those for controls given only radiolabelled ethanolamine or choline but no diethanolamine. It was found that diethanolamine, administered as a single dose, did not inhibit the incorporation of ethanolamine or choline into hepatic phospholipids in vivo and in fact even resulted in increased renal incorporation of ethanolamine, in particular. Total incorporation of ethanolamine measured 24 hours after administration of the chemicals was 20% and 2.7% of the administered dose for the liver and kidney, respectively. Choline incorporation into phospholipids in the liver and kidney was 7% of and 0.8% of the administered dose, respectively. Diethanolamine incorporation was considerably lower: 2% in liver and 0.2% in kidney. The half-lives for the disappearance of the radiolabelled chemicals from the phospholipids were also markedly different for the two natural components compared with diethanolamine. Whereas in liver the respective values for ethanolamine and choline were 1.6 and 1.7 days, it took 3.5 days for 50% of the initial radioactivity to be eliminated from the liver in the case of diethanolamine. The same picture was seen in the kidney, where the corresponding values were 2.3, 2.1 and 4.2 days for ethanolamine, choline and diethanolamine, respectively. In summary, the findings showed that diethanolamine was incorporated into phospholipids and that it was eliminated more slowly from its phospholipid derivatives than were choline or ethanolamine from theirs. Moreover, repeated administration of diethanolamine inhibits the synthesis of ethanolamine and choline phospholipids, consequently resulting in an accumulation of phospholipids containing the atypical component diethanolamine. Because phospholipids perform specific functions in cellular metabolism, particularly as membrane constituents, this may give rise to toxic cellular effects (Barbee and Hartung, 1979 b).

Also studied was the effect of diethanolamine on the activity of enzymes involved in the biosynthesis of phosphatidylcholine. Female Sprague-

Dawley rats (60 to 80 days old, weighing 140 to 180 g) were divided into groups of 3 rats and treated as follows. The first group was dosed intraperitoneally with 0.5 mmol/180 g body weight (equivalent to 290 mg/kg body weight) of diethanolamine per day on 3 consecutive days. The second group was dosed intraperitoneally with 1 mmol/170 g body weight (equivalent to 580 mg/kg body weight) of diethanolamine per day, again on 3 consecutive days. Both groups received diethanolamine dissolved in physiological saline solution, and concurrent control groups were included that were treated with the solvent alone. The third group was fed a liquid amino acid diet for one week without choline, methionine, vitamin B_{12} or folic acid and containing 1% diethanolamine. Again, a concurrent control was given the same diet without diethanolamine. At the end of the treatment period, the animals were sacrificed, their livers were removed and homogenised and the microsomal fraction of the homogenate was isolated. This fraction was assayed for the specific activities of phosphatidylethanolamine methyltransferase, phosphatidylmonoethylethanolamine methyltransferase, phosphatidyldimethylethanolamine methyltransferase and choline phosphotransferase, expressed as pmol phosphatidylcholine formed per minute and per mg of microsomal protein. Total liver microsomal activity was also given. The 6 treated animals in the first group given diethanolamine intraperitoneally for 3 days exhibited a significant decrease in the liver microsomal activity of choline phosphotransferase and a significant increase in the liver microsomal activity of phosphatidylethanolamine methyltransferase, as compared with the values determined for 4 control animals. The activity of phosphatidyldimethylethanolamine methyltransferase was unaffected. The same effects were found for the liver microsomes from the 8 animals in the second group dosed orally with diethanolamine for 3 days (6 control animals). In this case, however, the specific activity of phosphatidyldimethylethanolamine methyltransferase was also significantly increased, whereas total liver microsomal activity of this enzyme was not significantly increased. The animals in the third group, fed the deficient diet with diethanolamine for 7 days, exhibited a significant decrease in the liver microsomal activity of phosphatidylethanolamine methyltransferase and phosphatidylmonomethylethanolamine methyltransferase as well as a significant decrease in total liver microsomal activity of phosphatidyldimethylethanolamine methyltransferase in the absence of an effect on the latter enzyme's specific activity. Assays were performed on liver microsomes from 3 animals in comparison with 3 controls fed the same diet without di-

ethanolamine. It was concluded from the results for groups 1 and 2 that diethanolamine affected phosphatidylcholine biosynthesis. The major metabolic pathway involving choline phosphotransferase was inhibited. The second, less important metabolic pathway involving methyl transferases was stimulated. The effects were inverted in animals fed the diet containing diethanolamine, a finding which the investigators attributed to the enhanced incorporation of diethanolamine in place of ethanolamine (Hoffman et al., 1983).

In addition, the effect of diethanolamine on microsomal drug-metabolising enzymes was investigated in rats. Adult male Sprague-Dawley rats were given intraperitoneal treatment with diethanolamine as either a single dose of 1000 mg/kg body weight, 5 consecutive daily doses of 250, 500 or 750 mg/kg body weight, or a daily dose of 100 mg/kg body weight, administered over a period of 14 days. In all cases, there were concurrent untreated controls. Part of the animals were sacrificed 24 hours after the last administration, whilst others were kept for a recovery period of 14 days in order to determine hexobarbitone sleeping time. Hydroxylation of acetanilide and N-demethylation of aminopyrine were determined by rat liver 13000 x g supernatants (no further details of the method). There was a delay in onset of inhibition of the two enzymes following single administration of diethanolamine at 1000 mg/kg body weight. Five administrations of diethanolamine at dose levels of 250, 500 mg/kg or 750 mg/kg inhibited hydroxylation by 35, 61 or 64%, respectively, as compared with results for control animals. Respective values noted for the inhibition of N-demethylation were 55, 80 and 84% at the same dose levels. Hexobarbitone sleeping time was markedly prolonged in these animals. Prolongation was 55, 169 and 222% of the controls. Animals treated with diethanolamine at 100 mg/kg body weight per day for 14 days showed inhibition of hydroxylation and N-demethylation by 52 and 68%, respectively, relative to controls. Reversal of the inhibition was very slow. Inhibition of both enzymes was 50% even after 14 days without diethanolamine treatment. In contrast to its effect in vivo, diethanolamine did not cause inhibition of hydroxylation or N-demethylation when added to a normal in-vitro system (no further details). These data suggest that diethanolamine-induced inhibition of hepatic metabolism proceeds by an indirect mechanism (Foster et al., 1971; Foster, 1972).

Further studies of the mechanism by which diethanolamine inhibits the activity of hepatic microsomal enzymes revealed a statistically significant cor-

relation between the dose-related enzyme inhibition and decreases in hepatic microsomal cytochrome P-450. Microsomal cytochrome b5 and protohaeme content were also decreased. The latter suggests a decrease in haeme synthesis and might explain the production of anaemia by diethanolamine. Partial reversal of the diethanolamine-induced inhibition of hepatic microsomal drug-metabolising enzymes was accomplished by in-vitro addition of phosphatidylcholine to assays containing liver microsomes from rats pretreated with diethanolamine. This finding suggested that diethanolamine might unphysiologically alter phospholipids necessary for functioning of this enzyme system. Finally, it was demonstrated that phenobarbitone induction of hepatic microsomal enzymes as well as increases in cytochrome P-450 content and NADPH-cytochrome c reductase were blocked by diethanolamine when the two compounds were administered to rats simultaneously (Foster, 1972).

Also investigated were the effects of diethanolamine on isolated rat hepatocytes in vitro. The livers were obtained from male Sprague-Dawley rats (weighing 190 to 325 g). The hepatocytes were isolated by whole-liver perfusion with collagenase. The hepatocytes were incubated at diethanolamine concentrations of 0.1, 1 or 10 mM (equivalent to 1.05, 10.5 or 105 µg/ml, respectively) for 2 or 5 hours at 37 °C. Subsequently, cell counts and trypan blue viability counts were performed in the aliquots and enzymatic activities released by the hepatocytes during the incubation period were determined for aspartate aminotransferase and lactate dehydrogenase. In addition, the urea synthesis capability of hepatocytes and intracellular adenosine triphosphate levels were measured. Two-hour incubation of hepatocytes in the presence of diethanolamine failed to reduce the percent cell viability or increase aspartate aminotransferase release at the concentration levels tested when compared with the untreated controls. The highest diethanolamine concentration resulted in a statistically significant increase in lactate dehydrogenase activity to the medium. Five-hour incubation of hepatocytes in the presence of diethanolamine was associated with a dose-related increase in the release of aspartate aminotransferase and lactate dehydrogenase to the medium. The urea synthesis capability of hepatocytes and intracellular adenosine triphosphate levels were dose-dependently decreased. The number of intact cells was not ascertained. The investigators regarded their findings as evidence of the cytotoxicity of diethanolamine in vitro (Story et al., 1983).

8 Experience in humans

A test series carried out in order to compare different patch-test systems for skin irritation testing in humans also included diethanolamine (99% pure). The chemical was tested in the undiluted form using the 4 test systems “Webril”, “Hill Top”, “Finn” and “Van der Bend”, which were simultaneously attached to the upper outer arm of the 15 volunteers for 4 hours. Assessments were performed by visual inspection at 1, 24, 48 and 72 hours. Diethanolamine showed no marked differences in the 4 testing systems and generated only a low level of response. There was mild, if any, oedema and erythema. One subject exhibited a marked effect 4 days after treatment (no further details; York et al., 1995).

A series of nearly 200 control patch tests with 1- and 5-percent solutions of diethanolamine on eczematous patients yielded only negative results (no further details; Crow et al., 1968).

Patch tests were carried out in 32 patients who were sensitive to ethylenediamine in order to determine the skin-sensitising potential of a number of structurally related chemicals, among them diethanolamine. A 1-percent solution of diethanolamine produced a positive effect in one patient (no further details; Balato et al., 1986).

One report concerns 3 cases of metalworkers who developed an allergy to alkanolamineborates as ingredients in cutting oils. All 3 patients were patch-tested with diethanolamine, *inter alia*. A 1-percent solution of diethanolamine in buffer was without effect in all cases (no further details; Bruze et al., 1995).

Seventeen metalworkers with contact allergic eczema of the hand underwent a closed-patch test for, *inter alia*, any allergic reaction to diethanolamine. Two of the patients developed a positive reaction to coconut oil acid diethanolamine condensate. Occupational exposure to these substances could not be proven (Koch, 1996).

Out of a total of 295 patients examined in one or two patch test series for the metalworking industry and recorded by the Informationsverbund Dermatologischer Kliniken (IVDK; “Information Network of Dermatological Clinics”) from January 1990 to March 1991, there were 3 unequivocal cases of allergic response to a 2% solution of diethanolamine in petrola-

tum. An additional 21 cases showed equivocal reactions which were not clearly attributable to an allergic reaction. Coconut oil acid diethanolamine condensate containing considerable amounts of free diethanolamine (18.2%) was also tested (see Table 8). A 0.5-percent solution of the chemical in petrolatum produced a positive reaction in one case and questionable responses in 13 cases (Uter et al., 1993).

A further IVDK survey covering the period between April 1991 and December 1993 recorded 1104 patients who had also participated in the “Metallverarbeitung” (metalworking) test series and been tested for allergic responses to diethanolamine (2% in petrolatum). Of these patients, 18 showed a positive reaction while 28 showed a questionable response. Coconut oil acid diethanolamine condensate produced 6 positive and 12 questionable responses (Uter et al., 1996).

In the period from 1992 to 1999, the IVDK recorded 4339 patients who had been tested for contact allergy to diethanolamine. Of these patients, 73 (1.7%) showed positive reactions. When analysis was limited to a subgroup of patients with jobs in metalworking occupations, 60 out of a total sample of 2053 persons (2.9%) had positive responses, and further limitation to those who were metalworkers with a metal-cutting occupation and hence in direct contact with cooling lubricants, reduced sample size to 353 persons, 35 (10%) of whom showed positive reactions. These data show a clear trend suggesting that an increase in potential exposure to diethanolamine was associated with an increase in the incidence of allergies. This led to the conclusion that diethanolamine can act as a contact allergen in humans under appropriate conditions of exposure (IVDK, 2000).

A 54-year-old man who handled cutting oil every day developed dermatitis of both hands and forearms after 6 weeks. Patch tests revealed that he was sensitive to monoethanolamine, diethanolamine and triethanolamine, cutting oil, mercuric chloride, benzoyl peroxide and phenylmercuric acetate. Diethanolamine produced a positive result at a concentration of 0.1% in water. A patch test, which was repeated 4 weeks later, showed no reaction to diethanolamine at a concentration level of 0.05% in water, but produced a clear reaction at 1 and 2% in petrolatum. According to manufacturer’s specifications, the cutting oil contained no diethanolamine. The investigators pointed out the possibility of cross-reactivity and contamination of the mono- and triethanolamine-based constituents with diethanolamine (no further details; Blum and Lischka, 1997).

One report describes the case of a 47-year-old atopic man who had had occupation-related exposure to coolants for 24 years in connection with metal grinding and had developed contact dermatitis of the hands, forearms and face. When patch-tested, he showed an allergic reaction to a number of constituents of the coolant, including diethanolamine (no further details; Owen et al., 2000).

A case was reported of occupational asthma, the induction of which was attributed to diethanolamine. A 39-year-old metalworker whose work involved handling various diethanolamine-containing cutting fluids exhibited corresponding clinical signs related to his work. The diagnosis was confirmed by a chamber provocation test employing cutting fluids which were heated to simulate the conditions at work. The provocation test was carried out in the same manner at two different diethanolamine concentration levels, both of which were below the applicable threshold limit values for workplace exposure. As was the case with the cutting fluids, there was a delayed asthmatic reaction which was dose-dependent in severity (no further details; Piipari et al., 1996, 1998).

In a literature study which compiled the odour thresholds for air and water dilutions of 214 industrial chemicals, the values given for diethanolamine in air and water were 0.27 ppm and 22000 ppm, respectively (no further details; Amoore and Hautala, 1983).

When addressing the question of the carcinogenic potential of diethanolamine in humans, it is important to consider that the chemical's N-nitroso derivative, N-nitrosodiethanolamine, has been demonstrated to be carcinogenic in several studies in rats (Preussmann et al., 1982; Berger et al., 1990). It must be taken into account that diethanolamine, upon entering the body, is converted to N-nitrosodiethanolamine in the stomach in the presence of nitrate or nitrite. Interestingly, the connection between the exposure to N-nitrosodiethanolamine and the development of malignant cancer in humans has recently been acknowledged in a court case. A lathe operator who had been exposed to cooling lubricant containing 18% sodium nitrite and approx. 20% triethanolamine had died of cancer of the colon at the age of 34 years. His urine had been found to contain N-nitrosodiethanolamine, which was considered the cause of the disease (Lißner and Schoof, 1999; Schoof, 1999). The German TRGS 615 "Verwendungsbeschränkungen für Korrosionsschutzmittel, bei deren Einsatz N-Nitrosami-

ne auftreten können", i.e. the Technical Rules for Hazardous Substances, No. 615, which governs the restrictions on the use of corrosion inhibitors involving potential exposure to N-nitrosamines, covers *inter alia* the use of diethanolamine in order to prevent the formation of N-nitrosodiethanolamine (TRGS 615, 2003).

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has assigned diethanolamine to category 3A of carcinogenic substances in the List of MAK and BAT values. Category 3A comprises "Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data. The classification in Category 3 is provisional. Substances for which the criteria for classification in Category 4 or 5 are fulfilled but for which the database is insufficient for the establishment of a MAK or BAT value". The Commission has designated the substance with "Sh" and "H" on account of its skin-sensitising potential and the risk of percutaneous absorption, respectively (DFG, 2003; Greim, 2003).

The threshold limit value (TLV) for diethanolamine has been given as 2 mg/m³ for the USA (ACGIH, 2002).

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