Diethanolamine

[111-42-2]	
Supplement 2007	
MAK value (2006)	**1 mg/m^3 E**
Peak limitation (2006)	**Category I, excursion factor 1**
Absorption through the skin (2000)	**H**
Sensitization (2001)	**Sh**
Carcinogenicity (2006)	**Carcinogen Category 3B**
Prenatal toxicity (2006)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Chemical name (CAS)	

The documentation for diethanolamine dates back to 1981 (documentation "Diethanolamin", only available in German). A supplement on all end points is available from 2000 (documentation "Diethanolamine" 2000), and a supplement on the allergenic effect is available from 2001 (documentation "Diethanolamine" 2001). There is also an IARC report (IARC 2002) and a Toxicological Assessment by the BG Chemie (2005). This supplement only lists the recent data relevant for assessment.

Mechanism of Action

Nitrosamine formation

Studies in mice were not able to provide evidence of a possible participation of *N*-nitrosodiethanolamine in the carcinogenicity of diethanolamine.

Effects on phospholipid metabolism

Diethanolamine is metabolized in the same way as physiological ethanolamine, i.e. it is *O*-phosphorylated, *N*-methylated and *N*,*N*-dimethylated. Furthermore, dietha-

The MAK-Collection Part I, MAK Value Documentations 2015
DFG, Deutsche Forschungsgemeinschaft

nolamine and its phosphorylated and methylated metabolites are converted to non-physiological phosphoglyceride and sphingomyelin analogues and incorporated into cell membranes. Since the half-lives of non-physiological diethanolamine phospholipids are longer than those of natural phospholipids, repeated administration of diethanolamine leads to their accumulation in cell membranes throughout the body, but mainly in the liver, kidneys and spleen as well as in the heart, brain and blood. Accumulation of non-physiological phospholipids affects the physio-chemical properties of the cell membrane. This causes functional disturbances of the cell membrane and the observed organ toxicity to the liver, kidneys and spleen (Mathews et al. 1995). Effects on the blood count, inhibition of microsomal enzyme systems, impairment of mitochondrial functions (documentation "Diethanolamine" 2000) and of intracellular signal transductions and differentiations by the disturbed formation of second messengers (Leung et al. 2005) are also attributed to this mechanism.

Absorption, incorporation and accumulation of diethanolamine in phospholipids, particularly in phosphatidylcholine, were also detected in human liver sections in vitro (Mathews et al. 1995); therefore, this mechanism must be regarded as relevant for humans, too. The authors refer to the similarity of the effects observed after administration of diethanolamine in animal studies with the clinical manifestations of the Niemann-Pick disease. The disorder is characterized by an enzyme defect of sphingomyelinase, which leads to a disturbed degradation of sphingomyelins and their accumulation in the cell. This results in alterations of the cell function and impairs the function of the liver, spleen, lungs and central nervous system (Mathews et al. 1995).

Effects on choline homoeostasis

Ethanolamine is *O*-phosphorylated to phosphatidylethanolamine and *N*-methylated to phosphatidylcholine in the liver under physiological conditions. The subsequent cleavage of phosphatidylcholine to form choline and 1,2-sn-diacylglycerol is the only pathway for the *de novo* synthesis of choline in adult mammals (Leung et al. 2005). In rats, diethanolamine is methylated and cleaved via the same metabolic pathway, but it does not form any choline. Furthermore, diethanolamine selectively inhibits choline uptake in cells, and the aberrant diethanolamine-containing phospholipids reduce or inhibit the formation of phosphatidylcholine (Lehman-McKeeman and Gamsky 1999, 2000). This results in choline deficiency in mice (Lehman-McKeeman et al. 2002) and rats (Leung et al. 2005) after repeated administration of diethanolamine.

Diethanolamine-induced choline deficiency and dietary choline deprivation lead to a decrease of the choline metabolites phosphatidylcholine and *S*-adenosylmethionine and to an increase of *S*-adenosylhomocysteine, i.e. demethylated *S*-adenosylmethionine. In addition, dietary choline deficiency induces an accumulation of 1,2-sn-diacylglycerol in the liver (fatty liver), which is not found after administration of diethanolamine. Dietary choline deprivation also leads to the activation of protein kinase C and anomalies in signal transduction mediated by protein kinase C (Leung et al. 2005) and to increased cell proliferation, apoptosis and hepatocellular carcinomas mainly in rats, but also in mice (Mellert et al. 2004). Since liver and kidney tumours were observed in mice, but not in rats that react particularly sensitively to a dietary choline deficiency (see documentation "Diethanolamine" 2000), mechanistic differences between choline-induced liver tumourigenesis in rats and mice and diethanolamine-induced liver and kidney tumourigenesis in mice must be assumed, but have not been investigated to date.

Effects on methylation reactions

Choline can be oxidized to betaine, which functions as a methyl donor for the regeneration of methionine. Methionine is converted to *S*-adenosylmethionine, the active methylation agent for many enzymatic reactions (Leung et al. 2005). If *S*-adenosylmethionine and phosphatidylcholine are not sufficiently available, the probability of disturbances of metabolic processes and a loss of growth control increases. Deficiency of *S*-adenosylmethionine leads to hyper- and hypomethylations of the DNA in specific gene regions. Moreover, *S*-adenosylhomocysteine, i.e. demethylated *S*-adenosylmethionine, is a potent inhibitor of all methylases (Newberne 2002).

Accordingly, dermal application of diethanolamine to $B6C3F_1$ mice led to a decrease of *S*-adenosylmethionine and an increase of *S*-adenosylhomocysteine (Lehman-McKeeman et al. 2002). In vitro, incubation of mouse hepatocytes ($B6C3F_1$) with diethanolamine also resulted in altered DNA methylation patterns, the guanine- and cytosine-rich gene regions being affected most. Hypomethylations were mainly observed, and the methylation pattern induced by diethanolamine was similar to that resulting from choline deficiency. According to the authors, hypomethylation might promote liver tumourigenesis (Bachman et al. 2005).

Studies of the mechanism of action

In vitro

Possible species differences in the sensitivity to diethanolamine and the influence of choline were investigated in vitro in cryopreserved human hepatocytes and primary hepatocytes of $B6C3F_1$ mice and F344 rats. Incubation with up to cytotoxic diethanolamine concentrations of 500 µg/ml for 24 hours led to a dose-related increase of DNA synthesis, which was detected in the form of increased BrdU incorporation. The increase was not significant only at the lowest concentration of 5 µg/ml. Increased DNA synthesis was also observed after incubation of rodent hepatocytes in medium containing reduced choline. DNA synthesis was not increased either after diethanolamine addition or after choline depletion in medium. However, since the cryopreserved human hepatocytes were not adequately characterized as compared with the freshly isolated rodent hepatocytes, it cannot be decided whether human hepatocytes generally do not demonstrate any effects induced by diethanolamine or choline or whether the cryopreserved human hepatocytes were no longer sufficiently differentiated or vital as compared with the freshly isolated rodent hepatocytes.

In mouse and rat hepatocytes, 2-fold to 50-fold excess choline in medium reduced diethanolamine-induced DNA synthesis, sometimes below control levels. Gene expression analysis of mouse and rat hepatocytes following diethanolamine treatment showed increases or up-regulation of genes coding for cyclin D2, E, E2F1 or Cdk2L and decreases or down-regulation of further genes coding for p19, p53, NFxB, p21 cip, Hsf 1, p27 kip 2, caspase 3, bad or bax. The expression profile suggests a stimulation of the cell cycle and a reduction in apoptosis (Kamendulis 2002, 2006; Kamendulis and Klaunig 2005).

Other working groups also observed these alterations in gene expression caused by diethanolamine treatment (Duerksen-Hughes et al. 1999; Hayashi et al. 2003; Thompson et al. 2000).

In vivo

Groups of female and male B6C3F$_1$ mice were exposed to diethanolamine in 95% ethanol, which was applied to the shaved dorsal skin of the interscapular region at doses of 0 or 160 mg/kg body weight and day for 1 week. After 1 week, 8 animals per group and sex were sacrificed and another group was observed for 3 weeks without treatment to assess reversibility. After 1 week of treatment, liver cell proliferation was increased about three times mainly in zone 3, the central vein region (Rappaport et al. 1954), whereas a 2.4-fold increase was observed in the two kidney zones (cortex or outer medulla) (Table 1 and Table 2). In addition, there was a slight liver weight increase, which was statistically significant in the females. The incidence of apoptosis was not altered. Cell proliferation in liver and kidneys was reversible in this study after 3 weeks (BASF 2002 b; Mellert et al. 2004).

In another study, groups of 10 male B6C3F$_1$ mice were dermally exposed to diethanolamine concentrations of 0 or 160 mg/kg body weight and day, dissolved in 96% ethanol, for 1, 4 or 13 weeks. The absolute and relative liver and kidney weights were significantly increased after 13-week exposure. The proliferation rates in the liver and kidneys were increased as early as after 1 week (Table 3 and Table 4). These effects persisted throughout the study period, but their intensity lessened slightly in the course of 4 and 13 weeks. After diethanolamine exposure, a higher incidence of mitotic cells was found in the renal cortex, but the number of cells affected was not sufficient to derive a conclusive evaluation. No relevant cytotoxic alterations to the liver or kidney cells were observed (BASF 2001; Mellert et al. 2004).

In another dermal study, groups of 8 male B6C3F$_1$ mice were exposed to diethanolamine in 95% ethanol at doses of 0, 10, 20, 40, 80, 160, 630 or 1250 mg/kg body

Table 1 3-week recovery period (BASF 2002 b; Mellert et al. 2004;)

DEA (mg/kg b.w. and day)	Recovery period (weeks)	Sex	Hepatocytes in the S phase (% BrdU-labelled cells)				Number of apoptotic cells (zones 1; 2; 3)
			zones 1; 2; 3	zone 1	zone 2	zone 3	
0	–	♂	100	100	100	100	11
	–	♀	100	100	100	100	20
	3	♂	100	100	100	100	3
	3	♀	100	100	100	100	14
160	–	♂	300**	138	171*	997**	5
	–	♀	271**	268**	200**	573**	29
	3	♂	74	84	77	76	4
	3	♀	81*	120	63**	99	12

zone 1: periportal zone; zone 2: between zones 1 and 3; zone 3: central vein region;
*$p < 0.05$; **$p < 0.01$

weight and day for 1 week or 13 weeks to determine a dose-response relationship. All animals of the two high dose groups were sacrificed as early as after 1 week because of severe skin ulcerations. The absolute and relative liver weights were increased in all treatment groups after 1 week and 13 weeks. The liver weight increases of maximally 15% were about the same in a dose range between 10 and 160 mg/kg body weight and day; at 26 and 32%, they were marked and dose-related at the higher doses. The kidney weights were slightly increased after 1 week and somewhat more elevated after 13 weeks, but there was no dose-response relationship at either time. After 13-week treatment, a marked increase in the cell proliferation rate in the liver and kidneys, which became higher with increasing doses, was observed from the lowest dose (Table 5 and Table 6). This rise was obvious as a trend in both organs as early as after 1 week. Zone 3 was mainly affected in the

Table 2 Cell proliferation and apoptosis in the kidneys of male $B6C3F_1$ mice after 1-week dermal application of diethanolamine (DEA) and a 3-week recovery period (BASF 2002 b; Mellert et al. 2004)

DEA (mg/kg b.w. and day)	Recovery period (weeks)	Kidney cells in the S phase (% BrdU-labelled cells)		Number of apoptotic cells
		cortex	outer medullary zone	cortex
0	–	100	100	2
	3	100	100	1
160	–	237**	235**	2
	3	71*	61	6

*$p < 0.05$; **$p < 0.01$

Table 3 Cell proliferation and apoptosis in the liver of male $B6C3F_1$ mice after 1-, 4- or 13-week dermal application of diethanolamine (DEA) (BASF 2001; Mellert et al. 2004;)

DEA (mg/kg b.w. and day)	Treatment (weeks)	Hepatocytes in the S phase (% BrdU-labelled cells)				Number of apoptotic cells (zones 1; 2; 3)
		zone 1; 2; 3	zone 1	zone 2	zone 3	
0	1	100	100	100	100	8
	4	100	100	100	100	13
	13	100	100	100	100	19
160	1	161*	53	130	324**	6
	4	159	139	85	245**	13
	13	140*	98	101	216**	25

zone 1: periportal zone; zone 2: between zones 1 and 3; zone 3: central vein region;
*$p < 0.05$; **$p < 0.01$

liver, and the cortex and outer medullary zone in the kidneys. There was no definite effect on the incidence of apoptosis except for a marked increase in the kidneys of the animals of the highest dose group. The hepatocytes revealed eosinophilia in the portal zone (zone 1) from 80 mg/kg body weight and day, which was characterized as mitochondrial hyperplasia (BASF 2002 a; Mellert et al. 2004).

The influence of choline on cell proliferation and apoptosis in the liver and kidneys after diethanolamine treatment was investigated in male $B6C3F_1$ mice. Groups of 8 animals were exposed to diethanolamine in 96% ethanol, which was pipetted onto the shaved skin of the interscapular region at doses of 0, 10 or 160 mg/kg body weight and day for 1 week or 4 weeks. Further groups consisting of 8 animals were treated in the same way, but they also received feed supplemented with choline at a concentration of 20 000 mg/kg. The addition of choline alone led to a slight, non-significant increase in the proliferation rate mainly in zone 3 of the liver as well as in the cortex and outer medulla of the kidneys (Table 7 and Table 8).

Table 4 Cell proliferation and apoptosis in the kidneys of male $B6C3F_1$ mice after 1-, 4- or 13-week dermal application of diethanolamine (DEA) (BASF 2001; Mellert et al. 2004;)

DEA (mg/kg b.w. and day)	Treatment (weeks)	Kidney cells in the S phase (% BrdU-labelled cells)		Number of apoptotic cells (cortex)
		cortex	outer medullary zone	
0	1	100	100	3
	4	100	100	4
	13	100	100	19
160	1	446**	746**	2
	4	223**	224**	8
	13	181**	180*	20

*$p < 0.05$; **$p < 0.01$

Table 5 Dose-response relationship of cell proliferation and apoptosis in the liver of male B6C3F$_1$ mice after 1- or 13-week dermal application of diethanolamine (DEA) (BASF 2002 a; Mellert et al. 2004;)

DEA (mg/kg b.w. and day)	Treatment (weeks)	Hepatocytes in the S phase (% BrdU-labelled cells)				Number of apoptotic cells (zones 1; 2; 3)
		zones 1; 2; 3	zone 1	zone 2	zone 3	
0	1	100	100	100	100	0
	13	100	100	100	100	7
10	1	162	174	157	154	11**
	13	190*	189	155	241*	2
20	1	83	67	86	94	5
	13	213**	174	119	389**	0
40	1	151	114	160	171	5
	13	127	156	69	198	1
80	1	247*	140	142	674**	16*
	13	131	115	52	266**	5
160	1	513**	145	176	1909**	1
	13	173**	256*	78	261**	6
630	1	1386**	1248**	575	3646**	8
1250	1	2221**	2874**	1017	4523**	7

zone 1: periportal zone; zone 2: between zones 1 and 3; zone 3: central vein region;
* $p < 0.05$; ** $p < 0.01$

Table 6 Dose-response relationship of cell proliferation and apoptosis in the kidneys of male B6C3F$_1$ mice after 1- or 13-week dermal application of diethanolamine (DEA) (BASF 2002 a; Mellert et al. 2004;)

DEA (mg/kg b.w. and day)	Treatment (weeks)	Kidney cells in the S phase (% BrdU-labelled cells)		Number of apoptotic cells	
		cortex	outer medullary zone	cortex	outer medullary zone
0	1	100	100	5	1
	13	100	100	5	2
10	1	117	147	3	0
	13	137*	192*	8	1
20	1	161**	220**	9	1
	13	150*	172**	7	1
40	1	231**	297**	7	0
	13	125*	184*	12	1
80	1	278**	460**	7	2
	13	184**	174*	13*	0

Table 6 (Continued)

DEA (mg/kg b.w. and day)	Treatment (weeks)	Kidney cells in the S phase (% BrdU-labelled cells)		Number of apoptotic cells	
		cortex	outer medullary zone	cortex	outer medullary zone
160	1	516**	1020**	13	2
	13	198**	239**	7	3
630	1	911*	3352**	13	6
1250	1	866**	5920**	28**	25**

*$p < 0.05$; **$p < 0.01$

Diethanolamine had no effect on the proliferation rate at a dose of 10 mg/kg body weight and day after 1 week or after 4 weeks. Since only the BrdU-positive cells were counted at this dose, and not the negative ones, no values are listed in Table 7 or Table 8. The diethanolamine dose of 160 mg/kg body weight and day led to absolute and relative liver and kidney weight increases and to elevated liver and kidney cell proliferation rates. Contrary to the expectations from the in vitro findings, according to which excess choline in medium lowered DNA synthesis clearly below control values and reduced diethanolamine-induced DNA synthesis to control levels (Kamendulis and Klaunig 2005), excess dietary choline caused a slight

Table 7 Influence of choline on cell proliferation and apoptosis in the liver of male B6C3F$_1$ mice after 1- or 4-week dermal application of diethanolamine (DEA) under dietary supplementation with choline (BASF 2003)

DEA (mg/kg b.w. and day)	choline (20000 mg/kg diet)	Treatment (weeks)	Hepatocytes in the S phase (% BrdU-labelled cells)				number of apoptotic cells (zones 1; 2; 3)
			zones 1; 2; 3	zone 1	zone 2	zone 3	
0	–	1	100	100	100	100	2
0	+	1	127	115	128	143	2
160	–	1	213*	63	99	852**	6
160	+	1	252**	117	100	989**	3
0	–	4	100	100	100	100	2
0	+	4	154	156	147	163	1
160	–	4	152*	90	64	384**	5
160	+	4	163*	63*	77	433**	4

zone 1: periportal zone; zone 2: between zones 1 and 3; zone 3: central vein region;
*$p < 0.05$; **$p < 0.01$

Table 8 Influence of choline on cell proliferation and apoptosis in the kidneys of male B6C3F$_1$ mice after 1- or 4-week dermal application of diethanolamine (DEA) under dietary supplementation with choline (BASF 2003)

DEA (mg/kg b.w. and day)	Choline (20000 mg/kg diet)	Treatment (weeks)	Kidney cells in the S phase (% BrdU-labelled cells)		Number of apoptotic cells	
			cortex	outer medullary zone	cortex	outer medullary zone
0	–	1	100	100	8	5
0	+	1	132	143*	4	1
160	–	1	379**	551**	12	2
160	+	1	418**	684**	11	3
0	–	4	100	100	12	1
0	+	4	137	127	5	0
160	–	4	190**	166*	10	0
160	+	4	221**	153**	13	0

*$p < 0.05$; **$p < 0.01$

increase of cell proliferation in all 3 zones of the liver in vivo, but this was not statistically significant. After combined administration of diethanolamine and excess choline, there was a slightly elevated, statistically significant number of BrdU-positive cells in zone 3 of the liver and in both kidney areas, and the kidney weight was additionally increased after 4 weeks. However, no increase in the cell proliferation rate in the liver was found after 4 weeks (BASF 2003). The causes of the different findings in vitro and in vivo have not been clarified. In vitro studies in CHO cells provided evidence that diethanolamine inhibits choline uptake via its specific transport system and may thus lead to intracellular choline deficiency in spite of a sufficient amount of exogenous choline (Lehman-McKeeman and Gamsky 1999; see Supplement documentation "Diethanolamine" 2000). However, this did not seem to play a role in this study since excess dietary choline alone can lead to slightly increased cell proliferation.

Dermal treatment of groups of 6 male B6C3F$_1$ mice with diethanolamine in 96% ethanol at doses of 0, 10, 20, 40, 80 or 160 mg/kg body weight and day over a period of 4 weeks led to a significant depletion of phosphocholine (PCho) from 20 mg/kg body weight and day, a significant reduction of glycerophosphocholine (GPC) from 40 mg/kg body weight and day and a significant lowering of choline (Cho) and S-adenosylmethionine (SAM) from 80 mg/kg body weight and day as well as to significantly increased S-adenosylhomocysteine (SAH). levels At 160 mg/kg body weight and day, phosphatidylcholine (PC) was also significantly lowered (Table 9). The NOAEL for the disturbance in choline homoeostasis induced by diethanola-

Table 9 Development of choline metabolites in the liver after 4-week dermal application of diethanolamine in male $B6C3F_1$ mice and a 2-week recovery period (Lehman-McKeeman et al. 2002)

Parameter	Diethanolamine (mg/kg b.w. and day)						
	0	10	20	40	80	160	160-R[1)]
b.w. (g)	26.6 ± 0.8	25.9 ± 0.4	26.6 ± 0.1	25.8 ± 0.4	26.1 ± 0.4	27.1 ± 0.6	25.8 ± 0.8
rel. liver wght.	5.6 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	6.2 ± 0.1*	5.5 ± 0.1
PCho (nmol/g liver)	1220 ± 44	1192 ± 77	994 ± 78*	959 ± 70*	831 ± 52*	615 ± 37*	1224 ±114
GPC (nmol/g liver)	372 ± 34	463 ± 34	303 ± 32	275 ± 14*	281 ± 23*	193 ± 20*	318 ± 38
Cho (nmol/g liver)	155 ±14	137 ± 12	152 ± 11	126 ± 7	94 ± 14*	106 ± 13*	180 ± 21
PC (µmol/g liver)	19.6 ± 1.0	17.7 ± 1.0	17.0 ± 0.4	18.0 ± 1.0	17.4 ± 0.8	16.8 ± 0.4*	17.7 ± 0.7
SAM (nmol/g liver)	83.9 ± 1.1	84.1 ± 4.1	84.7 ± 3.9	82.1 ± 7.2	65.1 ± 6.4*	53.3 ± 4.3*	84.9 ± 1.0
SAH (nmol/g liver)	48.1 ± 1.9	52.2 ± 6.2	49.1 ± 1.8	52.6 ± 1.4	61.8 ± 4.4*	58.5 ± 2.7*	46.1 ± 4.2

Key: Cho: choline; GPC: glycerophosphocholine,
PC: phosphatidylcholine; PCho: phosphocholine;
SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine;
[1)] recovery group with 2-week post-exposure observation period
* $p < 0.05$

mine was 10 mg/kg body weight and day in this study. The biochemical processes were completely reversible 2 weeks after the end of exposure (Lehman-McKeeman et al. 2002).

As compared with mice that received a conventional diet, the 2-week administration of a choline-free diet to $B6C3F_1$ mice affected choline homoeostasis and choline-dependent biochemical processes more than dermal application of diethanolamine (Table 10). Body and liver weights were not altered, nor was there any evidence of fatty degeneration in the liver (Lehman-McKeeman et al. 2002). When male C57BL/6 mice were dermally exposed to diethanolamine at 160 mg/kg body weight and day, there was a significant reduction of phosphocholine, but other parameters, in particular S-adenosylmethionine, were only slightly or not at all. The authors interpreted this as a strain-specific sensitivity as compared with the changes observed in $B6C3F_1$ mice (Lehman-McKeeman et al. 2002).

Dermal application studies also demonstrated that using 95% ethanol as a solvent affected biochemical processes in hepatocytes and decreased hepatic betaine levels after 4-week dermal application. However, choline levels were not altered. Therefore, it must be assumed that ethanol additionally impaired the choline metabolism (Lehman-McKeeman et al. 2002).

Table 10 Development of choline metabolites in the liver of male B6C3F$_1$ mice after 2-week administration of a conventional diet (control) or a choline-deficient diet (Lehman-McKeeman et al. 2002)

Parameter	Control	Choline-deficient diet
b.w. (g)	24.8 ± 0.8	24.9 ± 0.6
rel. liver weight	5.1 ± 0.1	4.8 ± 0.1
PCho (nmol/g liver)	1146 ± 100	297 ± 53*
GPC (nmol/g liver)	615 ± 83	460 ± 27*
Cho (nmol/g liver)	139 ± 4	95 ± 18*
PC (µmol/g liver)	16.6 ± 1.6	15.2 ± 0.6*
SAM (nmol/g liver)	69.2 ± 4.2	56.8 ± 3.3*
SAH (nmol/g liver)	31.8 ± 2.3	41.1 ± 2.4*

Key: Cho: choline; GPC: glycerophosphocholine; PC: phosphatidylcholine;
PCho: phosphocholine; SAH: S-adenosylhomocysteine;
SAM: S-adenosylmethionine
* $p < 0.05$

Summary of the studies of the mechanism of action

The development of non-physiological diethanolamine phospholipids, followed by membrane disorders, may result in organ damage with permanent cell regenerations, which may then also lead to a clonal spread of initiated cells.

Choline homoeostasis is disturbed by diethanolamine treatment in rats and mice both in vitro and in vivo.

In vitro studies in rat and mouse hepatocytes have demonstrated that diethanolamine-induced DNA synthesis or cell proliferation is associated with choline deficiency. However, this has not been substantiated in in vivo studies to date. The causes of this have not yet been clarified.

The carcinogenicity studies have also raised questions about the mechanism of action. Although choline deficiency led to a higher incidence of liver tumours in rats and mice, such an increase was only observed in mice in the carcinogenicity study with diethanolamine carried out by the NTP. The diethanolamine amounts absorbed by rats may have been too low for an induction of systemic effects (see page 20, Section “Animal studies, Carcinogenicity”).

Currently, the most plausible assumption seems to be that hypomethylation induced by choline deficiency leads to tumourigenesis since a decrease of *S*-adenosylmethionine, an important co-factor of methylation reactions, was demonstrated in B6C3F$_1$ mice after diethanolamine administration. In contrast, this effect was not observed in C57Bl/6 mice with a lower spontaneous rate of liver tumours. However, this hypothesis does not explain the development of kidney tumours in mice.

Further key mechanisms that might contribute to the carcinogenic effect of diethanolamine are lipid peroxidation and the aneugenic effect that were observed in *Drosphila melanogaster*.

To sum up, it can be stated that the mechanism or the mechanisms contributing to the carcinogenicity of diethanolamine in mice are not regarded as having been sufficiently clarified on the basis of the data available to date. However, previous studies have clearly shown that mutagenic and clastogenic effects do not play a role. The relevance of the aneugenic effect observed in *Drosophila melanogaster* has not yet been clarified.

Currently, it cannot be conclusively assessed whether the liver and kidney tumours observed in mice are relevant to humans, they may only be of minor importance. Rats and mice require more dietary choline than humans since rodents oxidize choline faster than humans.

Toxicokinetics and Metabolism

Uptake

The ability of diethanolamine to penetrate the skin under simulation of consumer application conditions for washing powder and cosmetic products was investigated in vitro in the excised skin (split skin) of fuzzy rats (Sprague Dawley variant). For this purpose, ^{14}C-diethanolamine was applied in a diffusion cell at a concentration of 3 mg/cm^2 (0.5 μCi) for 24 hours. An oil-in-water emulsion was used as a vehicle (concentration not specified). Absorption was 1.4% after 24 hours; about 4% remained in the skin, no difference being observed between stratum corneum and epidermis or dermis. After 6 hours, 0.5% of the applied dose was detected in the receptor fluid. There was no change after prolonging the observation period to 72 hours (Yourick et al. 2006).

A study carried out under in-use conditions investigated the penetration of ^{14}C-diethanolamine (about 0.5 μCi) from representative cosmetic consumer products such as shampoos, hair dyes and body lotions through human skin in vitro in a diffusion cell. Depending on the composition of the product and time of application, most of the absorbed amount was found in the stratum corneum and dermis or epidermis. After 5-minute application of shampoos (diethanolamine at 4.2 or 7.7 μg/cm^2; 0.05% diethanolamine) or 30-minute application of hair dyes (diethanolamine at about 100 μg/cm^2; 0.6% diethanolamine), 2.5–3.6% had penetrated the skin, but only 0.1% was found in the receptor fluid as a measure of systemic availability. After 24-hour application of 2 body lotions (3 μg/cm^2; diethanolamine at about 1 μg/cm^2; 0.02% diethanolamine), 6 and 14.8% diethanolamine had penetrated the skin. The receptor fluid contained 0.6 and 1.2%. When the application period was extended to 72 hours, diethanolamine accumulated in the skin at a level of about 30%, but penetration into the receptor fluid remained the same at 1% (Kraeling et al. 2004).

Another study carried out under in-use conditions with standard formulations of cosmetic products such as shampoos, bath essences and hair dyes confirmed the very low penetration of diluted diethanolamine (maximally 0.1%) through human skin samples in vitro. Between 0.02 and 0.7% of the dose penetrated regardless of the exposure period of 10 minutes to 48 hours. This corresponds to 1 to 48 ng/cm^2 standardized to 24 hours (Brain et al. 2005).

Distribution and elimination

In order to investigate possible differences in toxicokinetics after administration of high and low diethanolamine doses, groups of female Sprague Dawley rats were administered 10 or 100 mg/kg body weight once via intravenous injection. Ninety-six hours after injection of the low and high dose, 69 and 56% of the radioactivity, respectively, were detected in the tissue, and most of it in the eviscerated animal body (28 and 35%), liver (21 and 17%) and kidneys (7 and 5%). Related to organ weight, the major fraction was found in the kidneys at 26 µg equivalents/g tissue for the low dose and 199 µg equivalents/g tissue for the high dose. In the liver, the values were 15 and 136 µg equivalents/g tissue for the low and high dose, respectively. Excretion was mainly via the urine and was 25 and 36% of the dose after 96 hours, excretion of the higher dose being faster. The observed biphasic elimination was very rapid in the first phase with half-lives of 3.5 and 2.4 hours for both dose groups. The subsequent second phase was almost constant and proportional to the dose. The peak concentration in the plasma and erythrocytes was detected after 5 minutes, the fraction in the erythrocytes being twice as high. After very rapid initial elimination from the blood with half-lives of 6 and 35 minutes, accumulation in the erythrocytes was found in the period between 6 and 96 hours, respectively, which was interpreted as a sign of possible incorporation into the phospholipids of the erythrocyte membrane. The half-lives of elimination from the blood in the second phase were 169 and 154 hours. Clearance from the blood was 84 ml/hour and kg body weight for the low dose and 242 ml/hour and kg body weight for the high dose (Mendrala et al. 2001).

Effects in Humans

Sensitizing effects on the skin

Further publications have been made available since the appearance of the supplement on sensitization from 2001 (documentation “Diethanolamine” 2001).

Among 143 metal workers with occupationally induced skin changes tested between 1998 and 2001 in the clinics of the Information Network of German Departments of Dermatology (IVDK: Informationsverbund Dermatologischer Kliniken), 6 patients showed a positive reaction to a 2% preparation of diethanolamine in petrolatum (Geier et al. 2004 b). However, most of these workers had already been included in the cohort described in the supplement from 2001 (documentation "Diethanolamine" 2001). Only one person (0.6%) from a cohort of 174 patients who had been exposed to various waterborne metalworking fluids in industry showed a positive reaction to a 2% diethanolamine preparation in petrolatum in the patch test carried out between April 2000 and July 2002 (Geier et al. 2003).

In 2002 and 2003, 6 of 200 metal workers tested in the clinics of the IVDK showed a positive reaction to a 2% preparation of diethanolamine in petrolatum (5 simple reactions and 1 twofold positive reaction). In addition, 6 questionable, but no irritant reactions were observed (Geier et al. 2004 a).

Sensitizing effects on the airways

There are no data available for sensitizing effects on the airways.

Carcinogenicity

In 1998, NIOSH published an evaluation of mortality studies among workers exposed to various metalworking fluids. They had been exposed to water, oil, sulfonates, non-ionic surfactants, ethanolamines, alkaline substances, biocides and microbial products. NIOSH concluded that for workers who had been exposed before 1975 there was sufficient evidence of increased incidences of tumours of various localizations such as the larynx, rectum, pancreas, skin, scrotum and urinary bladder. Mirer (2003) from the International Union, United Automobile, Aerospace and Agricultural Implement Workers of America (UAW) then further evaluated these data. He confirmed the findings obtained by NIOSH and moreover referred to an increased incidence of stomach cancer and liver tumours. The author concluded that there was biological plausibility for diethanolamine being the cause of the liver tumours among workers exposed to metalworking fluids since diethanolamine induced an increased incidence of liver tumours in an NTP study in mice (see Supplement 2000 "Diethanolamine") (Mirer 2003). The Commission does not agree with this assessment since it was not investigated whether exposure to individual substances such as diethanolamine correlates with individual tumour localizations and since the relevance of diethanolamine-induced liver tumours in mice to humans has not yet been clarified.

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

Inhalation

Since no NOAEC was demonstrated for local irritation to the respiratory tract in an earlier study (documentation "Diethanolamine" 2000), groups of 10 male and 20 female Wistar rats were exposed to diethanolamine at concentrations of 0, 1.5, 3 and 8 mg/m^3 for 3 months, 6 hours per day, 5 days per week. The animals were head-only exposed to an aerosol-vapour mixture. Groups of 10 females were observed for another 3 months without treatment to investigate the reversibility. Clinical examinations, organ weight determinations and a gross-pathological examination were carried out. Histopathology was restricted to the respiratory tract and liver since NOAECs had already been observed for all other parameters in the previous study. Diethanolamine was mainly present as an aerosol at a mass median aerodynamic particle diameter between 0.6 and 0.7 µm. No clinical signs, no impairment of body weight gain or any substance-induced organ weight changes were observed. At the end of exposure, slight signs of irritation at the upper respiratory tract in the form of focal squamous metaplasia at the base of the epiglottis were found in 9/10 animals of both sexes in the highest concentration group. They were accompanied by inflammatory cell infiltrations in 3 rats of both sexes. All findings were completely reversible within 3 months. Reversible squamous metaplasia was also found at this localization among 3/10 males of the middle concentration group. None of the affected animals revealed any inflammatory process. The authors of the study do not consider the slight and reversible findings observed at 3 mg/m^3 to be adverse toxic effects and therefore assess this concentration as the NOAEC; accordingly, 1.5 mg/m^3 is regarded as the NOEC (BASF 2002 c). However, the Commission does not agree with this assessment. The squamous metaplasia observed at 3 mg/m^3 is assessed as a substance-induced effect (LOAEC) and 1.5 mg/m^3 is thus regarded as the NOAEC.

Ingestion

The NTP carried out studies on a possible effect on the immune system after subacute oral diethanolamine administration to rats and mice.

Groups of 48 female F344 rats were administered diethanolamine concentrations of 0, 50, 100 or 200 mg/kg body weight and day by gavage for 14 days. The solutions were prepared in sterile, bidistilled water. At the end of administration, 8 rats per group were evaluated for standard toxicology, and various immunological end points including lymphocyte surface markers, spleen IgM antibody-forming cell response to sheep erythrocytes, spleen cell proliferative responses to mitogens, mixed lymphocyte response to allogeneic spleen cells, natural killer cell activity, macrophage activity and peritoneal cell differentials were investigated in the other groups. Body weight gain was reduced in relation to the dose from 50 mg/kg body weight and day; this decrease was significant from 100 mg/kg body weight and day. The absolute and relative kidney weights were increased slightly from 50 mg/kg body weight and day and significantly from 100 mg/kg body weight and day, the urea levels being significantly increased even from 50 mg/kg body weight and day. The absolute and relative liver weights were elevated in the high dose group. The red blood count was impaired in relation to the dose. Reticulocytes as well as haemoglobin and haematocrit were decreased in all dose groups and erythrocytes were reduced in the high dose group. The mixed lymphocyte response to allogeneic spleen cells showed an increase at 200 mg/kg body weight and day. A decrease in the natural killer cell response was recorded in the high dose group. The peritoneal macrophage subpopulations showed a heterogeneous pattern since both an increase and a decrease in the activity were observed from the middle dose. The peritoneal lavage cell differentials showed a trend to an increase of the lymphocyte percentage, but the difference was only significant at the high dose as compared with the control group. A decrease of the neutrophilic granulocytes was observed from the low dose. In contrast, no significant alteration was found in the differential blood count of the peripheral blood; there was a trend to a decrease only in the cell count in the lymphocytes and an increase in the eosinophils. Therefore, the findings in the peritoneal lavage cells are of unclear toxicological relevance. It was not possible to derive a NOAEL from this study (NTP 1992 a).

In a study very similar in design and scope to that of the rat study, groups of 40 female $B6C3F_1$ mice were administered diethanolamine by gavage at concentrations of 0, 100, 300 or 600 mg/kg body weight and day for 14 days. In addition, 3 tests were carried out for host resistance to *Listeria monozytogenes*, *Streptococcus pneumonia* and B16F10 melanoma cells, and the antibody-forming cell response to the injection of sheep erythrocytes was investigated after treatment with 0, 300, 600 or 800 mg/kg body weight and day. Liver weights were increased from 300 mg/kg body weight and day. The red blood count was affected at this dose and above in the form of a decrease in reticulocytes and reduced haemoglobin and haematocrit levels; at the high dose, the erythrocyte count was also decreased. Among the immunological parameters, the number of B lymphocytes was increased and the fraction of $CD4^+CD8$ T lymphocytes reduced at the high dose. A dose-dependent reduction of the antibody production as a response to the injection of sheep erythrocytes was observed from 300 mg/kg body weight and day. Natural killer cell response was not affected with the exception of a slight lowering at a cell ratio of 25:1 at the high dose. Among peritoneal macrophages, only the fraction of cells resident in cultures rather than the fraction of cells detached by peptone showed reduced cytotoxicity in the high treatment group, pre-treatment with activation factors having no effect. Diethanolamine treatment led to a decrease in host resistance to *Streptococcus pneumonia* and B16F10 melanoma cells, but there was no definite relation to the dose in any case (NTP 1992 b).

To sum up, it can be stated that immunological parameters were altered both in rats and mice. However, since the parameters differed between the two species, it is difficult to assess the relevance of these immunological findings. Effects on erythropoiesis were observed consistently in both species. They were found in rats from the low dose of 50 mg/kg body weight and day and in mice from 300 mg/kg body weight and day and are consistent with the findings obtained in studies with repeated oral administration in which specific microcytic anaemia occurred in rats without any lesions in the bone marrow being observed. Anaemia observed in rats was therefore attributed to the incorporation of diethanolamine into the membrane of the erythrocytes (documentation "Diethanolamine" 2000).

In the supplement from 2000 (documentation "Diethanolamine" 2000), a NOAEL was derived for diethanolamine of below 20 mg/kg body weight and day for rats and 100 mg/kg body weight and day for mice after subchronic exposure via the drinking water. Administration by gavage resulted in a NOAEL of 20 mg/kg body weight and day for rats and 50 mg/kg body weight and day for mice. After subchronic dermal application, the NOAELs for rats and mice were below the lowest tested doses of 32 and 80 mg/kg body weight and day, respectively; they are quantitatively similar to those determined in the oral studies since ingestion of the substance by licking is assumed.

Reproductive toxicity

Developmental toxicity

A number of studies on prenatal developmental toxicity were described in the supplement from 2000 (documentation "Diethanolamine" 2000). The results of these studies and those of another recent study with prenatal exposure and postnatal examinations are summarized in Table 11.

A NOAEC of 50 mg/m^3 was obtained for maternal toxicity and developmental toxicity in a prenatal inhalation developmental toxicity study in rats with exposure from days 6 to 15 of gestation. At 200 mg/m^3, the dams showed vaginal haemorrhages and the foetuses had an increased incidence of rudimentary cervical ribs. After oral administration of diethanolamine to rats from days 6 to 15 of gestation, a NOAEL of 50 mg/kg body weight and day was obtained for maternal toxicity in a range-finding study; no foetotoxic effects were observed at 200 mg/kg body weight, but presumably no skeletal or visceral investigations were carried out.

After occlusive dermal application of diethanolamine to rats and rabbits, rats showed maternal toxicity in the form of blood count changes even at the lowest dose of 150 mg/kg body weight and day, but skeletal alterations in the foetuses were only observed at 1500 mg/kg body weight and day. A NOAEL of 500 mg/kg body weight and day was obtained for developmental toxicity. Rabbits revealed maternal toxicity at 350 mg/kg body weight and day; this dose did not lead to any prenatal developmental toxicity (see documentation "Diethanolamine" 2000).

Table 11 Developmental toxicity studies of diethanolamine

Species, strain, No. of animals	Exposure	Findings	References
Prenatal toxicity			
rat, Wistar, 25 ♀ per group	**GDs 6–15,** 0, 10, 50, 200 mg/m³, 6 h/d, via the nose	**50 mg/m³**: dams and foetuses: NOAEC; **200 mg/m³:** dams: vaginal haemorrhages; foetuses: skeletal variations (in particular rudimentary cervical ribs) ↑	documentation “Diethanolamine” 2000
rat, SD, no other details	**GDs 6–15,** 0, 50, 200, 500, 800, 1200 mg/kg b.w. andday, gavage (range-finding study)	**50 mg/kg b.w.:** dams: NOAEL **from 200 mg/kg b.w.**: dams: b.w. gain ↓; total resorptions in 1 animal; foetuses: NOAEL (for resorptions, survival rate and b.w. of the foetuses) **≥ 500 mg/kg b.w.:** dams: highly toxic or lethal	documentation “Diethanolamine” 2000
rat, CD (SD), 25 ♀ per group	**GDs 6–15,** 0, 150, 500, 1500mg/kg b.w. and day, 6 h/d, dermal (occlusive)	**150 mg/kg b.w.**: dams: haematological effects; **from 500 mg/kg b.w.**: dams: slight skin irritation; abs. and rel. kidney weights ↑; foetuses: NOAEL **1500 mg/kg b.w.**: dams: b.w. gain ↓; severe skin irritation; foetuses: skeletal variations ↑	Marty et al. 1999 documentation “Diethanolamine” 2000
rabbit, white New Zealand, 15 ♀ per group	**GDs 6–18**, 0, 35, 100, 350mg/kg b.w. and day, 6 h/d, dermal (occlusive)	**35 mg/kg b.w.**: dams: NOAEL **from 100 mg/kg b.w.:** dams: b.w. gain ↓; **350 mg/kg b.w.:** dams: skin irritation; feed consumption ↓; b.w. gain ↓; liver and kidney weights slightly ↑ (not significant); coloration of the kidneys; no haematological effects; foetuses: NOAEL	Marty et al. 1999; documentation “Diethanolamine” 2000
mouse, CD1,50 ♀	**GDs 6–15**, 0, 450 mg/kg b.w. and day, gavage (screening test)	**450 mg/kg b.w. and day**: dams: no clinically evident effects; pups: number of live offspring, survival and b.w. gain until PND 3 ↓	documentation “Diethanolamine” 2000

Table 11 (Continued)

Species, strain, No. of animals	Exposure	Findings	References
Postnatal toxicity after prenatal exposure			
rat, SD, 12 ♀ per group	**GDs 6–19**, 0, 50, 125, 200, 250, 300 mg/kg b.w. and day, gavage; rearing of the offspring until PND 21	**50 mg/kg b.w. and day**: dams and offspring: NOAEL **from 125 mg/kg b.w. and day**: dams: kidney weights ↑; water consumption ↑; pups: mortality ↑ (PNDs 0 – 4) from 200 mg/kg b.w. and day: dams: b.w. gain and feed consumption ↓; morbidity/mortality ↑; post-implantation losses; pups: b.w. gain ↓	Price 1999; Price et al. 2005

Key: GD: gestation day; PND: postnatal day; SD: Sprague Dawley

In another recent postnatal development toxicity screening study with prenatal exposure, an aqueous diethanolamine solution was administered by gavage to groups of 12 Sprague Dawley rats from days 6 to 19 of gestation at doses of 0, 50, 125, 200, 250 or 300 mg/kg body weight and day. The development of the pups was recorded up to the end of lactation on postnatal day 21. Treatment led to maternal morbidity and mortality from 200 mg/kg body weight and day. Thus, 1 dam each of the groups receiving 200 and 250 mg/kg and all dams of the highest treatment group had to be sacrificed prematurely in a moribund state. Other signs of maternal toxicity occurred from 125 mg/kg body weight and day in the form of increased kidney weights on day 21 post partum and increased water consumption. Body weight gain was reduced from doses of 200 mg/kg body weight and day, feed consumption was decreased and post-implantation losses were increased. Developmental toxicity occurred from 125 mg/kg body weight and day in the form of slightly increased pup mortality between days 0 and 4 post partum. Body weight gain of the pups was also reduced from 200 mg/kg body weight and day. No external malformations were recorded. The NOAEL for maternal and developmental toxicity was 50 mg/kg body weight and day (Price 1999; Price et al. 2005). Postnatal maturation or behaviour was not investigated.

Genotoxicity

In vitro

Diethanolamine was not mutagenic or clastogenic in the available in vitro tests (Salmonella mutagenicity test, test for mitotic recombination in *E. coli* and *Saccharo-*

myces cerevisiae, mouse lymphoma test, test for chromosome aberrations in CHO, RL1 and RL4 cells and sister chromatid exchange in CHO cells) (see documentation "Diethanolamine" 2000).

In vivo

A micronucleus test in the peripheral erythrocytes of mice revealed no genotoxicity of diethanolamine either (see documentation "Diethanolamine" 2000).

Four-day-old *Drosophila melanogaster* females were fed with filter paper soaked in aqueous diethanolamine solutions (5, 10, 20, 40 and 80%) and subsequently mated with untreated males. An increased incidence of non-disjunctions as evidence of an aneugenic potential was found in the oocytes of the brood after 24 hours (Munoz and Mazar Barnett 2003). However, it is known that the described aneugenic effect cannot necessarily be transferred to higher organisms (Aardema et al. 1998; Parry and Sors 1993) and it is thus only of limited relevance.

Carcinogenicity

Short-term tests

In a cell transformation assay with cultivated embryonic hamster cells, diethanolamine induced no cell transformations in a concentration range between 25 and 500 µg/ml. In another cell transformation test with SHE cells, up to 5 times more morphologically transformed colonies were observed both after 24-hour (max. 4500 µg/ml) and after 7-day incubation (max. 2500 µg/ml), but there was no concentration-response relationship (see documentation "Diethanolamine" 2000).

A cell transformation test in SHE cells published since that time confirmed that there was a concentration-dependent increase of morphologically transformed cells after 7-day incubation of diethanolamine at concentrations of up to 500 µg/ml. No cell transformations were found after the addition of 30 mM choline to the medium. In contrast, choline had no effect on the transformations induced by benzo(*a*) pyrene as the positive control (Lehman-McKeeman and Gamsky 2000).

Long-term studies

In a dermal carcinogenicity study carried out by the NTP with diethanolamine in F344 rats and B6C3F$_1$ mice, an increased incidence of liver tumours was observed in mice of both sexes from the lowest dose of 40 mg/kg body weight and above. In addition, a higher incidence of kidney tumours was observed in male mice from 40 mg/kg body weight and day. This increase was significant from 80 mg/kg body weight and day. Rats showed no increased tumour incidences (see S documentation

"Diethanolamine" 2000). The lack of an increased tumour rate in rats is remarkable since dietary choline deficiency induces liver tumours mainly in rats. One reason for this might be that the amount of diethanolamine taken up was not high enough. Thus, only local rather than systemic effects were observed in rats, and the maximum diethanolamine doses of 64 mg/kg body weight and day applied to males and 32 mg/kg body weight and day applied to females were clearly lower than the maximum doses of 160 mg/kg body weight and day applied to mice. Moreover, since the thickness of the skin is greater in rats than in mice, an about three times lower tissue concentration can be assumed in rats (Lehman-McKeeman et al. 2002). The NTP carried out another dermal carcinogenicity study with a coconut diethanolamine condensate containing about 18% free diethanolamine. In this study, mice also showed a significantly increased incidence of hepatocellular adenoma, carcinoma and hepatoblastoma (control ♂: 29/50; ♀: 33/50; 100 mg/kg body weight ♂: 39/50; ♀: 46/50; 200 mg/kg body weight ♂: 49/50; ♀: 48/50) as well as kidney adenoma and carcinoma in male mice (1/50; 1/50; 9/50). In addition, a higher incidence of renal tubular adenoma and carcinoma was observed in female rats of the low dose group (control: 0/50; 50 mg/kg body weight: 4/50; 100 mg/kg body weight: 1/50) (NTP 2001).

Manifesto (MAK value, classification)

No increased tumour incidences were observed in a dermal carcinogenicity study of the NTP in F344 rats. However, an elevated number of liver and kidney tumours occurred in $B6C3F_1$ mice from the lowest dose of 40 mg/kg body weight and day (see documentation “Diethanolamine” 2000). The mechanistic studies carried out mainly in mice on the basis of these findings demonstrated that diethanolamine led to a disturbance in choline homoeostasis with increased cell proliferation both in vitro and in vivo. Hypomethylation induced by choline deficiency was discussed as a cause of the tumourigenesis, but other factors such as lipid peroxidation and a possibly aneugenic effect might also be involved. It has not yet been clarified whether the liver and kidney tumours observed in mice are relevant to humans. Diethanolamine cannot be classified in Carcinogen Category 4 since the mechanism or mechanisms contributing to its carcinogenicity in mice have currently not been sufficiently clarified. Therefore, diethanolamine is classified in Carcinogen Category 3B. No MAK value can be derived since diethanolamine revealed no mutagenic or clastogenic effect.

Local irritation proved to be the most sensitive end point. In an inhalation study carried out in Wistar rats for a period of 3 months, initial signs of irritation to the respiratory tract in the form of squamous metaplasia were observed at 3 mg/m^3. The NOAEC was 1.5 mg/m^3. Since the findings observed at 3 mg/m^3 were minimal and moreover only occurred in a few male but not in female rats, the MAK value is established at 1 mg/m^3.

On the basis of irritation as the most sensitive end point, diethanolamine is classified in Peak Limitation Category I. Since no data are available for effects in humans, an excursion factor of 1 has been established.

Depending on the dose, skin penetration rates between 0.11 and 137 µg/cm^2 and hour were calculated from studies with dermal exposure. In vitro, human skin penetration rates of 5.7 µg/cm^2 and hour were determined for the undiluted substance and of 12.7 µg/cm^2 and hour for the 37% aqueous solution (see documentation "Diethanolamine" 2000). If the MAK value of 1 mg/m^3 is observed, about 10 mg is absorbed at an inhaled volume of 10 m^3 in 8 hours. In contrast, a dermal absorption of 25 mg is calculated from an in vitro absorption rate of 12.7 µg/cm^2 and hour assuming 2000 cm^2 of exposed skin and 1-hour exposure. Therefore, substantial contribution of skin absorption to body burden is expected and the "H" designation remains.

The previous designation with "Sh" (documentation "Diethanolamine" 2001) has been retained.

Prenatal developmental toxicity studies in rats led to a NOAEC of 50 mg/m^3 after inhalation and a NOAEL of 50 mg/kg body weight and day after ingestion. Another study in rats with prenatal oral exposure and postnatal examinations also resulted in a NOAEL of 50 mg/kg body weight and day. A developmental toxicity study in rabbits provided no evidence of prenatal toxicity at a maternally toxic dose of 350 mg/kg body weight and day. Because of the large divergence from the MAK value of 1 mg/m^3, diethanolamine is classified in Pregnancy Risk Group C.

Since diethanolamine is not suspected of having a germ cell mutagenic effect, it is not classified as a germ cell mutagen.

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completed 22.06.2006