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N,N-Dimethylformamide

MAK Value Documentation, supplement – Translation of the German version from 2019

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated *N,N*-dimethylformamide [68-12-2] taking into account the increased respiratory volume at the workplace (see List of MAK and BAT Values, Sections I b and I c).

N,N-Dimethylformamide is a liver toxin and the maximum concentration at the workplace (MAK value) of 5 ml/m³ was set using data from a two-year study in mice showing liver cell hypertrophy and single cell necrosis at the lowest concentration tested of 25 ml/m³. In this study, rats were less susceptible as regards the liver toxicity of *N,N*-dimethylformamide. Species differences in toxicokinetics are a plausible explanation for the higher toxicity in mice. As human metabolism of *N,N*-dimethylformamide is quantitatively similar to that of rats, their susceptibility is expected to be similar to that of rats. On the basis of the NOAEC (no observed adverse effect concentration) of 25 ml/m³ for rats, the MAK value of 5 ml/m³ is retained even taking into account the increased respiratory volume at the workplace. Peak Limitation Category II and the excursion factor of 2 are confirmed.

The assignment of *N,N*-dimethylformamide to Pregnancy Risk Group B is retained. In an earlier assessment, it was concluded that exposure to a concentration of up to 1 ml/m³ is not expected to lead to developmental toxicity. This prerequisite for an assignment of *N,N*-dimethylformamide to Pregnancy Risk Group C is confirmed, also taking into account the increased respiratory volume at the workplace.

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MAK value (2005)	**5 ml/m³ (ppm) ≙ 15 mg/m³**
Peak limitation (2011)	**Category II, excursion factor 2**
Absorption through the skin (1969)	**H**
Sensitization	**–**
Carcinogenicity (2015)	**Category 4**
Prenatal toxicity (2017)	**Pregnancy Risk Group B[a)]**
Germ cell mutagenicity	**–**
BAT value (2018)	**20 mg *N*-methylformamide plus *N*-hydroxymethyl-*N*-methylformamide/l urine 25 mg *N*-acetyl-*S*-(methylcarbamoyl)-*L*-cysteine/g creatinine in urine**
log K_{OW} at 25 °C	–0.85 (ECHA 2017)
Vapour pressure at 20 °C	3.77 hPa (ECHA 2017)
1 ml/m³ (ppm) ≙ 3.03 mg/m³	**1 mg/m³ ≙ 0.329 ml/m³ (ppm)**

[a)] for the note on the prerequisite for group C at 1 ml/m³, see "*Manifesto (MAK value/classification)*".

In 2016, the Commission began using a revised approach for assessing substances with a MAK value based on systemic effects and derived from inhalation studies in animals or studies with volunteers at rest. This new approach takes into account that the respiratory volume at the workplace is higher than under these experimental conditions. However, this does not apply to gases or vapours with a blood:air partition coefficient < 5 (see List of MAK and BAT Values, Sections I b and I c). According to the formula of Buist et al. (2012), the blood:air partition coefficient of *N,N*-dimethylformamide is about 25,000. This supplement evaluates whether the MAK value for *N,N*-dimethylformamide and the note on the prerequisite for Pregnancy Risk Group C need to be changed as a result of the higher respiratory volume at the workplace.

Mechanism of Action

The mechanism of the hepatotoxic and hepatocarcinogenic effects was described in the supplements of 2006 (translated 2011, Hartwig 2011) and 2016 (translated 2017, Hartwig and MAK Commission 2017 a).

Oxidative stress and DNA double strand breaks were assumed to be the causes for the liver toxicity of *N,N*-dimethylformamide. After incubation with *N,N*-dimethylformamide, the increased formation of reactive oxygen species and DNA double strand breaks, determined as γH2AX foci, was observed in the human liver cell line HL-7702 at concentrations of 6.4 mM and above, as well as the increased formation of 8-OH-deoxyguanosine at 40 mM and above. Cytotoxicity occurred at concentrations of 16 mM and above (Wang et al. 2016).

Toxicokinetics and Metabolism

N,N-Dimethylformamide is toxified through oxidation by CYP2E1, and methyl isocyanate is considered to be the decisive toxic metabolite. From the reaction of methyl isocyanate with glutathione, a conjugate is formed, which is

degraded to *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) and excreted with the urine. AMCC is itself toxic, as it is able to carbamoylate proteins (Hartwig 2011).

After exposure to 500 ml *N,N*-dimethylformamide/m³ for 6 hours, a concentration of about 1000 mg *N,N*-dimethylformamide/l (about 13.7 mM) was attained in the plasma of rats and mice (Hartwig 2011; Hundley et al. 1993 a). Exposure of monkeys to 500 ml/m³ for 6 hours resulted in *N,N*-dimethylformamide plasma levels of only 150 to 250 mg/l (Hartwig 2011; Hundley et al. 1993 b).

Effects in Humans

In a cross-sectional study with 220 persons who were exposed to a median *N,N*-dimethylformamide concentration of 3.13 mg/m³ (determined by personal sampling; mean value: 6.21 ± 7.60 mg/m³) and 175 control persons, the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transpeptidase (GGT) in the blood were determined during the prescribed occupational health surveillance programme. The internal exposure to *N,N*-dimethylformamide was determined as the sum of *N*-methylformamide and *N*-hydroxymethyl-*N*-methylformamide (NMF and HMMF; median 4.83 mg/l, mean value: 7.75 ± 8.82 mg/l), AMCC, (median 4.84 mg/g creatinine, mean value: 9.42 ± 10.4 mg/l) and 3-methyl-5-isopropylhydantoin (median 60.5 nmol/g globin, mean value: 83.8 ± 83.1 nmol/g globin). The alcohol consumption was similar in the exposed and control persons. The exposed workers were divided into two groups with exposure concentrations ≥ 15 mg/m³ (AMCC median: 20.3 mg/g creatinine) and < 15 mg/m³ (AMCC median: 4.15 mg/g creatinine). A positive correlation was not found between any of the liver enzymes determined and the external or internal exposure. In the exposed persons, the frequency of alcohol intolerance (about 50%) was higher than in the control persons (1%) (Kilo et al. 2016).

In a study in China with 72 persons exposed to *N,N*-dimethylformamide at an average concentration of 18.6 mg/m³ (9.8–36.2 mg/m³), and 72 healthy control persons, the liver enzyme activities in the exposed workers were significantly increased compared with the levels in the control persons. AST, ALT and c-glutamyl transpeptidase (c-GT) in the serum were determined. AMCC in the urine was determined as a marker for internal exposure both in the exposed (mean value: 28.32 ± 8.07 mg/l) and in the control persons (mean value: 2.21 ± 0.47 mg/l). In 9 of the exposed persons, the AMCC concentration was higher than 40 mg/l. The external exposure was determined during the prescribed health surveillance programme carried out four times per year. Data for the alcohol consumption of the two groups are not available, persons whose alcohol consumption was higher than 25 g/day were, however, excluded (He et al. 2015). From the AMCC mean values, it can be concluded that these workers were exposed to higher concentrations than those in the study by Kilo et al. (2016).

In a study with 698 Chinese workers exposed to *N,N*-dimethylformamide and 188 control persons, 9.17% of the exposed persons and 4.26% of the controls showed signs of liver toxicity, defined as increased activities of ALT, AST or GGT. The exposed persons were employed at three workplaces with different levels of *N,N*-dimethylformamide. The external exposure was estimated from historical data to be more than 30 mg/m³ for the workplace with highest exposure. The internal exposure was determined by the excretion of NMF (median 1.75 mg/l) and AMCC (median 44.09 mg/l) with the urine as well as by *N*-methylcarbamoyl-haemoglobin (NMHb) in the blood (median 46 nmol/g globin). The exposed workers were subdivided into three groups according to their internal exposure for each of the three biomarkers. Lower confidence limits of the benchmark dose (BMDL) for liver toxicity in men and women of 14 mg NMF/l, 155 mg AMCC/l and 93.3 nmol NMHb/g globin were calculated. The authors point out that *N,N*-dimethylformamide is metabolized via CYP2E1, and that there are genetic differences between various ethnic populations. For this reason, the benchmark calculations are not transferable to collectives other than Han Chinese (Wu et al. 2017). The ratio of the medians of NMF to AMCC is very different to that in the study by Kilo et al. (2016).

In another Chinese study, the liver function of 512 workers exposed to *N,N*-dimethylformamide at an average yearly concentration of 7 mg/m³ (3.3–21.6 mg/m³), and of 521 workers not exposed was investigated. ALT and AST

activity in the serum of more than 40 U/l was defined as an indication of liver toxicity. This applied to 11.89% of the exposed and 6.87% of the control persons. The cumulative exposure was up to 94.43 mg/m³ × years. After adjustment for age, sex, smoking, alcohol consumption, high blood pressure and liver diseases, a threshold exposure of 7.3 mg/m³ × years was calculated for a significantly increased incidence of liver toxicity using mathematical modelling of the dose–response relationship (Qi et al. 2017). The exposure was not determined on a personal basis, but only via ambient air monitoring, and there was no biomonitoring. It is not clear how the value for the calculated threshold of cumulative exposure can be extrapolated to a concentration-related threshold value for *N,N*-dimethylformamide.

To summarize, the study by Kilo et al. (2016) is considered relevant for evaluation as, in contrast to the study by Qi et al. (2017), also the internal exposure was determined in this study and, unlike in all other studies from China, the current external exposure was determined by means of personal air monitoring. Furthermore, it is more comprehensive than the study by He et al. (2015).

Animal Experiments

In 2005, the MAK value was lowered to 5 ml/m³ based on the results of a 2-year study in CD rats and CD-1 mice (Table 1) from which a LOAEC (lowest observed adverse effect concentration) of 25 ml/m³ for liver cell hypertrophy in mice (Hartwig 2011; Malley et al. 1994) was obtained and a $BMDL_{05}$ of 7.8 ml/m³ was calculated. In the mice, the liver cell hypertrophy was associated with only a marginal increase in relative liver weights of 2% at 25 ml/m³. The determination of sorbitol dehydrogenase (SDH), ALT, AST and ALP in the serum of the rats revealed increased SDH activity at 100 and 400 ml/m³. The other enzyme values were not reported. These enzymes were not determined in the mice (Malley et al. 1994).

Tab. 1 Liver findings in the carcinogenicity study by Malley et al. (1994)

Author:		Malley et al. 1994			
Substance:		*N,N*-dimethylformamide (> 99.5%)			
Species:		**mouse (Crl:CD-1 Br)**			
Administration route:		inhalation			
Duration:		18 months, 5 days/week, 6 hours/day			
Concentration		0 ml/m³	25 ml/m³	100 ml/m³	400 ml/m³
Number of mice examined	♂	60	62	60	59
	♀	61	61	61	63
Animals with liver changes					
Centrilobular hypertrophy	♂	0	8*	41*	52*
	♀	0	6	19*	54*
Single cell necrosis	♂	24	59*	68*	87*
	♀	29	44*	70*	76*
Kupffer cell hyperplasia/pigment accumulation	♂	22	52*	60*	86*
	♀	51	57	71*	89*
Liver foci					
Mixed cell foci	♂	0	3	13*	19*
	♀	0	0	3	3
Eosinophilic foci	♂	2	8	10*	8
	♀	0	2	5	6

Tab. 1 *(continued)*

Author:		Malley et al. 1994			
Substance:		*N,N*-dimethylformamide (> 99.5%)			
Species:		**rat (Crl:CD Br)**			
Administration route:		inhalation			
Duration:		2 years, 5 days/week, 6 hours/day			
Concentration		0 ml/m³	25 ml/m³	100 ml/m³	400 ml/m³
Number of rats examined	♂	57	59	58	60
	♀	60	59	59	62
Animals with liver changes					
Centrilobular hypertrophy	♂	0	0	5*	30*
	♀	0	0	3*	40*
Single cell necrosis	♂	2	2	3	30*
	♀	0	0	5*	18*
Accumulation of lipofuscin/haemosiderin	♂	4	4	17*	58*
	♀	8	7	22*	61*
Liver foci					
Clear cell foci	♂	11	8	22*	35*
	♀	5	5	14	24*
Eosinophilic foci	♂	33	38	24*	45
	♀	22	12	25	40*

*$p < 0.05$

The authors of the study regarded the changes at 25 ml/m³ as minimal and found the NOEC (no observed effect concentration; they did not use the term NOAEC) to be just below 25 ml/m³.

Evaluation of the liver toxicity

The Commission regards 25 ml/m³ as the LOAEC for mice and as the NOAEC for rats. In the mice, at concentrations of 25 ml/m³ and above, apart from liver cell hypertrophy, also single cell necrosis and, as a sequel, hyperplasia of the Kupffer cells occurred in the liver with a concentration-dependent increase in the incidence.

Species differences – liver toxicity

The CD-1 mouse is more sensitive to hepatotoxicity than the CD rat (Table 1; Malley et al. 1994).

The 13-week study by Senoh et al. (2003) (Hartwig and MAK Commission 2017 a) allows a comparison of species sensitivities as regards liver enzyme activities, liver cell hypertrophy and single cell necrosis in F344/DuCrj rats and Crj:BDF1 mice to be made (see Tables 2 and 3).

Tab. 2 Serum values of liver enzymes and histopathological liver findings in the 13-week study by Senoh et al. (2003) in F344/DuCrj rats

		concentration (ml/m³)					
		0	50	100	200	400	800
Serum values							
AST (IU/l)	♂	77	72	73	71	64	199
	♀	69	67	68	69	71	112
ALT (IU/l)	♂	45	44	44	46	51	296**
	♀	37	36	37	39	45*	136**
GGTP (IU/l)	♂	1	1	1	1	0	1
	♀	1	1	1	2	4**	16**
ALP (IU/l)	♂	281	249	251	236	247	256
	♀	201	189	186	208	245**	242**
LDH (IU/l)	♂	136	120	136	136	128	449
	♀	141	161	158	153	194	333**
Animals with liver changes							
Single cell necrosis	♂	0	0	0	8/10**	10/10**	10/10**
	♀	0	0	0	8/10**	9/10**	10/10**
Centrilobular hypertrophy	♂	0	0	0	3/10	8/10**	9/10**
	♀	0	0	0	0	8/10**	10/10**

* $p \leq 0.05$; ** $p \leq 0.01$
ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGTP: gamma-glutamyl transpeptidase, LDH: lactate dehydrogenase

Tab. 3 Serum values of liver enzymes and histopathological liver findings in the 13-week study by Senoh et al. (2003) in Crj:BDF1 mice

		concentration (ml/m³)					
		0	50	100	200	400	800
Serum values							
AST (IU/l)	♂	47	45	42	52	47	151
	♀	67	77	72	77	82	88
ALT (IU/l)	♂	20	22	23	30	38	216**
	♀	24	35	41	59**	52**	89**
ALP (IU/l)	♂	175	160	156	158	166	218
	♀	310	264	254	243	248	277
LDH (IU/l)	♂	261	187	175	248	203	625
	♀	236	263	243	266	349	381
Animals with liver changes							
Single cell necrosis	♂	0	0	0	0	1/10	6/10*
	♀	0	0	0	0	0	5/10*
Focal necrosis	♂	0	0/10	4/10	2/10	3/10	4/10
	♀	0	1/10	6/10*	5/10*	7/10*	1/10
Centrilobular hypertrophy	♂	0	4/10*	10/10**	10/10**	10/10**	10/10**
	♀	0	0	0	0	0	7/10*

* $p \leq 0.05$; ** $p \leq 0.01$
ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, gamma-glutamyl transpeptidase in mice not determined

The NOAECs for liver enzyme activity and histopathological liver findings in this study are listed in Table 4.

Tab. 4 NOAECs (ml/m³) for liver findings in the 13-week study by Senoh et al. (2003)

	Liver enzyme activity	Liver cell hypertrophy	Single cell necrosis	Focal necrosis
rat ♂	400	100	100	800
rat ♀	200	200	100	800
mouse ♂	400	< 50	200	50
mouse ♀	100	400	400	50

The NOAECs for a statistically significant increase in liver enzyme activity are, except in the female mice, higher than those for liver cell hypertrophy and necrosis. This means that the incidences of single cell necrosis and liver cell hypertrophy may already be increased when the liver enzyme activities are not increased. Also in this study, the mouse, in this case only the male mouse, is more sensitive than the rat with regard to hypertrophy, though not with regard to single cell necrosis. As regards focal necrosis, the mouse, on the other hand, is more sensitive, as this occurred at and above 100 ml/m³, whereas focal necrosis was not found in rats.

In the carcinogenicity study by Senoh et al. (2004) described in the 2016 supplement (Hartwig and MAK Commission 2017 a), liver tumours were found in almost all Crj:BDF1 mice at and above the lowest concentration tested of 200 ml/m³, whereas the same concentration produced liver tumours in only 4/50 male F344/DuCrj rats. The mice in this study were also found to have a higher spontaneous incidence of liver tumours than the rats, as the incidences in the controls show: 8/50 of the male and 3/49 of the female mice had liver tumours, whereas only 1/50 male and 1/50 female rats had liver tumours. This means that liver tumours develop more easily from spontaneously occurring initiated cells in the Crj:BDF1 mouse than in the F344/DuCrj rat. With hepatotoxic substances, liver tumours can develop from spontaneously occurring initiated cells as a result of regenerative cell proliferation. As seen from the liver enzyme activities in the serum (Tables 5 and 6) in the 2-year study, *N,N*-dimethylformamide was considerably more hepatotoxic in Crj:BDF1 mice than in F344/DuCrj rats at the same exposure concentration.

Tab. 5 Serum values of liver enzymes in F344/DuCrj rats in the 2-year study by Senoh et al. (2004)

		concentration (ml/m³)			
		0	200	400	800
AST (IU/l)	♂	73	128	195**	497**
	♀	120	173**	158	230**
ALT (IU/l)	♂	32	56**	96**	195**
	♀	56	81**	79*	110**
GGTP (IU/l)	♂	8	21**	31**	45**
	♀	4	10**	13**	104**
ALP (IU/l)	♂	189	330	352**	395**
	♀	125	148	208*	306**
LDH (IU/l)	♂	174	193	201	850**
	♀	241	216	245	267

* $p \leq 0.05$; ** $p \leq 0.01$
ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGTP: gamma-glutamyl transpeptidase, LDH: lactate dehydrogenase

Tab. 6 Serum values of liver enzymes in Crj:BDF1 mice in the 2-year study by Senoh et al. (2004)

		concentration (ml/m³)			
		0	200	400	800
AST (IU/l)	♂	107	561**	553**	731**
	♀	411	991**	1981**	1748**
ALT (IU/l)	♂	51	501**	668**	829**
	♀	139	781**	1748**	1289**
ALP (IU/l)	♂	145	641**	771**	1209**
	♀	213	1098**	2115**	2168**
LDH (IU/l)	♂	415	4559**	3497**	2115**
	♀	847	3588**	6452**	3299**

** $p \leq 0.01$
ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, gamma-glutamyl transpeptidase in mice not determined

The difference in sensitivity is more marked than in the 13-week study (Senoh et al. 2003). Together with the particular susceptibility of the mouse for the development of liver tumours, the higher sensitivity of this species for liver tumour formation induced by *N,N*-dimethylformamide can be explained as resulting from the higher hepatotoxicity of *N,N*-dimethylformamide in the mouse.

Species differences – toxicokinetics

Sprague Dawley rats and CD-1 mice were given intraperitoneal injections of *N,N*-dimethylformamide of up to 2000 mg/kg body weight with and without acetone pretreatment via the drinking water. Without pretreatment, *N,N*-dimethylformamide did not cause hepatotoxicity in rats and mice. After acetone pretreatment, *N,N*-dimethylformamide caused hepatotoxicity only in the mice. CYP2E1 is induced by acetone, which increases the demethylation of *N,N*-dimethylformamide and metabolism to methyl isocyanate. The acetone treatment alone increased the rate of demethylation of *N,N*-dimethylformamide by liver microsomes of rats and mice. The increase in the V_{max} in mice (10-fold compared with the value in controls) was higher than in rats (7-fold compared with the value in controls). In rats, the K_M value for microsomes was, however, 10 times as high as that in mice. The higher hepatotoxicity of *N,N*-dimethylformamide in the mouse was explained by the fact that the oxidative conversion in mice takes place much more effectively than in rats due to the low K_M value. In controls without acetone treatment, the V_{max} values in the mouse and rat liver microsomes are about equal. However, the K_M value was not determined, as the specific activities were too low. With the purified CYP2E1 of acetone-treated mice and rats, the V_{max} was of a similar level, but the K_M value for rats, like for the microsomes, was about 10 times as high. The study demonstrates that the toxicity of *N,N*-dimethylformamide in mice is increased when CYP2E1 is induced by acetone, and that *N,N*-dimethylformamide is much more efficiently metabolized by mouse CYP2E1 than by rat CYP2E1. With the metabolite NMF no hepatotoxicity occurred in Sprague Dawley rats, whereas it produced strong hepatotoxicity in CD-1 mice (Chieli et al. 1995).

The apparent K_M and V_{max} values for the formation of HMMF from *N,N*-dimethylformamide and the formation of the glutathione conjugate *S*-(*N*-methylcarbamoyl)glutathione (SMG) from NMF and HMMF in liver microsomes from Sprague Dawley rats and humans were similar. The glutathione conjugate is formed with methyl isocyanate, which is produced from the oxidation of both NMF and HMMF (Table 7; Hartwig 2011; Mraz et al. 1993). From this it may be concluded that in humans and in Sprague Dawley rats the metabolic oxidation of formamides and the formation of the glutathione conjugate takes place with similar efficiency, and that humans are about equally as sensitive to *N,N*-dimethylformamide as Sprague Dawley rats (Amato et al. 2001).

Tab. 7 Kinetic parameters for the metabolic oxidation of formamides and the formation of the glutathione conjugate (SMG) in liver microsomes from Sprague Dawley rats and humans (Mraz et al. 1993)

		$appK_M$ (mM)		$appV_{max}$ (nmol/mg protein/min)	
Substrate	**Product**	**rat**	**humans**	**rat**	**humans**
N,N-Dimethylformamide	HMMF	0.20 ± 0.06	0.12 ± 0.06	0.54 ± 0.20	0.57 ± 0.49
NMF	SMG	4.28 ± 1.35	3.92 ± 2.11	0.34 ± 0.08	0.24 ± 0.17
HMMF	SMG	2.52 ± 0.34	1.25	0.016 ± 0.006	0.033

$appK_M$: apparent Michaelis-Menten constant, $appV_{max}$: apparent maximum velocity, HMMF: *N*-hydroxymethyl-*N*-methylformamide, NMF: *N*-methylformamide, SMG: *S*-(*N*-methylcarbamoyl)glutathione

In another study with microsomes from BALB/c and CBA/CA mice as well as Sprague Dawley rats and humans, the rates of SMG formation from 10 mM NMF were determined. They were about equal in both strains of mice and were 0.60 ± 0.08, 0.32 ± 0.11 and 0.34 ± 0.24 nmol/mg protein/minute in BALB/c mice, Sprague Dawley rats and humans, respectively. In microsomes from BALB/c mice, the rate of SMG formation from 10 mM HMMF was 1.4 nmol/mg protein after 20 minutes (0.070 nmol/mg protein/minute), and then remained constant. In human microsomes, it was 0.046 nmol/mg protein/minute, and the formation of SMG still increased after 20 minutes. The conversion of *N,N*-dimethylformamide to SMG could not be observed with either human or mouse microsomes (Cross et al. 1990). The K_M values and the conversion of *N,N*-dimethylformamide to HMMF were not reported, so that it is difficult to compare this study with those of Chieli et al. (1995) and Mraz et al. (1993).

It was assumed that humans are more sensitive to *N,N*-dimethylformamide than rats and mice as, after 8-hour whole-body exposure to 20 ml *N,N*-dimethylformamide/m³, volunteers excreted a greater proportion of AMCC with the urine (14.5% of the absorbed amount) than Sprague Dawley rats and BALB/c mice after intraperitoneal injection of *N,N*-dimethylformamide. However, the dermal absorption of *N,N*-dimethylformamide from the gaseous phase, which may account for up to 50% of the total absorbed amount (Hartwig and MAK Commission 2017 a), was not included in this estimate. For this reason, the percentage excreted as AMCC, is in fact smaller. After oral administration, 10% of the *N,N*-dimethylformamide dose was excreted in the form of AMCC by one volunteer. The different exposure routes can therefore also play a role (Hartwig 2011; Mraz et al. 1989). The data suggest that humans metabolize a higher percentage via the reactive metabolite methyl isocyanate than rats and mice. However, BALB/c mice and Sprague Dawley rats excrete about an equal percentage of AMCC. At low doses (0.1 mmol/kg body weight, intraperitoneal), Sprague Dawley rats excrete even more AMCC than BALB/c mice (5.2% compared with 1.6%) (Mraz et al. 1989). This does not agree with the higher hepatotoxicity and carcinogenicity of *N,N*-dimethylformamide in mice than in rats.

BALB/c mice were, however, considered to be a relative insensitive mouse strain as regards hepatotoxicity caused by *N,N*-dimethylformamide, which would agree with the low percentage of AMCC excreted. In Sprague Dawley rats, the sensitivity differed in two studies (Mraz et al. 1989). No data for the excretion of AMCC are available from the carcinogenicity studies with F344 and CD rats and CD-1 and Crj:BDF1 mice, so that it is not clear whether metabolism via methyl isocyanate in these strains is quantitatively similar to that in BALB/c mice and Sprague Dawley rats. After comparing the metabolic efficiency and formation of the glutathione conjugate, it can be concluded that humans are similar to Sprague Dawley rats as regards methyl isocyanate formation. As Sprague Dawley rats are less sensitive to the hepatotoxicity of *N,N*-dimethylformamide than CD-1 mice, it can be assumed that also humans are less sensitive than CD-1 mice.

In a 13-week study with Cynomolgus monkeys, no signs of hepatotoxicity were found in the histological examination and also no changes in the serum activities of ALT, AST and SDH after inhalation exposure to *N,N*-dimethylformamide concentrations of up to 500 ml/m³ for 6 hours per day, on 5 days per week. However, only 3 animals per sex were exposed (Hartwig 2011; Hurtt et al. 1992).

After exposure to 500 ml/m³, markedly higher concentrations of *N,N*-dimethylformamide were found in the plasma of rats and mice than in monkeys (see *Section "Toxicokinetics and Metabolism"*). This was cited by the authors as a reason for the lower toxicity of *N,N*-dimethylformamide in monkeys. In the range of 20 to 100 ml/m³, the toxicokinetics of the substance is similar in humans and monkeys (Hundley et al. 1993 b).

Species differences – conclusions

N,N-Dimethylformamide becomes toxic through oxidation by CYP2E1. The mouse is more sensitive than the rat as regards the hepatotoxicity and hepatocarcinogenicity of *N,N*-dimethylformamide (Malley et al. 1994; Senoh et al. 2003, 2004).

Compared with Sprague Dawley rats, CD-1 mice are more sensitive with regard to hepatotoxicity and they activate *N,N*-dimethylformamide more efficiently via CYP2E1 (Chieli et al. 1995). The V_{max} and K_M for the oxidation of *N,N*-dimethylformamide and the formation of the glutathione conjugate from NMF and HMMF with methyl isocyanate are similar in the liver microsomes of humans and Sprague Dawley rats (Mraz et al. 1993). For this reason, it can be expected that humans are less sensitive than the CD-1 mice used in the carcinogenicity study (Malley et al. 1994).

However, no PBPK (physiologically based pharmacokinetic) model for humans is available. As regards hepatotoxicity, monkeys seem to be less sensitive than rats and mice. One reason for this could be the lower level in plasma in monkeys compared with that in mice and rats at equal external concentrations of *N,N*-dimethylformamide.

Manifesto (MAK value/classification)

In a workplace study, hepatotoxicity in the form of increased liver enzymes in the blood was not found after exposure to *N,N*-dimethylformamide concentrations up to 15 mg/m³ (5 ml/m³) (Kilo et al. 2016). The critical effects are centrilobular liver cell hypertrophy and single cell necrosis in the liver of Crl:CD-1-BR mice, as found in a carcinogenicity study after whole-body exposure to 25 ml *N,N*-dimethylformamide/m³ for 6 hours per day, on 5 days per week (Malley et al. 1994). These histopathological effects cannot be determined in workplace studies. It can nevertheless be expected from the results of animal studies that they can occur at lower concentrations than those at which changes in liver-specific enzymes in the blood are usually detected.

MAK value. The starting points for the derivation of a MAK value are the $BMDL_{05}$ for centrilobular liver cell hypertrophy, the LOAEC of 25 ml/m³ for single cell necrosis in the liver in Crl:CD-1-BR mice and the NOAEC of 25 ml/m³ in rats (Malley et al. 1994).

In view of the species differences between rats, mice and humans, it can be expected that humans are less sensitive than the CD-1 mice used in the carcinogenicity study as regards the hepatotoxicity of *N,N*-dimethylformamide (Malley et al. 1994) and that the NOAEC for rats is the more suitable starting point for the derivation of the MAK value.

For the calculation of the MAK value, the increased respiratory volume at the workplace must be taken into account. The increased respiratory volume at the workplace compared with that in animal studies leads to an increase in absorption by a factor of 1.5, and the longer daily exposure at the workplace (8 hours instead of 6 hours) by a factor of 1.33 (Hartwig and MAK Commission 2017 b). In the critical carcinogenicity study, the mice were exposed whole-body. *N,N*-Dimethylformamide is, however, absorbed from the gaseous phase in about equal proportions through the skin and via the lungs (Hartwig and MAK Commission 2017 a). The proportion of the increased respiratory volume in the total exposure via the dermal and inhalation routes is thus reduced from 1.5 to 1.25, assuming that absorption through the skin in the case of increased physical activity remains the same. Together with the longer daily exposure at the workplace, therefore, the NOAEC from the animal study is 1.7 times (1.25 × 1.33) as high as the corresponding concentration under workplace conditions.

Based on the BMDL for centrilobular liver cell hypertrophy in mice of 7.8 ml/m³, a concentration of 4.5 ml/m³ is obtained if the increased respiratory volume (1:1.7) and the particular sensitivity of the mouse are taken into consideration. The usual margin applied when extrapolating data from animal studies to humans is not used in this case. The previous MAK value of 5 ml/m³ has therefore been retained. Assuming the NAEC (no adverse effect concentration) for single cell necrosis in the liver to be one third of the LOAEC of 25 ml/m³, that is 8.3 ml/m³, this also results in a MAK value of 5 ml/m³.

Based on the NOAEC for rats of 25 ml/m³ and taking into account the extrapolation of the data from the animal study to humans (1:2) and the increased respiratory volume (1:1.7), a concentration of 7.4 ml/m³ is obtained. This confirms the MAK value of 5 ml/m³.

A MAK value calculated from the NOAEC of 500 ml/m³ obtained in the 13-week study with monkeys (Hurtt et al. 1992) would be higher; here, however, the lower number of animals must be taken into account. For this reason, the calculation is less reliable than with data from the carcinogenicity studies using 60 rats or 60 mice per sex and group.

Peak limitation. In the 2012 supplement on peak limitation (Hartwig 2012, available in German only) *N,N*-dimethylformamide was assigned to Peak Limitation Category II due to its systemic effects. As the half-life of *N,N*-dimethylformamide is in the range of 1 to 2 hours, an excursion factor of 2 was set. There are no new data available. The excursion factor 2 has therefore been retained.

Prenatal toxicity. The assignment of *N,N*-dimethylformamide to Pregnancy Risk Group B has been retained. In the 2017 supplement (Hartwig and MAK Commission 2017 c), it was noted as the prerequisite for Pregnancy Risk Group C that prenatal toxicity is not to be expected after exposure to *N,N*-dimethylformamide at a level of 1 ml/m³. Even if the increased respiratory volume is taken into consideration (1:1.7 see *"MAK value"*), the difference between the NOAEC of 31 ml/m³ and the concentration of 1 ml/m³ is sufficiently large. The note regarding the prerequisite for Pregnancy Risk Group C has therefore also been retained.

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