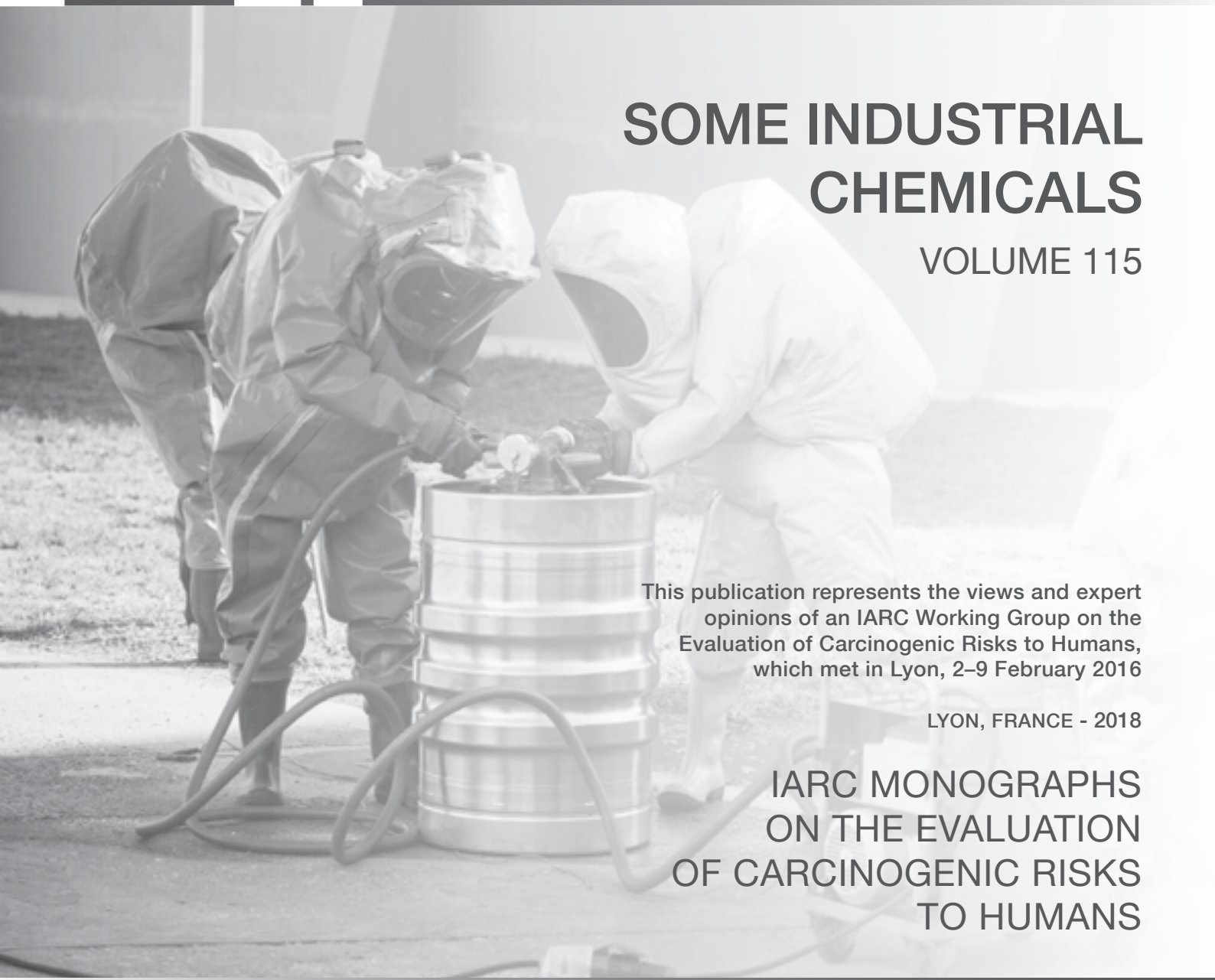
IARC MONOGRAPHS



SOME INDUSTRIAL CHEMICALS

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International Agency for Research on Cancer

World Health Organization

N,N-DIMETHYLFORMAMIDE

N,N-Dimethylformamide was considered by the *IARC Monographs* Working Group in 1989 and 1998 (IARC, 1989, 1999). New data have become available and have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 68-12-2

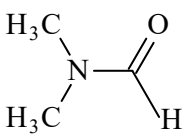
Chem. Abstr. Serv. Name: *N,N*-Dimethylformamide

IUPAC Systematic Name: *N,N*-Dimethylformamide

Synonyms: *N,N*-Dimethylmethane amide, formic acid dimethyl amide, *N*-formyldimethylamine

Acronym: DMF

1.1.2 Structure and molecular formula, and relative molecular mass



Molecular formula: C_3H_7NO

Relative molecular mass: 73.09

1.1.3 Physical and chemical properties of the pure substance

Description: Colourless to slightly yellow liquid with a faint amine-like odour (HSDB, 2015)

Degradation: Dimethylamine can be released from *N,N*-dimethylformamide and, in the presence of nitrogen oxides, carcinogenic nitrosamines can be formed. Other potential degradation products are ammonia, carbon monoxide, carbon dioxide, amines and formaldehyde.

Density (at 20 °C): 0.95 g/cm^3 (IFA, 2015)

Octanol/water partition coefficient: log K_{ow}, –1.01 (HSDB, 2015)

Melting point: –61 °C (IFA, 2015)

Boiling point: 153 °C (IFA, 2015)

Vapour pressure (at 20 °C): 0.377 kPa (2.83 mm Hg) (IFA, 2015)

Vapour density: 2.51 (air = 1) (HSDB, 2015)

Solubility: Entirely soluble in water at 20 °C (IFA, 2015)

Flammable limits: Lower explosion limit: 2.2 vol. %; upper explosion limit: 16 vol. % (IFA, 2015)

Flash point: 58 °C (IFA, 2015)

Ignition temperature: 440 °C (IFA, 2015)

Conversion factor (101 kPa, 20 °C): 1 ppm = 3.04 mg/m^3 (IFA, 2015).

1.2 Production and use

1.2.1 Production

N,*N*-Dimethylformamide is predominantly produced in a single-step reaction between dimethylamine and carbon monoxide under pressure at high temperatures and in the presence of basic catalysts such as sodium methoxide. The crude product contains methanol and *N*,*N*-dimethylformamide with increased purity (up to 99.9%) is obtained by multiple distillations (HSDB, 2015). Alternatively, it can be produced by a two-step process in which methyl formate is prepared separately and, in a second step, reacts with dimethylamine under similar conditions as those described for the single-step reaction. No catalysts are involved in the process (HSDB, 2015).

N,*N*-Dimethylformamide is listed as a high production volume chemical by the Organisation for Economic Co-operation and Development (OECD), indicating that this chemical is produced or imported at levels greater than 1000 tonnes per year in at least one member country or region (OECD, 2004, 2009). The OECD 2007 list of high production volume chemicals was compiled on the basis of submissions from eight member countries (including Australia, Canada, Japan, and the USA) in addition to the list provided by the European Union (OECD, 2009).

N,*N*-Dimethylformamide is also listed as a high production volume chemical in the USA by the Environmental Protection Agency (EPA) indicating that > 1 million pounds [~450 tonnes] were produced in or imported into the USA in 1990 and/or 1994 (HSDB, 2015).

The annual production volume of *N*,*N*-dimethylformamide (excluding imports) in the USA remained constant between 1986 and 2002, and was reported to be between 50 and 100 million pounds [approximately 20 000 and 45 000 tonnes] (HSDB, 2015). The annual production and import volume of nine USA companies was about 50 million pounds [~25 000 tonnes] (HSDB, 2015) and the total annual production volume in the European Union ranged from 50 000 to 100 000 tonnes in 2000 (SCOEL, 2006). No production volumes could be traced for Asia, but more than 300 suppliers of *N*,*N*-dimethylformamide could be identified globally, including > 200 in Asia, > 60 in the USA, and > 30 in the European Union (ChemBook, 2015).

1.2.2 Use

N,*N*-Dimethylformamide is used predominantly as an aprotic solvent in the manufacture of polyacrylonitrile fibres, and trends in its production parallel those of the polyacrylic fibre industry (HSDB, 2015). It is also used in the manufacture of high quality polyurethane and polyamide coatings (e.g. for leather or artificial leather fabrics), which are otherwise difficult to solubilize, and where a solvent with a slow rate of evaporation is needed. *N*,*N*-Dimethylformamide is commonly used as a solvent in the electronics industry, in pesticides, in industrial paint-stripping applications, and as a reaction and crystallizing solvent in the pharmaceutical industry. It has limited use as a selective solvent for the separation of aliphatic hydrocarbons such as the extraction of acetylene or butadiene from hydrocarbon streams (HSDB, 2015).

1.3 Measurement and analysis

Multiple methods exist for the analysis of *N*,*N*-dimethylformamide and its metabolites in air, water, urine, and blood. The methods are largely based on gas chromatography (GC) and high-performance liquid chromatography (HPLC) with various detection systems such as

flame ionization, nitrogen-sensitive, or mass-sensitive detection.

N,*N*-Dimethylformamide is most commonly measured in air by adsorbing on a silica gel and analysis by GC/flame ionization detection. The level of detection is approximately 0.05 mg per sample in a 15-L air sample or higher at a flow rate of 0.01–1 L/min (NIOSH, 1994).

Several diffusive sampling devices have been described for exposure assessment of *N*,*N*-dimethylformamide in the air of the workplace (Tanaka et al., 2002; Baglioni et al., 2007).

Because of the potential for dermal uptake, exposure to *N*,*N*-dimethylformamide should be measured by biomonitoring. Major metabolites in urine are *N*-hydroxymethyl-*N*-methylformamide (HMMF), *N*-methylformamide and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) (Gescher, 1993). HMMF degrades at injection temperatures > 250 °C, which allows the determination of total *N*-methylformamide in biological samples (Kawai et al., 1992).

An HPLC method also has been described that has a pre-heat phase to convert HMMF to *N*-methylformamide. The reported detection limit was 0.5 mg/L (Tranfo et al., 1999). As an alternative, HMMF can be converted chemically to *N*-methylformamide in the presence of potassium carbonate during sample preparation (Mráz & Turecek, 1987). The limits of detection are usually approximately 1 mg/L for total *N*-methylformamide and sufficient for exposure assessment in *N*,*N*-dimethylformamide-exposed workers (Mráz et al., 1987).

Recently, a method for simultaneously analysing total *N*-methylformamide and AMCC by capillary GC and nitrogen-selective detector with an injector temperature > 250 °C has been described (Käfferlein & Angerer, 2005). Under these conditions, the limits of detection were 1 mg/L of urine for total *N*-methylformamide and 0.5 mg/L of urine for AMCC.

The measurement of AMCC in urine can also form part of a multi-analysis of various mercapturic acids of organic compounds rather than measuring AMCC alone (Schettgen et al., 2008; Schettgen, 2010; Alwis et al., 2012). [The Working Group noted that AMCC can also be detected in the urine of the general population as a result of its endogenous production in the body (Käfferlein & Angerer, 1999).]

1.4 Occurrence and exposure

1.4.1 Natural occurrence

N,*N*-Dimethylformamide is not known to occur as a natural product.

1.4.2 Environmental occurrence

Industrial releases of *N*,*N*-dimethylformamide into the air appear to be considerably larger than those into other environmental media. *N*,*N*-Dimethylformamide is expected to exist almost entirely in the vapour phase in ambient air. When emitted into air, most of the *N*,*N*-dimethylformamide released remains in that compartment where it is degraded by chemical reactions with hydroxyl radicals; its half-life in the air is estimated to be in the range of few days. Some atmospheric *N*,*N*-dimethylformamide can reach the aquatic and terrestrial environment, presumably during periods of rain. Releases into the water or soil are expected to be followed by relatively rapid biodegradation (half-life, 18–36 hours) (IPCS, 2001; HSDB, 2015).

Measurements of *N*,*N*-dimethylformamide in air and water are presented in Table 1.1.

(a) Air

In Lowell, MA, USA, *N*,*N*-dimethylformamide was detected in the air over an abandoned chemical waste reclamation plant (mean, 7 µg/m^3), a neighbouring industry (> 150 µg/m^3), and a residential area (24 µg/m^3) (Amster et al., 1983; cited in IPCS, 2001). In ambient air samples collected in the north-eastern USA in 1983, levels

Table 1.1 Environmental exposure to *N,N*-dimethylformamide

Country	Duration	Sites/situation		DMF exposure											Reference
				DMF in air (μg/m³)				NMF in urine (mg/g creatinine)			DMF in surface water (μg/L)				
				No.	Mean	SD	Range	No.	Mean	SD	No.	Mean	SD	Range	
China	1-year follow-up	"Unpolluted" area in Longwan		366[a]	98.3	109.7	4.4–678.3								Wang et al.(2014)
	Cross-sectional	Near synthetic leather factories in Longwan	A	25	297.5	95		25	7.7	6.2					
			B	39	430	122.5		39	6.7	2.7					
			C	22	180	0		22	1.5	1.7					
			D	23	565	516.2		23	23.4	24.9					
			E	24	270	84.9		24	1.8	0.8					
Japan	Cross-sectional	All over Japan		105[b]	0.092[c]		0.016–0.49				47[d]	0.27[c]		ND–0.53	Ministry of the Environment Japan (2012)
USA		North-eastern					ND–14								Kelly et al. (1994)
USA		Massachusetts/ polluted area					7– > 150								Amster et al. (1983) cited in IPCS (2001)

[a] Monitoring daily throughout 2008
[b] Three samples were measured from 35 sites in Japan. *N,N*-Dimethylformamide was detected in all samples
[c] Geometric mean
[d] One sample was measured from 47 sites in Japan. *N,N*-Dimethylformamide was below the detection limit (0.019 μg/L) in 10 samples out of 47
DMF, *N,N*-dimethylformamide; ND, not detected; NMF, *N*-methylformamide; SD, standard deviation

of *N*,*N*-dimethylformamide ranged from not detected to 14 µg/m³ (Kelly et al., 1994).

Airborne concentrations of *N*,*N*-dimethylformamide, measured near synthetic leather factories in China where it is used, ranged from 180 to 565 µg/m³. In the same region, but away from the factories, mean concentration in 366 samples was 98.3 µg/m³ (Wang et al., 2014). Airborne concentrations of *N*,*N*-dimethylformamide measured at 35 sites all over Japan in 2008 ranged from 0.016 to 0.49 µg/m³ (Ministry of the Environment Japan, 2012).

(b) Water

Concentrations of *N*,*N*-dimethylformamide in water from rivers, lakes, and bays were measured throughout Japan and, in a total of 47 samples from 47 sites, ranged from not detected to 0.53 µg/L (Ministry of the Environment Japan, 2012).

1.4.3 Exposure of the general population

A study on biological monitoring in the general population living in areas near synthetic leather factories in China investigated the consistency between the concentration of *N*,*N*-dimethylformamide in outdoor air and levels of urinary *N*-methylformamide (Wang et al., 2014). The mean concentration of urinary *N*-methylformamide ranged from 1.5 to 23.4 mg/g creatinine. Under the most intensive exposure (> 450 µg/m³ of *N*,*N*-dimethylformamide in the air), the maximum value of urinary *N*-methylformamide was 41.03 mg/g creatinine. The correlation between urinary *N*-methylformamide and air sampling was strong ($P < 0.01$) with a coefficient of 0.80 (Wang et al., 2014; Table 1.1).

1.4.4 Occupational exposure

According to the 1981–83 United States National Occupational Exposure Survey, as many as 125 000 workers in the USA were potentially exposed to *N*,*N*-dimethylformamide (NOES, 1997).

Exposures to *N*,*N*-dimethylformamide were assessed in workplaces by both air monitoring and biomonitoring (urinary *N*-methylformamide) beginning in the 1970s (Table 1.2).

1.4.5 Exposure assessment in epidemiological studies

In a retrospective cohort study (Chen et al., 1988a, b), at an acrylic-fibre production plant in the USA, exposure to *N*,*N*-dimethylformamide was assessed semi-quantitatively by an "exposure classification committee" comprising 15 employees. They classified all jobs into three categories: "low" exposure (no direct contact with liquids, workplace concentrations of *N*,*N*-dimethylformamide in air consistently below 10 ppm and no odour of *N*,*N*-dimethylformamide), "moderate" exposure (intermittent contact with liquids containing more than 5% *N*,*N*-dimethylformamide and workplace concentrations of *N*,*N*-dimethylformamide in air occasionally (more than once a week) above 10 ppm) and "high" exposure (frequent contact with liquids containing more than 5% *N*,*N*-dimethylformamide, workplace concentrations of *N*,*N*-dimethylformamide in air often above 10 ppm and breathing protection often required for 15–60 min). Monitoring data on *N*,*N*-dimethylformamide were not available for the period; exposure was estimated for 1950–70 and exposure intensity for each job was assumed to be constant during this time period.

In a nested case–control study in four plants (Walrath et al., 1989), a much more detailed exposure assessment was performed. Data on air measurements of *N*,*N*-dimethylformamide

Table 1.2 Occupational exposure to *N,N*-dimethylformamide

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Personal air sampling										
China	7	Leather production		43	27.7					Cai et al. (1992)
		Polyurethane production		65	11.9					
		Shoe sole production		17	2.1					
		Laboratory A		23	1.2					
		Laboratory B		59	0.6					
		Leather printing		52	7.6					
		Resin production		59	1.8					
Germany	1	Polyacrylic fibre		63	5.3[b]	ND–485.7	92	13.1	ND–108.7	Käfferlein et al. (2000)
Germany	1	Polyacrylic fibre	Total	118	12.5	ND–115.2	125	4.7[b,c]	0.4–62.3[c]	Wrbitzky & Angerer (1998)
			Wet-spinning	30	22.2	0.9–115.2	30	8.9[b,c]	0.4–54.0[c]	
			Dry-spinning	25	19.5	2.4–112.2	28	6.7[b,c]	0.9–62.3[c]	
			Finishing	51	4.3	ND–41.6	55	3.0[b,c]	0.6–19.9[c]	
			Dyeing	12	7.6	0.3–29.8	12	5.5[b,c]	0.8–17.2[c]	
Italy	2	Synthetic leather		100	22.0	8.0–58.0				Cirla et al. (1984)
Italy	1	Synthetic leather		125	13.5	0.4–75.2	125	17.1[d]	1.5–114.2	Imbriani et al. (2002)
Japan	1	Synthetic resin	Blending	3	7.0[d,e]	6.1–9.1				Sakai et al. (1995)[f]
			Kneading	3	14.6[d,e]	6.1–24.3				
			Extruding	3	14.9[d,e]	12.2–18				
			Ageing	3	4.3[d,e]	3.0–9.1				
			Total				10	24.7[c]		
Japan	1	Synthetic resin	Summer	128	5.2[d]		128	4.1[d]		Miyauchi et al. (2014)
			Winter	142	3.0[d]		142	1.4		

Table 1.2 (continued)

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Republic of Korea	15	Fibre coating		57	14.9	0.3–48.9	57	17.5[d]	0.4–97.9	Yang et al. (2000)
		Synthetic leather		108	25.8[d]	4.0–168.7	108	32.8[d]	0.8–235.8	
		Paint		13	3.5[d]	0.3–22.5	13	4.2[d]	1.2–12.7	
		Synthetic fibre		116	2.4[d]	0.3–32.2	116	8.3[d]	1.2–91.4	
		Synthetic leather		4	2.1[d]	0.6–11.6	4	7.8[d]	0.4–54.9	
		Synthetic fibre		2	0.5[d]	0.3–1.0	2	3.7[d]		
		Fibre coating		11	3.7[d]	0.5–21.0	11	4.4[d]	0.4–19.5	
		Synthetic leather		19	13.0[d]	2.1–49.2	19	15.8[d]	4.7–65.4	
		Paint		5	1.2[d]	0.3–8.1	5	3.2[d]	0.4–10.8	
Republic of Korea	9	Synthetic leather		116	26.8[d]		143	47.5[d]		Kim et al. (2004)
Taiwan, China	1	Synthetic leather and resin		176	35.3	0.3–263.3				Luo et al. (2001)
Taiwan, China	1	Synthetic leather		12	34.7		12	17.9		Chang et al. (2004a)
Taiwan, China	4	Various	Total	75	4.6[d]	0.1–58.9	75	0.47[d]	0.03–104.4	Chang et al. (2004b)
		Synthetic acrylic fibre factory		23	2.1[d]	0.1–17.5	23	0.09[d]	0.03–3.20	
		Synthetic leather factory A		8	19.9[d]	10.5–58.9	8	4.95[d]	1.32–21.01	
		Synthetic acrylic leather factory B		24	13.3[d]	2.4–50.7	24	14.3[d]	4.9–104.4	
		Circuit board factory		20	1.7[d]	0.3–29.9	20	0.05[d]	0.03–1.20	
Taiwan, China	4	Synthetic leather		59	12.4[d]	1.6–155.3		20.8[d]	1.35–178.6	Wang et al. (2004)
Taiwan, China	1	Synthetic leather		65	34.9	2.2–104.8				Chang et al. (2005b)
Taiwan, China	1	Synthetic leather		13	32.2[b]	20.2–104.8	13	13.8[b]	7.5–73.7	Shieh et al. (2007)

Table 1.2 (continued)

Country	Workplace			Airborne exposure DMF (mg/m³)			Urinary concentration total NMF (mg/L)[a]			Reference
	Plants (No.)	Industry	Type of work	No.	Mean	Range	No.	Mean	Range	
Air sampling in the workplace										
Belgium	1	Synthetic acrylic fibre	Mixer	54	32.7					Lauwerys et al. (1980)
			Spinning	54	13.4					
China	2	Synthetic resin	Resin line	12	15.0[d]	5.4–30.3				He et al. (2010)
			Control line	4	2.9[d]	2.1–4.7				
		Synthetic leather	Mixing	3	46.7[d]	42.5–51.2				
			Wet process	9	49.1[d]	13.1–199.8				
			Dry process	6	22.3[d]	14.9–72.4				
			Laboratory	7	3.3[d]	1.1–7.7				
			Product inspection	3	4.3[d]	1.3–17.2				
			Administration	4	1.5[d]	ND–2.2				
Italy	1	Synthetic leather	Washing	10	21.5[d]	5–40				Fiorito et al. (1997)
			Production	22	18.7[d]	2–35				

[a] Post-shift urine samples
[b] Median
[c] mg/g creatinine
[d] Geometric mean
[e] Stationary sampling
[f] Temperature of the injector port < 250 °C for biomonitoring/method unclear
DMF, *N*,*N*-dimethylformamide; ND, not detected; NMF, *N*-methylformamide

and urinary concentrations of *N*-methylformamide were used whenever possible. For plant A, 167 personal *N*,*N*-dimethylformamide samples (1979–86), 107 urinary *N*-methylformamide samples (1975–79) and 14 *N*,*N*-dimethylformamide area samples (1975–80) were available. For plants B and C, respectively, 2916 and 2718 personal or area air samples of *N*,*N*-dimethylformamide (1974–86) were available; ~61 000 urinary (1980–86) and ~12 000 air *N*-methylformamide measurements (1975–86) were used for the exposure assessment. For plant D, 2361 *N*,*N*-dimethylformamide samples (1974–86) and 2037 *N*-methylformamide samples (1978–1986) were available. Two estimates of 8-hour time-weighted average (TWA) exposure to *N*,*N*-dimethylformamide (average and peak exposure) were assigned to each job title based on the measurements; however, the final exposure classification was similar to the classification for the cohort study described in Chen et al. (1988a). Jobs were ranked as having "low" (1.0–< 2.0 ppm of *N*,*N*-dimethylformamide in air), "moderate" (2.0–< 10.0 ppm) and "high" (≥ 10.0 ppm) exposure. Controls were ranked as "none" or "present" if not exposed or exposed to < 1 ppm of *N*,*N*-dimethylformamide in air, respectively. Contrary to the retrospective cohort study, a job could be ranked differently over time because of the use of time-resolved monitoring data.

1.5 Regulations and guidelines

Depending on the country, 8-hour TWA occupational exposure limits have been set at 15 mg/m³ (5 ppm) or 30 mg/m³ (10 ppm) for *N*,*N*-dimethylformamide in the air (GESTIS, 2015). For example, the permissible exposure limit issued in the USA by the Occupational Safety and Health Administration is 30 mg/m³, whereas the threshold limit value recommended by the Scientific Committee for Occupational Exposure Limits of the European Union is 15 mg/m³ (SCOEL, 2006; GESTIS, 2015).

The European Union and the Occupational Safety and Health Administration label *N*,*N*-dimethylformamide with a skin notation (SCOEL, 2006; OSHA, 2016). As a consequence, biological limit values, in terms of measuring metabolites of *N*,*N*-dimethylformamide in urine, have been recommended to take into account all routes of exposure (inhalation, dermal, oral). The Scientific Committee for Occupational Exposure Limits recommends a biological limit value of 15 mg *N*-methylformamide per litre urine in end-of-shift samples, based on the association between exposure to 5 ppm airborne *N*,*N*-dimethylformamide and excretion of *N*-methylformamide in urine at the end of shift (SCOEL, 2006). Several countries of the European Union list *N*,*N*-dimethylformamide as a reproductive toxicant. For example, in Germany, *N*,*N*-dimethylformamide is categorized in group B of reproductive toxicants because embryonic or fetal damage cannot be excluded if women are exposed to the compound during pregnancy, even when the respective threshold limit values are observed (DFG, 2015).

According to the risk phrases of the Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations, *N*,*N*-dimethylformamide is harmful if inhaled (H332) and if skin contact occurs (H312). It also can cause serious eye irritation (H319) and may damage fertility or the unborn child (H360D) (ECHA, 2016).

N,*N*-Dimethylformamide has recently been categorized as a group 4 carcinogen by the Commission for the Investigation of Health Hazards at the Workplace in Germany (DFG, 2015).

EPA (1990) promulgated a reference concentration for *N*,*N*-dimethylformamide in the air of 0.03 mg/m³.

2. Cancer in Humans

2.1 Aircraft repair

See Table 2.1

The human carcinogenicity of *N*,*N*-dimethylformamide was questioned by Ducatman et al. (1986) beginning with an investigation of a cluster of three cases of testicular germ cell cancer that occurred between 1981 and 1983. The three testicular cancers (< 1 expected) occurred among 153 white civilian repairmen who were exposed to *N*,*N*-dimethylformamide while repairing the exterior surfaces and exterior electrical components of the F4 Phantom jet aircraft at a single United States naval air rework facility in North Carolina, USA. The finding led to an evaluation of testicular cancer in two groups of civilian aircraft repair workers, which was reported in the same paper. The two groups of workers were at a United States naval air rework site in California. Similar to the North Carolina workers, the first of these groups also repaired the F4 Phantoms among other naval aircraft and were exposed to *N*,*N*-dimethylformamide, while workers in the second group performed re-work on a variety of naval aircraft, but not the F4 Phantoms, and were not exposed to *N*,*N*-dimethylformamide. At the California facility, four of the 680 men with a history of working on F4 phantoms were diagnosed with testicular germ cell cancers. The men in this groups of exposure workers had an estimated 19 040 person–years of experience from 1970 to 1983, and that finding represented an apparent increase of fourfold [4.21; 95% confidence interval (CI), 1.15–10.78] in incidence compared with national incidence rates ($P < 0.02$). [It was not clear whether the expected numbers reported in the paper were age-adjusted.] In contrast, none of the 446 men in the unexposed population (who did not repair the F4 Phantom) had a diagnosis of testicular germ cell cancer. Within the North Carolina and California populations involved in F4 Phantom repairs, the men with a diagnosed cancer (five seminomas and two embryonal cell cancers) had long histories of aircraft repair before their diagnosis.

Naval air rework facilities (dedicated to military aircraft repair) presented opportunities for repair workers to be exposed to surface coatings and associated emulsifiers and surfactants including "Teflon" paints and dyes, solvents and metals (Ducatman et al., 1986). A relatively uncontrolled process that was unique to F4 Phantom repair at the two facilities involved open air spraying of an 80% *N*,*N*-dimethylformamide solution onto in-situ electrical cables in quantities sufficient to dissolve the elastomeric surface coatings of the cables, with the goal of performing needed maintenance on the bare cables in place. The process was open and no specific ventilation was employed: it was reported to involve substantial inhalation and dermal exposure. At the time of the investigation, the process had been out of use for more than a decade. A formal exposure model was not created. Among the seven cases at both facilities (age range, 30–46 years), three reported direct responsibility for the setting up and use of this process and the others recalled that they were exposed in proximity. [The Working Group was aware that this group of military aircraft mechanics had respiratory and dermal exposure to *N*,*N*-dimethylformamide. Limitations of the study included the lack of quantitative exposure measurements and the fact that follow-up for cancer incidence included only the years when the workers were employed.]

The above findings in civilian repairmen working for the United States Navy motivated an evaluation of testicular cancer hospitalizations of United States active duty military personnel from 1974 to 1979 (Garland et al., 1988). This study found 143 incident cases of testicular cancer in currently active duty personnel. No excess risk was observed overall but, among aviation support technicians, the standardized incidence ratio was 6.2 (95% CI, 1.9–13.0; 5 cases). [The Working

Table 2.1 Cohort studies of cancer and exposure to *N,N*-dimethylformamide

Reference, location enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ducatman et al. (1986) Naval air rework facility North Island (the 2nd facility investigated, which did not contain the original cluster), CA, USA 1970–1983	680 exposed, 446 not exposed; male civilian employees at a US naval air rework facility Exposure assessment method: direct observation; onsite survey, linkage to local cancer registry, outreach to local physicians and hospitals, and review of medical records	Testis	All exposed workers	4	[4.21 (1.15–10.78)]	NR	Strengths: the investigation was prompted by a specific hypothesis related to a cluster investigation at a previous facility; there was a nested case–control comparison to men who had similar jobs but never used the same process Limitations: statistical comparison was to national SEER data; only active workers included; the implicated exposure process had stopped before the investigation, and no exposure model was created
CDC (1989) Fulton County, NY, USA 1 January 1975 to 31 December 1987	80 male workers; males employed in the finishing line of a leather tannery from 1975–1987 Exposure assessment method: environmental monitoring; medical record confirmed cases and linkage to the New York State cancer registry	Testis	All workers in the cluster plant	3	40.5 (8.1–118.4)	Age, sex, period, location	Exposure monitoring was performed after the relevant exposure had ceased; a historical survey attempted to assess the previous history of high exposure by interrogating the presence of alcohol intolerance during the period of the DMF operation Strengths: additional follow-up of Fulton County testicular cancer cluster Limitations: statistical calculations include the initial cancer cluster; the exposure monitoring did not include the process of interest

Table 2.1 (continued)

Reference, location enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chen et al. (1988a) Fibre-producing industrial facility, USA 1 January 1956 to 31 December 1984	3859 total: 2530 DMF only; 1329 to DMF and acrylonitrile; and 1130 with exposures to neither. Data are tabulated for the cohort with any exposure to DMF; male workers with potential exposure to DMF between 1950 and 1970 Exposure assessment method: assessment by employee committee; corporate cancer registry, begun in 1956	Upper aerodigestive tract: buccal cavity & pharynx	All DMF cohort	11	[1.67 (0.83–2.98)]	Age, sex, period	SRRs were generally elevated, but not statistically significant for the DMF-only cohort Strengths: cancer incidence study; exposure matrix Limitations: exposure measurements only after 1970
		All cancers combined	All DMF cohort	88	[0.92 (0.73–1.13)]		
		Prostate	All DMF cohort	10	[1.92 (0.92–3.54)]		
		Malignant melanoma	All DMF cohort	7	[1.55 (0.56–3.21)]		
		Lung	All DMF cohort	21	[0.97 (0.60–1.48)]		
		Testis	All DMF	1	[0.59 (0.01–3.28)]		
		Upper aerodigestive tract: buccal cavity & pharynx	Low/ moderate DMF	5	[4.17 (1.35–9.72)]		
			High DMF	6	[3.0 (1.10–6.5)]		
			< 5 yrs DMF exposure	4	[3.64 (0.99–9.31)]		
			≥ 5 yrs DMF exposure	7	[3.18 (1.28–6.56)]		

Table 2.1 (continued)

Reference, location enrolment/follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chen et al. (1988b) Fibre-producing industrial facility, USA 1 January 1950 to 31 December 1982	See incidence study by Chen et al. (1988a) Male workers with potential exposure to DMF between 1950 and 1970 Exposure assessment method: assessment by employee committee; corporate mortality data	All cancers combined	All DMF cohort	75	[0.98 (0.77–1.23)]	Age, sex, period, location	Same population and methods as Chen et al. (1988a) Strengths: local worker comparison group Limitations: mortality only; no testicular cancer deaths were reported (mortality is not the optimal end-point for investigating this cancer)
			DMF only cohort	38	[0.95 (0.67–1.30)]		
		Upper aerodigestive tract: buccal cavity & pharynx	All DMF cohort	3	[1.88 (0.39–4.48)]		
			DMF only cohort	2	[2.5 (0.30–9.0)]		
		Lung	All DMF cohort	33	[1.24 (0.85–1.74)]		
		Lung	DMF-only cohort	19	[1.40 (0.85–2.20)]		

CI, confidence interval; DMF, *N*,*N*-dimethylformamide; NR, not reported; SEER, Surveillance, Epidemiology and End Results; SRR, standardized relative risk; US, United States; yr, year

Group considered it unlikely that active duty aviation support technicians in general would have had significant exposure to the unique process involving *N*,*N*-dimethylformamide.]

2.2 Leather workers

See Table 2.1 and Table 2.2

Levin et al. (1987) reported a cluster of three cases of testicular germ cell cancer in workers who worked on a spray line in a leather tannery (Fulton County, NY, USA) where *N*,*N*-dimethylformamide was used. They were aged 25–36 years at the time of diagnosis, which occurred between 1982 and 1984, and had worked for 8–14 years on the spray line before diagnosis. Other than *N*,*N*-dimethylformamide and "dyes", this report did not discuss other potential exposures.

In a further investigation, a team including personnel from the United States National Institute of Occupational Safety and Health performed air sampling of the spray process for partially tanned hides at the same facility (CDC, 1989). At the time of the investigation, the facility had ceased to use *N*,*N*-dimethylformamide in the tanning process and therefore none was detected in air sampling. This investigation established that *N*,*N*-dimethylformamide had been used historically in the spraying process. The exposures identified included dyes, pigments, surface coatings and solvents, including *N*,*N*-dimethylformamide. Sampling after the *N*,*N*-dimethylformamide process had stopped detected several glycol ethers, including 2-butoxyethanol, at up to 10.9 ppm. A cohort study of testicular cancer in 80 workers in the index tannery found a standardized incidence ratio of 40.5 (95% CI, 8.1–118.4) compared with state Surveillance, Epidemiology and End Results data. In addition, this report featured a case–control study, performed at the county level, of testicular cancer cases in male residents aged 20–54 years from January 1974 to March 1987. The three men reported in the Levin et al. (1987) report were included, as well as an additional seven cases. A group of 129 male controls of similar age who had other cancer diagnoses from the same registry was used. Five of the 10 cases had histories of leather-related employment, resulting in an odds ratio of 5.8 (95% CI, 1.5–22.0) (CDC, 1989). [The Working Group noted that the inclusion a posteriori of an initially detected disease cluster within a small population calculation can contribute to an expected outcome of an elevated standardized incidence ratio.]

Workers from the index tannery workplace were also enrolled in a prospective testicular cancer screening programme. The National Institute of Occupational Safety and Health investigators reported that 51 out of 83 workers participated and, as of 1990, no additional cases of testicular cancer had been found (Calvert et al., 1990).

2.3 Chemical manufacturing

2.3.1 Cohort study

See Table 2.1

A major chemical manufacturer conducted retrospective cohort cancer incidence (Chen et al., 1988a) and cancer mortality (Chen et al., 1988b) studies of workers at an acrylic-fibre production plant in the USA whose employment provided exposure to *N*,*N*-dimethylformamide alone (2530 workers), acrylonitrile alone (16 workers), neither (1130 workers) or both (1329 workers). The two groups with any exposure to *N*,*N*-dimethylformamide were then combined into a single historical cohort (3859 workers).

Monitoring data were reportedly unavailable for most of the historical period (1950–1970) considered in these reports, and exposures were classified as "low", "moderate" or "high" by a committee of workers. Diagnostic information on cancer was obtained from the manufacturer's internal cancer registry and was therefore

Table 2.2 Case–control studies of cancer and exposure to *N,N*-dimethylformamide

Reference, location enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/deaths	Risk estimate	Covariates controlled	Comments
CDC (1989) Fulton County, NY, USA Testicular cancer cases from county-level analysis of New York State cancer registry data	Cases: 10; New York State cancer registry Controls: 129; New York State cancer registry Exposure assessment method: based on recorded usual occupation at diagnosis	Testis	Leather tannery workers	5	5.8 (95% CI, 1.5–22.0)	Date, year of diagnosis	Strengths: added information (two additional cases) to earlier cluster investigation Limitations: the original cluster was included in the case numerator
Walrath et al. (1989) Four industrial facilities of one employer 1956–1985	Cases: 39 buccal cavity & pharynx, 6 liver, 43 prostate, 11 testis, 39 skin; employer cancer registry Controls: 276; other workers from cohort Exposure assessment method: expert assessment; existing monitoring data were characterized by job title, for mean and peak DMF; a facility history was developed for each of four facilities	Liver/ hepatocellular carcinoma	Minimum latency 10 yrs	NR	6.10 (90% CI, 0.38–72.00)	Age, sex, plant, pay class	Strengths: improved description of JEM for this employer Limitations: exposure monitoring data not available for the earlier years: extrapolations were made
		Liver/ hepatocellular carcinoma	> 10 yr duration	NR	2.74 (90% CI, 0.16–47.3)	Age, study site, pay class, year of diagnosis, age at diagnosis	
		Upper aerodigestive tract: buccal cavity & pharynx	All categories	15	0.89 (90% CI, 0.35–2.29)	Age, sex, plant, pay class	
		Prostate	All categories	17	1.47 (90% CI, 0.66–3.30)	Age, sex, plant, pay class	
		Malignant melanoma	All categories	16	1.70 (90% CI, 0.52–5.51)	Age, sex, plant, pay class	
		Testis	All categories of exposure	3	0.99 (90% CI, 0.22–4.44)	Age, sex, plant, pay class	
		Testis	Moderate exposure only	2	11.60 (90% CI, 0.47–286)	Age, study site, pay class, year of diagnosis, age at diagnosis	

CI, confidence interval; DMF, *N,N*-dimethylformamide; JEM, job–exposure matrix; NR, not reported; SEER, Surveillance, Epidemiology and End Results; US, United States; yr, year

limited to the time of employment. Person–years were calculated from first exposure or first employment from 1956 to 1984. Comparisons of corporate registry data were then made to national Surveillance, Epidemiology and End Results cancer registry data for 1973–1977. Thirty-four incident cases of all types of cancer were recorded in the cohort of wage workers exposed to *N*,*N*-dimethylformamide only (Chen et al., 1988a). Only one case of testicular cancer was found compared with 1.7 expected [standardized relative risk, 0.59; 95% CI, 0.01–3.28] in the cohort exposed to *N*,*N*-dimethylformamide only. Buccal cavity and pharyngeal cancers (combined) were significantly increased (8 observed, 1 expected [standardized relative risk, 8.00; 95% CI, 3.45–15.76]) in the cohort exposed to *N*,*N*-dimethylformamide only, but not in the cohort exposed to both *N*,*N*-dimethylformamide and acrylonitrile. No dose–response relationship was observed related to the classified exposure level.

Chen et al. (1988b) used the same methods to study mortality from all causes and cancer in the same population. Compared with internal company rates, non-statistically significant excesses of buccal cavity and pharyngeal cancers (combined) and lung cancer were reported (Chen et al., 1988a). [The exposure characterization for this *N*,*N*-dimethylformamide-manufacturing cohort was more complete than that in the previously cited studies in aircraft repair and leather work and provides an impression that exposures were better controlled than those for aircraft and leather workers. The *N*,*N*-dimethylformamide solution at the manufacturing sites is reported to have generally been up to 5% in concentration and intermittently > 5% – less concentrated than the 80% solutions used by aircraft repairmen and leather workers – and the observation that respirators were supposed to have been used if atmospheric concentrations were > 10 ppm of *N*,*N*-dimethylformamide does not appear to pertain to the aircraft repair and leather operations previously described.]

2.3.2 Case–control study

See Table 2.2

The same chemical manufacturer subsequently performed a broader case–control study on exposure to *N*,*N*-dimethylformamide among male active employees at four of its manufacturing facilities including the one studied by Chen et al. (1988a, 1988b), from 1956 to 1985, based on cancer cases reported to the company cancer registry (Walrath et al., 1989). The study population included only cases identified among currently employed workers. For each case, two controls from the entire cohort were matched to the case worker based on year of employment, year of birth, sex, plant, and payroll/salary class. The study provided a more detailed exposure assessment than that used in the preceding cancer incidence and mortality studies (Chen et al., 1988a, 1988b), including measurements of *N*,*N*-dimethylformamide in the air and its urinary metabolites. Average and peak exposures were considered. When data were not available, an extrapolation was made using exposure data from later years. The exposure classifications were more comprehensive but ultimately similar to those used in the cohort study (Chen et al., 1988a): "low", "medium", "high" and none. The geometric mean concentration of *N*,*N*-dimethylformamide in the air ranged from 1 to 2 ppm [3–6 mg/m^3] for jobs in the low exposure group to > 10 ppm [30 mg/m^3] for jobs in the high exposure group. Air concentrations > 50 ppm [149 mg/m^3] were possible in the high exposure category. Mantel-Haenszel and multiple logistic regression methods were used to calculate odds ratios. The odds ratio for testicular cancer for ever being exposed was 0.99 (90% CI, 0.22–4.44; 3 exposed cases). Odds ratios for testicular cancer by level of exposure were 0.86 (90% CI, 0.09–8.56) for low

and 11.6 (90% CI, 0.47–286; 2 exposed cases, 2 exposed controls) for moderate exposure. No cases occurred in the high exposure category. The same data set was also considered with regard to different durations of exposure. The odds ratio for testicular cancer was 1.28 (90% CI, 0.14–7.12) for those with < 10 years exposure and no cases occurred among those with ≥ 10 years exposure. Buccal cavity and pharyngeal cancer were not found to be significantly increased when all facilities were combined, nor were any other studied cancer sites. [The Working Group noted that interpretation of these studies was limited by the small numbers of testicular cancer cases and by the restriction to cases occurring among active workers.]

3. Cancer in Experimental Animals

N,*N*-Dimethylformamide was first reviewed by the *IARC Monographs* Working Group (IARC, 1989) when it had been tested for carcinogenicity by oral administration and by subcutaneous injection in one rat strain (Druckrey et al., 1967) and by intraperitoneal injection in another strain (Kommineni, 1972). All of these studies were judged to be inadequate for an evaluation and were still considered to be inadequate by the current Working Group because of the small number of animals, the short duration of treatment and the incomplete reporting of the results. *N*,*N*-Dimethylformamide was subsequently reviewed by a second Working Group (IARC, 1999) when it had also been tested for carcinogenicity by inhalation in one study in mice and one study in rats, with no significant increase in tumour incidence (Malley et al., 1994).

Studies that were judged to be adequate for an evaluation (including studies released since the previous *IARC Monographs*) are summarized below.

3.1 Mouse

See Table 3.1

Inhalation

Groups of 78 male and 78 female Crl:CD-1 (ICR) BR mice (age, 55 days) were exposed to *N*,*N*-dimethylformamide (purity, 99.9%) by whole-body inhalation at concentrations of 0, 25, 100, and 400 ppm for 6 hours per day on 5 days per week for 18 months. Five male and five females per group were killed at 2 weeks, 3 months and 12 months to evaluate cell proliferation in the liver. Survival in all exposure groups was similar to that of controls. Higher body weights and body-weight gain were observed in the male and female mice exposed to 100 and 400 ppm compared with controls and were considered to be compound-related. The males exposed to 100 and 400 ppm and females exposed to 400 ppm had higher liver weights relative to body weight at necropsy. No significant increase in tumour incidence was observed in any dose group. All treated males had significant increases in centrilobular hepatocellular hypertrophy and hepatic single cell necrosis. Centrilobular hepatocellular hypertrophy was also significantly increased in the females exposed to 100 and 400 ppm and hepatic single cell necrosis in all treated females (Malley et al., 1994). [The Working Group noted the short duration of the study.]

In a well-conducted study that complied with good laboratory practice, groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were exposed to *N*,*N*-dimethylformamide (purity, > 99.8%) by whole-body inhalation at concentrations of 0, 200, 400, and 800 ppm for 6 hours per day on 5 days per week for 104 weeks. No significant difference in survival was observed between the exposed groups and the controls. The survival rate of females exposed to 800 pm decreased marginally (but not significantly) after the 78th week because of the development of liver tumours.

Table 3.1 Studies of carcinogenicity with *N,N*-dimethylformamide in mice and rats

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Crl:CD-1 (ICR) BR (M) 55 days 18 mo Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 78, 78, 78, 78 56%, 68%, 60%, 59%	*Liver or testis* No increase in tumour incidence	NS	Principal limitations: short duration of the study 5 males per group were killed at 2 wks, 3 mo, and 12 mo, for evaluation of cell proliferation in the liver
Full carcinogenicity Mouse, Crl:CD-1 (ICR) BR (F) 55 days 18 mo Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 78, 78, 78, 78 68%, 57%, 62%, 76%	*Liver or mammary gland* No increase in tumour incidence	NS	Principal limitations: short duration of the study 5 males and 5 females per group were killed at 2 wks, 3 mo, and 12 mo, for evaluation of cell proliferation in the liver
Full carcinogenicity Mouse, Crj:BDF1 (M) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 37, 33, 37, 40	*Liver* Hepatocellular adenoma: 6/50, 36/50*, 41/49*, 41/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appears to have complied with GLP
		Hepatocellular carcinoma: 2/50, 12/50*, 16/49*, 16/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma: 0/50, 13/50*, 7/49*, 4/50	*$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 8/50, 42/50*, 46/49*, 44/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF1 (F) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 49, 50, 50, 49 29, 30, 21, 22	*Liver* Hepatocellular adenoma: 1/49, 42/50*, 47/50*, 48/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appears to have complied with GLP
		Hepatocellular carcinoma: 3/49, 25/50*, 32/50*, 35/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma: 0/49, 0/50, 4/50, 0/49	NS	
		Hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined): 3/49, 45/50*, 49/50*, 49/49*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
Full carcinogenicity Rat, Crl:CD BR (M) 47 days 2 yrs Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 87, 87, 87, 87 27%, 34%, 40%, 44%	*Liver, testis or mammary gland* No increase in tumour incidence	NS	Principal limitations: survival for all dose groups and controls was < 45% 5 males per group were killed at 2 wks, 3 mo, and 12 mo for evaluation of cell proliferation in the liver; 10 males per group were killed for interim pathological evaluation at 12 mo
Full carcinogenicity Rat, Crl:CD BR (F) 47 days 2 yr Malley et al. (1994)	Inhalation Purity, 99.9% NA 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 87, 87, 87, 87 35%, 23%, 19%, 39%	*Liver or mammary gland* No increase in tumour incidence	NS	Principal limitations: survival for all dose groups and controls was less than 40% Five females per group were killed at 2 wks, 3 mo, and 12 mo for evaluation of cell proliferation in the liver; 10 females per group were killed for interim pathological evaluation at 12 mo

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose regimen No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (M) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 42, 38, 40, 37	*Liver* Hepatocellular adenoma: 1/50, 3/50, 13/50*, 20/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well conducted study that appeared to comply with GLP The authors stated that multiple occurrences of hepatocellular tumours were found in the liver of DMF-exposed rats, in contrast to the occurrence of a single tumour in the liver of the animals of the control group
		Hepatocellular carcinoma: 0/50, 1/50, 0/50, 24/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined): 1/49, 4/50, 13/50*, 33/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	
Full carcinogenicity Rat, F344/DuCrj (F) 6 wks 104 wks Senoh et al. (2004)	Inhalation Purity, > 99.8% NA 0, 200, 400, 800 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 42, 38, 38, 30	*Liver* Hepatocellular adenoma: 1/49, 1/50, 6/50, 16/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	Principal strengths: well-conducted study that appeared to comply with GLP The authors stated that multiple occurrences of hepatocellular tumours were found in the liver of DMF-exposed rats, in contrast to the occurrence of a single tumour in the liver of the animals of the control group
		Hepatocellular carcinoma: 0/49, 0/50, 0/50, 5/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.05$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined): 1/49, 1/50, 6/50, 19/50*	Positive trend: $P < 0.01$, Peto's test; *$P < 0.01$, Fisher exact test	

d, day; DMF, *N*,*N*-dimethylformamide; F, female; GLP, good laboratory practice; M, male; mo, month; NA, not applicable; NS, not significant; ppm, parts per million; wk, week; yr, year

Body weights of all treated males and of females exposed to 800 ppm were decreased by more than 10% compared with controls. The incidence of hepatocellular adenoma (males: 6/50 controls, 36/50 at 200 ppm, 41/49 at 400 ppm, and 41/50 at 800 ppm; females: 1/49 controls, 42/50 at 200 ppm, 47/50 at 400 ppm, and 48/49 at 800 ppm), hepatocellular carcinoma (males: 2/50 controls, 12/50 at 200 ppm, 16/49 at 400 ppm, and 16/50 at 800 ppm; females: 3/49 controls, 25/50 at 200 ppm, 32/50 at 400 ppm, and 35/49 at 800 ppm) and hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in all groups of treated males and females ($P < 0.01$, Fisher's exact test) in a dose-related manner ($P < 0.01$, Peto's trend test). There was also a significant increase in the incidence of hepatoblastoma in males exposed to 200 and 400 ppm (0/50 controls, 13/50* at 200 ppm, 7/49* at 400 ppm and 4/50 at 800 ppm; * $P < 0.01$, Fisher's exact test). The incidence of hepatoblastoma in females was 0/49 controls, 0/50 at 200 ppm, 4/50 at 400 ppm and 0/49 at 800 ppm. Multiple hepatocellular adenomas and carcinomas were found in the livers of treated mice, and the tumour tissues often occupied almost all areas of the entire liver. No *N*,*N*-dimethylformamide-related neoplastic or non-neoplastic lesions were found in any other organ except the liver (Senoh et al., 2004). [The strengths of this study included the use of multiple doses, a large number of animals per group and the testing of two sexes.]

3.2 Rat

3.2.1 Inhalation

See Table 3.1

Groups of 87 male and 87 female Crl:CD BR rats (age, 47 days) were exposed to *N*,*N*-dimethylformamide (purity, 99.9%) by whole-body inhalation at concentrations of 0, 25, 100, and 400 ppm for 6 hours per day on 5 days per week for 2 years. Five male and five females per group were killed at 2 weeks, 3 months, and 12 months to evaluate cell proliferation in the liver, and 10 males and 10 females per group were killed for interim pathological evaluation at 12 months. Exposure to the highest concentration reduced body-weight gain in both sexes. Survival in all treated groups was similar to that of controls; however, survival in all treated groups and controls was less than 45%. The males treated with 100 and 400 ppm and females treated with 400 ppm had higher liver weights relative to body weight at necropsy. No significant increase in tumour incidence was observed in any treated group. The incidence of minimal to mild centrilobular hepatocellular hypertrophy was increased in males and females treated with 100 and 400 ppm (Malley et al., 1994). [The Working Group noted the poor survival of all groups, including controls.]

In a well-conducted study that complied with good laboratory practice, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were exposed to *N*,*N*-dimethylformamide (purity, > 99.8%) by whole-body inhalation at concentrations of 0, 200, 400, and 800 ppm for 6 hours per day on 5 days per week for 104 weeks. Survival rates of treated males did not differ significantly from those of controls. Survival of the females treated with 800 ppm was significantly decreased compared with that of controls after the 9th week. Body weights of the males and females treated with 400 and 800 ppm were decreased by more than 10% compared with their respective controls. Exposure to *N*,*N*-dimethylformamide caused a dose-related increase ($P < 0.01$, Peto's trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in males and females. The incidence of hepatocellular adenoma was significantly increased in male rats exposed to 400 and 800 ppm (1/50 controls, 3/50 at 200 ppm, 13/50* at 400 ppm

Table 3.2 Incidence of hepatocellular tumours in male F344/DuCrlCrj rats exposed to *N,N*-dimethylformamide by inhalation and/or in drinking-water

Parameter	Inhalation (ppm)								
	0			200			400		
	Drinking-water (ppm)								
	0	800	1600	0	800	1600	0	800	1600
Number of rats examined	50	50	50	50	50	50	50	50	50
Surviving animals	41	34	40	36	36	41	37	43	38
Adenoma[a]	1	6*	8*	15*	28**	45**	26*	43**	46**
Carcinoma[a]	0	0	4*	1	6**	14**	2	12**	14**
Adenoma or carcinoma (combined)[a]	1	6*	12*	16*	30**	46**	26*	45**	47**

[a] Number of tumour-bearing animals
* Significantly different from untreated control group, at $P < 0.05$ by chi-square test
** Significantly different from untreated control group, each drinking-water-alone group and each inhalation-alone group with matching concentrations, respectively, at $P < 0.05$ by chi-square test
Adapted from Ohbayashi et al. (2009), *J Toxicol Sci*, 34(1):53–63.

and 20/50* at 800 ppm; *$P < 0.01$, Fisher's exact test) and in females exposed to 800 ppm (1/49 controls, 1/50 at 200 ppm, 6/50 at 400 ppm and 16/50* at 800 ppm; *$P < 0.01$, Fisher's exact test). The incidence of hepatocellular carcinoma was significantly increased in both sexes exposed to 800 ppm (males: 0/50 controls, 1/50 at 200 ppm, 0/50 at 400 ppm, and 24/50* at 800 ppm; females: 0/49 controls, 0/50 at 200 ppm, 0/50 at 400 ppm and 5/50** at 800 ppm; *$P < 0.01$, **$P < 0.05$, Fisher's exact test). Multiple occurrences of hepatocellular tumours were found in the liver of exposed rats and no multiplicity of hepatocellular tumours was observed in the control groups. No *N,N*-dimethylformamide-related neoplastic or non-neoplastic lesions were found in any other organ except the liver (Senoh et al., 2004). [The strengths of this study included the use of multiple doses, a large number of animals per group, and testing in males and females.]

3.2.2 Inhalation and drinking-water (combined)

See Table 3.2

In a well-conducted study that complied with good laboratory practice, groups of 50 male Fischer 344/DuCrlCrj rats (age, 6 weeks) were exposed to *N,N*-dimethylformamide (purity, > 99.5%) by whole-body inhalation at concentrations of 0, 200, and 400 ppm for 6 hours per day on 5 days per week, and given *N,N*-dimethylformamide-formulated drinking-water at 0, 800, or 1600 ppm (w/w) ad libitum for 24 hours per day on 7 days per week for 104 weeks. No significant difference in survival was found between the untreated control group and the two inhalation-only groups, the two oral administration-only groups or the four combined exposure groups. The terminal body weight was significantly decreased in the inhalation-only group, both oral administration-only groups and all four combined exposure groups compared with controls. Exposure to *N,N*-dimethylformamide caused a significant increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in the four combined exposure groups, the two oral administration-only groups and the two inhalation-only groups compared with the untreated controls, a

significant increase in the incidence of hepatocellular carcinoma in the four combined exposure groups and in the oral administration-only group exposed to 1600 ppm compared with the untreated controls, and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in the four combined exposure groups compared with each of the oral administration-only and inhalation-only groups with matching concentrations (see Table 3.2 for tumour incidences and statistics). The incidence of hepatocellular tumours induced by the combined exposures tended to exceed the dose–response relationship that would be expected under the assumption that the incidence of hepatocellular tumours induced by the single-route exposures through inhalation and ingestion are additive [the authors also stated that the combined exposures were found to produce multiple occurrences of hepatocellular adenomas compared with the single-route exposures but did not show these data] (Ohbayashi et al., 2009). [The strengths of this study included the use of multiple doses and a large number of animals per group. The Working Group noted that only one sex was used and that no mention or discussion was made of any observations in any tissue or organs other than the liver.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

(a) Absorption, distribution, and excretion

N,*N*-Dimethylformamide is readily absorbed after inhalation, dermal and oral exposure. The absorption and elimination of *N*,*N*-dimethylformamide was studied after inhalation of vapour (Mráz & Nohová, 1992a), and after percutaneous absorption of both vapour and liquid (Mráz & Nohová, 1992b). In the inhalation study (Mráz & Nohová, 1992a), retention of *N*,*N*-dimethylformamide in the lungs was 90% in 10 volunteers (5 men and 5 women) exposed for 8 hours to concentrations of 10, 30, or 60 mg/m^3. After a single exposure, for the metabolites that accounted for 49% of the dose, the half-lives of urinary excretion and urinary recoveries were: *N*-methylformamide, 4 hours (22%); formamide, 7 hours (13%); and AMCC, 23 hours (13%). A small amount (0.3% of the dose) of unchanged *N*,*N*-dimethylformamide was also detected with a half-life of excretion of 2 hours. With repeated exposure to 30 mg/m^3 for 8 hours per day on 5 consecutive days, a significant accumulation of AMCC was observed with an equilibrium being reached on day 4. [The Working Group noted that *N*-methylformamide and formamide, which were determined by gas chromatography, were at least in part formed from corresponding hydroxymethyl derivatives by thermal decomposure (formaldehyde release) in the hot sample chamber of the gas chromatograph.]

After a 4-hour exposure to *N*,*N*-dimethylformamide vapour (50 mg/m^3), 13–36% of the total dose (depending on air humidity and temperature) was absorbed through the skin in 8 volunteers (3 men and 5 women). Absorption

of liquid *N*,*N*-dimethylformamide was studied (i) by dipping one hand up to the wrist for 2, 10, 15, and 20 minutes; and (ii) by a patch experiment, in which *N*,*N*-dimethylformamide was placed on a patch of teflon foil, which was then attached to the forearm of the volunteers for 8 hours. The rate of percutaneous absorption in the dipping experiment was 9.4 ± 4 mg/cm^2 per hour (mean ± standard deviation (SD); $n = 4$). In the patch experiment, the urinary excretion of metabolites was not delayed compared with the short-term dipping experiments, but the metabolites amounted to only half of those excreted after inhalation at the same absorbed dose levels (Mráz & Nohová, 1992b). A similar value of percutaneous absorption rate (11 mg/cm^2 per hour), based on in-vitro experiments on excised human skin, was reported (Bortsevich, 1984). The same study showed that aqueous *N*,*N*-dimethylformamide is poorly absorbed; < 1% in 4 hours was absorbed from 15% aqueous *N*,*N*-dimethylformamide, whereas absorption of pure liquid *N*,*N*-dimethylformamide was 51% in 4 hours (Bortsevich, 1984). Absorption of *N*,*N*-dimethylformamide vapour through the skin was further evaluated in an experiment on 13 male volunteers exposed for 4 hours through whole-body exposure (but inhaling clean air via a respirator) as well as lung-only inhalation (at intervals of 96 hours or more) to *N*,*N*-dimethylformamide at concentrations below 10 ppm. Based on the comparison of excreted amounts of *N*-methylformamide, it was estimated that the skin and the lung absorption amounted to 40.4% and 59.6%, respectively. The biological half-life of urinary *N*-methylformamide after dermal exposure (4.75 ± 1.63 hours) was significantly longer than that after respiratory exposure (2.42 ± 0.63 hours) (Nomiyama et al., 2001a).

An even higher contribution of skin absorption amounting to 71% of the total dose of *N*,*N*-dimethylformamide was found under conditions of actual occupational exposure by Wang et al. (2007) at airborne concentrations below 10 ppm.

Internal exposure determined by urinary *N*-methylformamide was monitored in two groups of occupationally exposed workers across a working week to determine the total body burden. Twenty-five workers in a synthetic leather factory and 20 workers in a copper laminate circuit board factory were recruited (Chang et al., 2005a). The average airborne concentration of *N*,*N*-dimethylformamide was similar for both groups (about 4 ppm) but dermal exposure to *N*,*N*-dimethylformamide of the synthetic-leather workers was significantly higher. A significant pattern of linear accumulation was found across a 5-day work cycle for synthetic leather workers but not for copper laminate circuit board workers. [The Working Group noted that dermal exposure to *N*,*N*-dimethylformamide over 5 consecutive days can result in its accumulation in the body.]

In a recent study of the percutaneous absorption of *N*,*N*-dimethylformamide in 193 occupationally exposed workers, the mean breathing zone concentrations in both summer and winter were below the occupational exposure limit of 10 ppm. However, the urinary levels of *N*-methylformamide and AMCC were 2.6 and 1.6 times higher, respectively, in the summer (at 34.1 ± 2.6 °C; mean ± SD) than in the winter (at 17.1 ± 4.8 °C; mean ± SD) (Tsuda et al., 2014).

(b) Metabolism

In an early GC study by Kimmerle & Eben (1975a), *N*-methylformamide and formamide were reported to be major urinary metabolites. However, further studies showed that these metabolites were actually HMMF and *N*-hydroxymethylformamide (HMF), respectively, which released formaldehyde under the conditions of GC (in the sample chamber) (Brindley et al., 1983; Scailteur et al., 1984; Kestell et al., 1986). AMCC was identified for the first time in the urine of a volunteer who inhaled

an unspecified dose of *N*,*N*-dimethylformamide (Mráz & Tureček, 1987).

[The Working Group noted that the above mentioned data indicated the formation of electrophilic metabolites, namely methyl isocyanate and/or another *N*-methylcarbamoylating species and formaldehyde.]

More recently, a liquid chromatography-tandem mass spectrometry method was developed for simultaneous determination of HMMF, *N*-methylformamide, and AMCC and was applied to the analysis of 13 urine samples taken from workers occupationally exposed to *N*,*N*-dimethylformamide. The molar excretion ratio of HMMF:*N*-methylformamide:AMCC was approximately 4:1:1. HMMF was confirmed as the most abundant urinary metabolite in humans (Sohn et al., 2005).

Interactions of *N*,*N*-dimethylformamide with ethanol were also studied. In an early study, four volunteers were exposed to *N*,*N*-dimethylformamide at concentrations of 50–80 ppm [152–243 mg/m^3] for 2 hours with or without preceding oral administration of ethanol. Persons who received ethanol excreted slightly elevated urinary concentrations of *N*,*N*-dimethylformamide during the first 4 hours and lower blood levels of *N*-methylformamide (Eben & Kimmerle, 1976).

A haemoglobin adduct of methyl isocyanate, a proposed metabolic intermediate of *N*,*N*-dimethylformamide and *N*-methylformamide, with *N*-terminal valine (*N*-(*N*-methylcarbamoyl)valine) was identified in the blood of 35 workers in the polyacrylic fibre industry exposed to *N*,*N*-dimethylformamide. The exposure was assessed by measuring urinary *N*-methylformamide which was between 1.3 and 46.5 mg/L (mean, 17.0 mg/L; median, 9.8 mg/L). The methyl isocyanate adduct was released by modified Edman degradation and converted to 3-methyl-5-isoprolylhydantoin, the concentrations of which in globin samples ranged from 26.1 to 412.0 nmol/g of globin (Käfferlein & Angerer, 2001). More recently, a new lysine adduct, N_ε-(*N*-methylcarbamoyl)lysine, was identified in globin samples of humans occupationally exposed to *N*,*N*-dimethylformamide (Mráz et al., 2006).

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

The toxicokinetics of *N*,*N*-dimethylformamide in rats, mice and cynomolgus monkeys was extensively studied by Hundley et al. (1993a, b). Male and female cynomolgus monkeys were exposed by whole-body inhalation to *N*,*N*-dimethylformamide at concentrations of 30, 100, or 500 ppm [90, 300, or 1500 mg/m^3] for 6 hours per day on 5 days per week for 13 consecutive weeks. Disproportionate increases were observed in *N*,*N*-dimethylformamide plasma area under the concentration curve (AUC) values of 19–37-fold in male monkeys and 35–54-fold in females as the atmospheric concentrations increased fivefold from 100 to 500 ppm. Plasma half-lives of *N*,*N*-dimethylformamide were 1–2 hours and those of *N*-methylformamide (actually HMMF + *N*-methylformamide) were significantly longer (4–15 hours). Plasma *N*-methylformamide concentrations exceeded those of *N*,*N*-dimethylformamide 0.5 hour after the beginning of each exposure. HMMF formed 56–95% of the urinary metabolites, regardless of exposure level and duration of the study (Hundley et al., 1993b). The AUC values and peak plasma levels for *N*,*N*-dimethylformamide in rats and mice following a single exposure to 500 ppm (Hundley et al., 1993a) were substantially greater than the respective values in monkeys after a similar exposure. In contrast, the enhancement of metabolism by repeated exposure to *N*,*N*-dimethylformamide at 500 ppm, which was observed in rats and mice, was not clearly demonstrated in monkeys (Hundley et al., 1993b).

In another study, cynomolgus monkeys were exposed to *N*,*N*-dimethylformamide at 500 ppm

[1500 mg/m^3] for 6 hours per day on 5 days per week for 2 weeks either by head-only or whole-body inhalation (one monkey per exposure route), plasma samples taken 0.5–18 hours after the first exposure showed *N*,*N*-dimethylformamide AUC values that were three times higher in the monkey exposed by whole-body inhalation, indicating considerable absorption by non-inhalation route(s). The same comparison of plasma samples taken after the final (10th) exposure revealed an *N*,*N*-dimethylformamide AUC value that was sixfold for the monkey exposed by whole-body inhalation (Hurtt et al., 1991).

Rapid absorption was observed in pregnant Sprague-Dawley rats treated on gestation days 12 and 18 with a single oral dose of 100 mg/kg bw [^{14}C]-*N*,*N*-dimethylformamide. The radioactivity in plasma peaked within 1 hour after treatment. A major part of the dose (60–70%) was excreted in the urine and 3–4% in the faeces. Levels of radioactivity in embryonic and fetal tissues were nearly equal to those in maternal plasma up to 8 and 24 hours, respectively, but were higher thereafter. In lactating rats treated with a single oral dose of 100 mg/kg bw [^{14}C]-*N*,*N*-dimethylformamide on lactation day 14, *N*,*N*-dimethylformamide, HMMF, and *N*-methylformamide were found in the milk at concentrations equal to those in the plasma (Saillenfait et al., 1997).

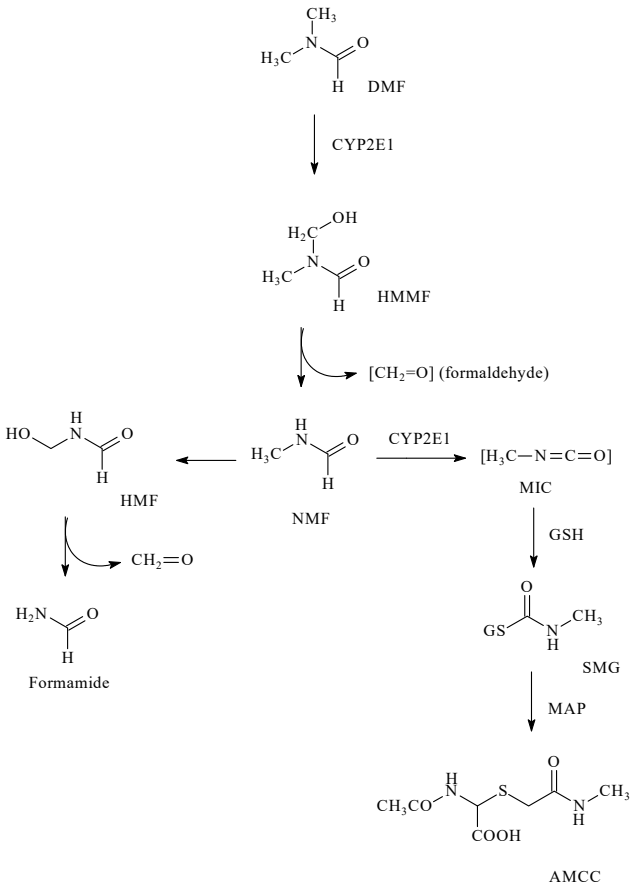
A single ethanol drinking episode significantly enhanced the dermal absorption of *N*,*N*-dimethylformamide in a study using the skin of ethanol-dosed and control Wistar rats to test the penetration of *N*,*N*-dimethylformamide in vitro (Brand et al., 2006).

(b) Metabolism

The metabolism of *N*,*N*-dimethylformamide has been extensively reviewed by Gescher (1993). In both humans and animals, *N*,*N*-dimethylformamide is primarily oxidized by cytochrome P450 (CYP) 2E1 to HMMF. Although relatively stable in neutral or mildly acidic solutions, HMMF may release formaldehyde under physiological conditions to produce *N*-methylformamide. In early studies, *N*-methylformamide was identified by GC as a major metabolite in rats and dogs (Kimmerle & Eben, 1975b). Further metabolic oxidation of *N*-methylformamide leads to HMF, which has been identified as a minor urinary metabolite in rats (Tulip et al., 1989). Unequivocal evidence exists that HMF is formed after the metabolism of *N*-methylformamide in rats and mice (Tulip et al., 1986; Threadgill et al., 1987; Cross et al., 1990). In a close analogy with HMMF, HMF undergoes thermal decomposition to formamide when analysed by GC; therefore, GC data on formamide actually usually include HMF and formamide (Mráz & Tureček, 1987; Mráz et al., 1987; Mráz & Nohová, 1992a).

The mercapturic acid AMCC is formed by a toxicologically relevant pathway via glutathione (GSH) conjugation with a presumed electrophilic *N*-methylcarbamoylating intermediate, probably methyl isocyanate or its chemical equivalent formed by oxidation of *N*-methylformamide and/or HMMF. The GSH conjugate, *S*-(*N*-methylcarbamoyl)glutathione undergoes mercapturic acid pathway to yield AMCC, which is then excreted in the urine. AMCC was found in the urine of rats, mice, Syrian hamsters (Mráz et al., 1989) as well as in human urine (Mráz & Tureček, 1987; Mráz & Nohová, 1992a, b). The pattern of *N*,*N*-dimethylformamide metabolites is qualitatively the same in various rodent species and in humans (Fig. 4.1). Experiments with liver microsomes from rats and mice treated with inducers of CYP2E1 and with CYP2E1 purified from rat and mouse liver microsomes indicated the pivotal role of CYP2E1 in the oxidation of *N*,*N*-dimethylformamide to HMMF as well as in the formation of the key reactive intermediate, which was proposed to be methyl isocyanate (Gescher, 1993; Mráz et al., 1993; Chieli et al., 1995). The key role of CYP2E1 was later confirmed in an in-vitro study on *N*,*N*-dimethylformamide dealkylation. Among several forms of human

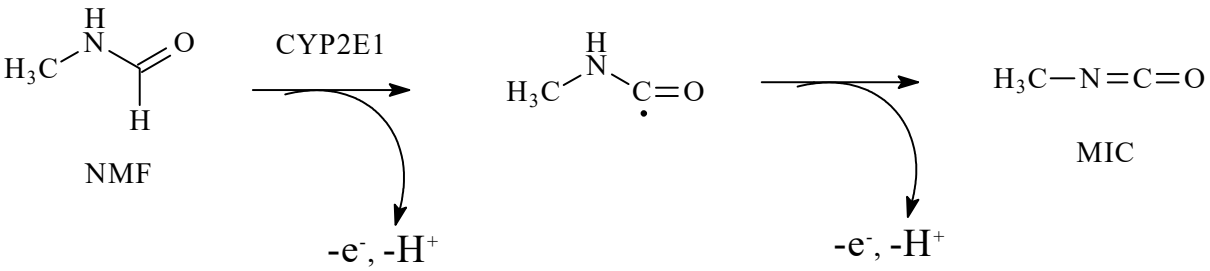
Fig. 4.1 Metabolism of *N*,*N*-dimethylformamide



AMCC, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine; CYP2E1, cytochrome P450 2E1; DMF, *N*,*N*-dimethylformamide; GSH, glutathione; HMF, *N*-hydroxymethylformamide; HMMF, *N*-hydroxymethyl-*N*-methylformamide; MAP, mercapturic acid pathway; MIC, methyl isocyanate; NMF, *N*-methylformamide; SMG, *S*-(*N*-methylcarbamoyl)glutathione
Adapted with permission from Gescher (1993) and Mráz et al. (1993)

recombinant CYPs, namely, CYP1A1, 1A2, 2B6, 2C10, 3A4 and 2E1, only CYP2E1 oxidized *N*,*N*-dimethylformamide (Amato et al., 2001). Methyl isocyanate or its chemically equivalent metabolite is formed via *N*-methylformamide as an intermediate, although HMMF is also a substrate of CYP2E1. However, the amount of *S*-(*N*-methylcarbamoyl)glutathione generated from HMMF in vitro was much smaller than that formed from *N*-methylformamide under identical conditions (Mráz et al., 1993). The mechanism by which the key reactive intermediate is formed was studied by kinetic isotope effect measurements in experiments in mice. The conversion of *N*-methylformamide to urinary AMCC and biliary *S*-(*N*-methylcarbamoyl)glutathione was found to be subject to large primary kinetic isotope effects when hydrogen was replaced by deuterium in the formyl group (k_H/k_D , 4.5 ± 1.0 and 7 ± 2, respectively) indicating the cleavage of the formyl C–H bond is the rate limiting step (Threadgill et al., 1987). The proposed mechanism of the key step in the metabolic activation of *N*,*N*-dimethylformamide through the oxidation of *N*-methylformamide is shown in Figure 4.2. Methyl isocyanate was also reported to be a main

Fig. 4.2 Proposed mechanism of generation of the key electrophilic carbamoylating species, methyl isocyanate



MIC, methyl isocyanate; NMF, *N*-methylformamide
Adapted from Gescher (1993)

product of *N*-methylformamide photo-oxidation with hydroxyl radicals under aerobic conditions and the carbonyl centred radical was proposed as a precursor to methyl isocyanate based on quantum chemical calculations (Bunkan et al., 2015). This observation represents indirect support for the above-mentioned mechanism (Fig. 4.2).

Adducts with globin at *N*-terminal valine and N_{ε}-lysine, *N*-(*N*-methylcarbamoyl)valine and N_{ε}-(*N*-methylcarbamoyl)lysine, respectively, were identified in rats exposed to high doses of *N*,*N*-dimethylformamide (1000 mg/kg bw) (Mráz et al., 2004) as well as in humans exposed to *N*,*N*-dimethylformamide (Mráz et al., 2002, 2006). As cysteine sulfhydryl groups are much stronger nucleophiles than amino acid amine groups, *N*-methylcarbamoylation is likely to occur also at the sulfhydryl groups of cysteine to yield reactive thiocarbamate species capable of transferring the *N*-methylcarbamoyl moiety to other nucleophilic sites (transcarbamoylation). [The Working Group noted that, due to the reactivity of the thiocarbamate moiety, *N*-methylcarbamoylated cysteine residues may have easily escaped detection in the analyses of adducted globin. The *N*-methylcarbamoylating species, either methyl isocyanate or a *N*-methylcarbamoylated cysteine residue, may carbamoylate nucleophilic sites in proteins and in the DNA.]

The effects of the co-administration of ethanol and *N*,*N*-dimethylformamide on the metabolism of *N*,*N*-dimethylformamide, *N*-methylformamide and ethanol were investigated in several early studies. Increases in the concentrations of *N*,*N*-dimethylformamide, *N*-methylformamide, ethanol or acetaldehyde in blood were observed after co-exposure. These results were attributed to the inhibition by *N*,*N*-dimethylformamide of the activity of alcohol dehydrogenase observed both in vitro and in vivo (Eben & Kimmerle, 1976; Hanasono et al., 1977; Sharkawi, 1979) and of aldehyde dehydrogenase observed in vivo (Elovaara et al., 1983). The effect of the competitive inhibition of CYP2E1, which seems to be very probable in the light of more recent observations (Mráz et al., 1993; Chieli et al., 1995), was not considered at the time of these early studies.

In conclusion, three metabolic events appear to play key roles in *N*,*N*-dimethylformamide toxicity: (i) *N*-methylcarbamoylation mediated by methyl isocyanate and/or its chemical equivalents; (ii) free radical damage caused by carbamoyl radicals; and (iii) the probable formation of formaldehyde by oxidative demethylation (Fig. 4.1 and Fig. 4.2). The main enzyme responsible for metabolic activation is CYP2E1.

4.2 Mechanisms of carcinogenesis

The evidence on the key characteristics of carcinogens (Smith et al., 2016), concerning whether *N*,*N*-dimethylformamide induces oxidative stress; alters cell proliferation, cell death, or nutrient supply; is genotoxic; or modulates receptor-mediated effects – is summarized below.

4.2.1 Oxidative stress

(a) Humans

(i) Exposed humans

N,*N*-Dimethylformamide-exposed Chinese workers (n = 104) and 101 controls were studied for oxidative and antioxidative status. The *N*,*N*-dimethylformamide concentration in workplace air was within the range of 3.3–12.4 mg/m^3. The *N*-methyl-carbamoylated haemoglobin adduct (NMHb) in blood was chosen as a biomarker measured as the Edman degradation product, 3-methyl-5-isopropylhydantoin (MVH). The MVH level in exposed workers was 19.69 ± 12.52 mg/kg, and MVH was not detected in the control group. The activity of superoxide dismutase (SOD) in exposed workers (125.30 ± 21.23 U/mL) was significantly higher than the control group (118.35 ± 18.48 U/mL). However, the activity of SOD showed different trends with increasing MVH levels. When MVH ≤ 24 mg/kg, the SOD activity increased with the increasing of MVH level. When MVH > 24 mg/kg, SOD activity decreased with increasing MVH level. No significant differences were observed in glutathione-*S*-transferase, malondialdehyde or 3-nitrotyrosine levels among the two groups. It was concluded that *N*,*N*-dimethylformamide exposure did not cause obvious lipid and/or protein peroxidative damage (Cheng et al., 2014).

(ii) Human cells in vitro

HL-7702 normal human liver cells were used to study *N*,*N*-dimethylformamide-induced oxidative stress. Reactive oxygen species (ROS)-induced fluorescence was determined by flow cytometry using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) and propidium iodide to identify viable cells. ROS levels increased with *N*,*N*-dimethylformamide dose (0–40 mM) to a maximum of ~1.5-fold in cells treated for 24 hours. The levels of 8-hydroxy-2′-deoxyguanosine measured by enzyme-linked immunosorbent assay (ELISA) increased to ~1.5-fold after incubation with *N*,*N*-dimethylformamide at 40 and 100 mM for 24 hours (Wang et al., 2015).

N,*N*-Dimethylformamide (60 mM) induced differentiation of human HL-60 promyelocytic leukaemia cells, and these cells acquired polymorphonuclear leukocyte functions and the ability to generate ROS when stimulated (Speier & Newburger, 1986). HL60 cells are known to contain CYP2E1 (Nagai et al., 2002), which can metabolize *N*,*N*-dimethylformamide. Treatment of HL-60 cells with *N*,*N*-dimethylformamide resulted in a decline in total SOD, while catalase activity declined slightly. Oxidized glutathione (GSSG) reductase activity and reduced glutathione (GSH) and GSSG levels declined slightly over time. Concomitantly with the decrease in antioxidant enzymes during the course of *N*,*N*-dimethylformamide-induced differentiation of human HL-60 cells, treatment with phorbol myristate acetate (PMA, an ROS stimulating agent) increased the ability of the cells to generate hydrogen peroxide (Speier & Newburger, 1986). At a higher dose, *N*,*N*-dimethylformamide (80 mM) induced granulocytic cell differentiation of human HL-60 promyelocytic leukaemia and PLB-985 human myeloid leukaemia cells. *N*,*N*-Dimethylformamide (80 mM) treatment increased glutathione peroxidase enzymatic activity, gene expression (mRNA) and protein levels in HL60 and PLB-985 cells (Shen et al., 1994).

N,*N*-Dimethylformamide (0.5%) induced granulocytic differentiation in PLB-985 human myelomonoblastic leukaemia cells after 6 days of treatment. Further treatment with PMA (1 μM) and formyl-methionyl-leucine phenylalanine (800 nM), ROS stimulating agents, induced ROS detected by nitroblue tetrazolium staining in the cells (Katschinski et al., 1999).

Both *N*,*N*-dimethylformamide and its metabolite *N*-methylformamide reduced total GSH levels in a concentration–response manner (0–200 mM) after incubation in DLD-1 Clone A human colon carcinoma cells (Cordeiro & Savarese, 1984). In a follow-up study using the same experimental conditions, both reduced GSH and GSSG were measured separately. In concentration–response studies with both *N*,*N*-dimethylformamide and *N*-methylformamide, it was found that that the levels of GSSG remained relatively constant over 0–200 mM, while the levels of reduced GSH decreased with increasing concentrations of *N*,*N*-dimethylformamide or *N*-methylformamide (Cordeiro & Savarese, 1986).

(b) Non-human mammalian systems

(i) In vivo

N,*N*-Dimethylformamide treatment of Sprague-Dawley rats after intraperitoneal injection (500 mg/kg bw per day for three consecutive days) had no effect on the hepatic levels of thiobarbituric acid reactive substances (TBARS). *N*,*N*-Dimethylformamide decreased the hepatic GSH content to 61% of control values (Kim et al., 2010).

Malondialdehyde (MDA) levels (measured as TBARS) increased four- to ninefold in the liver, and four- to sevenfold in the kidney of male Wistar rats, 24 and 48 hours after intraperitoneal injection with a single dose of *N*,*N*-dimethylformamide (1.5 g/kg bw). The activities of the hepatic antioxidant enzymes decreased at both time-points: SOD (~50%), catalase (~50%), and glutathione peroxidase (~58%). The hepatic levels of reduced GSH and vitamin C also decreased 55% and ~62%, respectively. The levels of SOD, catalase, glutathione peroxidase, reduced GSH, and vitamin C each decreased 40–60% in the kidney (Jyothi et al., 2012). In a spectroscopic and modelling study, Kalyani et al. (2014) reported a destabilization of SOD, which might explain the decreases in activity reported by Jyothi et al. (2012).

ICR mice (both sexes) treated with *N*,*N*-dimethylformamide daily by gavage for 90 days had significant increases in MDA levels measured as TBARS in heart homogenates and in liver homogenates (at the 0.32, 0.63, and 1.26 g/kg bw dose levels) compared with controls. SOD activity levels decreased significantly in heart homogenates and liver homogenates (at the 0.63 and 1.26 g/kg bw dose levels) compared with controls. GSH levels decreased only in liver homogenates at 0.32, 0.63, and 1.26 g/kg bw (Rui et al., 2011).

Male Sprague-Dawley rats were dosed with *N*-methylformamide or *N*,*N*-dimethylformamide by intraperitoneal injection (1 mL/kg bw). Only *N*-methylformamide depleted the hepatic GSH level by about 30% 1 hour after injection. Twenty-four hours after the treatment, the GSH level returned to the control value (Scailteur & Lauwerys, 1984).

GSH levels were measured in liver, kidney, heart, lung and spleen tissues from male CBA/CA mice dosed with single doses of *N*-methylformamide (400 mg/kg bw), *N*-ethylformamide (495 mg/kg bw) or formamide (dose not reported) after 1 hour. *N*-Methylformamide, but not *N*-ethylformamide or formamide, decreased hepatic GSH levels by 59.8%. Of the other tissues investigated only the kidneys exhibited GSH depletion (21.6%). In a dose–response study, hepatic GSH levels in male BALB/c mice decreased with increasing dosage of *N*-methylformamide (0–400 mg/kg bw) 1 hour after intra-

peritoneal injection of *N*-methylformamide (Gescher et al., 1982).

N-Methylformamide (200 mg/kg bw) injected intraperitoneally into BALB/c mice depleted total hepatic GSH to 21% of control levels 2 hours after administration and induced hepatotoxicity. In CBA/CA and BDF1 mice, the same dose of *N*-methylformamide depleted total hepatic GSH levels to 53% of control levels and did not cause hepatotoxicity (Pearson et al., 1987).

N-Methylformamide treatment of male BALB/c mice (single intraperitoneal injection of 50–200 mg/kg bw) induced a dose-dependent decrease in hepatic non-protein sulfhydryls (reduced GSH is the major non-protein sulfhydryl in cells). Higher doses (up to 800 mg/kg bw) did not induce a further decline. A time-course study revealed that 4 hours after a dose of 300 mg/kg bw, non-protein sulfhydryls were reduced to 25% of control values. *N*-Methylformamide did not significantly decrease the levels of hepatic non-protein sulfhydryls in male Sprague-Dawley rats 4 hours after an intraperitoneal injection of 1000 mg/kg bw (Tulip & Timbrell, 1988).

Changes in gene expression were examined in the livers of male BALB/c mice dosed with a single intraperitoneal injection (300 mg/kg bw) of *N*-methylformamide. Comparison of the gene expression patterns of *N*-methylformamide versus saline control identified a series of significantly altered genes associated with oxidative stress (e.g. upregulation of aldehyde oxidase 1 *Aox1,* heat shock protein *Hspb1, Hspa8,* and *Hsp105*) (Mutlib et al., 2006).

N,*N*-dimethylformamide induced CYP2E1 protein levels in liver microsomes from male Sprague Dawley rats after intraperitoneal injection of *N*,*N*-dimethylformamide at 0, 450, 900 or 1800 mg/kg bw once per day for 3 days. The activities of *p*-nitrophenylhydroxylase (a measure of CYP2E1 enzymatic activity) in the *N*,*N*-dimethylformamide-treated groups were significantly higher than that of the control group after 3 days of treatment. Under the same experimental conditions there were no significant increases in CYP1A1 or CYP2B1/2 proteins or increases in their associated enzymes ethoxyresorufin-*O*-deethylase (EROD) or pentoxyresorufin-*O*-dealkylase (PROD) activities, respectively (Koh et al., 1999). The effects of *N*,*N*-dimethylformamide on the induction of CYP2E1 protein levels, *p*-nitrophenylhydroxylase, glutathione *S*-transferase and glutathione peroxidase activities were studied in the livers of male Sprague-Dawley rats after intraperitoneal injection of *N*,*N*-dimethylformamide at 0, 450, 900, or 1800 mg/kg bw once per day for 3 days. The levels of CYP2E1 analysed by Western immunoblot increased and *p*-nitrophenylhydroxylase activity increased ~3.7-fold after 3 days of treatment. Glutathione *S*-transferase levels increased slightly with *N*,*N*-dimethylformamide treatment while *N*,*N*-dimethylformamide had no effect on glutathione peroxidase levels (Kim & Chung, 2013). In another report, CYP2E1 protein was induced ~1.5-fold in liver homogenates from rats treated with *N*,*N*-dimethylformamide (500 mg/kg bw daily for 3 days) (Kim & Kim, 2011). It has been reported that CYP2E1 induction can lead to the over production of ROS and oxidative stress (Caro & Cederbaum, 2004; Gonzalez, 2005). [A possible explanation for the ability of *N*,*N*-dimethylformamide to induce hepatotoxicity is the production of ROS by *N*,*N*-dimethylformamide through CYP2E1 induction and uncoupling of the catalytic cycle (release or escape of the superoxide anion radical during the course of the P450 catalytic cycle) (Gonzalez, 2005) combined with *N*,*N*-dimethylformamide and the depletion of GSH by *N*-methylformamide.]

(ii) In vitro

N-Methylformamide decreased total intracellular GSH levels in murine TLX5 lymphoma cells in a concentration-related fashion up to 160 mM after a 48-hour incubation (Bill et al., 1988)

(c) Non-mammalian systems

N,*N*-Dimethylformamide induced oxidative stress in the proteobacteria, *Paracoccus* sp. SKG. Glutathione *S*-transferase, catalase, and SOD activities increased in a concentration–response manner. Cellular levels of methionine as well as protein levels of the enzyme methionine sulfoxide reductase, which catalyses the reduction of both free and protein-bound methionine sulfoxide residues back to methionine (Ezraty et al., 2005) were increased (Kirankumar et al., 2014).

[The Working Group noted that both *N*,*N*-dimethylformamide and *N*-methylformamide have been shown to alter indices of oxidative stress. *N*,*N*-Dimethylformamide induced ROS in human liver cells and increased malondialdehyde levels in the livers of mice and rats. *N*,*N*-dimethylformamide altered the levels of glutathione peroxidase and SOD in human tumour cells and lowered the levels of hepatic GSH in the livers of mice and rats. *N*-Methylformamide reduced the levels of GSH in tumour cells and in the livers of mice and rats and increased the expression of genes associated with oxidative stress in the livers of treated mice.]

4.2.2 Altered cell proliferation or death

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human tumour cells implanted into experimental animals

N,*N*-Dimethylformamide and *N*-methylformamide inhibited the growth of two human colon cancer cell lines xenografted into female nude mice. Mice received subcutaneous transplants of HCT-15 or DLD-2 human colon cancer cells and were treated intraperitoneally with *N*,*N*-dimethylformamide or *N*-methylformamide, 2219 and 374 mg/kg bw given daily for 21 days, respectively. HCT-15 tumour growth was inhibited 65% by *N*,*N*-dimethylformamide compared with controls. In two independent experiments with *N*,*N*-dimethylformamide, DLD-2 tumour growth was inhibited by 45 and 67%. *N*-Methylformamide treatment produced 43 and 75% growth inhibition of HCT-15 and DLD-2 tumours, respectively (Dexter et al., 1982). Nude mice (15 mice per group) were subcutaneously injected with SHG-44 human glioma cells grown in the absence or presence of *N*,*N*-dimethylformamide. Pretreatment of SHG-44 cells with *N*,*N*-dimethylformamide reduced the growth rate of the xenografts (Li et al., 1997).

CD-1 male nude mice (eight mice per group) were injected subcutaneously with human colon carcinoma HT-29 cells or HT-29 cells grown in medium containing 1% (170 mM) *N*-methylformamide (designated HT29-NMF cells) inducing cell differentiation. HT29-NMF cells had an increased latency period in the appearance of palpable tumours compared with HT-29 cells and tumour weights were lower in mice carrying the HT29-NMF cell xenographs compared with mice carrying the HT29 cell xenographs, indicating slower growth induced by *N*-methylformamide (D'Agnano et al., 1992).

(iii) Human cells in vitro

N,*N*-Dimethylformamide has been shown to inhibit the growth of human tumour cells in culture in many studies.

The growth of RKO colon carcinoma cells (Brattain et al., 1984) treated with *N*,*N*-dimethylformamide (0.7%) resulted in an 88% reduction in cell number compared with untreated control cells (Zipfel et al., 1993). Similarly, cell proliferation of MOSER human colon carcinoma cells, transforming growth factor-β (TGF-β)-resistant MOSER human colon carcinoma cells and HCT 116 human colon carcinoma cells was reduced by 1%

N,*N*-dimethylformamide; there was essentially minimal cell growth for each cell line (Mulder et al., 1988).

DLD-1 and HCT-15 human colon carcinoma cell lines and several subclones of DLD-1 cells (Clone A, Clone D) were treated with *N*,*N*-dimethylformamide (0.8%) which induced morphological changes in the cells and alterations in their growth properties. *N*,*N*-Dimethylformamide also induced differentiation and maturation to a less malignant phenotype (Dexter et al., 1979; Dexter & Hager, 1980). Growth in the presence of *N*,*N*-dimethylformamide also caused a marked reduction in the tumorigenicity of the cells in vivo. While 20 of 20 nude mice injected with DLD-1 cells developed tumours in 10–14 days, only one nude mouse out of ten inoculated with *N*,*N*-dimethylformamide-treated DLD-1 cells developed a tumour in the third month after the injection (Dexter et al., 1979; Dexter & Hager, 1980).

N-Methylformamide increased the doubling times of DLD-1 Clone A cells in culture in a concentration–response fashion (Dibner et al., 1985). *N*-Methylformamide (1%, 170 mM) treatment of human HT-29 colon carcinoma cells induced cell differentiation and reduced cell proliferation. *N*-Methylformamide treatment induced a G_0/G_1 phase accumulation, with a higher percentage of treated cells in G_0/G_1 phase compared with controls (D'Agnano et al., 1992). *N*,*N*-Dimethylformamide (0.25, 0.5, 0.75, and 1%) produced a concentration-dependent inhibitory effect on cell proliferation in monolayers of human glioma SHG-44 cells Li et al. (1997). The percentage of DNA fragmentation and apoptotic nuclei increased in cultures of human colonic carcinoma HT-29 cells after treatment with *N*,*N*-dimethylformamide (1%) for 72 hours (Heerdt et al., 1996).

The effects of *N*,*N*-dimethylformamide on hyperthermia-induced apoptosis, DNA fragmentation and cell cycle effects were studied in PLB-985 human myelomonoblastic leukaemia cells. Hyperthermia (42 or 43 °C, 1 hour) induced apoptosis and DNA fragmentation in PLB-985 cells and led to a decrease in the number of G_0/G_1 cells as determined by flow cytometry. *N*,*N*-Dimethylformamide (0.5%) treatment of these cells caused differentiation along the granulocytic pathway and prevented the induction of apoptotic death (Katschinski et al., 1999).

In the normal human liver cell line, HL-7702, *N*,*N*-dimethylformamide (0, 50, 100, 150, and 200 mM) for 12 hours increased the apoptotic rate of hepatocytes in a concentration-related manner as measured by flow cytometry. *N*,*N*-Dimethylformamide induced a steady decrease of the expression of the anti-apoptotic Bcl-2 protein shown by Western blotting and its level was lower than the control group. The expression of the pro-apoptotic Bax protein showed no significant differences between the different *N*,*N*-dimethylformamide concentration groups. Increasing the *N*,*N*-dimethylformamide concentration decreased the ratio of Bcl-2/Bax; at 200 mM this ratio was significantly lower than that of the controls. Procaspase-3 protein was observed at 150 and 200 mM, which demonstrated that there was active caspase-3 (Lu et al., 2008).

(b) Non-human mammalian systems

(i) In vivo

Dose related increases in cell proliferation were seen in male F344/DuCrlCrj rats (SPF) exposed to *N*,*N*-dimethylformamide by both inhalation (0, 200, or 400 ppm for 6 hours per day, 5 days per week, for 4 weeks) and in drinking-water (0, 800, 1600, or 3200 ppm for 24 hours per day, 7 days per week, for 4 weeks). Maximal induction up to 9.7-fold was reported at the 200 ppm inhalation/3200 ppm oral dose level. Rats treated with *N*,*N*-dimethylformamide only by inhalation or only by oral dosing also showed

modest dose-related increases in cell proliferation (Ohbayashi et al., 2008).

The effects of *N*,*N*-dimethylformamide on several hepatic enzymes associated with apoptosis were investigated in Sprague-Dawley rats after intraperitoneal injections of 500 mg/kg bw per day for 3 consecutive days. *N*,*N*-Dimethylformamide induced a marginal increase in the ratio of the protein levels of Bax to Bcl-xL (an apoptosis inhibitor), and had no effects on poly (ADP-ribose) polymerase (PARP) cleavage or caspase-3 activity (Kim et al., 2010).

Fourteen of 17 CE/J mice receiving intraperitoneal injections of murine rhabdomyosarcoma cells (pre-cultured in media containing *N*,*N*-dimethylformamide (1%) for 10 days) did not develop tumours after 6 months. This was compared with 21 mice receiving intraperitoneal injections of untreated rhabdomyosarcoma cells, which died of tumour between 11 and 31 days (Dexter, 1977).

The growth of murine M5076 ovarian sarcoma cells injected intramuscularly into female BDF_1 mice was inhibited by daily intraperitoneal injections of *N*-methylformamide (25, 50, 100, or 200 mg/kg bw) for 17 days. In similar types of studies, formamide (200 mg/kg bw) or *N*-methylformamide (200 mg/kg bw) administered to female BDF_1 mice daily during the course of 17 days markedly reduced the tumour volume of M5076 tumours. Daily dosing with *N*-methylformamide (300 mg/kg bw) or formamide (300 mg/kg bw) for 9 days by intraperitoneal injections reduced murine sarcoma 180 tumour volume in female BDF_1 mice injected with this tumour (Gescher et al., 1982).

N-Methylformamide (300 mg/kg bw) administered intraperitoneally daily for 6 days slowed the growth of FSA fibrosarcoma, and HCA-I hepatocarcinoma tumour xenographs and totally inhibited the growth of the MCA-K mammary carcinoma tumour xenographs in CH_3Hf/Kam mice. However, the effects were transient; tumours resumed their pretreatment growth rate upon cessation of the treatment (Iwakawa et al., 1987b). In a similar study, CH_3Hf/Kam mice were injected intramuscularly with a single-cell suspension of the FSA fibrosarcoma. Treatment with *N*-methylformamide (300 mg/kg bw) only slightly inhibited the tumour growth when administered for 8 days (Iwakawa et al., 1987a).

Changes in gene expression associated with cell proliferation were reported in the livers of male BALB/c mice dosed with a single intraperitoneal injection (300 mg/kg bw) of *N*-methylformamide (Mutlib et al., 2006).

(ii) In vitro

N,*N*-Dimethylformamide (1%, 170 mM) decreased the growth of both murine rhabdomyosarcoma cells (Dexter, 1977), AKR-2B mouse embryo fibroblast cells and their methylcholanthrene-transformed counterpart AKR-MCA cells in culture (Hoosein et al., 1988).

N-Methylformamide (43 to 170 mM) decreased the growth of TLX5 murine lymphoma cells and changed the distribution of these cells in the cell cycle determined by flow cytofluorimetry, increasing the proportion of G_1 cells and reducing the proportion of cells in the S and G_2/M phases. *N*-Methylformamide (0–160 mM) also inhibited the ability of TLX5 cells to grow in soft agar (Bill et al., 1988).

N-Methylformamide (0.5 or 1%) reduced the clonogenicity of MCA-K (mammary carcinoma), but not FSA (fibrosarcoma) or HCA-I cells (hepatocarcinoma) in vitro (Iwakawa et al., 1987b).

One study reported that *N*,*N*-dimethylformamide increased apoptosis. Treatment of immortalized Rat-1 fibroblasts in monolayer with *N*,*N*-dimethylformamide at 50–175 mM for 24 hours induced cell death by apoptosis with early apoptotic changes observed in the cells, cell shrinkage with nuclear condensation, and the formation of membrane and DNA fragmentation (Boyle & Hickman, 1997).

[The Working Group noted that *N*,*N*-dimethylformamide and *N*-methylformamide have been used in preclinical cancer chemotherapeutic trials. Experimental studies showed that *N*,*N*-dimethylformamide and *N*-methylformamide inhibited the growth of cancer cells in culture, and cancer cell lines implanted into mice. In these studies, cancer cells were cultured in the presence of *N*,*N*-dimethylformamide or *N*-methylformamide and injected into mice, or mice carrying cancer xenographs were dosed with *N*,*N*-dimethylformamide or *N*-methylformamide. *N*,*N*-Dimethylformamide also altered the malignant phenotype of cancer cells and reduced cell growth in vitro. One study in rats showed that *N*,*N*-dimethylformamide increased hepatic cell proliferation. *N*,*N*-Dimethylformamide increased apoptosis in tumour cells and in a normal human liver cell line.]

4.2.3 Genetic and related effects

(a) Humans

(i) Exposed humans

See Table 4.1

In the analytical study of Hennebrüder & Angerer (2005), urine samples taken from male workers exposed to *N*,*N*-dimethylformamide vapours (exposure level not given) were analysed by a sensitive LC/MS method for DNA adducts. N^4-(*N*-methylcarbamoyl)cytosine (N4-NMCC), a DNA adduct, which was already known to be formed from MIC (Segal et al., 1989), a probable metabolic intermediate of *N*,*N*-dimethylformamide, was detected. In 10/32 urine samples collected from occupationally exposed subjects N4-NMCC was detected in concentrations of 31–172 ng/L (detection limit was 8 ng/L). No N4-NMCC was detected in urine samples taken from 24 subjects with no record of *N*,*N*-dimethylformamide exposure (Hennebrüder & Angerer, 2005).

Significant differences in the comet extent, tail extent, Olive tail moment, and tail DNA/total DNA (%) were reported by Chen (2004) in workers who were occupationally exposed to *N*,*N*-dimethylformamide (6–7 years of exposure) as compared with controls ($P < 0.05$).

Changes in mitochondrial DNA in blood leukocytes, namely, a common deletion $\Delta mtDNA^{4977}$ and mtDNA copy numbers, were reported in a study on 13 male synthetic-leather factory workers. Exposure to *N*,*N*-dimethylformamide was followed by air sampling (median, 10.59 ppm; range, 6.65–34.38 ppm), as well as by urine analysis for the biomarkers "NMF" (actually HMMF+NMF; median, 13.77 mg/L; range, 7.47–73.64 mg/L) and AMCC (median, 40.70 mg/L; range, 6.76–442.24 mg/L). The values found in subjects exposed to *N*,*N*-dimethylformamide were significantly higher than those in controls, which were matched by age, seniority, smoking, and alcohol drinking habits. Moreover, both parameters appeared to be exposure-dependent when subjects exposed to *N*,*N*-dimethylformamide at concentrations higher and lower than 10 ppm (permissible exposure limit) were compared. The frequencies of $\Delta mtDNA^{4977}$ were significantly elevated also in subjects with high urinary levels of AMCC (≥ 40 mg/L). On the other hand elevated mtDNA copy number was found in subjects with relatively high urinary "NMF" concentrations (≥ 15 mg/L) considered as a short-term marker of exposure to *N*,*N*-dimethylformamide (Shieh et al., 2007).

Rates of sister-chromatid exchange were determined in peripheral blood lymphocytes of 22 non-smoking women exposed to *N*,*N*-dimethylformamide working in a synthetic-leather manufacturing plant for 1.1–9.9 years. The exposed group was divided into three subgroups according to the exposure level, which was 0.3, 0.7, and 5.8 ppm (time-weighted average, TWA) in the low, intermediate, and high exposure group, respectively. A co-exposure by low

Table 4.1 Genetic and related effects of *N,N*-dimethylformamide in exposed humans

Biological sample	Description of exposed and controls	End-point	Test	Results	Agent, dose (LED or HID)	Comments	Reference
Urine	32 male workers in acrylic fibre industry and 24 control from general population	DNA damage	N^4-(*N*-methylcarbamoyl) cytosine by LC-ESI-MS/MS	+	DMF		Hennebrüder & Angerer (2005)
Peripheral blood cells	Workers with 6–7 yrs of exposure to DMF	DNA damage	DNA strand breaks (comet assay)	+ ($P < 0.05$)	DMF		Chen (2004)
Leukocytes	13 male synthetic leather factory workers; 13 control subjects from the administrative department of the same factory	DNA damage	End-point deletion ΔmtDNA^{4977}, mitochondrial mtDNA copy number	+	DMF	Smoking taken into account	Shieh et al. (2007)
Lymphocytes	22 non-smoking women; synthetic leather production, 3 exposure groups; 22 sex-, age- and residence-matched controls	Chromosomal damage	Sister-chromatid exchange	+ ($P < 0.01$)	DMF + toluene, 0.7 ppm (TWA)	Exposure-related effect; co-exposure with toluene in the intermediate exposure group	Seiji et al. (1992)
Lymphocytes	26 male viscose rayon plant workers; 26 matched controls and 6 industrial controls (administrative workers)	DNA and chromosomal damage	UDS, chromosomal aberrations, aneuploidy, sister-chromatid exchange	(+) ($P < 0.01$)	DMF + acetonitrile	Effect of smoking separated; effects of DMF and acrylonitrile not separated [synergism is likely]	Major et al. (1998)
Lymphocytes	40 occupationally exposed workers, 31 unexposed persons	Chromosomal damage	Chromosomal aberrations	(+) $P < 0.05$	DMF, 150 mg/m^3 (TWA)	Smoking not reported	Koudela & Spazier (1981)
Lymphocytes	56 workers in printed circuit-board manufacture; no unexposed controls	Chromosomal damage	Sister-chromatid exchange	(–)	DMF + epichlorohydrin, 33.6 ± 3.9 ppm, long-term	No association with DMF exposure but strong association with exposure to epichlorohydrin	Cheng et al. (1999)
Lymphocytes	29 workers synthetic leather manufacture; no unexposed controls	Chromosomal damage	Sister-chromatid exchange	(–)	DMF, 34.6 ± 6.3 ppm, long-term	Smoking and GST genotypes taken into account	Cheng et al. (1999)

+, positive; (+) or (–), positive or negative in a study with limited quality; DMF, *N,N*-dimethylformamide; GST, glutathione *S*-transferases; HID, highest ineffective dose; LC-ESI-MS/MS, liquid chromatography and electrospray ionization mass spectrometry; LED, lowest effective dose; NMF, *N*-methylformamide; TWA, time-weighted average; UDS, unscheduled DNA synthesis; yr, year

concentrations of toluene (0.9 ppm, TWA) was reported for the intermediate exposure group but not for other groups. Age-, sex-, and residence-matched controls from the same factory (administrative staff) were used. A dose-dependent increase in sister-chromatid exchanges was found in the exposed group (rates of 8.26, 7.23, and 5.67 for the high, intermediate, and low exposure subgroup, respectively) and the mean of exposed group was significantly higher than that of controls ($P < 0.01$). However, the mean sister-chromatid exchange rate value in the low exposure group appeared to be lower than that in controls ($P < 0.05$) (Seiji et al., 1992).

Two other field studies in humans exposed to *N*,*N*-dimethylformamide in vivo found increases in chromosomal aberrations (Major et al., 1998; Koudela & Spazier, 1981), aneuploidy, sister-chromatid exchange, and unscheduled DNA synthesis (Major et al., 1998). [The Working Group noted that the genotoxic effects observed may not be related to *N*,*N*-dimethylformamide exposure because of co-exposure with high concentrations of acrylonitrile (Major et al. 1998), and because no data were available on smoking and co-exposure with numerous other chemicals occurring at the workplace (Koudela & Spazier, 1981).]

Cheng et al. (1999) studied sister-chromatid exchange in peripheral blood lymphocytes of 85 plant workers occupationally exposed to *N*,*N*-dimethylformamide and/or epichlorohydrine. Airborne *N*,*N*-dimethylformamide concentrations during the work-shifts ranged from 1 to 83 ppm (TWA). An association between sister-chromatid exchange frequencies and epichlorohydrin exposure levels, but not *N*,*N*-dimethylformamide exposure levels, was found. No unexposed control group was used and the exposure history of the subjects studied was not reported.

(ii) Human cells in vitro

See Table 4.2

In vitro, *N*,*N*-dimethylformamide gave negative results in a human hepatocyte primary culture DNA repair assay based on autoradiographic detection of unscheduled DNA synthesis at concentrations up to 10 mM (McQueen et al., 1988).

Positive results were reported when phosphorylated histone H2AX (γH2AX) was used as a biomarker of double-strand breaks in human liver cell DNA treated with *N*,*N*-dimethylformamide (1.5–100 mM) (Xuan et al., 2008; Wang et al., 2015).

The levels of 8-hydroxy-2′-deoxyguanosine were increased 1.5-fold in human liver cells HL-7702 exposed to *N*,*N*-dimethylformamide at 40 and 100 mM (Wang et al., 2015).

Negative results for *N*,*N*-dimethylformamide (up to 10 mM, 731 μg/mL) were obtained in a study of genotoxic insult evaluated by GreenScreen HC *GADD45α-GFP* genotoxicity assay (Hastwell et al., 2006).

No increase in the frequency of sister-chromatid exchange was observed in human lymphocytes incubated in vitro with *N*,*N*-dimethylformamide at concentrations up to 1.1 M for 24, 48, or 72 hours (Antoine et al., 1983).

(b) Experimental systems

See Table 4.3

Studies on genetic and related effects in various experimental systems in vivo (e.g. rats, mice, and Syrian hamsters) and in vitro (including human lymphocytes, rat and mouse hepatocytes, mouse lymphoma cells, Chinese hamster ovary cells, yeast, *Salmonella typhimurium*, and *Escherichia coli*) were comprehensively reviewed in a previous *IARC Monograph*, when the data on *N*,*N*-dimethylformamide were evaluated by the Working Group in 1999. At the time, the results were mostly negative or inconclusive, and did not provide evidence supporting a genotoxic mechanism (IARC, 1999). For instance, *N*,*N*-dimethylformamide gave negative results

Table 4.2 Genetic and related effects of *N,N*-dimethylformamide in human cells in vitro

Species	Cell type	End-point	Test	Results	Dose (LED or HID)	Reference
Human	Hepatocytes	DNA damage	UDS	–	10 mM [731 µg/mL]	McQueen et al. (1988)
Human	Hepatocytes	DNA damage	γH2AX	+	1.56 mM [114 µg/mL]	Xuan et al. (2008)
Human	Liver HL-7702 cells	DNA damage	γH2AX	+	6.4 mM [468 µg/mL]	Wang et al. (2015)
Human	Liver HL-7702 cells	DNA damage	8-OHdG (ELISA)	+	40 mM [2924 µg/mL]	Wang et al. (2015)
Human	Lymphoblastoid TK6 cell line	DNA damage	*GADD45α-GFP* assay	–	10 mM [731 µg/mL]	Hastwell et al. (2006)
Human	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	–	1.1 M [80 400 µg/mL]	Antoine et al. (1983)

+, positive; –, negative; ELISA, enzyme-linked immunosorbent assay; *GADD45α*, growth arrest and DNA damage-inducible 45α gene; GFP, green fluorescent protein; HID, highest ineffective dose; LED, lowest effective dose; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; UDS, unscheduled DNA synthesis

for micronuclei in mice (Antoine et al., 1983) and in the white/white$^+$ eye mosaic test in Drosophila (Vogel & Nivard, 1993).

Recent studies of genotoxicity in vitro have shown predominantly negative results. *N,N*-Dimethylformamide administered by gavage at the dose levels of 50, 100, and 200 mg/kg bw once per day for 14 consecutive days to rats significantly increased lymphocyte comet tail, average tail length, and tail moment (Xing et al., 2014). However, negative results for *N,N*-dimethylformamide at concentrations up to 257 mM were obtained in an *RAD54-GFP* assay on yeast (*Saccharomyces cerevisiae*). *RAD54* is a DNA repair gene induced by a variety of DNA lesions above the constitutive level (Knight et al., 2007). *N,N*-Dimethylformamide (4% v/v) was also not mutagenic in the *umu*-test on *Salmonella typhimurium* TA1535 (Degirmenci et al., 2000).

In a study on the combined action of *N,N*-dimethylformamide and hydrogen peroxide in the presence of Cu(II), *N,N*-dimethylformamide (0.5–4%) markedly enhanced DNA strand breaks and 8-hydroxy-2′-deoxyguanosine induced by 50 µM H_2O_2 and 20 µM Cu(II) (Midorikawa et al., 2000).

Formaldehyde, a necessary product of *N,N*-dimethylformamide demethylation, and MIC, a likely metabolic intermediate of *N,N*-dimethylformamide, can also cause chromosomal damage (Goswami, 1986; Tice et al., 1987; IARC, 2006).

4.2.4 Receptor-mediated effects

(a) Humans

Sperm motility was reduced in 12 workers exposed to *N,N*-dimethylformamide in a synthetic-leather factory compared with 8 non-exposed sociodemographically matched controls (Chang et al., 2004a). Sperm motility was related to urinary *N*-methylformamide level, but not airborne *N,N*-dimethylformamide.

In occupationally exposed workers, effects of *N,N*-dimethylformamide on coagulation were probably related to change on the membrane receptor of platelets and to a modification in phospholipid components. A follow-up experiment in vitro with platelets indicated that effects on aggregation were most likely induced by epinephrine and adenosine diphosphate (Imbriani et al., 1986).

Table 4.3 Genetic and related effects of *N*,*N*-dimethylformamide in experimental systems

Species, strain (sex), tissue	End-point	Test	Results	Dose (LED or HID)	Reference
Rat, Wistar (F), lymphocytes	DNA damage	DNA strand breaks (comet)	+	50 mg/kg bw gavage; 1 ×/day, 14 days	Xing et al. (2014)
Mouse Balb/C (M), bone marrow	Chromosomal damage	Micronuclei	–	2000 mg/kg bw i.p.; dosing 30 h after treatment	Antoine et al. (1983)
Drosophila, Cross C-1, strains y and w	Mutation	Somatic mutation and recombination test (SMART)	–	10 mM [731 µg/mL]	Vogel & Nivard (1993)
Saccharomyces cerevisiae GenT01/GenC01 (control strain)	DNA damage	*RAD54-GFP* assay	–	257 mM [18 800 µg/mL]	Knight et al. (2007)
Salmonella typhimurium TA1535	DNA damage	*umu*-test	NT	4% (v/v), tested with (but not without) metabolic activation	Degirmenci et al. (2000)

+, positive; –, negative; bw, body weight; GFP, green fluorescent protein; F, female; HID, highest ineffective dose; i.p., intraperitoneal; M, male; NT, not tested; *RAD54*, gene coding for a DNA repair/recombination protein; v/v, volume per volume

In human colon carcinoma and other cell lines, *N*,*N*-dimethylformamide stimulated mitogenesis via the epidermal growth factor (EGF) receptor and increased TGF-β receptors, with results variable across cell type (Levine et al., 1985a, b; Levine et al., 1989; Levine & Chakrabarty, 1992). *N*,*N*-dimethylformamide also significantly induced the number of urokinase receptors in a human colon carcinoma cell line (Boyd et al., 1988).

(b) Experimental systems

A 13-week toxicity study in female and male cynomolgus monkeys treated with *N*,*N*-dimethylformamide at 30–500 ppm by inhalation showed a slight trend towards increasing estrous cycle (Hurtt et al., 1992).

Some experimental studies did report receptor perturbations at relatively high (in the range of 7 to 15%) dose levels. While using *N*,*N*-dimethylformamide to increase the solubility of estrogen receptor (ER) agonists, it was observed that *N*,*N*-dimethylformamide was able to interfere with ER-binding kinetics and could cause receptor inactivation on the binding site directly or the ER-estradiol complex (Sasson & Notides, 1988). In addition, *N*,*N*-dimethylformamide was also able to act as a weak ER agonist in fish (Ren et al., 1996). *N*,*N*-Dimethylformamide also influenced the sex ratio and the gonadosomatic index in rainbow trout (van den Hurk & Slof, 1981).

4.2.5 Other mechanisms

In the polyclonal human ovarian adenocarcinoma cell line HOC-7, 0.5% *N*,*N*-dimethylformamide prolonged the cell doubling time, downregulated c-Myc protein expression and caused development of the HOC-7 cells to a less malignant cell phenotype (Somay et al., 1992). In addition, *N*,*N*-dimethyl-formamide has also been found to induce the EGF receptor, but the overall inhibitory effect on c-Myc expression by *N*,*N*-dimethylformamide has been suggested to dominate its eventual anti-proliferative effect in these HOC-7 cells (Grunt et al., 1993).

4.3 Data relevant to comparisons across agents and end-points

For all compounds evaluated in the present volume, including *N*,*N*-dimethylformamide, analyses of high-throughput screening data generated by the Tox21 and ToxCast™ research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) are presented in the *Monograph* on 1-bromopropane in the present volume.

4.4 Susceptibility to cancer

No studies with cancer as an outcome that investigated susceptibility were available to the Working Group.

There were susceptibility studies concerning other end-points. A study showed that workers with *GSTT1* null genotype are more susceptible to *N*,*N*-dimethylformamide exposure-associated abnormal liver function (Luo et al., 2005). Two other studies showed no associations between polymorphism of *CYP2E1* and the levels (Nomiyama et al., 2001b) or half-lives (Nomiyama et al., 2001c) of urinary *N*-methylformamide. A total of 66 male workers exposed to *N*,*N*-dimethylformamide were investigated (Luo et al., 2005). Compared with the *GSTT1*-positive genotype workers, the adjusted odds ratio and 95% confidence intervals for abnormal liver function test were 4.41 (95% CI, 1.15–16.9) for the *GSTT1* null genotype workers. Compared with the *N*,*N*-dimethylformamide low-exposure group with *GSTT1*-positive genotype workers, the odds ratio (adjusted for hepatitis B virus (HBV) status) of abnormal liver function test was 12.38 (95% CI, 1.04–146.9) for the *N*,*N*-dimethylformamide high-exposure group with *GSTT1*-null genotype workers. Multiplicative interactions between *N*,*N*-dimethylformamide exposure and *GSTT1* genotype were demonstrated.

There was no significant difference between slopes of single linear regression model for *N*,*N*-dimethylformamide and *N*-methylformaldehyde in *1C homozygotes and *1D-hetero- and -homozygotes, in a study of the *1C/*1D CYP2E1 polymorphism in 20 male and 24 female workers (Nomiyama et al., 2001b).

A total of 123 workers exposed to *N*,*N*-dimethylformamide were investigated for genotypes of *CYP2E1* and half-lives of urinary *N*-methylformamide (Nomiyama et al., 2001c). The workers comprised 77 c1 homozygotes, 45 c2 heterozygotes, and 1 c2 homozygotes. The half-lives of c1 homozygotes were not significantly different from those of the c2 heterozygotes and there were no differences between *N*-methylformamide half-lives for subjects with or without the c2 allele.

4.5 Other adverse effects

Occupational exposure to *N*,*N*-dimethylformamide induced liver injury in exposed workers (Wrbitzky, 1999; Wang et al., 2014). Exposure to *N*,*N*-dimethylformamide induced various symptoms, such as abdominal pain, nausea, vomiting or diarrhoea, as well as jaundice and disulfiram-type reaction (Potter, 1973; Fiorito et al., 1997). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (ALT > AST generally) and γ-glutamyl transpeptidase were increased (Potter, 1973; Nomiyama et al., 2001d). HBV carrier status and increased body mass index had synergistic effects with *N*,*N*-dimethylformamide exposure in causing liver abnormalities (Luo et al., 2001). A patient who was exposed to *N*,*N*-dimethylformamide by a suicide attempt showed elevation in AST, ALT, and total bilirubin and decrease in prothrombin time (Buylaert et al., 1996).

In experimental systems, there were many studies in rats (e.g. Tanaka, 1971; Kim & Chung, 2013), mice (e.g. Craig et al., 1984; Rui et al., 2011), hamsters (Ungar et al., 1976), cats or rabbits (Massmann, 1956) exposed to

N,*N*-dimethylformamide by inhalation (Craig et al., 1984; Senoh et al., 2003), inhalation and in drinking-water (Ohbayashi et al., 2008), gavage (Rui et al., 2011), and intraperitoneal injection (Ungar et al., 1976; Kim & Chung, 2013), which showed hepatotoxicity (Malley et al., 1994; Senoh et al., 2003). The main histopathological changes were centrilobular hepatocellular necrosis (Ungar et al., 1976; Kim & Chung, 2013), centrilobular hepatocellular hypertrophy (Tanaka, 1971; Rui et al., 2011), massive necrosis associated with centrilobular fibrosis (Senoh et al., 2003), focal necrosis (Senoh et al., 2003), centrilobular single cell necrosis (Malley et al., 1994; Senoh et al., 2003), and lipofuscin/hemosiderin accumulation in Kupffer cells (Malley et al., 1994). Serum sorbitol dehydrogenase (Malley et al., 1994; Lynch et al., 2003), ALT (Tanaka, 1971; Rui et al., 2011), AST (Tanaka, 1971; Rui et al., 2011), lactate dehydrogenase (Tanaka, 1971; Rui et al., 2011), cholesterol (Lynch et al., 2003; Senoh et al., 2003), phospholipid (Senoh et al., 2003), and total bile acids (Lynch et al., 2003) were increased.

In rodents, exposure to *N*,*N*-dimethylformamide by inhalation (Kimmerle & Machemer, 1975), gavage (Saillenfait et al., 1997), in drinking-water (Fail et al., 1998), or by epicutaneous administration (Hansen & Meyer, 1990) reduced fertility (Hansen & Meyer, 1990), and the number of fetuses (Hansen & Meyer, 1990), decreased maternal weight (Hansen & Meyer, 1990; Saillenfait et al., 1997) and fetal weight (Hansen & Meyer, 1990; Saillenfait et al., 1997) as well as inducing skeletal variations (Saillenfait et al., 1997) or malformations (Fail et al., 1998).

5. Summary of Data Reported

5.1 Exposure data

N,*N*-Dimethylformamide is a chemical with a high production volume that is mainly used in the manufacture of acrylic fibres, and in the synthetic-leather industry. *N*,*N*-Dimethylformamide is also used as a solvent in the electronics industry, in pesticide formulations, and as a component of paint strippers. Exposures of the general population have been reported in China among residents living near synthetic-leather factories. *N*,*N*-Dimethylformamide has been detected in the air in the vicinity of a waste site in the USA, and in environmental samples of air and water in Japan. Occupational exposures to *N*,*N*-dimethylformamide have been measured among workers employed in acrylic-fibre and synthetic-leather industries.

5.2 Human carcinogenicity data

Evidence on the carcinogenicity of *N*,*N*-dimethylformamide in humans was available from studies of aircraft-repair workers, leather-tannery workers, and chemical manufacturing workers.

One study described a cluster of three cases of cancer of the testes among 153 workers repairing F4 Phantom jet aircraft in the USA. These workers participated in or worked in proximity to a single process that involved uncontrolled exposure to *N*,*N*-dimethylformamide. The finding of the cluster investigation motivated a further study at a different aircraft-repair facility, where one unit repaired the F4 Phantom aircraft (also with exposure to *N*,*N*-dimethylformamide) and one unit repaired other aircraft (without exposure to *N*,*N*-dimethylformamide). In the exposed group, four cases of cancer of the testes were observed, compared with none in the unexposed group.

Shortly thereafter, a cluster of three cases of cancer of the testes was observed in workers who used *N*,*N*-dimethylformamide at a leather tannery in New York state, USA. A subsequent case–control study of cancer of the testis in the county where the tannery was located, which included the 3 original cases and 7 others, supported the existence of a cluster among leather workers.

A cohort study in one plant producing acrylic fibres in the USA, and a subsequent case–control study including that plant and three others, all of which used *N*,*N*-dimethylformamide and other chemicals, found 11 cases of cancer of the testes, of which three had been exposed to *N*,*N*-dimethylformamide, with no observed excess. It is likely that the exposures to *N*,*N*-dimethylformamide in these manufacturing plants were lower than those of leather-tanning and aircraft-repair workers.

The data from studies of aircraft-repair workers and leather workers provided evidence for a positive association between exposure to *N*,*N*-dimethylformamide and cancer of the testes; however, chance and confounding by other occupational exposures could not be ruled out.

5.3 Animal carcinogenicity data

N,*N*-dimethylformamide was tested for carcinogenicity in two studies by whole-body inhalation in male and female mice, two studies by whole-body inhalation in male and female rats, and one study by whole-body inhalation plus oral (drinking-water) administration (combined) in male rats. The Working Group determined that one study by oral administration, one study by subcutaneous injection, and one study by intraperitoneal injection in rats were inadequate for the evaluation.

In a 2-year study that complied with good laboratory practice (GLP), treatment of mice with *N*,*N*-dimethylformamide by inhalation caused a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined), in males and females in all dose groups, with a significant positive trend. There was also a significant increase in the incidence of hepatoblastoma in males. Multiple hepatocellular adenomas and carcinomas were found in the liver of exposed mice. In the second study by inhalation in mice, no significant increase in tumour incidence was reported in any dose group.

In a 2-year GLP study in rats treated by inhalation, exposure to *N*,*N*-dimethylformamide caused a significant positive trend in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), in males and females; the incidences of hepatocellular adenoma and hepatocellular carcinoma were significantly increased in males and females at the highest dose. Multiple occurrences of hepatocellular tumours were found in the livers of exposed rats. In the second study by inhalation in rats, no significant increase in tumour incidence was reported in any dose group.

In the GLP study of inhalation plus oral administration (combined) in male rats, *N*,*N*-dimethylformamide caused a significant increase in the incidences of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined) in the groups treated by inhalation plus oral administration, oral administration only, and inhalation only. It also caused a significant increase in the incidence of hepatocellular carcinoma in one group treated by oral administration only and in all groups treated by inhalation plus oral administration.

5.4 Mechanistic and other relevant data

With respect to the key characteristics of human carcinogens, there is *strong* evidence that *N*,*N*-dimethylformamide is metabolically activated, including in exposed humans. *N*,*N*-Dimethylformamide is readily absorbed after inhalation, dermal, and oral exposure. Several urinary metabolites, including the mercapturic acid, have been identified in humans. Carbamoylated adducts formed from methyl isocyanate and/or another carbamoylating metabolic intermediate of *N*,*N*-dimethylformamide have been identified in globin (both lysine and *N*-terminal valine adducts). A carbamoylated cytosine adduct has also been detected in vivo in the urine of occupationally exposed humans. Additionally, formaldehyde is a probable metabolite from oxidative demethylation of *N*,*N*-dimethylformamide.

There is *strong* evidence that *N*,*N*-dimethylformamide induces oxidative stress. A study of occupationally-exposed workers showed a distinct increase in the activity of superoxide dismutase in blood compared with the controls. Experimental studies in vivo and in vitro confirmed that *N*,*N*-dimethylformamide is capable of increasing oxidative stress in rodents, as well as in human liver, leukaemia and colon carcinoma cells.

There is *strong* evidence that *N*,*N*-dimethylformamide alters cell proliferation. No data were available in exposed humans. Experimental studies in vitro have shown an anti-proliferative and differentiating effect in human cancer cells, while it induced cell proliferation in normal human and rodent liver cells.

There is *moderate* evidence that *N*,*N*-dimethylformamide is genotoxic. Chromosomal and DNA damage has been observed in several studies in occupationally exposed humans, but results were equivocal. The results of studies of genotoxicity in various experimental systems in vivo and in vitro were mostly negative or inconclusive.

There is *weak* evidence that *N*,*N*-dimethylformamide modulates receptor-mediated effects. So far there is little mechanistic evidence that *N*,*N*-dimethylformamide exerts any toxic effects through a specific receptor in humans or in experimental systems. Some experimental studies did report perturbations of the receptors for estrogen, transforming growth factor β, and epidermal growth factor.

There were few data on other key characteristics of carcinogens (alters DNA repair or causes genomic instability, induces epigenetic alterations, induces chronic inflammation, is immunosuppressive, or causes immortalization).

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of *N*,*N*-dimethylformamide. A positive association has been observed between exposure to *N*,*N*-dimethylformamide and cancer of the testes.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*,*N*-dimethylformamide.

6.3 Overall evaluation

N,*N*-Dimethylformamide is *probably carcinogenic to humans (Group 2A).*

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