Danish Ministry of the Environment
Environmental Protection Agency

N,N-Dimethyl-formamide

Evaluation of health hazards and proposal of a health-based quality criterion for ambient air

Environmental Project No. 1543, 2014

Title:

N,N-Dimethylformamide

Author:

Elsa Nielsen

Ole Ladefoged

Division of Toxicology and Risk Assessment.

National Food Institute, Technical University of Denmark

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

Authored in 2007
Published in 2014

ISBN no.

978-87-93026-97-1

Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to N,N-Dimethylformamide and proposal of a health based quality criterion for ambient air. This resulted in 2007 in the present report, which was prepared by Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Working Environment Authority,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
The Danish Environmental Protection Agency

The Danish Environmental Protection Agency
Copenhagen, January 2014

General description

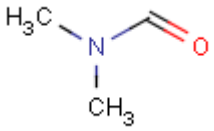
This assessment of N,N-dimethylformamide (DMF) is primarily based on the following reviews and criteria documents: OECD (2003), CICAD (2001), IARC (1999), DECOS (1995), WHO (1991), and IRIS (2005).

1.1 Identity

IUPAC name: *N*,*N*-Dimethylformamide

Molecular formula: C_3H_7NO

Structural formula:



Molecular weight: 73.09

CAS-no.: 68-12-2

Synonyms: Dimethylformamide
DMF
DMFA
Formdimethylamide
N-Formyldimethylamine

1.2 Physical / chemical properties

Description: Colourless to very slightly yellow liquid with a faint amine odour.

Purity: Commercial DMF may contain trace amounts of methanol, water, formic acid, and dimethylamine.

Melting point: -60.5 °C

Boiling point: 153.5 °C

Density: 0.9445 g/ml (at 25°C)

Vapour pressure: 2.65 mmHg (353 Pa) (at 20°C)

Concentration of saturated vapours: 14 800 mg/m^3 (at 25°C and 760 mmHg)
10 600 mg/m^3 (at 20°C and 760 mmHg)

Vapour density: 2.51 (air = 1)

Conversion factor:	1 ppm = 3.04 mg/m^3 (at 20°C and 760 mmHg) 1 mg/m^3 = 0.329 ppm
Flash point:	58 °C (closed cup), 67 °C (open cup)
Flammable limits:	2.2 -16 (v/v% in air) (70 – 500 g/m^3)
Autoignition temp.:	445 °C
Solubility:	Water: miscible in all proportions (at 20°C) Miscible with ether, ketones, aromatic hydrocarbons, ethanol.
$logP_{octanol/water}$:	-0.85, -1.01, 0.13
Henry's constant:	7.47 x 10^{-5} hPa x m^3 / mol (at 25 °C)
pK_a-value:	-
Stability:	DMF is hygroscopic and easily absorbs water from a humid atmosphere. DMF does not change under light or oxygen and does not polymerise spontaneously. At temperatures > 350 °C, decomposition to dimethylamine and carbon dioxide may occur.
Incompatibilities:	Contact with carbontetrachloride and other halogenated hydrocarbons, particularly when in contact with iron or with strong oxidising agents may cause fires and explosions.
Odour threshold, air:	0.12 - 0.15 mg/m^3 for the most sensitive people (WHO 1991) 6.8 mg/m^3 (Amoore & Hautala 1983)
Odour threshold, water:	50 mg/l (Amoore & Hautala 1983)
Taste threshold, water:	-
References:	WHO (1991), IARC (1999), OECD (2003), CICAD (2001), DECOS (1995)

.

1.3 Production and use

DMF is usually manufactured by a one-stage reaction of carbon monoxide with dimethylamine or by a two-stage reaction with methylformate and dimethylamine. DMF can also be manufactured from carbon dioxide, hydrogen, and dimethylamine, in the presence of halogen-containing transition metal compounds. (WHO 1991, DECOS 1995).

The production volume in the EU was 50000 to 100000 tonnes/year, in Asia 100000 to 500000 tonnes/year, and in North America 50000 to 100000 tonnes/year (OECD 2003).

DMF has been termed the universal solvent. It is predominantly used as a solvent in the synthesis of fine chemicals, in polyacrylonitrile fibre production, in polyurethane coating, an in electronics industry. Minor uses include various applications like varnishing, surface coating, polyamide coating, absorbents, cleaners, and extractants. The former use of DMF as a solvent in crop protection agent formulations has been abandoned. (OECD 2003).

In the Danish product register (PRA 2006), there were a total number of 19 products containing DMF and the total use volume of DMF was approximately 75 tonnes per year. Product types were solvents, intermediates, and paint, lacquers and varnishes.

1.4 Environmental occurrence and fate

DMF does not occur naturally (WHO 1991, CICAD 2001). According to CICAD (2001), DMF is a possible product of the photochemical degradation of dimethylamine and trimethylamine, both are commonly occurring natural substances.

Industrial releases of DMF into air appear to be considerably larger than releases to other environmental media (CICAD 2001).

1.4.1 Air

Atmospheric DMF is predominantly present in the vapour phase and is expected to be readily transported from air into surface water or soil pore water during rainfall. Chemical degradation of DMF in air is likely due to reaction with hydroxyl radicals. The degradation half-life in air has not been determined but data suggest that the half-life is at least 8 days. Photochemical decomposition is not expected to occur. (CICAD 2001, WHO 1991).

In the U.S., DMF has been detected in the air over an abandoned chemical waste reclamation plant (0.007 mg/m^3), a neighbouring industry (> 0.15 mg/m^3), and a residential area (0.024 mg/m^3) (Amster et al. 1983 – quoted from CICAD 2001, WHO 1991).

Levels were generally < 0.02 mg/m^3 at a hazardous waste site in unsettled wind conditions, up to 9 mg/m^3 at nearby industrial sites, and < 0.02 mg/m^3 in adjoining residential areas (Clay & Spittler 1983 – quoted from CICAD 2001, WHO 1991).

Ambient air samples collected in the northeastern USA in 1983 ranged from <0.00002 to 0.0138 mg/m^3 (Kelly et al. 1993,1994 – quoted from CICAD 2001).

In Germany, a concentration of ≥ 0.000005 mg/m^3 has been detected (Figge et al. 1987 – quoted from CICAD 2001).

1.4.2 Water

When released into the water, DMF remains in the dissolved form and is not expected to adsorb to organic fractions of sediments or suspended organic matter, or to transfer to biota or the atmosphere. Biodegradation appears to be the primary degradation process in surface water. Under experimental conditions, DMF was degraded, either aerobically or anaerobically, by various microorganisms and algae

in activated sludge, over a wide range of concentrations. The overall rate of chemical degradation is expected to be very slow in surface water and photochemical decomposition is unlikely in water. The photooxidation half-life of DMF in water has been estimated experimentally at 50 days and is expected to be even longer in the natural environment. The rate of hydrolysis is expected to be very slow. (CICAD 2001, WHO 1991).

In 3 of 23 groundwater samples collected in the USA, concentrations ranged from 0.05 to 0.2 mg/l, with an average value of 0.117 mg/l (Syracuse Research Corporation 1988 – quoted from CICAD 2001).

DMF has been detected in the water of heavily industrialised river basins only rarely, at concentrations below 0.01 mg/l (WHO 1991).

1.4.3 Soil

When released into the soil, DMF is incorporated into the pore water. DMF will be degraded by biological processes or leached into groundwater. If it reaches the groundwater, DMF will be slowly degraded anaerobically. Chemical degradation is expected to be slow and volatilisation from moist soils is expected to be limited. (CICAD 2001).

No data have been located concentrations of DMF in soil.

1.4.4 Foodstuffs

No data have been located.

1.4.5 Bioaccumulation

No bioaccumulation was observed in carp during an 8-week bioaccumulation test (CICAD 2001, WHO 1991).

1.5 Human exposure

No data have been located on exposure of the general population to DMF from environmental sources or foodstuffs.

In a Canadian multimedia study, DMF was not detected in indoor air samples from 50 residences (detection limit 0.0034 mg/m^3) or in tap water samples (detection limit 0.00034 mg/l) (Conor Pacific Environmental 1998 – quoted from CICAD 2001).

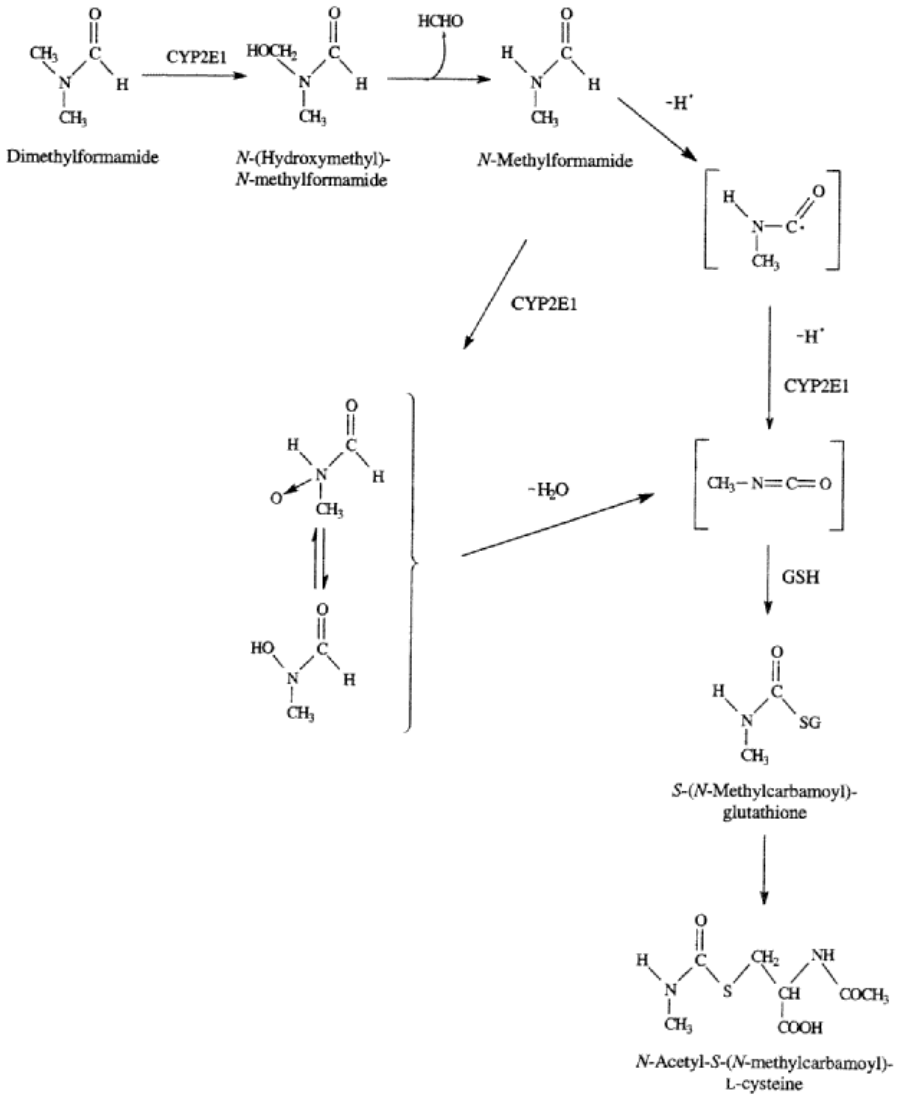
DMF is a component in certain consumer products (OECD 2003). In the EU, DMF is not allowed in consumer products in concentrations ≥ 0.5% as DMF is classified for reproductive toxicity in category 2 (Repr. Cat. 2; R61).

2 Toxicokinetics

2.1 Metabolism

There have been numerous studies on the metabolism of DMF in the past 25 years. The liver is the main site of biotransformation of DMF. The proposed metabolic pathway is illustrated in Figure 2.1 and summarised below based on OECD (2003), IARC (1999), CICAD (2001).

Figure 2.1 Proposed metabolic pathway of DMF (from IARC 1999)



Data indicate that two metabolic pathways can be distinguished: 1) hydroxylation of the carbon atom of one of the *N*-methyl groups of DMF, and 2) oxidation of the formyl moiety.

1) The major pathway in humans and animals is the hydroxylation of one of the *N*-methyl groups, which is mediated by a cytochrome P450-dependent reaction (CYP2E1). The resulting *N*-hydroxymethyl-*N*-methylformamide (HMMF) is the main urinary metabolite. Mono-*N*-methylformamide (MMF) was once considered as being the predominant urinary metabolite of DMF; later it was revealed that HMMF is unstable in many analytical manipulations and readily decomposes to MMF. For this reason, HMMF was underestimated, or not detected, and MMF was overestimated in a number of early studies.

2) The minor pathway (of lower capacity) is the oxidation of the formyl moiety of HMMF resulting in MMF, which is then partially conjugated with the sulfhydryl-containing molecules, glutathione (GSH) or cysteine, forming S-(*N*-methyl-carbamoyl)-glutathione (SMG) or S-(*N*-methyl-carbamoyl)-cysteine (SMC), respectively. SMG and SMC undergo further transformation to yield the mercapturic acid *N*-acetyl-S-(*N*-methyl-carbamoyl)-L-cysteine (AMCC). In the biotransformation of MMF to SMG, an intermediary formation of a reactive species (methyl isocyanate (MIC) has, according to IARC (1999), been postulated but not proven. MMF can also undergo hydroxylation of the *N*-methyl group (CYP2E1) resulting in *N*-(hydroxymethyl)-formamide (HMF), which is further degraded to formamide. HMF is, similarly to HMMF, unstable in many analytical manipulations and readily decomposes to formamide and is measured as such in the urine.

2.2 Absorption, distribution and excretion

2.2.1 Inhalation

Volunteers (5 men, 5 women) were exposed to DMF vapour at concentrations of 10, 30, or 60 mg/m^3 for 8 hours or to 30 mg/m^3 for 8 hours/day on 5 consecutive days. The uptake from the respiratory tract was 90% and the various urinary metabolites accounted for 49% of the retained dose. After single exposure to 30 mg/m^3, the half-lives of excretion and the urinary recoveries of the metabolites were: DMF, 2 hours (0.3% of the dose); HMMF, 4 hours (22.3% of the dose); HMF, 7 hours (13.2% of the dose); and AMCC, 23 hours (13.4% of the dose). Following repeated exposure, AMCC accumulated in urine; urinary elimination 16 hours following the fifth exposure was approximately 14% HMMF, 32% HMF, and 54% AMCC. (Mráz & Nohová 1992 – quoted from IARC 1999, DECOS 1995, OECD 2003).

Volunteers (5 men, 5 women) exposed to DMF by inhalation (60 mg/m^3 for 8 hours) excreted 16-49% of the dose as ‘MMF’ (representing the total of HMMF and MMF, see section 2.1), 8-24% as ‘formamide’ (representing the total of HMF and formamide, see section 2.1), 10-23% as AMCC, and 1-2% as DMF. (Mráz et al. 1989 – quoted from IARC 1999, DECOS 1995, CICAD 2001; Mráz et al. 1993 – quoted from OECD 2003).

After respiratory exposure to DMF, lung retention in workers in an artificial leather factory was 72% (Brugnone 1980 – quoted from WHO (1991); no further details have been provided.

Rats and mice were exposed to 10, 250, or 500 ppm (30, 750 or 1500 mg/m^3) DMF for single 1-, 3-, or 6-hour periods, or for 6 hours/day, 5 days/week for 2 weeks. DMF was not detected in plasma after the 10-ppm dose. Steady-state plasma levels were approached after 6 hours of exposure to 250 ppm. At 500 ppm, plasma levels increased 2-fold in rats and 3-fold in mice between 3 and 6 hours of exposure. The area under the plasma concentration curve (AUC) values after a single 6-hour exposure increased disproportionately (8-fold and 29-fold increases in rats and mice, respectively) compared with the increase in DMF exposure concentration (from 250 to 500 ppm). Multiple exposures to 500 ppm resulted in lower AUC values for both rats (3-fold reduction) and mice (18-fold reduction) compared with the AUC values following a single 500-ppm exposure indicating an increased capacity of rats and mice to metabolise DMF after repeated exposure. The plasma levels of 'MMF' (representing the total of HMMF and MMF, see section 2.1) increased with time of exposure to single 250 ppm but did not increase further at 500 ppm. HMMF represented over 90% of the total of DMF and determined metabolites.
When cynomolgus monkeys were subjected to whole-body exposure to 30, 100, or 500 ppm (90, 300, or 1500 mg/m^3) for 6 hours/day on 5 days/week for 13 weeks, there were also disproportionate increases in plasma AUC of 19- to 37-fold in males and of 35- to 54-fold in females as the concentrations increased from 100 to 500 ppm. Plasma half-lives ranged from 1-2 hours for DMF and 4-15 hours for 'MMF' (representing the total of HMMF and MMF, see section 2.1). The plasma concentration of 'MMF' was higher than that of DMF at 0.5 hours. HMMF was the main urinary metabolite (56-95%), regardless of exposure level or duration of exposure. DMF was not readily excreted in the urine.
(Hundley et al. 1993a,b – quoted from IARC 1999, CICAD 2001).

Kimmerle & Eben (1975 – quoted from WHO 1991) studied DMF and MMF concentrations in the blood of rats and dogs as well as elimination of DMF, MMF and formamide after single and repeated respiratory exposure.
After a single exposure (6015 mg/m^3, 3 hours), DMF was still detectable in the blood of male rats up to 2 days after the end of exposure and MMF levels continued to increase for the 2 days following exposure. At lower concentrations (63 and 438 mg/m^3), DMF levels in the blood decreased rapidly and DMF and MMF were not detectable 3 hours after the end of exposure at the low concentration. In the urine, no MMF was found 24 hours after exposure to 63 mg/m^3 for 3 hours or 87 mg/m^3 for 6 hours, whereas exposure to 513 mg/m^3 for 6 hours or to 6015 mg/m^3 for 3 hours resulted in excretion of MMF in the urine during the 24-hour period following the start of exposure, and DMF was also detected at the high dose level.
In male dogs, blood concentrations of DMF also decreased rapidly following a single 6-hour exposure (60 or 513 mg/m^3), whereas MMF could be detected in the blood at higher concentrations and for a longer period of time after exposure.
When male rats were exposed repeatedly to 1050 mg/m^3 (6 hours/day, 5 days), the blood levels of DMF and MMF declined to 'not detectable' before each consecutive exposure. Urinary levels of MMF remained almost constant for the first 3 days and then slightly decreased; excretion of formamide was much lower than excretion of MMF.
When male dogs were exposed repeatedly to 177 mg/m^3 (6 hours/day, 5 days), MMF accumulated in the blood. Increasing concentrations of MMF and formamide were excreted in the urine, whereas excretion of DMF was very low.
In female dogs exposed repeatedly to 69 mg/m^3 (6 hours/day, 5 days), the MMF concentration in the blood remained almost constant, returning to a low level before each new exposure. No urinary accumulation of MMF or formamide was observed.

When male and female dogs were exposed repeatedly to 63 mg/m^3 (6 hours/day, 5 days/week for 4 weeks), DMF levels declined to 'not detectable' before each new exposure; there was no accumulation of MMF. The weekly average concentrations of MMF were slightly higher in males than in females. MMF and formamide concentrations in the urine remained almost constant during the exposure period; male dogs generally excreted slightly higher levels of metabolites than female dogs.

In rats exposed to 1690 or 6700 mg/m^3 for 4 hours, blood levels of MMF for the first 3 hours following exposure were lower at the high concentration than at the lower concentration. DMF and MMF were distributed uniformly throughout the tissues. The authors suggested that high DMF concentrations inhibited DMF metabolism. (Lundberg et al. 1983 – quoted from WHO 1991, CICAD 2001).

When the sum of all three *N*-methylcarbamoylthioesters in plasma (SMG, SMC, AMCC) was assessed as a function of exposure concentration in rats (after inhalation), the following pattern was obtained: Following exposure to 25 and 84 ppm (75 and 255 mg/m^3), there was a linear relation between DMF exposure and the sum of the thioesters. At 25 ppm, the steady state levels for the sum of the thioesters (around 50 µmol/l) was obtained after 12 hours of exposure and stayed at the level during a continuing exposure for up to 48 hours. After exposure termination, the thioesters were excreted with a half-life of approximately 2.8 hours. At 84 ppm, the steady state level of the sum of the thioesters was around 200 µmol/l and the excretion half-life was approximately 2.2 hours. At 213 ppm (640 mg/m^3), no thioesters were found until 6 hours following a 72 hours exposure time. (Filser et al. 1994 – quoted from OECD 2003).

Peak plasma DMF levels and the DMF AUC values were markedly higher following whole-body exposure compared with head-only exposure in monkeys exposed to 500 ppm (1500 mg/m^3) 6 hours/day, 5 days/week for 2 weeks (Hurt et al. 1991 – quoted from DECOS 1995).

2.2.2 Oral intake

In female rats administered a single oral dose of ^{14}C-labelled DMF at 100 mg/kg b.w. on day 12 or 18 of pregnancy, plasma radioactivity was relatively constant from 0.5-4 hours after dosing (about 0.5% of the dose), and declined rapidly thereafter. Approximately 4% of the dose was present in the liver at 0.5 hours after dosing (both gestation times), with 8 and 13% in the gastrointestinal tract, and 0.7 and 0.8% in the kidneys, respectively. By 48 hours, only the liver (0.5 and 0.6%) and intestine (0.2 and 0.3%) retained any significant activity. In animals exposed on day 12 of gestation, approximately 1.5% of the dose was present in the uterus, placenta, embryo, and amniotic fluid at between 0.5 and 4 hours, which rapidly declined to less than 0.1% at 24 hours. In animals exposed on day 18 of gestation, foetal tissues accounted for 6% of the administered dose. Analyses indicated that DMF and metabolites were readily transferred to the embryonic and foetal tissues, where levels were generally equal to those in maternal plasma. DMF accounted for most of the radioactivity in plasma or tissues until 4-8 hours (61-77% for the first 4 hours and 73-93% for the first 8 hours after treatment on days 12 and 18, respectively) and then decreased corresponding with an increase in the levels of HMMF and MMF. HMMF accounted for 40-47% at 8 hours (day 12) and for 41-55% at 16 hours (day 18); the equivalent figures for MMF were 9-13% and 16-18%, respectively. The amounts of AMCC and formamide in plasma or tissues were < 4% at all time points. Around 60-70% of the radioactivity was excreted in

urine and 3-4% in faeces at 48 hours. (Saillenfait et al. 1997 – quoted from CICAD 2001).

After oral administration of 200 to 4000 mg/kg b.w. of DMF to rats, mean blood levels ranged from 40 to 1870 mg/l. After administration of 40 to 2000 mg/kg b.w., about 6% of the dose was excreted within 24 hours. (Sanotsky et al. 1978 – quoted from WHO 1991).

2.2.3 Dermal contact

Percutaneous absorption *in vivo* has been examined from liquid DMF and from DMF vapour (Mráz & Nohová 1992 – quoted from IARC 1999, DECOS 1995). Volunteers were exposed to liquid DMF by dipping one hand up to the wrist in DMF for 2 to 20 minutes or by applying 2 mmol DMF over an area of 100 cm^2 on the forearm (approximately 1.5 mg/cm^2). In both cases, the absorption rate was 9 mg/cm^2/hour.
Percutaneous uptake of DMF vapour was evaluated in volunteers (wearing light clothing and breathing fresh air through masks) exposed to 50 mg/m^3 for 4 hours. Uptake increased with increasing ambient temperature and humidity and accounted for 13-36% of urinary HMMF excreted during combined inhalation and percutaneous exposure to the same concentration of DMF vapour.

2.2.4 Other routes

Male rats, mice and Syrian hamsters were given approximately 7, 50 or 500 mg/kg b.w. of DMF (solution in saline) once by intraperitoneal injection. Measurable amounts of metabolites were detected in the urine in rats up to 60 hours, in mice up to 24 hours, and in hamsters up to 36 hours after dosing. 'MMF' (representing the total of HMMF and MMF, see 2.1) was the major metabolites in all three rodent species. The percentage of the dose, which was metabolised to 'MMF' varied between 8.4 and 47.3%. Between 7.9 and 37.5% was excreted as 'formamide', and between 1.1 and 5.2% as AMCC. DMF was a minor urinary metabolite. (Mráz et al. 1989 – quoted from IARC 1999, DECOS 1995, CICAD 2001; Mráz et al. 1993 – quoted from OECD 2003).

2.2.5 Species differences

In comparative analyses of the two studies by Hundley et al. (1993a,b – quoted from IARC 1999, CICAD 2001 – see section 2.2.1), the authors suggested that toxicokinetic differences may, in part, contribute to the observed species differences in toxicity. The AUC values and peak plasma levels of DMF for rats and mice following a single 500 ppm (1500 mg/m^3) exposure was substantially greater than the respective values in monkeys following a similar exposure. Whereas repeated exposure to 500 ppm in rats and mice enhanced metabolism, as indicated by diminished AUC values for DMF and increased plasma concentrations of MMF, this effect was not clearly demonstrated in monkeys.

Mráz et al. (1989 – quoted from IARC 1999, DECOS 1995, CICAD 2001) compared the extent of the biotransformation *in vivo* of DMF to the three urinary metabolites ('MMF', 'formamide' and AMCC) in humans and rodents based on the studies in rats, mice and hamsters administered DMF intraperitoneally (see section 2.2.4) and the study in human volunteers exposed to DMF by inhalation of 60 mg/m^3 for 8 hours (see section 2.2.1). This comparison suggests that there is a

quantitative difference between the metabolic pathway of DMF to AMCC in humans and rodents. In humans, the percentage of the dose excreted in urine as AMCC is higher (10-23%) than in rodents (1.1-5.2%).

2.2.6 Interaction between DMF and ethanol

Mutual interaction occurs between the degradation of DMF and ethanol. Ethanol and probably the metabolite acetaldehyde inhibit the breakdown of DMF and conversely, DMF inhibits the metabolism of ethanol and acetaldehyde. Furthermore, ethanol induces cytochrome P450 2E1, which facilitates the initial hydroxylation of DMF. (OECD 2003, CICAD 2001).

2.3 Mode of action

Two metabolic pathways for DMF can be distinguished: 1) hydroxylation of one of the *N*-methyl groups and 2) oxidation of the formyl moiety (see section 2.1). The former pathway results in the major metabolite HMMF and presumably constitutes a detoxification route; the latter pathway leads to the formation of SMG (and SMC), which may undergo further transformation to give AMCC and may be is associated with the toxicity of DMF. The mechanism by which DMF causes toxicity seems linked to formation of methyl isocyanate (MIC), an intermediary reactive species in the biotransformation of MMF to SMG, which, according to IARC (1999), has been postulated but not proven. (DECOS 1995).

Data from an *in vitro* study suggest the metabolite SMG and its sequel adducts (SMC and AMCC) to be responsible for the developmental toxic effects of DMF, see section 4.5.4 (Klug et al. 1998 – quoted from OECD 2003, CICAD 2001).

Data suggest a quantitative difference in the formation of SMG/AMCC in humans and rodents, most likely in the formation of the reactive carbamoylating intermediate, which acylates glutathione. In humans, the percentage of the dose excreted in urine as AMCC is higher (10-23%) than in rodents (1.1-5.2%). (OECD 2003, IARC 1999, DECOS 1995).

3 Human toxicity

3.1 Single dose toxicity

3.1.1 Inhalation and dermal contact

Several cases of acute accidental occupational poisoning with DMF have been reported. Over-exposure has occurred via the skin and/or inhalation. Usually, the symptoms appeared from several hours up to several days after the accident. The major symptoms were epigastric or abdominal pain accompanied by dizziness, nausea, anorexia, vomiting, fatigue, alcohol intolerance, and skin irritation. Clinical investigations have shown liver function disturbances, and radioisotope diagnostic tests and liver biopsies have revealed morphological changes in the liver. The patients recovered and liver function tests returned to normal. (Several studies quoted in CICAD 2001, WHO 1991, DECOS 1995, IRIS 2005).

3.1.2 Oral intake

Hepatic impairment (marked increases in serum levels of several hepatic enzymes together with fulminant hepatitis and jaundice) was reported in a woman who ingested about 600 mg/kg b.w. of DMF (in a formulation containing other ingredients) in a suicide attempt (Nicolas et al. 1990 – quoted from CICAD 2001).

3.2 Irritation

3.2.1 Skin irritation

The skin irritation potential of DMF was studied in 110 volunteers after a 48-hour application of a patch with 1, 2, 5, or 10% DMF in vegetable oil. Local irritation was observed in 6.3% of the volunteers with the 10% solution. (Bainova 1985 – quoted from DECOS 1995).

Skin irritation has also been reported in workers exposed to DMF, see section 3.1.1.

3.2.2 Eye and respiratory tract irritation

Irritation to the eyes and the respiratory tract has been reported in workers exposed to DMF, see section 3.4.2.

3.3 Sensitisation

One case of positive patch test reaction (0.1-1.0% DMF in petrolatum) was reported in a woman using DMF in her laboratory work; DMF (0.1% in petrolatum) was negative in 20 controls (Camarasa 1987 – quoted from OECD 2003).

3.4 Repeated dose toxicity

3.4.1 Inhalation

No treatment-related effects were reported in 8 volunteers exposed to 30 mg/m^3 DMF, 6 hours/day for 5 days. The volunteers were given a complete physical survey and laboratory testing, 7 days before and after exposure. The examinations comprised haematology, clinical chemistry (e.g., liver enzymes), and measurements of body temperature, heart rate, and pulmonary function. (Krivanek et al. 1978 – quoted from DECOS 1995).

3.4.2 Inhalation and dermal contact

In several studies of workers (quoted in OECD 2003, CICAD 2001, IARC 1999, WHO 1991, DECOS 1995, IRIS 2005), symptoms such as eye irritation, respiratory tract irritation, headache, anorexia, gastrointestinal disturbances, and sometimes hepatomegaly were reported; clinical investigations have shown increased serum levels for several liver enzymes.

Workers exposed to DMF at mean or median concentrations from about 10 ppm (30 mg/m^3) have reported headaches, dizziness, anorexia, nausea, abdominal pain, and alcohol intolerance (Several studies quoted in CICAD 2001).

In a recent study, some workers reported subjective symptoms following exposure to DMF at a median concentration of 1.2 ppm (3.6 mg/m^3) (Wrbitzky 1999 – quoted from CICAD 2001); no further details given in CICAD.

An epidemiological study was conducted of 100 workers, all males with a mean age of 36 years (range 21-56 years), exposed to DMF at a mean concentration of 22 mg/m^3 (Time Weighted Average (TWA), range 8-58 mg/m^3) for an average of 5 years (range 1 to 15 years). Study subjects were selected to minimise large variations in exposure and those with histories of possible accidental exposures were also excluded. The referent control group was 100 workers at the same or similar factories, without exposure to any solvents or toxic metals, matched by sex, age group, alcohol history, smoking habits, coffee intake, socio economic status, residence, and dietary customs. DMF-exposed workers complained more often of headache, dyspepsia, non-specific cardiac distress, and digestive impairment indicative of hepatic functional impairment. Symptoms of irritation that were significantly increased in DMF-exposed workers included watery eyes, cough, and dry throat. Exposed workers also exhibited significantly increased γ-glutamyl transpeptidase levels, and elevated (not significantly) levels of aspartate amino transferase (AST) and of alanine amino transferase (ALT). (Cirla et al. 1984 – quoted from IRIS 2005, CICAD 2001).

Hepatic function was studied in 54 workers exposed to low levels of DMF (Catenacci et al. 1984 – quoted from IRIS 2005, CICAD 2001). The workers were divided into two groups: the first group of 28 subjects was exposed to an 8-hour TWA concentration of 18 mg/m^3 (range, 12-25 mg/m^3) DMF, and the second group of 26 subjects to 3 mg/m^3 (range, 1-5 mg/m^3) DMF. The control group consisted of 54 workers never exposed to solvents. No significant differences in hepatic enzyme levels were observed between either of the exposed groups and the controls.

Significantly increased hepatic enzyme levels have been reported in workers exposed to DMF at geometric mean concentrations (8-hour sampling) of about 20 mg/m^3 (range 2-40 mg/m^3) (Fiorito et al. 1997 – quoted in CICAD 2001).

Hepatic pain and palpable liver have been reported in 4 of 13 workers exposed to 15.6 mg/m^3 DMF (and other solvents) for a few weeks to 4 years (Tomasini et al. 1983 – quoted from CICAD 2001).

Liver biopsies from workers 'heavily' exposed to DMF (and other solvents, exposure levels not reported), have revealed histopathological changes in the liver (less than 3 months exposure: hepatocellular necrosis, enlarged Kupffer cells, microvesicular steatosis, complex lysosomes, and pleomorphic mitochondria; 14-120 months exposure: fatty changes with occasional lipogranuloma) (Redlich et al. 1990 – quoted from CICAD 2001, IARC 1999, IRIS 2005, DECOS 1995).

Excess mortality from ischaemic heart disease has been reported in a historical cohort study of DMF-exposed workers in a US acrylonitrile fibre plant. There were 62 deaths versus 40.3 expected from company rates. A similar observation was made for a second group of workers at the plant who were potentially exposed to both DMF and acrylonitrile (65 deaths versus 48.3 expected from company rates). The increases were not significant in comparison with the state (South Carolina) rates. (Chen et al. 1988 – quoted from CICAD 2001).

3.4.3 Oral intake

No data have been located.

3.5 Toxicity to reproduction

No data have been located.

3.6 Mutagenic and genotoxic effects

No association between exposure to DMF and the frequency of sister chromatid exchange in peripheral lymphocytes of 85 workers from a resin synthesis plant was found. Nine workers had a low exposure (median, 15.6 mg/m^3; range, 2.7-15.9 mg/m^3) and 20 workers had a high exposure (median, 74 mg/m^3; range, 34-250 mg/m^3) (Cheng et al. 1999 – quoted from OECD 2003).

Increases in chromosome aberrations, sister chromatid exchange and UV-induced unscheduled DNA synthesis in peripheral lymphocytes of 26 viscose rayon plant workers (exposed to DMF and acrylonitrile during a 20-month period) have been noted. The frequency of premature centromere division in peripheral lymphocytes in 18 of the 26 workers did not show a significant increase compared to controls. Peak concentrations in the air at the start of the study ranged from 0.2 to 7.7 ppm (0.6-23 mg/m^3) for DMF and from 0 to 5.9 ppm (0-13 mg/m^3) for acrylonitrile. Seven months later, the concentrations ranged from 1.2 to 7.6 ppm (3.6-23 mg/m^3) and from 0.1 to 1.7 ppm (0.2-3.8 mg/m^3) for DMF and acrylonitrile, respectively; no data were available for twenty months. (Major et al. 1998, Major 1999 – both quoted from OECD 2003, CICAD 2001).

The frequencies of sister chromatid exchange in peripheral lymphocytes in female workers in a leather production factory were significantly higher than in controls at

DMF concentrations of 0.7 and 5.8 ppm (2.1 and 18 mg/m^3) but not at 0.3 ppm (0.9 mg/m^3). Workers were also exposed to toluene at concentrations up to 0.9 ppm (3.4 mg/m^3). (Seiji et al. 1992 – quoted from OECD 2003, CICAD 2001, IARC 1999).

The frequency of chromosomal gaps and breaks in peripheral lymphocytes was 1.4% in 20 workers exposed to DMF as well as to mono-, di-, and trimethylamine compared to 0.4% in controls. Workplace concentrations ranged from 5.8 to 27 mg/m^3 (mean 12.3 mg/m^3) for DMF and from 0.01 to 3.3 mg/m^3 (mean 0.63 mg/m^3) for dimethylamine. Possible effects of smoking were not taken into account. (Berger et al. 1985 – quoted from OECD 2003, CICAD 2001, IARC 1999).

The frequencies of chromosomal aberrations in peripheral lymphocytes were increased in workers exposed to DMF and several other chemicals. Frequencies were 1.49, 1.58, 1.59, 2.74 and 3.82% for DMF concentrations in ambient air of 11.7, 13.3, 16.7, 50, and 60 ppm, respectively (35, 40, 51, 152, and 182 mg/m^3). Aberration frequencies in controls ranged from 1.10 to 1.61%. Air concentrations of the co-exposures were not given. (Koudela & Spazier 1981 – quoted from OECD 2003, CICAD 2001, IARC 1999).

3.7 Carcinogenic effects

In a cohort study of 3859 workers with potential exposure to DMF (2530 workers) and to DMF and acrylonitrile (1329 workers) in an acrylonitrile fibre production facility in South Carolina, US, the incidences of cancer of the buccal cavity and pharynx, lung, prostate, stomach, nervous system, and bladder were considered in relation to exposure level and duration. The control group consisted of 1130 workers from the same plant. (Chen et al. 1988 – quoted from IARC 1999, CICAD 2001, WHO 1991, DECOS 1995).
Exposure levels were classified as low (below 30 mg/m^3), moderate (sometimes above 30 mg/m^3), or high (often above 30 mg/m^3).
For all workers exposed to DMF (alone or with acrylonitrile), the standardised incidence ratio (SIR) based on company rates for all cancers combined was 1.1 (95% CI, 0.9-1.4; 88 cases). One case of testicular cancer was observed among exposed workers (DMF alone or with acrylonitrile) versus 1.7 expected based on company rates; no testicular cancer was observed in the DMF-group. The SIR for cancer of the buccal cavity and pharynx was 3.4 (95% CI, 1.7-6.2; 11 cases) among workers exposed to DMF, based on company rates; no such excess was found among the workers exposed to both DMF and acrylonitrile. There was no relationship between cancer of the buccal cavity and pharynx and intensity or duration of exposure.
According to CICAD (2001) and DECOS (1995), there was also a significant increase in prostate cancer (10 observed versus 5.1 expected from company rates and 5.2 expected from national rates) among workers exposed either to DMF or to both DMF and acrylonitrile; the increase was not significant in the DMF-group. According to WHO (1991), there were no cases of liver cancer.

Mortality in 1950-1982 was evaluated in the same cohort among both active and pensioned employees. Expected numbers (adjusted for age and time period) were based on company rates. For all workers exposed to DMF only, the standardised mortality ratios (SMR) were 0.9 (38 observed versus 30.1 expected) for all cancers combined, 2.5 (2 observed versus 0.8 expected) for buccal cavity and pharynx cancers, and 1.4 (19 observed versus 13.5 expected) for lung cancer. No other cancer excesses were reported. (Chen et al. 1988 – quoted from IARC 1999, WHO 1991, DECOS 1995).

In a case-control study, cancers of the buccal cavity and pharynx (n=39), liver (n=6), prostate (n=43), testis (n=11), and malignant melanoma of the skin (N=39) were examined in approximately 8700 workers from four plants (a DMF production plant, two acrylic fibre plants that used DMF, and a plant using DMF as a solvent for inks) (Walrath et al. 1989 – quoted from IARC 1999, CICAD 2001, WHO 1991, DECOS 1995).
Potential exposure to DMF was classified as low or moderate. Geometric means for air measurements of DMF ranged from less than 3 mg/m^3 to about 30 mg/m^3. Odds ratios for 'ever exposed' were 0.9 (n=15; 90% CI, 0.4-2.3) for buccal cavity and pharynx cancers; 1.7 (n=16; 90% CI, 0.5-5.5) for malignant melanoma; 1.5 (n=17; 90% CI, 0.7-3.3) for prostate cancer; and 1.0 (n=3; 90% CI, 0.2-4.4) for testicular cancer. Two liver cancer cases and one control were exposed to DMF giving a logistic regression odds ratio of 6.1 (90% CI, 0.4-72). Odds ratios for malignant melanoma by level of exposure were 1.9 (90% CI, 0.5-7.3) for low and 3.1 (90% CI, 0.8-11.9) for moderate exposure. Odds ratios for testicular cancer by level of exposure were 0.9 (90% CI, 0.1-8.6) for low and 11.6 (90% CI, 0.5-286) for moderate exposure (2 exposed cases and 2 exposed controls).

Seven cases of testicular germ cell tumours (5 seminomas, 2 embryonal cell carcinomas) have been reported among about 830 men employed in aircraft repair. Three of the cases had with certainty been exposed to a solvent mixture containing 80% DMF (20% unspecified) and three cases had probably been exposed. (Ducatman et al. 1986 – quoted from IARC 1999, CICAD 2001, WHO 1991, DECOS 1995).

Three cases of embryonal cell carcinoma of the testis have been reported in workers at a leather tannery in the US. DMF as well as a wide range of dyes and solvents were used, including the testicular toxicants 2-ethoxyethanol and 2-ethoxyethanol acetate (Levin et al. 1987 –quoted from OECD 2003, IARC 1999, CICAD 2001, DECOS 1995). No additional cancers were reported in a screening effort undertaken to identify additional testicular cancers in 51 of the 83 workers at the leather tannery (Calvert et al. 1990 – quoted from OECD 2003, IARC 1999, WHO 1991, CICAD 2001).

Based on these studies, IARC (1999) has concluded that there is inadequate evidence in humans for the carcinogenicity of DMF.

4 Animal toxicity

4.1 Single dose toxicity

The acute toxicity has been investigated in a number of species following oral or dermal administration, or via inhalation. Animals given large single doses of DMF or exposed to high air concentrations showed general depression, anaesthesia, loss of appetite, decreased body weight, tremors, laboured breathing, convulsions, haemorrhage of the nose and mouth, liver injury, and coma immediately preceding death. In studies where tissue pathology was included, the prominent organ showing damage was the liver. At high single doses, liver effects observed included congestion and centrilobular necrosis of hepatocytes; lower doses resulted in altered liver function such as decreased excretion of cholic acid in the bile, bromosulfthalein retention, increased serum activities of aspartate aminotransferase (AST, also abbreviated ASAT, previously SGOT), alanine aminotransferase (ALT, also abbreviated ASAT, previously SGPT), leucine aminopeptidase (LAP), ornithine carbamoyltransferase (OCT), alkaline phosphatase, lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT), and significant increased cholesterol, triglyceride and bilirubin contents in the serum and liver homogenates. No obvious species differences were observed with regard to acute lethality, but young rats appeared more sensitive to DMF-induced lethality than older rats. (WHO 1991, CICAD 2001).

4.1.1 Inhalation

LC_{50}-values reported for rats ranged from 9400 to 15000 mg/m^3 and in mice from 6000 to 18300 mg/m^3 (WHO 1991, CICAD 2001, DECOS 1995).

In mice and rats, exposed to DMF via inhalation, signs of mucous membrane irritation were seen and lung damage was detected histologically (WHO 1991).

4.1.2 Oral intake

The oral LD_{50}-values reported for rats ranged from 2000 to 7500 mg/kg, in mice from 3700 to 6800 mg/kg, and were above 5000 mg/kg for rabbits (WHO 1991, CICAD 2001, OECD 2003, DECOS 1995).

4.1.3 Dermal contact

The dermal LD_{50}-values reported for rabbits ranged from >500 to 5000 mg/kg, in rats from 4000 to 17000 mg/kg, and were above 5000 mg/kg in mice (WHO 1991, CICAD 2001, DECOS 1995).

4.2 Irritation

DMF is reported to be irritating to the eyes, mucous membranes, and the skin (WHO 1991).

4.2.1 Skin irritation

Undiluted DMF was not irritating to the skin of rats (mean primary irritation score: 0) under occlusive conditions on abraded skin after a 24-hour exposure (TSCATS 1978 – quoted from OECD 2003).

A single application of 500 mg/kg resulted in transient irritation within 2 to 3 hours in mice, but no irritation in rats (Wiles & Narcisse 1971 – quoted from WHO 1991).

A single application of neat DMF of 1000 to 5000 mg/kg to the shaved skin of mice produced slight transient skin irritation at 2500 to 5000 mg/kg. No skin irritation was detected in rabbits treated similarly at 100 to 500 mg/kg (Kennedy 1986, Kiss 1979, Bainova 1985 – quoted in WHO 1991, CICAD 2001).

Repeated treatment with DMF at 960 or 1920 mg/kg for 28 days did not induce marked local dermal effects in rats (Bainova et al. 1985 – quoted from WHO 1991, CICAD 2001).

Dermal irritation was not seen in rabbits treated dermally with DMF at 2000 mg/kg for 6 hours daily, 15 times during a 4-week period (Kennedy 1986 – quoted from WHO 1991, CICAD 2001).

After repeated application of DMF to the skin of guinea-pigs for 21 days, the mean irritative dose was 31% DMF (Bainova 1985 – quoted from WHO 1991).

4.2.2 Eye irritation

Single doses of DMF instilled into the eyes of rabbits produced moderate corneal damage and conjunctival redness that was most pronounced at 2 to 3 days. By day 14, a mild degree of conjunctival redness, moderate corneal damage with an area of severe injury, slight surface distortion, and subsurface vascularisation were observed. (Kennedy & Sherman 1986 – quoted from WHO 1991, CICAD 2001). In another study, the same authors reported that, after a single DMF instillation, the eye inflammation subsided and disappeared by the 8^{th} day.

DMF was irritating to the eyes of rabbits following administration of 0.1 ml or one drop twice at an interval of 5 minutes, respectively (TSCATS 1978, BASF 1952 – quoted from OECD 2003). In the study by BASF (1952), severe signs of inflammation (redness, chemosis and purulent secretion) as well as transient opacity of the cornea in one of two animals were observed. In the study by TSCATS (1978), the primary irritation index (according to Draize) was 50.8 after 1 hour, 35.8 after 72 hours, 35.0 on day 4, and 3.3 on day 13; all animals showed large blisters on the inside of upper and lower lids at the 1- and 4-hour readings, blisters were decreased in size at the 24-hour reading, and disappeared at 48 hours.

A 25% solution of DMF in water, injected into the conjunctival sac of the rabbit, did not produce any effects. A 50% solution was slightly irritating and 75 to 100% produced more severe irritation. (Massmann 1956 – quoted from WHO 1991).

4.2.3 Respiratory irritation

Mice and rats, exposed to DMF via inhalation in acute toxicity studies, showed signs of mucous membrane irritation and lung damage was detected histologically (WHO 1991).

In rats exposed up to 6300 mg/m^3, a 30% reduction in the respiratory rate was observed (Dupont unpublished data – cited in DECOS 1995).

4.3 Sensitisation

DMF has been tested, using a maximization technique, on guinea-pigs to determine skin sensitisation; it did not induce any response (Bainova 1985 – quoted from WHO 1991).

DMF has been tested in the local lymph node assay in mice. Cell proliferation, based on [^{3}H]-thymidine incorporation in lymph nodes) was significantly increased in exposed groups receiving a daily topical application on the dorsum of both ears for 3 consecutively days compared with control groups. (Montelius et al. 1996 – quoted from CICAD 2001, Ulrich et al. 2001 – quoted from OECD 2003). According to OECD (2003), there was no clear indication of a sensitising potential of DMF.
In subsequent assays, thymidine incorporation in exposed mice was up to 3-fold higher than in control mice; however, the increase was not considered to be significant (Montelius et al. 1998 – quoted from CICAD 2001).

No difference in proliferation was detected in a lymph node assay in which lymph node cells from DMF-exposed mice were compared with those from control mice (Kimber & Weisenberger 1989 – quoted from CICAD 2001).

4.4 Repeated dose toxicity

The toxicity of DMF following repeated exposure has been extensively studied in a number of animal species including rat, mouse, rabbit, guinea-pig, cat, dog, and monkey, using the inhalation, oral and dermal routes. The studies have identified the liver as the predominant target organ for the toxicity of DMF. The substance may also cause damage to the haematological system and the kidneys as indicated in some animal studies.

A number of the studies included in the various reviews and criteria documents consulted for this assessment of DMF (OECD 2003, CICAD 2001, IARC 1999, DECOS 1995, WHO 1991, IRIS 2005) are not adequate for an evaluation of the repeated dose toxicity of DMF due to various limitations in study design or reporting, and these studies have not been included in this assessment.
The NOAELs and LOAELs presented in this section are those stated in the reviews and criteria documents.

4.4.1 Inhalation

The inhalation studies on DMF included in OECD (2003), CICAD (2001), IARC (1999), DECOS (1995), WHO (1991), and IRIS (2005) are summarised in Table 4.4.1 and supplementary information on selected studies is given in the text.

Rat, 27 days (Germanova et al. 1979 – quoted from WHO 1991, DECOS 1995): DMF was administered at 130 mg/m^3 for 4 hours daily or at 300 mg/m^3 in 5 peaks of 15 minutes at 40-minute intervals. The functional changes in kidneys and liver and the decrease (minimal) in blood pressure were more pronounced after additional single administration of DMF at 500 mg/kg on the 1st, 8th, and 27th days of the studies, as well as after intermittent exposure.

Rat, 28 days (Tanaka 1971 – quoted from WHO 1991, IRIS 2005, DECOS 1995): Young rats (3, 4, 5, 8, or 12 weeks old): Serum AST and ALT were increased (only significant in 3-week old rats). The histopathological changes in the liver (centrilobular degeneration, cloudy swelling and fatty changes) were more pronounced in the younger animals. No histological abnormalities were observed in other organs.
Young rats (3 weeks old): Serum AST and ALT were increased, in animals exposed 8 hours/day at all sacrifice times, and in animals exposed 1 hour/day after the first week only. Degenerative liver changes were observed at the 1st, 2nd, 3rd, and 4th week of test and were more pronounced after 8 hours than after 1 hour of exposure, and more pronounced after 1 week than after 4 weeks of exposure.

Rat, 12 weeks (Craig et al. 1984 – quoted from CICAD 2001, IRIS 2005, DECOS 1995):
Serum cholesterol was significantly increased in females from 1800 mg/m^3 and in males at 3600 mg/m^3. Serum alkaline phosphatase was reduced in a dose-related manner in males, beginning at 900 mg/m^3, but increased in a dose-related manner in females, significantly from 1800 mg/m^3. Data on organ weights were not presented. Histopathological changes were observed in the liver at 3600 mg/m^3 (areas of collapse, necrosis, accumulation of yellow-brown pigment in Kupffer cells, macrophages and hepatocytes, and a large variation in nuclear and cell size); at 900 and 1200 mg/m^3 (mainly characterised by a large variation in nuclear and cellular size, and were, according to WHO, "barely discernible" at 900 mg/m^3); no liver effects were observed at 450 mg/m^3. The NOEC was set, by CICAD, at 450 mg/m^3 based upon slight histopathological changes in the liver. IRIS identified a NOAEC of 450 mg/m^3 based upon the hepatic cell changes.

Rat, 13 weeks (NTP 1992):
Absolute and relative liver weights were increased significantly from 300 mg/m^3 in males and relative liver weights at all dose levels (from 150 mg/m^3) in females, although there was no clear dose-response, as weights declined at the highest dose. Serum cholesterol was significantly increased at all exposure levels with no clear dose-response. In males, at day 24, there was a significant dose-related increase in serum ALT at all exposure levels; at day 91, the increase was only significant at the highest exposure concentration (2400 mg/m^3). At day 91, a dose-related increase in serum sorbitol dehydrogenase (indicative of hepatic effects), which was significant from 600 mg/m^3. Minimal to moderate hepatocellular necrosis was observed from 1200 mg/m^3, with the lesion more severe in females. NTP concluded a NOAEL of 600 mg/m^3 "*…based on the absence of liver histopathology, although liver function assays and liver weights showed changes at all exposure levels*". OECD concluded that "*Although liver histopathology findings were absent, the NOAEC was 300 mg/m^3 based on the findings observed in the liver function assays, i.e., increased serum cholesterol.*"

Rat, 2 years (Malley et al. 1994 – quoted from CICAD 2001, IARC 1999, OECD 2003, DECOS 1995):
Whole-body exposure. A reduction in body weight gain was noted in rats at 1200 mg/m^3 and, to a lesser extent and towards the end of the study, in males at 300 mg/m^3. Survival was not affected. Haematological findings and urinary analyses

were normal. There was a concentration-related increase in serum sorbitol dehydrogenase activity from 300 mg/m^3. Microscopic examination revealed hepatic lesions including accumulation of lipofuscin/haemosiderin (significant in both sexes from 300 mg/m^3), foci of alterations (clear cell, significant in males from 300 mg/m^3 and in females at 1200 mg/m^3), centrilobular hepatocellular hypertrophy (significant in both sexes from 300 mg/m^3), and hepatic single-cell necrosis (females, significant from 300 mg/m^3). Microscopic examination of an extensive range of tissues from the high-dose animals, and of selected tissues from the lower dose groups, revealed no treatment-related lesions except in females, in which there was an increased incidence of uterine endometrial stromal polyps (1.7%, 5.1%, 3.4% and 14.8% for control, low-, mid- and high-dose females, respectively). The NOEC was, according to CICAD and OECD, set at 75 mg/m^3 based upon a significant increase in centrilobular hepatocellular hypertrophy (both sexes), hepatic accumulation of lipofuscin/haemosiderin (both sexes), and hepatic single-cell necrosis (females).

Mouse, 60 days (Craig et al. 1984 – quoted from WHO 1991):
Increased serum AST, ALT, alkaline phosphatase, and cholesterol.

Mouse, 12 weeks (Craig et al. 1984 – quoted from CICAD 2001, IRIS 2005, DECOS 1995):
Mortality was 10% at 1800 mg/m^3 and 40% at 3600 mg/m^3. Body weight gain was not affected. Data on organ weights were not presented. Hepatic changes were characterised by centrilobular cytomegaly and were observed in all exposed mice, the incidence and severity were related to dose. According to DECOS, the cytomegaly appeared to be more prominent in male than in female mice. Liver effects observed at the higher dose levels comprised single cell necrosis, haemosiderosis, and incidentally in the high-dose animals areas of collapse and coagulative necrosis. No histopathological changes were apparent in lungs, heart, thymus, spleen, pancreas, kidneys, or testes. The LOEC was set, by CICAD, at 450 mg/m^3. IRIS identified a LOAEC of 450 mg/m^3 based upon the hepatic cell changes.

Mouse, 13 weeks (NTP 1992):
Body weights were slightly reduced in high-dose females. Relative liver weight was significantly increased in both sexes and absolute liver weight in females at all exposure levels (from 150 mg/m^3), although the dose-response was not clear. Centrilobular hepatocellular hypertrophy (minimal to mild) was observed in all exposed males (from 150 mg/m^3) and in females from 300 mg/m^3. NTP concluded *"…hepatocellular hypertrophy or increased liver weights occurred at all exposure concentrations"*. The LOEC was set by CICAD at 150 mg/m^3 based upon increased relative liver weight in both sexes and hepatocellular hypertrophy in males. OECD concluded that *"Since in chronic inhalation studies in rats and mice (Malley et al. 1994) no increased incidence of hepatic tumours occurred, the hepatocellular hypertrophy can be regarded as the result of an adaptive process, thus the NOAEC for mice is expected to be about 1200 mg/m^3."*

Mouse, 18 months (Malley et al. 1994 – quoted from CICAD 2001, IARC 1999, OECD 2003, DECOS 1995):
Whole-body exposure. Survival was not affected. Haematological observations were normal. At termination, mid- and high-dose males and high-dose females had higher liver weights. Microscopic alterations in the liver were observed at all exposure levels and included accumulation of Kupffer cell hyperplasia/pigment (significant in males from 75 mg/m^3 and in females from 300 mg/m^3), centrilobular hepatocellular hypertrophy (significant in males from 75 mg/m^3 and in females from 300 mg/m^3), and hepatic single-cell necrosis (significant in both sexes from

75 mg/m^3). The LOEC was, according to CICAD and OECD, set at 75 mg/m^3 based upon a significant increase in centrilobular hepatocellular hypertrophy (males), hepatic accumulation of Kupffer cell hyperplasia/pigment (males), and hepatic single-cell necrosis (both sexes).

Monkey, 13 weeks (Hurtt et al. 1992 – quoted from CICAD 2001, IARC 1999, OECD 2003, DECOS 1995):
Whole-body exposure. Three animals per sex per group, and two additional males per group, which were maintained for a further 13-week observation period after exposure had ceased. Microscopic examination included a comprehensive range of organs and tissues in all animals. Sperm morphology and vaginal cytology were also evaluated in all animals. There were no overt signs of toxicity and no effects on body weight gain, haematology, clinical chemistry, urinalysis, organ weights, or histopathological effects attributable to DMF at exposure concentrations up to 1500 mg/m^3. OECD concluded that the NOAEC is 1500 mg/m^3. The authors concluded, according to CICAD and DECOS, that the monkey is much less sensitive than the rat or mouse.

Rat, mouse, rabbit, guinea-pig, dog, 58 days (Clayton et al. 1963 – quoted from IRIS 2005, DECOS 1995):
Animals were exposed to 60 mg/m^3 for 5.5 hours/day followed by 1300 mg/m^3 for 0.5 hours/day for 58 weekdays. This exposure regimen was designed to simulate peak exposures that occur in plant operations. No adverse clinical signs were observed except for one of the four dogs, which had a decrease in systolic blood pressure. This dog also had increased plasma cholesterol and exhibited degenerative myocardial changes at necropsy. Increased liver weight was only significant in mice.
According to DECOS, a number of pathological changes were noted in the exposed animals. Organs that were affected in each of the species were liver, pancreas, spleen, kidneys, adrenals, and thymus. However, a description of the histopathological changes has not been included in the paper, only an indication whether anatomical changes were present or not.

According to CICAD (2001), the inhalation studies performed by Massmann (1956), Clayton et al. (1963), Cai & Huang (1979) and Arena et al. (1982) are either poorly reported or limited in their scope.

4.4.2 Oral intake

The oral studies on DMF included in OECD (2003), CICAD (2001), DECOS (1995), and WHO (1991) are summarised in Table 4.4.2 and supplementary information on selected studies is given in the text.

Rat, 28 days, gavage (BASF 1977 – quoted from OECD 2003):
All high-dose (1900 mg/kg b.w./day) animals died, mostly at the beginning of the study. Reduced food consumption was noted for males at all dose levels and in females at the two highest dose levels (from 950 mg/kg b.w./day). Changes in clinical chemistry values indicative of altered liver function (e.g., increased

Table 4.4.1. Repeated dose toxicity studies on DMF, inhalation

Species / strain	Duration / Dose levels (mg/m^3)	Effects	NOAEC (mg/m^3)	LOAEC (mg/m^3)	Reference
Rat	27 days 130 - 4 h/d or 300 peaks	changes liver, kidney, ↓ blood pressure			Germanova et al. (1979 – in WHO, DECOS)
Rat / ♀ (3-12 weeks old)	28 days 600 - 8 h/d	enz, hist liver			Tanaka (1971 – in WHO, IRIS, DECOS)
Rat / ♀ (3 weeks old)	28 days 600 - 8 h/d or 1 h/d	enz, hist liver, no accumulation of hepatotoxicity			Tanaka (1971 – in WHO, IRIS, DECOS)
Rat 10/sex	58 weekdays 60 - 6h/d	↑ w liver, ↑ cholesterol, hist		60 (IRIS)	Clayton et al. (1963 – in WHO, IRIS, DECOS)
Rat / F344	12 weeks 0, 450, 900, 1800, 3600 - 6 h/d, 5 d/wk	3600: ↓ bw, ↑ cholesterol ♂ ≥ 1800: ↑ cholesterol ♀, ↑ enz ♀ ≥ 900: ↓ enz ♂, hist liver	450 (IRIS) 450 (NOEC CICAD)		Craig et al. (1984 – in CICAD, IRIS, DECOS)
Rat / F344 30/sex/group	13 weeks 0, 150, 300, 600, 1200, 2400 - 6 h/d, 5 d/wk	≥ 1200: ↓ bwg, hist liver ≥ 300: ↑ abs/rel w liver ♂ ≥ 150: ↑ cholesterol, ↑ enz, ↑ rel w liver ♀	600 (NTP) 300 (OECD)	600 (OECD)	NTP (1992)
Rat / Crl:CD BR 87/sex/group	2 years 0, 75, 300, 1200 - 6 h/d, 5 d/wk	1200: ↓ bwg, ↑ rel w liver ≥ 300: ↓ bwg ♂, enz, hist liver	75 (NOEC CICAD, OECD)	300 (LOEC CICAD, OECD)	Malley et al. (1994 – in CICAD, IARC, OECD, DECOS)
Mouse 11 ♀	58 weekdays 60 - 6h/d	↑ w liver, hist		60 (IRIS)	Clayton et al. (1963 – in WHO, IRIS, DECOS)
Mouse	60 days 450, 900, 1800, 3600 - 6 h/d	≥ 900: ↑ enz, cholesterol, anaemia, hist liver ≥ 450: ↑ w liver	< 450 (NOEL WHO)		Craig et al. (1984 – in WHO)
Mouse / B6C3F1	12 weeks 0, 450, 900, 1800, 3600 - 6 h/d, 5 d/wk	≥ 1800: mortality ≥ 450: hist liver		450 (IRIS, DECOS) 450 (LOEC CICAD)	Craig et al. (1984 – in CICAD, IRIS, DECOS)
Mouse / B6C3F1 10/sex/group	13 weeks 0, 150, 300, 600, 1200, 2400 - 6 h/day, 5 d/wk	2400: ↓ bw ♀ ≥ 300: hist liver ♀ ≥ 150: ↑ w liver, hist liver ♂	1200 (OECD)	150 (LOEC CICAD) 2400 (OECD)	NTP (1992)
Mouse / Crl:CD 1 (ICR)BR 78/sex/group	18 months 0, 75, 300, 1200 - 6 h/d, 5 d/wk	1200: ↑ rel w liver ♀ ≥ 300: ↑ rel w liver ♂ ≥ 75: hist liver		75 (LOEC CICAD, OECD)	Malley et al. (1994 – in CICAD, IARC, OECD, DECOS)

Rabbit 2/sex	58 weekdays 60 - 6h/d	↑ w liver, ↑ cholesterol, hist		60 (IRIS)	Clayton et al. (1963 – in WHO, IRIS, DECOS)
Rabbit	14 weeks 317 ± 37.8 - 6 h/d, 6 d/wk	bw changes, liver damage functionally/ structurally (congestion/ haemorrhage)			Cai & Huang (1979 – in WHO)
Rabbit	18 weeks 22 ± 1.6 - 6 h/d, 6 d/wk	no changes liver parameters or ECG			Cai & Huang (1979 – in WHO)
Guinea-pig 10 ♂	58 weekdays 60 - 6h/d	hist			Clayton et al. (1963 – in WHO, IRIS, DECOS)
Cat	10 weeks 3000 - 6 h/d, 6 d/wk	no effects (detailed examination of liver and kidney)			Hoffman et al. (1960 – in DECOS)
Cat	120 days 300, 690, 1350 – 8 h/d, 6 d/wk	weight loss, liver degeneration/ necrosis, changes brain/ myocardium/kidney, iron deposition spleen, bronchopneumonia, no changes haematology or ECG		300 (IRIS)	Massmann (1956 – in WHO, IRIS, DECOS)
Dog	28 days 63 - 6 h/d, 5 d/wk	no changes enz	63 (NOEL WHO)		Kimmerle & Eben (1975 – in WHO, DECOS)
Dog 4 ♂	58 weekdays 60 - 6h/d	↑ w liver, ↑ cholesterol, ↓ blood pressure, hist		60 (IRIS)	Clayton et al. (1963 – in WHO, IRIS, DECOS)
Cynomolgus monkey	2 weeks 1500 - 6 h/d, 5 d/wk	no effects			Hurtt et al. (1992 – in IARC, DECOS)
Cynomolgus monkey 3/sex/group	13 weeks 0, 90, 300, 1500 - 6 h/d, 5 d/wk	no effects	1500 (OECD) >1500 (DECOS)		Hurtt et al. (1992 – in CICAD, IARC, OECD, DECOS)

↓: reduced
↑: increased
♂ / ♀: male / female
bw / bwg: body weight / body weight gain
enz: enzyme activity / activities or levels
hist: histopathological changes
w: weight
abs: absolute
rel: relative
SD: Sprague Dawley
h: hour(s)
d: day(s)
wk: weeks

enzyme activities) and altered kidney function (elevated urea (females) and creatinine values), and histopathological changes in the liver (acute to subacute haemorrhagic liver dystrophy with necrosis) were observed at the two highest dose levels. OECD concluded that the NOAEL is 240 mg/kg b.w./day based upon the increased relative liver weights in both sexes and increased relative kidney weights in males at the higher dose levels.

Rat, 49 days, drinking water (Elovaara et al. 1983 – quoted from WHO 1991, DECOS 1995):
The relative liver weight was significantly increased at all dose levels. In liver and kidneys, increased (dose-related) glutathione, microsomal UDP glucuronosyl transferase, and ethoxy coumarin *O*-demethylase activities. No changes in liver microsomal cytochrome P-450 or ADPH-cytochrome reductase activity.

Rat, 90 days, diet (Kennedy & Shermann 1986 – quoted from WHO 1991; Haskell Laboratory 1960, Kennedy & Shermann 1986 – quoted from CICAD 2001; TSCATS 1960 – quoted from OECD 2003; Llwellyn et al. 1974 – quoted from DECOS 1995)):
Rats were, according to WHO, OECD and DECOS, administered 200, 1000, or 5000 mg/kg diet (equivalent to 12, 60, or 300 mg/kg b.w./day), and, according to CICAD and DECOS, 0, 10, 50, or 250 mg/kg b.w./day.
The following paragraph is based on CICAD: Reduced weight gain and increase in serum cholesterol (statistical analyses were not presented) were observed in high-dose animals. Mild effects in the liver (enlargement of hepatic cells) and haematological effects (anaemia, leucocytosis) were observed in mid- and high-dose animals. The NOEL was set at 10 mg/kg b.w./day based upon a significant increase in relative liver weights in males.
The following paragraph is based on OECD: In mid- and high-dose animals, increased (slight) relative liver weights, increased cholesterol, elevated phospholipid values, leucocytosis, and a decrease in the red blood cell count were observed. In high-dose animals, depressed body weight gain and reduced food consumption, slight anaemia, and mild liver injury was also observed. OECD concluded that the NOAEL is 12 mg/kg b.w./day as the increased relative liver weights at the higher dose levels were dose-related.
The following paragraph is based on DECOS: In high-dose animals, reduced growth, increased absolute and relative liver weights, slight anaemia, hypercholesterolaemia, slight liver injury, and leucocytosis were observed. The NEL was considered to be 50 mg/kg b.w./day.

Rat, 15 weeks, diet (Becci et al. 1983 – quoted from WHO 1991, CICAD 2001, DECOS 1995):
Rats were, according to WHO and DECOS, administered 215, 750, or 2500 mg/kg diet, and, according to CICAD and DECOS, 0, 18/20, 61/69, or 210/235 mg/kg b.w./day.
The following paragraph is based on CICAD: This study involved a larger group size than the 90-day dietary study described just above and a more comprehensive tissue examination. Growth was inhibited but no tissue lesions were observed. A significant increase in relative liver weight was observed in females from 69 mg/kg b.w./day. The NOEL was set at 20 mg/kg b.w./day based upon increased relative liver weights in females.
The following paragraph is based on DECOS: A significant reduction in body weight gain and food consumption was observed in the high-dose group and was considered a consequence of decreased palatability of the DMF diets. A dose-related, statistically significant increase in relative liver weight was observed from 61/69 mg/kg b.w./day; at the lowest dose level (18/22 mg/kg b.w./day), the relative liver weight was still increased, though not significantly higher than in controls.

The dose-related increase in relative liver weight was considered by the authors to be a normal phenomenon (physiological adaptation) required for the biotransformation of DMF. Histopathology did not reveal any abnormalities attributable to treatment.

Mouse, 17 weeks, diet (Becci et al. 1983 – quoted from WHO 1991, CICAD 2001, DECOS):
Mice were, according to WHO and DECOS, administered 160, 540, 1850 mg/kg diet, and, according to CICAD and DECOS, 0, 22/28, 70/96, or 246/326 mg/kg b.w./day.
The following paragraph is based on CICAD: There were no overt signs of toxicity and no notable effects on blood morphology, blood biochemistry, or urinary parameters. There was a dose-related increase in relative liver weight as all doses, but this was statistically significant only in the mid- and high-dose females and in the high-dose males. Microscopic examination of an extensive range of organ and tissues revealed only mild effects on the liver in the majority of high-dose animals. The NOEL was set at 28 mg/kg b.w./day based upon increased relative liver weights in females.
The following paragraph is based on DECOS: Relative spleen, thyroid and adrenal weights were significantly increased without showing any histopathological changes attributable to treatment. The dose-related increase in relative liver weight was considered by the authors to be a normal phenomenon (physiological adaptation) required for the biotransformation of DMF. The slight hepatocytomegaly in high-dose animals was not considered by the authors to be a toxic effect.

Dog, 13 weeks, diet (BASF 1984 – quoted from WHO 1991, CICAD 2001):
The protocol included measurements of food consumption and body weight gain, hearing tests, ophthalmoscopic examination, clinical laboratory investigations, measurement of organ weights, and histopathological observations.

4.4.3 Dermal contact

The dermal studies on DMF included in DECOS (1995), WHO (1991), are summarised in Table 4.4.3 and supplementary information on selected studies is given in the text.

Rat, 28 days (Bainova et al. 1981, Bainova 1985 – quoted from WHO 1991, DECOS 1995):
Dermal application of 960 mg/kg daily or 1920 mg/kg intermittently; two alternative intermittent regimens were used: 1) 1920 mg/kg/day for 2 days, followed by no treatment for 2 days, and 2) 1920 mg/kg every second day. Functional, biochemical, and pathomorphological changes were observed in the liver on the 4^{th}, 8^{th}, 14^{th}, and 28^{th} day of the tests. Changes were more pronounced after intermittent exposure.

Rat, 30 days (Bainova & Antov 1980 – quoted from WHO 1991, DECOS 1995):
Dose-related changes AST, ALT, alkaline phosphatase, γ-glutamyl transpeptidase (gamma-GT), lipid fractions in serum and liver homogenates.

Table 4.4.2. Repeated dose toxicity studies on DMF, oral administration

Species / strain	Duration / Dose levels	Effects mg/kg bw/day	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Reference
Rat	14 days, drinking water 102, 497, 1000 mg/litre (7.8/7, 39/36, 75/66 mg/kg bw/day - DECOS)	≥ 7-8: dose-related deviations cerebral/glial cell enz; no behav changes	< 7-8 (NEL DECOS)		Savolainen (1981 – in WHO, DECOS, CICAD)
Rat / ♂	14 days, drinking water 0, 14, 70, 140 mg/kg bw/day	≥ 14: ↑ rel w liver (dose-related, significant), enz			Elovaara et al. (1983 – in CICAD)
Rat / SD	28 days, gavage, 5 days/week 240, 475, 950, 1900 mg/kg bw/day	1900: ↑ mortality 950: ↓ bw ♀, ↑ enz, ↑ rel w kidney ♀, hist liver ≥ 475: ↓ bw ♂, ↑ rel w liver, ↑ rel w kidney ♂	240 (OECD)	475 (OECD)	BASF (1977 – in OECD)
Rat	49 days, drinking water 100, 500, 1000 mg/litre (7.8/7, 39/36, 75/66 mg/kg bw/day - DECOS)	≥ 7-8: ↑ rel w liver, ↑ enz	< 7-8 (NEL DECOS)		Elovaara et al. (1983 – in WHO, DECOS)
Rat / Crl:CD	90 days, diet 200, 1000, 5000 mg/kg diet (equiv 12, 60, 300 mg/kg bw/day –- WHO, OECD)	300: ↓ bwg, ≥ 60: ↑ rel w liver, haematol, ↑ cholesterol, hist liver 250: ↓ bwg, ↑ abs/rel w liver, haematol, ↑ cholesterol, hist liver (DECOS)	12 (NOEL WHO) 10 (NOEL CICAD) 12 (OECD) 50 (NEL DECOS)	50 (LOEL CICAD) 60 (OECD)	Kennedy & Sherman (1986 – in WHO, CICAD) Haskell Laboratory (1960 – in CICAD) TSCATS (1960 – in OECD) Llwellyn et al. (1974 – in DECOS)
Rat / Wistar	15 weeks, diet 0, 215, 750, 2500 mg/kg diet (0, 18/20, 61/69, 210/235 mg/kg bw/day – CICAD, DECOS)	↑ abs/rel w liver (dose-related) (WHO) 210: ↑ rel w liver ♂ ≥ 69: ↑ rel w liver ♀ (CICAD) 210: ↓ bwg, ↑ abs w liver ≥ 61: ↑ rel w liver (DECOS)	18/20 (WHO) 20 (NOEL CICAD) <18/20 (NEL DECOS)	69 (LOEL CICAD)	Becci et al. (1983 – in WHO, CICAD, DECOS)
Mouse / CD 1	17 weeks, diet 0, 160, 540, 1850 mg/kg diet (0, 22/28, 70/96, 246/326 mg/kg bw/day – CICAD, DECOS)	↑ abs/rel w liver (dose-related), no hist or enz changes (WHO) 246/326: hist liver 22/28: ↑ rel w liver (CICAD) 246/326: enz (↑ ALT), ↑ w adrenal/ thyroid/ spleen, hist liver 22/28: ↑ abs/rel w liver (DECOS)	246-326 (NOEL WHO) 28 (NOEL CICAD) <22/28 (NEL DECOS)	96 (LOEL CICAD)	Becci et al. (1983 – in WHO, CICAD, DECOS)
Dog / Beagle 4/sex/group	13 weeks, diet 0, 1.4, 7.0, 34.8 mg/kg bw/day	no effects	34.8 (NOEL CICAD)		BASF (1984 – in CICAD)

↓: reduced
↑: increased
♂ / ♀: male / female
bw / bwg: body weight / body weight gain
enz: enzyme activity / activities or levels
hist: histopathological changes
w: weight
abs / rel: absolute / relative
behav: behavioural
equiv: equivalent to

Table 4.4.3. Repeated dose toxicity studies on DMF, dermal administration

Species / strain	Duration / Dose levels	Effects	NOAEL mg/kg bw/day	Reference
Rat	28 days 960 mg/kg bw/day or 1920 mg/kg bw/day intermittently	≥ 960: hepatotoxicity		Bainova et. al (1981), Bainova (1985) (in WHO, DECOS)
Rat	30 days 215, 430, 960, 4800 mg/kg bw/day	dose-related changes enz liver	215 (NOEL WHO) 215 (DECOS)	Bainova & Antov (1980 – in WHO, DECOS)
Rat	30 days 215, 320, 960, 4800 mg/kg bw/day	≥ 320: dose-related changes enz liver, kidney, myocardium	215 (NOEL WHO) 215 (DECOS)	Bainova (1985 – in WHO, DECOS)
Rabbit	7 days 50, 100% in water - 3 times/day	100%: mortality day 5-8, changes enz, hist liver		Huang et al. (1981 – in WHO, DECOS)
Rabbit	9 days 2000 mg/kg bw/day	anorexia, cyanosis, mortality, liver necrosis		Kennedy & Sherman (1986 – in WHO, DECOS)
Guinea-pig	7 days 50, 75, 100% in water - 3 times/day	≥ 75%: mortality day 2-4 50%: mortality day 4-9, loss bw, liver damage		Huang et al. (1981 – in WHO, DECOS)

bw: body weight
enz: enzyme activity / activities or levels
hist: histopathological changes

4.5 Toxicity to reproduction

The toxicity of DMF to reproduction has been studied in one continuous breeding study in mice using the oral route (drinking water), and extensively in developmental toxicity studies in a number of animal species including rat, mouse, and rabbit, using the inhalation, oral (gavage) and dermal routes.

A number of the studies included in the various reviews and criteria documents consulted for this assessment of DMF (OECD 2003, CICAD 2001, IARC 1999, DECOS 1995, WHO 1991, IRIS 2005) are not adequate for an evaluation of the toxicity of DMF to reproduction due to various limitations in study design or reporting, and these studies have not been included in this assessment.
The NOAELs and LOAELs presented in this section are those stated in the reviews and criteria documents.

4.5.1 Inhalation

In male rats exposed at 90 or 900 mg/m^3 6 hours/day over 5 days, no gross or histopathological changes were observed in reproductive organs after 6 weeks. Pairing of the exposed males with unexposed females for 6 weeks after exposure resulted in a reduced number of viable foetuses per dam in the low-dose group only. (Lewis et al. 1979 – quoted from CICAD 2001, WHO 1991).

In rats, examination of the sperm and histology of the testes and ovaries did not show any pathological signs. Male rats were exposed to 49-51 mg/m^3 or 584-616 mg/m^3 for 4 hours/day for 2, 4, or 8 days; female rats were exposed to 2.3 or 10.7 mg/m^3 for 4 hours/day for 30 days. (Sheveleva et al. 1979 – quoted from WHO 1991).

No testicular or ovarian lesions were observed in rats or mice after exposure for 12 weeks to DMF at concentrations of up to 3600 mg/m^3 (Craig et al. 1984 – quoted from WHO 1991), see also Table 4.4.1.

In a 13-week inhalation (whole-body) study with cynomolgus monkeys exposed to DMF at concentrations of 0, 90, 300, or 1500 mg/m^3 for 6 hours/day on 5 days/week, no significant effect on semen volume, percentage of motile sperm, sperm count, or abnormal sperm morphology was observed (Hurtt et al. 1992 – quoted from IARC 1999).

The inhalation studies on the developmental toxicity of DMF included in OECD (2003), DECOS (1995), WHO (1991), and IRIS (2005) are summarised in Table 4.5.1 and supplementary information on the studies is given in the text.

Rat, GD; rabbit, GD 7-19 (Hellwig et al. *in press* – quoted from WHO 1991; BASF 1974 – quoted from IRIS 2005; Praetorius 1978 – quoted from IRIS 2005; Hellwig et al. 1991 – quoted from OECD 2003):
Rat: A series of inhalation studies have been carried out in which the exposure periods did not fully cover the critical period of the gestation phase.
In one set, exposure to 660 or 1560 mg/m^3 for 6 hours/day on gestation days 4-8 (18 animals per group) caused decreased maternal weight gain, decreased foetal weights, retardations and increased embryolethality.
In another set, 861 mg/m^3 for 6 hours/day on gestation days 0-1, 4-8, 11-15, and 18-19; or 0-3, 6-10 and 13-18 (30 animals, 20 animals subjected to caesarean section on gestation day 20, the offsprings of the other 10 animals wee raised until

post-natal day 21) resulted in decreased maternal weight gain from the beginning of the treatment, decreased foetal weights, resorptions, and increased numbers of variations and retardations. No malformations were noted.
Rabbit: At 1350 mg/m^3, animals showed a slight retardation in body weight gain. The foetal weights were significantly lower, and there was a significant increase in malformations, mostly hernia umbilicalis (7/86 foetuses, in 4/15 litters). Some soft-tissue malformations (missing gall bladder) were also recorded (not significantly increased) as well as anomalies of the sternum, an increase in split vertebra, and a number of variations. At 450 mg/m^3, maternal body weights were slightly retarded and the corrected body weight gain was marginally, but significantly, decreased. One case of hernia umbilicalis was recorded among 75 foetuses, and an increase in sternal variations were observed.

Rat, GD 6-15 (Biodynamics 1978 – quoted from IRIS 2005):
A significant reduction in implantations and foetuses was observed in the dams exposed to 90 mg/m^3. Since the resorption rate was not significantly increased, the authors concluded that the reduction in the number of foetuses in this group could be attributed to an unexplainable decrease in both ovulation and implantation rates.

4.5.2 Oral intake

In a multi-generation study (continuous breeding) in Swiss mice (10-20/group), DMF was administered in the drinking water at concentrations of 0, 1000, 4000, or 7000 mg/litre. (Fail et al. 1998). Litters from F_0 animals were sacrificed immediately. At week 16, pairs were separated and the final litters reared to post-natal day 21, then entered into an F_1 fertility assessment. A crossover mating trial was also carried out with the high-dose F_0 animals.
Estimates of actual doses were:
1000 mg/l: 182-188 mg/kg b.w./day for F_0 males and 256-193 mg/kg b.w./day for F_0 females at week 1 and week 27, respectively; 239-213 mg/kg b.w./day for F_1 males and 315-268 mg/kg b.w./day for F_1 females at week 12 and week 16, respectively.
4000 mg/l: 675-545 mg/kg b.w./day for F_0 males and 845-730 mg/kg b.w./day for F_0 females at week 1 and week 27, respectively; 1006-888 mg/kg b.w./day for F_1 males and 1172-1024 mg/kg b.w./day for F_1 females at week 12 and week 16, respectively.
7000 mg/l: 1071-1026 mg/kg b.w./day for F_0 males and 1276-1578 mg/kg b.w./day for F_0 females at week 1 and week 27, respectively; 1943-1684 mg/kg b.w./day for F_1 males and 2160-1948 mg/kg b.w./day for F_1 females at week 12 and week 16, respectively.
In the F_0 animals, reduced body weight was observed in high-dose females, and increased relative liver weight (both sexes), increased kidney/adrenal weights (females), and increased epididymal weight (males) were observed at all dose levels. Histopathological examination of livers revealed centrilobular hepatocellular hypertrophy in mid- and high-dose groups; no histopathological changes were observed in the male reproductive organs. F_0 animals included reduced fertility and fecundity in mid- and high-dose groups. Reproductive effects in the A decreased number of high-dose females had normal oestrus cycles. F_1 pup post-natal survival and pup weight was reduced in the mid- and high-dose groups. Pups born to DMF-treated pairs had external malformations or other abnormalities, including craniofacial and sternebral malformations (domed heads and haematomas along the nose and on the head). Those pups affected most severely died shortly after birth. Those animals less affected did grow to maturity, and examination after necropsy indicated that the malformations present at birth had persisted through young adulthood. During the continuous breeding phase, the proportion of litters

Table 4.5.1. Reproductive toxicity studies on DMF, inhalation

Species / strain	Duration / Dose levels (mg/m³)	Effects	NOAEC (mg/m³)	LOAEC (mg/m³)	Reference
Rat	see text 0, 660, 861, 1560 6 h/day	≥ 861 ↓ bwg maternal, embryolethality ≥ 660: ↓ w/length foetal	<861 (ME, dev) (DECOS)	660 (IRIS)	Hellwig et al. (*in press* – in WHO) Helwigg et al. (1991 – in DECOS) BASF (1974 – in IRIS)
Rat / LE 23/group	GD 6-15 0, 54, 516 6 h/day	516: ↓ w foetal	54 (IRIS) 516 (ME, TE) 54 (FE) (OECD)	516 (IRIS)	Kimmerle & Machemer (1975 – in WHO, IRIS, OECD, DECOS)
Rat / SD 19/group	GD 6-15 0, 90, 900 6 h/day	900: ↓ bwg maternal, ↓ w foetal, ↑ (slightly) incidence skeletal (ossification) variations (from 60 to 75%) 90: ↓ implantations/foetuses	90 (IRIS, DECOS) 90 (ME, FE) 900 (TE) (OECD)	900 (IRIS)	Keller & Lewis (1981 – in WHO, DECOS) BioDynamics (1978 – in IRIS) TSCATS (1978 – in OECD)
Rabbit 15/group OECD TG 414	GD 7-19 0, 150, 450, 1350 6 h/day	1350: ↓ w foetal ≥ 450: ↓ bwg maternal, ↑ variations, TE	150 (IRIS) 150 (ME, FE, TE) (OECD) 150 (ME, dev) (DECOS)	450 (IRIS)	Hellwig et al. (*in press* – in WHO) Hellwig et al. (1991 – in OECD, DECOS) Praetorius (1989 – in IRIS)

↓: reduced
↑: increased
bwg: body weight gain
w: weight
LE: Long Evans
SD: Sprague Dawley
GD: gestation days
ME: maternal toxicity
FE: foetotoxicity
TE: teratogenicity
dev: developmental toxicity

with one or more pups with an abnormal appearance was 10.5%, 90.0% and 77.8% for the low-, mid- and high-dose groups, respectively, compared to 7.9% for the control group. The lower incidence in the high-dose group compared to the mid-dose group was, according to the authors, due to the decreased fertility, increased prenatal death, and postnatal cannibalism observed in the high-dose group.
Animals selected for the F_1 parental generation showed reduced body weights in the mid- and high-dose groups, increased absolute and relative liver weights in both sexes at all dose levels, increased relative kidney/adrenal weights in mid- and high-dose females, decreased relative prostate weight at all dose levels, and centrilobular hepatocellular hypertrophy in both sexes at all dose levels; no histopathological changes were observed in the male reproductive organs. F_1 oestrus cycle length was significantly longer in the high-dose females compared to the control animals. The epididymal spermatozoa concentration was reduced in high-dose males. Reproductive effects in the F_1 animals included reduced mating index in the high-dose group; reduced fertility (number pregnant), increased average days to litter, and decreased number of live pups per litter and proportion of pups born alive in mid- and high-dose groups; and decreased live pup weight at all dose levels. F_2 pups born to DMF-treated F_1 pairs exhibited malformations similar to those observed for F_1 litters. The proportion of litters with one or more pups with an abnormal appearance was 0, 27.7%, 60% and 75% for the control, low-, mid- and high-dose groups, respectively.
In the crossover mating trial conducted with high-dose animals, groups with dosed females had fewer pups per litter, pup weights were lower, and pups exhibited malformations similar to those observed during the continuous breeding phase. The proportion of litters with one or more externally malformed pups was 12.5% for control male mated with control female, 0% for treated male mated with control female, and 90.0% for control male mated with treated female. These data suggest, according to the authors, that the female was the sex affected by DMF exposure.
According to the authors, the maximum tolerated dose for both generations was 1000 ml/l (average exposure of 219 mg/kg b.w./day); a NOAEL for DMF could not be established.
According to OECD (2003), 1000 mg/l (approximately 219 mg/kg b.w./day) was a NOAEL for fertility in both generations and for F_1 developmental toxicity, and a LOAEL for parental systemic toxicity in both generations and for F_2 developmental toxicity.

The histological examination of male rats treated orally with DMF in short-term studies (Becci et al. 1983, Kennedy & Sherman 1986 – both quoted from WHO 1991) with a variety of doses (see also Table 4.4.2) did not reveal any changes in the testes.

The oral studies on the developmental toxicity of DMF included in OECD (2003), DECOS (1995), and WHO (1991) are summarised in Table 4.5.2 and supplementary information on the studies is given in the text.

Rat, GD 6-15 (Hellwig et al. *in press* – quoted from WHO 1991; Hellwig et al. 1991 – quoted from OECD 2003):
A dose-dependent decrease in foetal weights and an increase in the number of retardations and variations were observed.
At the high dose, 63% of the implantations were resorbed and 12% of the surviving 85 foetuses were malformed (9 cases of diffuse anasarca, 2 cases of tail aplasia, 1 micrognathia); several foetuses had anomalies of the ribs, sternum, and vertebral column.
At the mid dose, some early foetal deaths was observed; malformations observed consisted of 2 cases of tail aplasia, 2 cases of cleft palate, 1 atresia ani, 1 anasarca, 1 open eye, and several foetuses with split and aplastic vertebrae.

At the low dose, a slight but significant and dose-related reduction of mean placental weight was observed, however, the number of live foetuses and foetal weights at this dose were comparable to the control or even higher.

Rat, GD 6-20 (Saillenfait et al. 1997 – quoted from OECD 2003):
Maternal toxicity from 100 mg/kg b.w./day in form of dose-dependent impairment of body weight gain and food consumption was observed. Foetotoxicity also occurred at these dose levels in form of a dose-related decrease in foetal body weight per litter and in increase in the total number of foetuses with skeletal variations (significant from 200 mg/kg b.w./day). The total number of skeletal variations was also slightly increased at 50 mg/kg b.w./day.

Mouse, GD 6-15 (Hellwig et al. *in press* – quoted from WHO 1991; Hellwig et al. 1991 – quoted from OECD 2003):
A dose-dependent decrease in foetal weights and in foetal growth, and an increase in the number of retardations and variations were observed. According to OECD, a dose-response relationship was not seen for reduced foetal body weight and growth. At the high dose, clear signs of teratogenicity was observed (significant increase in malformations, mostly related to the head (cleft palate, exencephaly, hydro-cephalus internus, aplasia of presphenoid), in 17/241 foetuses). At the low dose (193 µl/kg b.w./day), a slight (not significant, in 4/245 foetuses) increase in malformations (mostly cleft palate) was observed. No maternal effects were recorded.

Rabbit, GD 6-18 (Merkle & Zeller – quoted from WHO 1991, DECOS 1995; BASF 1976 – quoted from OECD 2003):
All 11 high-dose animals became pregnant and showed reduced food intake and body weight gain. Placental weights were significantly lower and 3 abortions occurred. The foetuses showed weight reduction. Malformations were observed among 16 foetuses in 7 litters and included hernia umbilicalis (7 cases), hydrocephalus internus (6 cases), eventratio simplex (3 cases), exophthalmus (2 cases), cleft palate (1 case), and malposition of limbs (1 case).
In the mid-dose group, 16/18 animals became pregnant. An increase in skeletal variations and retardations were present and hydrocephalus internus were reported (in 3 foetuses in 2 litters).
In the low-dose group, 10/12 animals became pregnant; one case of hydrocephalus internus was reported.

4.5.3 Dermal contact

No testicular lesions were noted in male rats after a 30-day dermal application of DMF (Bainova 1985 – quoted from WHO 1991), see also Table 4.4.3.

The dermal studies on the developmental toxicity of DMF included in OECD (2003), IARC (1999), DECOS (1995), WHO (1991), are summarised in Table 4.5.3 and supplementary information on the studies is given in the text.

Rat, GD 6-10, 13-15; rabbit, GD 6-28 (Hellwig et al. *in press* – quoted from WHO 1991; Hellwig et al. 1991 – quoted from OECD 2003):
Rat: DMF was administered as undiluted material in an uncovered dermal system. At 1000 µl/kg b.w./day, there was a slightly retarded weight gain among the dams and significant dermal irritation. The foetuses were slightly smaller. Malformations consisted of split thoracic vertebrae and anomalies of the ribs. The rate of the malformations in live foetuses was 0% (control group), 2.46%, 3.05%, and 5.46%

Table 4.5.2. Reproductive toxicity studies on DMF, oral administration

Species / strain	Duration / Dose levels	Effects mg/kg bw/d	NOAEL mg/kg bw/d	Reference
Rat / SD 26/group FDA TG	GD 6-15 gavage 0, 176, 533, 1600 µl/kg bw/day (167, 506, 1520 mg/kg bw/day)	≥ 506: ↓ bw maternal, embryolethality, ↓ w foetal, retardations, variations, malformations 167: ↓ w placenta	167 (ME, FE, TE) (OECD) 167 (ME), <167 (dev) (DECOS)	Hellwig et al. (*in press* – in WHO) Hellwig et al. (1991 – in OECD, DECOS)
Rat / SD	GD 6-20 gavage 0, 50, 100, 200, 300 mg/kg bw/day	≥ 100: ↓ bwg maternal, ↓ w foetal ≥ 50: variations	50 (ME, FE), 300 (TE) (OECD)	Saillenfait et al. (1997 – in OECD)
Mouse / NMRI 26/group FDA TG	GD 6-15 gavage 0, 193, 580 µl/kg bw/day (183, 551 mg/kg bw/day)	≥ 183: ↓ w foetal, malformations, retardations, variations	551 (ME), 183 (FE, TE) (OECD) >551 (ME), >183 (dev) (DECOS)	Hellwig et al. (*in press* – in WHO) Hellwig et al. (1991 – in OECD, DECOS)
Rabbit 26/group FDA TG	GD 6-18 gavage 0, 46.4, 68.1, 200 µl/kg bw/day (44.1, 65, 190 mg/kg bw/day)	190: ↓ bwg maternal, ↓ w placenta, ↓ w foetal ≥ 65: ↓ number implantations / live foetuses ≥ 44.1: malformations	65 (ME, FE), 44.1 (TE) (OECD) 44.1 (DECOS)	Merkle & Zeller (1980 – in WHO, DECOS) BASF (1976 – in OECD)

↓: reduced
↑: increased
bw: body weight
bwg: body weight gain
w: weight
GD: gestation days
SD: Sprague Dawley
ME: maternal toxicity
FE: foetotoxicity
TE: teratogenicity
dev: developmental toxicity

with increasing dose level. According to WHO, this may indicate a weak dose-related teratogenic effect.
Rabbit: DMF was applied in undiluted form under semi-occlusive conditions. A dose-dependent skin irritation occurred in all DMF treated animals. A slight (5-6%) but significant decrease in maternal body weights occurred in the highest dose group towards the end of the treatment period (GD 16-18); one dam showed abortion of day 21. An increase in skeletal (sternal) malformations was found in 15 foetuses in 7 litters, and 5 cases of missing gall bladder (in 2 litters). No malformations occurred in the mid-dose group whereas in the low-dose group, one foetus had a sternal anomaly, 2 foetuses had gall bladder agenesis, and one of the latter had a hypertrophic-dilative cardiac-aortic malformation. The 3 foetuses with malformations in the low-dose group were, according to OECD, regarded to be incidental, since no malformations were observed in the foetuses at the mid-dose.

Rat, GD 9-13; rabbit, GD 8-16 (Stula & Krauss 1977 – quoted from WHO 1991):
Rat: DMF was administered as undiluted material under non-occlusive conditions according to the following dose regimen: 600 mg/kg b.w. at day GD 9, 10+11, 11+12, 12+13; 1200 mg/kg b.w. at GD day 10+11; 1200 mg/kg b.w. at day GD 12+13; 2400 mg/kg b.w. at GD day 10+11; 400 or 200 mg/kg b.w. applied 6 times/day at GD day 11+12+13. There was clear evidence of embryolethality at 2400 mg/kg b.w., at 1200 mg/kg b.w., and at 400+200 mg/kg b.w. Maternal weight gain and average foetal weights were also suppressed. Foetal abnormalities were not observed.

Rabbit: DMF was applied in undiluted form to the intact skin, according to WHO, apparently under non-occlusive conditions. Embryolethality was 6% compared with 3% in controls.

Rat, GD 6-15 or 1-20 (Hansen & Meyer 1990 – quoted from IARC 1999).
DMF was applied in a porous dressing placed on shaved skin either on gestation days 6-15 or 1-20. Body weights and body weight gain, and pregnancy rates were reduced in those rats administered 2 ml/kg b.w./day DMF on days 6-15; a reduction in the number of live foetuses and in foetal weight, as well as an increase in post-implantation loss, were also observed at this dose level. Similar but more pronounced effects were observed in rats treated on days 1-20 with the same daily dose.

4.5.4 *In vitro* studies

In an *in vitro* study with limb bud organ cultures, DMF and its major metabolites (HMMF, NMF, SMG, SMC, AMMF, AMCC) were investigated for their developmental toxicity in the mouse. Neither DMF nor the predominant urinary metabolite HMMF as well as NMF and AMMF exhibited developmental activity, whereas the metabolite SMG and its sequel adducts (SMC and AMCC) showed potent developmental activity on growth and development of day 12 old mouse limb buds after 24 hours as well as 6 days in culture. The authors concluded that the developmental toxicity of DMF in different species is related to the magnitude of glutathione binding. (Klug et al. 1998 – quoted from OECD 2003, CICAD 2001).

Table 4.5.3. Reproductive toxicity studies on DMF, dermal administration

Species / strain	Duration / Dose levels	Effects mg/kg bw/day	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Reference
Rat 26/group	GD 6-10, 13-15 0, 100, 500, 1000 µl/kg bw/day (0, 94, 472, 945 mg/kg bw/day)	945: ↓ bw maternal, irritation, ↓ length foetal ≥ 94: malformations	472 (ME), <94 (dev) (DECOS)		Hellwig et al. (*in press* – in WHO) Hellwig et al. (1991 - in DECOS)
Rat	GD 9-13 0, 600, 1200, 2400 mg/kg bw	≥ 600: ↓ bwg maternal, ↓ w foetal, embryolethality			Stula & Krauss (1977 – in WHO)
Rat / Wistar	GD 6-15 or 1-20 0, 0.25, 1, 2 ml/kg bw/day (0, 236, 945, 1889 mg/kg bw/day)	1889: ↓ bw/bwg maternal, ↓ pregnancy rates / number live foetuses, ↑ post-implantation loss ≥ 945: ↓ w foetal	236 (dev), 945 (ME) (DECOS)	945 (IARC)	Hansen & Meyer (1990 – in IARC, DECOS)
Rabbit	GD 8-16 0, 200 mg/kg bw	embryolethality			Stula & Krauss (1977 – in WHO)
Rabbit 15/group OECD TG 414	GD 6-18 0, 100, 200, 400 mg/kg bw/day 6 h/day	400: ↓ bw maternal ≥ 100: irritation, malformations	200 (ME, FE, TE) (OECD) 200 (ME, dev) (DECOS)		Hellwig et al. (*in press* – in WHO) Hellwig et al. (1991 – in OECD, DECOS)

↓: reduced
↑: increased
bw: body weight
bwg: body weight gain
w: weight
GD: gestation days
ME: maternal toxicity
FE: foetotoxicity
TE: teratogenicity
dev: developmental toxicity

4.6 Mutagenic and genotoxic effects

According to IARC (1999) and WHO (1991), DMF was selected for study in the International Collaborative Program for the Evaluation of Short-term Tests for Carcinogens, in which 30 assay systems were included and more than 50 laboratories contributed data (de Serres & Ashby 1981 – cited in IARC 1999, WHO 1991). Since then, the database has been expanded.

This section is primarily based on IARC (1999), which has provided the most comprehensive review of the genotoxicity assays and studies with DMF, but other reviews and criteria documents (OECD 2003, CICAD 2001, DECOS 1995, WHO 1991) have been consulted as well. These reviews and criteria documents are broadly in concordance with the descriptions of the assays and studies in IARC.

4.6.1 *In vitro* studies

In most of the *in vitro* studies, DMF has been tested in both the presence and the absence of an exogenous metabolic system (IARC 1999).

DMF was reported to induce mutation in *Salmonella typhimurium* TA1538 and TA98 in one test (Trueman 1981) with metabolic activation, but the response occurred only at a single, intermediate dose. In all the other tests performed with *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) (18 assays) as well as with *Escherichia coli* WP2*uvrA* (6 assays), DMF did not induce gene mutation, and did not induce differential toxicity indicative of DNA damage in bacteria. (IARC 1999).

DMF induced aneuploidy in *Saccharomyces cerevisiae* D6 in both the presence and absence of an exogenous metabolic system (Parry & Sharp 1981) and gave positive results in another study for mitotic recombination in *Saccharomyces cerevisiae* D6 (Zimmerman & Scheel 1981), but most tests for gene mutation or mitotic recombination in yeast gave negative results (IARC 1999).

DMF was not mutagenic in L5178Y $tk^{+/-}$ mouse lymphoma cells in three studies, but an increased mutation frequency of about two-fold was observed at the highest dose level in one study (McGregor et al. 1988). Gene mutations were not induced in a single study with human fibroblasts. (IARC 1999).

Sister chromatid exchanges were not induced in Chinese hamster ovary cells or in human lymphocytes, and no chromosomal aberrations were induced in rodent cells. Chromosomal aberrations were reported to be induced in one study with cultured human lymphocytes at a dose level of 0.007 µg/ml (Koudela & Spazier 1979), but not in another study at a dose level of 80000 µg/ml. (IARC 1999).

DMF induced a slight increase in unscheduled DNA synthesis in primary rat hepatocyte cultures in one study (Williams 1977), but not in two other studies with rat hepatocytes, or in studies with mouse and Syrian hamster hepatocytes (IARC 1999).

No morphologically transformed colonies were observed in Syrian hamster embryo cell cultures (IARC 1999).

DMF inhibited intercellular communication, as measured by metabolic cooperation, between Chinese hamster V79 *hprt*$^{+/-}$ cells (Chen et al. 1984 – quoted from IARC 1999).

4.6.2 *In vivo* studies

DMF did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* in experiments where DMF was used as a solvent for other substances to be tested (IARC 1999).

DMF did not induce sister chromatid exchanges in mouse bone-marrow cells in a single study, or micronuclei in mouse bone-marrow cells in four different studies, in which intraperitoneal doses of up to 2000 mg/kg b.w. were used. In one study (Ye 1987, abstract only), micronuclei were reported to be induced at a dose of 1 mg/kg b.w., but, according to CICAD, a dose-response was not clear. (IARC 1999).

No dominant lethal effect was observed in rats (in groups of 10 animals) exposed by inhalation to 900 mg/m^3 DMF for 6 hours/day for five consecutive days (Lewis 1979 – quoted from IARC 1999 and CICAD 2001, BASF 1976 – quoted from OECD 2003).

No morphologically transformed colonies were observed in Syrian hamster embryo cell cultures after exposure of dams to DMF (3 ml/kg b.w.) by intraperitoneal injection (IARC 1999).

The IARC Working Group was also aware of inhalation studies with DMF conducted for the United States National Institute of Occupational Health involving exposure to 1200 mg/m^3 for 7 hours in a rat bone-marrow cell cytogenetic study, for 7 hours/day for five days in a rat bone-marrow cell cytogenetic study, a male rat dominant lethal assay, and a mouse sperm morphology assay; all results were negative (IARC 1999).

4.7 Carcinogenic effects

4.7.1 Inhalation

Rats (Crl:CD BR, 87/sex/group were exposed (whole-body) for 6 hours/day, 5 days/week, to 0, 75, 300, or 1200 mg/m^3 DMF vapour for 24 months (Malley et al. 1994 – quoted from CICAD 2001, IARC 1999, DECOS 1995). A reduction in body weight gain was noted in rats at 1200 mg/m^3 and, too a lesser extent and towards the end of the study, in males at 300 mg/m^3. Survival was not affected. No increase in tumours occurred, but in females, there was an increased incidence of uterine endometrial stromal polyps (1.7%, 5.1%, 3.4% and 14.8% for control, low-, mid- and high-dose females, respectively). Historical control data from the same laboratory indicated a highly variable incidence of endometrial stromal polyps (2-15% for 14 control groups, average 6.6%). According to CICAD, the authors concluded that DMF was not carcinogenic to rats under the conditions of exposure. Non-neoplastic findings are addressed in section 4.4.1.

Mice (Crl:CD 1 (ICR)BR, 78/sex/group were exposed for 6 hours/day, 5 days/week, to 0, 75, 300, or 1200 mg/m^3 DMF vapour for 18 months (Malley et al. 1994 – quoted from CICAD 2001, IARC 1999, DECOS). No increased tumour incidence was observed. According to CICAD, the authors concluded that DMF

was not carcinogenic to mice under the conditions of exposure. Non-neoplastic findings are addressed in section 4.4.1.

According to IARC (1999), there is evidence suggesting lack of carcinogenicity of DMF in experimental animals.

4.7.2 Oral intake

DMF was administered in drinking water to BD rats, according to CICAD, at approximately 10 or 20 mg/kg b.w./day for 500 or 250 days, respectively, or, according to DECOS at 75 (15 animals) or 150 (5 animals) mg/kg b.w./day (Druckrey et al. 1967 – quoted from CICAD 2001, DECOS 1995). No tumours were found. According to CICAD, this study was inadequate as a carcinogenicity study, e.g., the extent of tissue examination was not specified. According to DECOS, only a limited number of animals was used and the reporting of the results was minimal.

4.7.3 Dermal contact

No studies have been located.

5 Regulations

5.1 Ambient air

Denmark (C-value):	0.1 mg/m^3, Main Group 2 (MST 2002).
WHO:	-
US-EPA:	0.03 mg/m^3 The reference concentration (RfC) for chronic inhalation exposure is based on a LOAEC of 22 mg/m^3 for digestive disturbances and minimal hepatic changes suggestive of liver abnormalities observed in human occupational studies (Cirla et al. 1984, Catenacci et al. 1984). The LOAEC was adjusted to a LOAEC of 7.9 mg/m^3 for continuous exposure. An uncertainty factor of 10 was used for protection of sensitive human subpopulations, and a factor of 30 to account for use of a LOAEL, the lack of reproductive toxicity data, and the less than chronic duration of exposure. Last revised: 10/01/1990. (IRIS 2005).

5.2 Drinking water

Denmark:	-
WHO:	-
US-EPA:	-

5.3 Soil

Denmark:	-
The Netherlands:	-

5.4 Occupational Exposure Limits

Denmark:	10 ppm (30 mg/m^3), skin notation (At 2005)
Germany:	5 ppm (15 mg/m^3), skin notation (MAK 2006)
The Netherlands:	5 ppm (15 mg/m^3), skin notation The 8-hour TWA is based on the following considerations: a concentration of 15 mg/m^3 is a factor 10 lower than the NOAEL for developmental effects, a factor 5 lower than the concentration resulting in slight microscopic liver changes in mice in a 18-month

inhalation study, and a factor 4 below the concentration reported to result in reversible blood pressure changes in dogs. Moreover, there are no indications for compound-related adverse effects in humans at exposure concentrations of 15 mg/m^3 and lower. (DECOS 1995)

5.5 Classification

DMF is classified for acute toxicity (Xn;R20/21 – harmful by inhalation and in contact with skin), for irritative effects (Xi;R36 – irritating to eyes), and for reproductive effects (Rep2;R61 – may cause harm to the unborn child) (MM 2002).

5.6 IARC

DMF is not classifiable as to its carcinogenicity to humans (Group 3). There is inadequate evidence in humans for the carcinogenicity of DMF. There is evidence suggesting lack of carcinogenicity of DMF in experimental animals. (IARC 1999).

5.7 US-EPA

DMF has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential (IRIS 2005 – last revised 10/01/1990).

6 Summary and evaluation

6.1 Description

DMF is a colourless to very slightly yellow liquid with a faint amine odour. It has a relatively high vapour pressure (2.65 mmHg) and is miscible with water in all proportions. Odour thresholds reported in air range from 0.12 to 0.15 mg/m^3 for the most sensitive people.

6.2 Environment

DMF does not occur naturally. Industrial releases of DMF into air appear to be considerably larger than releases to other environmental media.

Atmospheric DMF is predominantly present in the vapour phase and is expected to be readily transported from air into surface water or soil pore water by wet deposition. Chemical degradation of DMF in air is likely due to reaction with hydroxyl radicals; the half-life is at least 8 days. There are few data concerning levels of DMF in air; concentrations of approximately 0.02 mg/m^3 have been measured in residential areas, near industrial sites.

6.3 Human exposure

No data have been located on exposure of the general population to DMF from environmental sources or foodstuffs.

In the EU, DMF is not allowed in consumer products in concentrations ≥ 0.5% as DMF is classified for reproductive toxicity in category 2 (Repr. Cat. 2; R61).

6.4 Toxicokinetics

DMF is readily absorbed via all exposure routes in humans and in experimental animals. Inhalation studies in human volunteers indicate that the uptake from the respiratory tract is almost complete (> 90%).
Following absorption, DMF and/or metabolites are distributed uniformly and are also transferred to the embryonic and foetal tissues, where levels generally were equal to those in maternal plasma.

DMF is rapidly metabolised, the main biotransformation site is the liver and excretion occurs for the larger part of the metabolites via the urine. The main metabolites in humans and in rodent species are HMMF, MMF and AMCC with HMMF being the major metabolite (see section 2.1 for details). Data indicate that two metabolic pathways for DMF can be distinguished: 1) hydroxylation of one of the *N*-methyl groups and 2) oxidation of the formyl moiety (see Figure 2.1). The former pathway results in the major metabolite HMMF; the latter pathway leads to the formation of SMG (and SMC), which may undergo further transformation to give AMCC. At higher doses, DMF inhibits its own metabolism, i.e., the formyloxidation to MMF, which is further biotransformed to AMCC.

Comparative analyses of studies in rodents (rats, mice) and monkeys indicate that toxicokinetic differences between rodents and monkeys may, in part, contribute to the observed species differences in toxicity. The AUC values and peak plasma levels of DMF for rats and mice (single exposure to 1500 mg/m^3) was substantially greater than the respective values in monkeys following a similar exposure. Whereas repeated exposure (1500 mg/m^3) in rats and mice enhanced metabolism, this effect was not clearly demonstrated in monkeys.
There are also indications that at comparable dose levels, the metabolite pattern in rodents and humans differs quantitatively from each other with respect to the formation of the metabolite AMCC, a metabolite that is possibly associated with the toxicity of DMF. After repeated inhalation of DMF, persons excreted AMCC at levels of 10-23% of the dose with a total half-life (i.e., DMF biotransformation and excretion) of 23 hours, whereas rodents only excreted up to 5% of a dose as AMCC in the urine.

6.5 Human toxicity

6.5.1 Single dose toxicity

Workers exposed, via the skin and/or inhalation, to high concentrations of DMF (exposure levels not given) have reported epigastric/ abdominal pain accompanied by dizziness, nausea, anorexia, vomiting, fatigue, alcohol intolerance, and skin irritation. Clinical investigations have shown liver function disturbance and liver biopsies have revealed morphological changes in the liver.

6.5.2 Irritation and sensitisation

Irritation of the skin, eyes and respiratory tract has been noted by workers exposed repeatedly to DMF. An epidemiological study has reported symptoms of eye and respiratory tract irritation at 22 mg/m^3 (TWA, range 8-58 mg/m^3).

One case of positive patch test reaction (0.1-1.0% in petrolatum) was reported in a woman using DMF in her laboratory work.

6.5.3 Repeated dose toxicity

No treatment-related effects were reported in 8 volunteers exposed to 30 mg/m^3 DMF, 6 hours/day for 5 days.

In several studies of workers, symptoms such as irritation to eyes and the respiratory tract, headache, dizziness, nausea, anorexia, vomiting, fatigue and alcohol intolerance, and sometimes hepatomegaly were reported; clinical investigations have shown increased serum levels for several liver enzymes. Liver biopsies from workers ‘heavily’ exposed to DMF (and other solvents, exposure levels not reported), have revealed histopathological changes in the liver.
An epidemiological study has reported subjective symptoms of mild liver dysfunction, irritation, and increased hepatic enzyme levels (significantly for one enzyme) at 22 mg/m^3 (TWA, range 8-58 mg/m^3). In another study, significantly increased hepatic enzyme levels were reported at geometric mean levels (8-hour sampling) of about 20 mg/m^3 (range 2-40 mg/m^3). In a third study, hepatic enzyme levels were not significantly increased in workers exposed to DMF at an 8-hour TWA concentration of 18 mg/m^3 (range, 12-25 mg/m^3). In a recent study, some

workers reported subjective symptoms following exposure to DMF at a median concentration of 3.6 mg/m^3.

6.5.4 Toxicity to reproduction

No data have been located.

6.5.5 Mutagenic and genotoxic effects

Increases in chromosome aberrations, sister chromatid exchange and UV-induced unscheduled DNA synthesis have been observed in peripheral lymphocytes of workers exposed to DMF and concomitantly to other organic solvents such as acrylonitrile, toluene, mono-, di-, and trimethylamine, and several other chemicals. No association of exposure to DMF and the frequency of sister chromatid exchange in peripheral lymphocytes was found in workers from a resin synthesis plant.

6.5.6 Carcinogenic effects

In a cohort study of 3859 workers with potential exposure to DMF and to DMF and acrylonitrile, the standardised incidence ratio (SIR) for all cancers combined was 1.1 for all workers. One case of testicular cancer was observed among all exposed workers versus 1.7 expected. The SIR for cancer of the buccal cavity and pharynx was 3.4 among workers exposed to DMF; there was no relationship between cancer and intensity or duration of exposure. Exposure levels were classified as low (below 30 mg/m^3), moderate (sometimes above 30 mg/m^3), or high (often above 30 mg/m^3). Mortality was evaluated in the same cohort among both active and pensioned employees. For all workers exposed to DMF only, the standardised mortality ratios (SMR) were 0.9 for all cancers combined, 2.5 for buccal cavity and pharynx cancers, and 1.4 for lung cancer; no other cancer excesses were reported.

In a case-control study, odds ratios for ‘ever exposed’ workers were 0.9 for buccal cavity and pharynx cancers, 1.7 for malignant melanoma, 1.5 for prostate cancer, 1.0 for testicular cancer, and 6.1 for liver cancer. Odds ratios for malignant melanoma by level of exposure were 1.9 and 3.1 for low and moderate exposure, respectively. Odds ratios for testicular cancer by level of exposure were 0.9 and 11.6 for low and moderate exposure, respectively. Geometric means for air measurements of DMF ranged from less than 3 mg/m^3 to about 30 mg/m^3.

Seven cases of testicular germ cell tumours have been reported among about 830 men employed in aircraft repair; three of the cases had with certainty been exposed to a solvent mixture containing 80% DMF (20% unspecified) and three cases had probably been exposed. Three cases of testicular cancer have been reported in workers at a leather tannery in the US in which DMF as well as a wide range of dyes and solvents were used; no additional cancers were reported in a screening effort undertaken to identify additional testicular cancers.

6.6 Animal toxicity

6.6.1 Single dose toxicity

Experimental animals exposed to high air concentrations of DMF or given large single doses showed general depression, anaesthesia, loss of appetite, decreased

body weight, tremors, laboured breathing, convulsions, haemorrhage of the nose and mouth, liver injury, and coma immediately preceding death. In mice and rats, signs of mucous membrane irritation were seen after inhalation. Liver and lung damage was shown (inhalation) upon histopathology. No obvious species differences were observed, but young rats appeared more sensitive than older rats.

LC_{50}-values reported for rats ranged from 9400 to 15000 mg/m^3 and in mice from 6000 to 18300 mg/m^3. The oral LD_{50}-values reported for rats, mice and rabbits were above 2000 mg/kg, and dermal LD_{50}-values reported for rabbits and rats above 500 mg/kg.

6.6.2 Irritation

Undiluted DMF, a single application, was not irritating to the skin of rats (no concentration given) or rabbits (up to 500 mg/kg), but resulted in transient skin irritation in mice (2500 to 5000 mg/kg).
Repeated treatment with DMF did not induce marked local dermal effects in rats (960 or 1920 mg/kg for 28 days) or dermal irritation in rabbits (2000 mg/kg for 6 hours daily, 15 times during a 4-week period). After repeated application of DMF to the skin of guinea-pigs for 21 days, the mean irritative dose was 31% DMF.

Single doses of neat DMF instilled into the eyes of rabbits produced severe signs of inflammation and moderate corneal damage that was most pronounced at 2 to 3 days after application; by day 14, a mild degree of conjunctival redness, moderate corneal damage, slight surface distortion, and subsurface vascularisation were observed. A 25% solution of DMF in water, injected into the conjunctival sac of the rabbit, did not produce any effects. A 50% solution was slightly irritating and 75 to 100% produced more severe irritation.

Mice and rats, exposed to DMF via inhalation in acute toxicity studies, showed signs of mucous membrane irritation. In rats exposed by inhalation at concentrations up to 6300 mg/m^3, a 30% reduction in the respiratory rate was observed.

6.6.3 Sensitisation

DMF did not induce any response in a maximization test on guinea-pigs. In a murine local lymph node assay, cell proliferation was significantly increased in exposed groups compared with control groups. In another lymph node assay, no difference in cell proliferation was detected between DMF-exposed mice and control mice.

6.6.4 Repeated dose toxicity

The toxicity of DMF following repeated exposure has been extensively studied in a number of animal species using the inhalation (rat, mouse, rabbit, guinea-pig, cat, dog, monkey), oral (rat, mouse, dog) and dermal (rat, rabbit, guinea-pig) routes. The studies have identified the liver as the predominant target organ for the toxicity of DMF and the effects observed included alterations in enzymes, increased cholesterol, increased weight and histopathological changes. Other effects observed included decreased body weight and body weight gain, and in some studies, decreased blood pressure and kidney effects. Monkeys appear to be much less sensitive than rodents, and young rats appear more sensitive than older rats.

Following inhalation, liver toxicity has been observed in rats at dose levels from 150 mg/m^3 (increased relative weight), in mice from 75 mg/m^3 (histopathology), in rabbits from 60 mg/m^3 (increased weight, histopathology, poorly reported studies), in guinea-pigs at 60 mg/m^3 (histopathology, poorly reported study), in cats from 300 mg/m^3 (histopathology) in one study whereas no effects were observed in another study at 3000 mg/m^3 in which detailed examination of the liver was claimed to have been performed, and in dogs at 60 mg/m^3 (histopathology, poorly reported study). In monkeys, no effects were observed at concentrations up to 1500 mg/m^3 for 13 weeks. For further details, see section 4.4.1 and Table 4.4.1.

Following oral administration (dietary, drinking water, gavage), liver toxicity has been observed in rats at dose levels generally from about 50-70 mg/kg b.w./day (increased relative weight, histopathology - DMF administered in the diet for 13-15 weeks). In one study with rats, liver effects (increased relative weight) were reported following 7-8 mg/kg b.w./day (DMF in drinking water for 49 days); however, no details have been provided regarding statistical significance or magnitude of increase. In mice, liver toxicity (increased relative weight) has been observed from about 100 mg/kg b.w./day (DMF administered in the diet for 17 weeks). In dogs, no liver toxicity (or other effects) were reported at dietary dose levels of up to about 35 mg/kg b.w./day. For further details, see section 4.4.2 and Table 4.4.2.

Following dermal administration, liver toxicity (indicated by changes in enzymes) has been observed in rats at dose levels from about 300 mg/kg b.w./day and histopathological changes from 960 mg/kg b.w./day (lowest dose level in the study); in rabbits at 2000 mg/kg b.w./day (necrosis - only dose level in the study); and in guinea-pigs following application of a 50% solution in water (liver damage). For further details, see section 4.4.3 and Table 4.4.3.

6.6.5 Toxicity to reproduction

No effects on organ weights or histopathological effects in the reproductive organs have been observed in medium-term or long-term studies in rats or mice following inhalation or oral exposure. In some of these studies, additional reproductive end-points were examined, including various sperm parameters; no adverse effects were reported.

In the continuous breeding study in mice, mild general toxicity (increased relative liver weight) was present at all doses tested and the maximum tolerated dose for both generations was 1000 mg/l of DMF in the drinking water (average exposure 219 mg/kg b.w./day). Significant reproductive toxicity (reduced fertility and fecundity characterised by reduced pregnancy and mating index, reduced number of litters, reduced average litter size, and for F_1 parental males by effects on prostate weight and epididymal spermatozoa concentrations) and developmental toxicity (reduced survival and growth of pups, malformations) was observed in both generations at 4000 mg/l (average exposure 820 mg/kg b.w./day) and 7000 mg/l (average exposure 1455 mg/kg b.w./day). Developmental toxicity in form of reduced F_2 pup weight was also noted at 1000 mg/l (average exposure 195 mg/kg b.w./day). Craniofacial and sternebral malformations were observed in offspring of both generations in the mid- and high-dose groups. Those pups affected most severely died shortly after birth. Those animals less affected did grow to maturity, and examination after necropsy indicated that the malformations present at birth had persisted through young adulthood. A crossover mating at 7000 mg/l identified F_0 females as the affected sex.

The developmental toxicity of DMF has been studied in a number of animal species using the inhalation (rat, rabbit), oral (rat, mouse, rabbit) and dermal (rat, rabbit) routes.

Following inhalation, developmental toxicity has been observed in rats at dose levels from 500-600 mg/m^3 (reduced foetal weight) and at 900 mg/m^3 (variations), and in rabbits from 450 mg/m^3 (variations, malformations) and at 1350 mg/m^3 (reduced foetal weight). Maternal effects (decreased body weight gain) were reported in rats at dose levels of about 900 mg/m^3 and in rabbits from 450 mg/m^3. For further details, see section 4.5.1 and Table 4.5.1.

Following oral administration (gavage), developmental toxicity has been observed in rats at dose levels from about 500 mg/kg b.w./day (embryolethality, reduced foetal weight, variations, malformations) in one study and from 100 mg/kg b.w./day (reduced foetal weight, variations) in another study; in mice from about 200 mg/kg b.w./day (reduced foetal weight, variations, malformations); and in rabbits from about 45 mg/kg b.w./day (malformations, at higher dose levels also embryolethality and reduced foetal weight). Maternal effects (decreased body weight / body weight gain) were also reported in rats at similar dose levels; in rabbits, maternal effects were reported at higher dose levels (from about 200 mg/kg b.w./day) whereas in mice, no maternal effects were reported. For further details, see section 4.5.2 and Table 4.5.2.

Following dermal administration, developmental toxicity (malformations) has been observed in rats at dose levels from about 100 mg/kg b.w./day (lowest dose level) in one study, from about 600 mg/kg b.w./day (lowest dose level) in another study (embryolethality, reduced foetal weight), and from about 950 mg/kg b.w./day in a third study (reduced foetal weight); and in rabbits at 200 mg/kg b.w./day (only dose level in this study) in one study (embryolethality) and from 100 mg/kg b.w./day (lowest dose level in this study) in another study (malformations). Maternal effects (decreased body weight / body weight gain) were reported at higher dose levels in both rats and rabbits. For further details, see section 4.5.3 and Table 4.5.3.

Data from an *in vitro* study suggest the metabolite SMG and its sequel adducts (S-methylcarbamoyl-cystein and the corresponding mercapturic acid AMCC) to be responsible for developmental toxic effects.

6.6.6 Mutagenic and genotoxic effects

DMF has been tested extensively in a broad range of *in vitro* and *in vivo* assays. The vast majority of the tests have shown negative results.

6.6.7 Carcinogenic effects

No increased tumour incidences were observed when DMF was tested in one study in rats and one study in mice for carcinogenic effects by inhalation (whole-body) exposure for 6 hours/day, 5 days/week, to 0, 75, 300, or 1200 mg/m^3 DMF vapour for 24 months (rats) or 18 months (mice). In female rats, there was an increased incidence of uterine endometrial stromal polyps (1.7%, 5.1%, 3.4% and 14.8% for control, low-, mid- and high-dose females, respectively); historical control data from the same laboratory indicated a highly variable incidence of endometrial stromal polyps (2-15% for 14 control groups, average 6.6%).

6.7 Evaluation

DMF is readily and almost completely absorbed following inhalation in humans (>90%) and in experimental animals, distributed uniformly, transferred to the developing foetus, rapidly metabolised (in the liver) and excreted with the larger part via the urine. The main metabolites in humans and in rodents are HMMF, MMF and AMCC with HMMF being the major metabolite. At higher doses, DMF inhibits its own metabolism, i.e., the formyloxidation to MMF, which is further biotransformed to AMCC. Comparative analyses of studies in rodents (rats, mice) and monkeys indicate that toxicokinetic differences (AUC values and peak plasma levels of DMF) may, in part, contribute to the observed species differences in toxicity. Data suggest a quantitative difference between the metabolic pathway of DMF to SMG/AMCC in humans (AMCC, 10-23% of a dose in the urine) and rodents (AMCC, 1-5% of a dose in the urine) at comparable dose levels. The formation of SMG/AMCC may be associated with the toxicity of DMF possibly via methyl isocyanate, an intermediary reactive species in the biotransformation of MMF to SMG, which, according to IARC (1999), has been postulated but not proven.

Workers acutely exposed to high concentrations of DMF have reported subjective symptoms; no exposure levels have been reported. Clinical investigations and liver biopsies have revealed that the liver is a target organ in humans.
The acute toxicity of DMF in a number of animal species, via inhalation or following oral or dermal administration, is relatively low, with concentrations generally in the g/m^3 range for inhalation exposure (above 6000 mg/m^3), and doses in the g/kg for the oral (above 2000 mg/kg) and dermal (above 500 mg/kg) routes. At high air concentrations or large doses, animals showed general signs of central nervous system depression, and signs of mucous membrane irritation were seen after inhalation. The liver and the lung (inhalation) were identified as target organs. Young rats appeared more sensitive than older rats.
In conclusion, DMF is considered to be of low acute toxicity following inhalation and acute toxicity is not considered a critical effect in relation to establishment of a quality criterion for DMF in ambient air.

Human data are limited to reports of irritation of the skin, eyes and respiratory tract in workers exposed to DMF. An epidemiological study has reported symptoms of eye and respiratory tract irritation at 22 mg/m^3 (TWA, range 8-58 mg/m^3).
Standard tests for dermal irritation by DMF have not been located. The available although limited data indicate that DMF produces transient skin irritation only at relatively high doses. OECD (2003) has concluded that DMF causes no skin irritation.
DMF (undiluted) produced severe signs of inflammation and moderate corneal damage in the eyes of rabbits that was most pronounced at 2 to 3 days after application. Also a 75% solution produced severe eye irritation in rabbits whereas a 50% solution was slightly irritating and a 25% solution did not produce any effects. OECD (2003) has concluded that DMF is irritating to the eyes. Mice and rats, exposed to DMF via inhalation in acute toxicity studies, showed signs of mucous membrane irritation. In rats exposed by inhalation at concentrations up to 6300 mg/m^3, a 30% reduction in the respiratory rate was observed; none of the other studies with repeated inhalation exposure has reported respiratory tract irritation.
In conclusion, DMF is an eye and respiratory tract irritant, effects, which are considered critical in relation to establishment of a quality criterion for DMF in ambient air. DMF is considered to have a very mild skin irritating potential, an

effect, which is not critical in relation to establishment of a quality criterion in ambient air.

Human data are limited to one case of a positive patch test reaction (0.1-1.0% in petrolatum) reported in a woman using DMF in her laboratory work.
Data on the skin sensitising potential in experimental animals is conflicting. DMF did not induce any response in a maximisation test with guinea pigs. In a murine local lymph node assay, cell proliferation was significantly increased in exposed groups whereas in another lymph node assay, no difference in cell proliferation was detected. According to OECD (2003), there was no clear indication of a sensitising potential of DMF in the positive lymph node assay. No clear conclusion regarding a skin sensitising potential of DMF can be drawn; however, the available data point at that DMF has no skin sensitising potential. No effect data have been located in order to evaluate whether DMF is a respiratory tract sensitiser; however, no structural alerts are identified.

Human data on repeated dose toxicity comprise predominantly studies in workers exposed to DMF as a vapour. DMF is absorbed both by inhalation and via dermal contact. Several studies have reported increases in subjective symptoms suggestive of mild liver dysfunction and changes in objective measurements of liver damage (serum enzyme levels and liver enlargement). Symptoms of irritation and of gastro-intestinal disturbances have also been reported in several of these studies. Only few of the studies have reported exposure levels and dose-response relationships. In one epidemiological study (which has accounted for confounding factors including alcohol ingestion, cigarette smoking and caffeine and the possibility of peak exposures to DMF was ruled out), subjective symptoms, eye and respiratory tract irritation, and increased hepatic enzyme levels (significant for one enzyme) were reported at a mean concentration of 22 mg/m^3. Another study has reported significantly increased hepatic enzyme levels at geometric mean levels of about 20 mg/m^3. However, in a third study, hepatic enzyme levels were not significantly increased at a mean concentration of 18 mg/m^3. In a recent study, some workers reported subjective symptoms (not further specified) following exposure to DMF at a median concentration of 3.6 mg/m^3. Overall, these studies point at a LOEC for effects in the liver of approximately 20 mg/m^3 based on increased enzyme levels, and for subjective symptoms of approximately 3-20 mg/m^3.

The repeated dose toxicity of DMF has been studied in a number of animal species using the inhalation (rat, mouse, rabbit, guinea-pig, cat, dog, monkey), oral (rat, mouse, dog) and dermal (rat, rabbit, guinea-pig) routes. The studies have identified the liver as the predominant target organ. Other effects observed included decreased body weight and body weight gain, and in some studies, decreased blood pressure and kidney effects; these effects generally occurred at higher dose levels than those resulting in liver effects.
Following inhalation, liver toxicity has been observed in rats, mice, rabbits, guinea-pigs, cats and dogs. In the most valid studies (sub-chronic and chronic studies), liver effects were observed at dose levels from 150 mg/m^3 in the rat, and from 75 mg/m^3 in the mouse. In the monkey, no effects were observed in the two available studies at concentrations up to 1500 mg/m^3 for 13 weeks; this indicates that the monkey is much less sensitive than the rodent species. Based on the inhalation studies, a NOAEC for repeated dose toxicity of 1500 mg/m^3 is considered for the monkey, and a LOAEC of 150 mg/m^3 the rat, and of 75 mg/m^3 for the mouse. See also Table 6.7.1.
Following oral administration (dietary, drinking water, gavage), liver toxicity has been observed in rats and mice. In the most valid studies (sub-chronic dietary studies), liver effects were observed at dose levels from about 50-70 mg/kg b.w./day in the rat and from about 100 mg/kg b.w./day in the mouse. In dogs, no

effects were reported at dietary dose levels of up to about 35 mg/kg b.w./day. Based on the oral studies, a NOAEL for repeated dose toxicity of 10 mg/kg b.w./day is considered for the rat, of 28 mg/kg b.w./day for the mouse, and of 35 mg/kg b.w./day for the dog. See also Table 6.7.1.
Following dermal administration, liver toxicity has been observed in rats, mice and guinea pigs. The studies are generally poorly reported, and exposure durations only last for up to 30 days. A dermal NOAEL for repeated dose toxicity of 430 mg/kg b.w./day is considered the rat as histopathological changes were observed from 960 mg/kg b.w./day. See also Table 6.7.1.

The inhalation studies indicate the monkey to be much less sensitive to the liver toxicity than the rodent in general. Overall, a LOAEC for repeated dose toxicity of 75 mg/m^3 is considered based on the 18-month study in mice in which histopathological changes in the liver were observed at the lowest dose level in the study (75 mg/m^3). As the available data do not allow a conclusion whether the sensitivity of rodents in comparison to the monkey is specifically related to rodents, the liver toxicity observed in rodents are considered relevant for humans.

Table 6.7.1 NOAEL/C or LOAEL/C for repeated dose toxicity

Species	NOAEL/C	LOAEL/C
		LOAEL/C
Inhalation	mg/m^3	mg/m^3
Rat	-	150
Mouse	-	75
Rabbit	60*	317*
Guinea-pig	-	60*
Cat	-	300*
Dog	-	60*
Monkey	1500	-
Oral	mg/kg b.w./day	mg/kg b.w./day
Rat	10-20	50-70
Mouse	28	96
Dog	34.8	-
Dermal	mg/kg b.w./day	mg/kg b.w./day
Rat	430	960
Rabbit	-	2000
Guinea-pig	-	50% solution in water

*: poorly reported studies, which are not considered adequate for establishing a NOAEL/C or LOAEL/C for liver toxicity, the critical effects following repeated exposure

No human data on reproductive toxicity have been located.

Examination (organ weights or histopathological effects) of the reproductive organs in a number of the repeated dose toxicity studies in experimental animals did not indicate these organs to be a target of DMF toxicity. In some of these studies, additional reproductive end-points were examined, including various sperm parameters; no adverse effects were reported.
In the recently performed continuous breeding study in mice, significant reproductive and developmental toxicity, including malformations, was observed in both generations following administration of DMF in drinking water from 4000 mg/l (average exposure 820 mg/kg b.w./day); developmental toxicity (reduced F_2 pup weight) was also noted at 1000 mg/l (average exposure 195 mg/kg b.w./day).

A crossover mating with high-dose F_0 animals (7000 mg/l – average exposure 1455 mg/kg b.w./day) identified the female as the affected sex. Mild general toxicity (increased relative liver weight) was present at all doses tested, with histopathological changes (centrilobular hepatocellular hypertrophy) also in mid- and high-dose groups. No histopathological changes were observed in the male reproductive organs. The sensitivity to toxicity seemed to be similar in each genera and between sexes. Based on this study, a NOAEL of 219 mg/kg b.w./day is considered for fertility, and a LOAEL of 219 mg/kg b.w./day for parental as well as for developmental toxicity.

The developmental toxicity of DMF has been studied in a number of animal species using the inhalation (rat, rabbit), oral (rat, mouse, rabbit) and dermal (rat, rabbit) routes. Developmental toxicity has been observed in all three species and following every of the three exposure routes.
Following inhalation, developmental toxicity (variations, malformations) occurred in rabbits at dose levels (from 450 mg/m^3), which also were reported to result in maternal toxicity (decreased body weight gain), whereas in rats, maternal effects (decreased body weight gain) were reported at a higher dose level (about 900 mg/m^3) than that (500-600 mg/m^3) resulting in developmental toxicity (reduced foetal weight). Based on the inhalation studies, a NOAEC for developmental toxicity of 54 mg/m^3 is considered for rats, and of 150 mg/m^3 for rabbits. See also Table 6.7.2.
Following oral administration (gavage), developmental toxicity (embryolethality, reduced foetal weight, variations, malformations) occurred in rats at dose levels from about 500 mg/kg b.w./day in one study and from 100 mg/kg b.w./day in another study), dose levels which also were reported to result in maternal toxicity (decreased body weight / body weight gain). In rabbits, maternal effects (decreased body weight gain) were reported at a higher dose level (about 200 mg/kg b.w./day) than that (about 45 mg/kg b.w./day) resulting in developmental toxicity (malformations). In mice, no maternal effects were reported, developmental toxicity (reduced foetal weight, variations, malformations) occurred at dose levels from about 200 mg/kg b.w./day. Based on the oral studies, a NOAEL for developmental toxicity of 50 mg/kg b.w./day is considered for rats and of 50 mg/kg b.w./day for mice, and a LOAEL of 45 mg/kg b.w./day for rabbits. See also Table 6.7.2.
Following dermal administration, maternal effects (decreased body weight / body weight gain) were reported in both rats and rabbits generally at higher dose levels than those resulting in developmental toxicity. Based on the dermal studies, a LOAEL for developmental toxicity of 100 mg/kg b.w./day is considered for both rats and rabbits. See also Table 6.7.2.

The oral studies indicate the rabbit to be more sensitive to developmental toxicity than the rat and the mouse; however, the inhalation and dermal studies indicate similar sensitivity for the rat and the rabbit. In some studies, developmental toxicity was observed at dose levels also resulting in maternal effects whereas in other studies, developmental toxicity was observed at lower dose levels than those resulting in maternal effects. No clear conclusion can be drawn based on the available studies and therefore, the developmental effects are considered as being critical for the purpose of setting a health based quality criterion in ambient air.
Overall, a NOAEC for developmental toxicity of 150 mg/m^3 is considered based on the OECD TG 414 study in rabbits although a lower NOAEC has been considered for rats; it should be noted, however, that in the rat study, the LOAEC for developmental toxicity was 516 mg/m^3 and in the rabbit study, 450 mg/m^3.
The metabolite SMG and its sequel adducts (SMS and AMCC) appear to be responsible for the developmental toxic effects. Data suggest a quantitative difference in the formation of SMG/AMCC between species; rodents have been

reported to excrete very low levels of AMCC in the urine (1.1-5.2%), whereas humans excreted considerably higher percentages of a dose as AMCC (10-23%).

Table 6.7.2 NOAEL/C or LOAEL/C for reproductive toxicity

Species	Developmental toxicity		Maternal toxicity	
	NOAEL/C	LOAEL/C	NOAEL/C	LOAEL/C
Inhalation	mg/m^3	mg/m^3	mg/m^3	mg/m^3
Rat	54	516	660	860
Rabbit	150	450	150	450
Oral	mg/kg b.w./day	mg/kg b.w./day	mg/kg b.w./day	mg/kg b.w./day
Rat	50	100	50	100
Mouse	183	219*	-*	219*
Rabbit	-	45	65	200
Dermal	mg/kg b.w./day	mg/kg b.w./day	mg/kg b.w./day	mg/kg b.w./day
Rat	-	100	100	600
Rabbit	-	100	200	400

The NOAEL/C or LOAEL/C for reproductive toxicity have been considered from the traditional developmental toxicity studies except those marked with an *, which have been considered based on the continuous breeding study.

Human data on genotoxicity comprise predominantly studies in workers exposed to DMF and concomitantly to other organic solvents such as acrylonitrile, toluene, mono-, di-, and trimethylamine, and in one study also to several other chemicals. Increases in chromosome aberrations, sister chromatid exchange and UV-induced unscheduled DNA synthesis have been observed in peripheral lymphocytes. In the most recent study, no association of exposure to DMF and the frequency of sister chromatid exchange in peripheral lymphocytes was found in workers from a resin synthesis plant. According to IARC (1999), reports on chromosomal damage in workers exposed to DMF either failed to take into account smoking as a bias factor or were documented incompletely.
DMF has been tested extensively in a broad range of *in vitro* and *in vivo* genotoxicity assays; the vast majority of the tests have shown negative results. According to IARC (1999), results have been consistently negative in well-controlled studies. According to OECD (2003), the single studies with positive results are not regarded to be plausible or consistent.
In conclusion, DMF is not considered to have a potential for inducing mutagenic or genotoxic effects.

Case reports of testicular cancer in aircraft repair and leather tannery facilities suggested possible association with DMF; further studies have failed to confirm this relationship. Mortality and cancer incidence studies, and case-control investigations of testicular cancer and of cancer in other organs and tissues, at several facilities with exposure to DMF showed no convincing associations. IARC (1999) has concluded that there is inadequate evidence in humans for the carcinogenicity of DMF.
DMF has been adequately tested for carcinogenicity by inhalation in one study in rats and one study in mice; no increased tumour incidences were observed. In female rats, an increased incidence of uterine endometrial stromal polyps was observed; however, historical control data from the same laboratory indicated a highly variable incidence of endometrial stromal polyps (2-15% for 14 control

groups, average 6.6%). IARC (1999) has concluded that there is evidence suggesting lack of carcinogenicity of DMF in experimental animals.
In conclusion, DMF is not considered to have a potential for inducing carcinogenic effects.

6.7.1 Critical effect(s) and NOAEC/LOAEC

The critical effects following inhalation exposure to DMF as a vapour are considered to be the irritation to the eyes and the respiratory tract reported by workers, the liver toxicity indicated in an epidemiological study of workers and evidenced by numerous studies in experimental animals, and the developmental toxicity reported in studies in experimental animals.

Eye and respiratory tract irritation has been reported by workers at a mean concentration of 22 mg/m^3 (TWA, range 8-58 mg/m^3). Mice and rats, exposed to DMF via inhalation in acute toxicity studies, showed signs of mucous membrane irritation. In rats exposed by inhalation at concentrations up to 6300 mg/m^3, a 30% reduction in the respiratory rate was observed; none of the other studies with repeated inhalation exposure has reported respiratory tract irritation. A LOAEC of 8 mg/m^3 is considered for irritative effects based on the human data.

The epidemiological studies of workers point to a LOEC for effects in the liver of approximately 20 mg/m^3 based on increased enzyme levels indicative of altered liver function.

A LOAEC for liver toxicity of 75 mg/m^3 is considered based on the 18-month study in mice in which histopathological changes in the liver were observed at the lowest dose level in the study (75 mg/m^3, corresponding to a continuous exposure of 75 x 6/24 x 5/7 = 13.4 mg/m^3). The inhalation studies indicate the monkey to be much less sensitive to the liver toxicity than the rodent in general. As the available data do not allow a conclusion whether the sensitivity of rodents in comparison to the monkey is specifically related to rodents, the liver toxicity observed in rodents are considered relevant for humans.

For reproductive toxicity, no human data have been located. A NOAEC for developmental toxicity of 150 mg/m^3 is considered based on the OECD TG 414 study in rabbits. It should be noted that at the LOAEC (450 mg/m^3) for developmental toxicity (increased incidences of variations and malformations) in this study, maternal effects (decreased body weight gain) were noted as well.

Overall, a LOAEC of 8 mg/m^3 is considered as the starting point for derivation of a health based quality criterion in ambient air. This LOAEC is considered as being relatively conservative and to take into account the liver toxicity reported in humans as well as in rodents. Adjustment of the LOAEC of 8 mg/m^3 to a LOAEC for continuous exposure is not considered relevant as irritative effects are generally related to the concentration of a substance in the air rather than to the total dose of the substance.

7 Quality criterion in ambient air

7.1 Critical effects and NOAEC/LOAEC

The critical effects following inhalation exposure to DMF as a vapour are considered to be the irritation to the eyes and the respiratory tract reported by workers, the liver toxicity indicated in an epidemiological study of workers and evidenced by numerous studies in experimental animals, and the developmental toxicity reported in studies in experimental animals.

A LOAEC of 8 mg/m^3 is considered as the starting point for derivation of a health based quality criterion in ambient air based on the irritation of the eyes and the respiratory tract reported by workers. Adjustment of the LOAEC of 8 mg/m^3 to a LOAEC for continuous exposure is not considered relevant as irritative effects are generally related to the concentration of a substance in the air rather than to the total dose of the substance. This LOAEC is considered as being relatively conservative and to take into account the liver toxicity reported in humans as well as in rodents.

7.2 Allocation

No data have been located on exposure of the general population to DMF from environmental sources or foodstuffs. Industrial releases of DMF into air appear to be considerably larger than releases to other environmental media. Low levels of DMF have been detected in ambient air and water; no measured concentrations have been located in soil or foodstuffs.
DMF may be a component in certain products for consumer use; however, in the EU, DMF may not be used in substances or preparations for consumer use in concentrations $\geq$ 0.5% implication a low exposure of the general population to DMF via consumer products.

Based on the available, although extremely limited data, the general population is predominantly exposed to DMF from ambient air. Therefore, allocation is not warranted.

7.3 Quality criterion in ambient air

The quality criterion in air QC_{air} is calculated based on a LOAEC of 8 mg/m^3 observed for irritative effects in humans (workers):

$$QC_{air} = \frac{LOAEC}{UF_I * UF_{II} * UF_{III}} = \frac{8\ mg/m^3}{1 * 10 * 10}$$

$$= 0.08\ mg/m^3$$

The uncertainty factor UF_I is set to 1 as human data are used. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 10 to account for use of a LOAEC, although being a conservative one, and the less than chronic duration of exposure.

A quality criterion of 0.08 mg/m^3 has been calculated. A C-value of 0.08 mg/m^3 is proposed and placing in Main Group 1 as DMF is classified for reproductive toxicity in category 2 (Repr. Cat. 2; R61).

For DMF, a low odour threshold has been reported in air, 0.12-0.15 mg/m^3 for the most sensitive people. The proposed C-value of 0.08 mg/m^3 is considered to protect most individuals of the general population from experiencing adverse odour nuisance from DMF in the ambient air.

7.3.1 C-value

0.08 mg/m^3, Main Group 1.

8 References

Amoore JE and Hautala E (1983). Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol **3**, 272-290.

At (2005). Grænseværdier for stoffer og materialer. Arbejdstilsynets At-vejledning C.0.1, april 2005.

CICAD (2001). N,N-Dimethylformamide. Concise International Chemical Assessment Document 31, World Health Organization, Geneva.

DECOS (1995). Formamide and dimethylformamide. Health based recommended occupational exposure limits. Report of the Dutch Expert Committee on Occupational Standards, a committee of the Health Council of the Netherlands. No. 1995/08WGD, The Hague, 20 December 1995.

Fail PA, George JD, Grizzle TB and Heindel JJ (1998). Formamide and dimethylformamide: reproductive assessment by continuous breeding in mice. Reprod Toxicol **12**, 317-332.

IARC (1999). Dimethylformamide. In: IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 71 Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide (Part two), Lyon, 545-574.

IRIS (2005). N,N-Dimethylformamide. In: Integrated Risk Information System. Database quest, last revised: 10/01/1990. US-EPA.

MAK (2006). Deutsche Forschungsgemeinschaft. MAK- und BAT-Werte-Liste 2006., Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte. Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Mitteilung 40, DFG, Wiley-VCH.

MM (2002). The Statutory Order from the Ministry of the Environment no. 430 of 3 June 2002, on the List of Chemical Substances.

MST (2002). B-værdivejledningen. Vejledning Nr. 2 2002, Miljøstyrelsen, Miljøministeriet.

NTP (1992). NTP Technical Report on toxicity studies of N,N-Dimethylformamide administered by inhalation to F344/N rats and B6C3F1 mice. NIH Publication No. 93-3345, November 1992, United States Department of Health and Human Services, Public Health Service, National Institute of Health.

OECD (2003). Dimethylformamide. OECD Screening Information Data Set (SIDS), UNEP publications, last update July 2003.

PRA (2006): The Danish Product Register, data from 7th February 2006.

WHO (1991). Dimethylformamide. Environmental Health Criteria 114. World Health Organisation, International Programme on Chemical Safety, Geneva.

N,N-Dimethylformamide

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to N,N-Dimethylformamide. This resulted in 2007 in the present report which includes a health-based quality criterion for the substance in ambient air.

Danish Ministry of the Environment
Environmental Protection Agency

Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00

www.mst.dk