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Recommendation from the Scientific Committee on

Occupational Exposure Limits

for N-Methyl-2-Pyrrolidone

8 hour TWA: 10 ppm (40 mg/m^3)

STEL (15 min): 20 ppm (80 mg/m^3)

Additional classification: "skin"

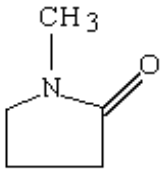
Biological Limit Value (BLV): 20 mg/g creatinine 2-hydroxy-N-methylsuccinimide (2-HMSI) in urine, measured morning-after-shift (18 hours)

or[1]

70 mg/g creatinine 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) in urine, measured 2-4 hours after the end of exposure/shift

Substance information

N-Methyl-2-Pyrrolidone



Synonyms : NMP, N-methylpyrrolidone, 1-methyl-2-pyrrolidone, 1-methyl-2-pyrrolidinone

EINECS No. : 212-828-1

EEC No. : 606-021-00-7

CAS No. : 872-50-4

Classification : Xi; R36/38 (19th ATP, Dir. 67/548/EC)[2]

M.Wt. : 99.13

Conversion factor (20°C, 101 kPa): 4.12 mg/m^3 = 1 ppm

[1] Either of these biological indicators may be used for biological monitoring purposes, depending on the analytical capability available, see text for further information

[2] The classification of NMP has been subsequently reconsidered by the Commission Working Group on Classification and Labelling of Dangerous Substances, Dir.67/548/EEC. This Working Group decided at its meeting in November 2003 that the substance should be reclassified as T, Repr. Cat 2: R61 – Xi; R36/37/38. This reclassification is implemented via the 30th Adaptation to Technical Progress of Dir. 67/548/EEC, which received a favourable opinion from the Committee on the Adaptation to Technical Progress of the Directives for the Elimination of Technical Barriers to Trade with Dangerous Substances and Preparations on 16 February 2007. The Directive has not yet been adopted by the Commission.

This evaluation is based on the Concise Chemical Assessment Document (CICAD) of 2001 (IPCS, 2001), the MAK report of 1998 (DFG, 1998), the UK Health and Safety Executive Risk Assessment document of 1997 (HSE, 1997), the Nordic Expert Group document of 1994 (Åkesson, 1994) and the references contained in these documents.

1. Physico-chemical properties:

N-Methyl-2-Pyrrolidone (NMP) is a water-miscible colourless liquid with a characteristic amine odour. The boiling point of NMP is 202°C at 101.3 Pa and its vapour pressure is 0.39 hPa at 20 °C and 0.45 hPa at 25 °C (IPCS, 2001). Log Kow is – 0.38 and the density is 1.028 g/cm^3. NMP is not flammable (flash point, closed cup, 90°C, open cup 95°C (Åkesson, 1994)).

2. Analytical methods

NMP in air is adsorbed onto solid sorbent or into absorption solution, followed by extraction of the NMP with an organic solvent. The NMP-containing extract can be analysed by gas chromatography (GC), using flame ionisation (FID) or nitrogen-phophorus detection (NPD), with a detection limit corresponding to 0.1 mg/m^3 NMP in air using FID detection and 0.01 mg/m^3 using NPD (Blome and Hennig, 1984; Andersson and Andersson, 1991; Åkesson and Paulsson, 1997). Alternatively, airborne NMP can be analysed on a continuous basis by photoacoustic IR spectrometry (INNOVA, 1412 Photo Acoustic Field Gas-Monitor) (Bader et al, 2007). Analysis of NMP in biological matrices such as blood and urine may be determined by HPLC methods (e.g. Wells et al, 1992) or alternatively may be extracted from the matrix by solvent extraction followed by GC using NPD or mass spectrometry detection. A detection limit for NMP in blood of 0.004 mg/l and in urine of 0.01 mg/l has been reported by Åkesson and co-workers (Åkesson and Paulsson, 1997), while Bader and co-workers have reported a limit of quantification of 0.01 mg/l for NMP in urine (Bader et al., 2007).

3. Occurrence/use and occupational exposure:

The primary use of NMP is as a solvent in a wide range of applications including the paints and petrochemical industries, for stripping and cleaning applications in the microelectronics industry, for the removal of graffiti, as a paint stripper and as a substitute for chlorinated solvents (IPCS, 2001). It is also used as an intermediate in the pharmaceutical, polymer and other chemical industries and as a formulating agent for plant protection and biocidal actives, and as a solvent for pigments, dyes and inks. Further uses include as a penetration enhancer for topically applied pharmaceuticals and as a vehicle in the cosmetics industry. It is increasingly used as a replacement for chlorinated solvents because of concern about the toxicological profile of some of the latter, e.g. it has been used to replace dichloromethane as a solvent in paint strippers.

Although NMP does not have a high vapour pressure, the pattern and wide range of uses results in some potential for occupational exposure by inhalation. Exposure may be to NMP as a vapour, as an aerosol or as a mixture of both, the relative proportions being dependent on temperature and relative humidity (DFG, 1998). At normal room temperature and humidity (60% relative humidity) and concentrations of NMP below 80 mg/m^3, aerosol formation is unlikely, however aerosol formation is potentiated at higher humidities and with increasing concentrations of NMP (DFG, 1998). Levels of up to 10 mg/m^3 NMP have been measured in the breathing zone of workers involved in the removal of graffiti (Anundi et al., 1993; Anundi et al., 2000), while workers in the microelectronics industry have been exposed to up to 6 mg/m^3 (Beaulieu and Schmerber, 1991). Much higher exposures (up to 280 mg/m^3) were reported in the microelectronics industry when NMP was used at a temperature of 80°C (Beaulieu and Schmerber, 1991). Exposures of up to 64 mg/m^3 have been measured in the breathing zone of paint-strippers, with peak exposures of up to 280 mg/m^3 (Åkesson and Jönsson, 2000a).

Dermal exposure to NMP in the occupational setting is also likely, given the pattern and wide range of uses. NMP is readily absorbed through the skin, and dermal exposure thus is considered to contribute significantly to the internal NMP dose. There are several older reports in the literature of toxic effects resulting from skin contamination through spills, inhalation of fumes may however have contributed to

the toxicity seen (DFG, 1998). Additionally, Bader and co-workers have reported dermal absorption of NMP from the vapour phase, equivalent to approximately ~ 30 % of the total inhalation dose in an experimental study in human volunteers, the design of which included a phase in which inhalational uptake was prevented by face shields (Bader et al., 2007).

4. Health Significance

4.1 Toxicokinetics

Human data

Human volunteer studies have shown that NMP is rapidly absorbed following exposure by the inhalation, dermal or oral route (Ursin et al., 1995; Åkesson and Paulsson, 1997; Åkesson and Jönsson, 1997; Åkesson and Jönsson, 2000b,c; Akrill et al., 2002; Jönsson and Åkesson, 2003; Åkesson et al., 2004; Bader et al., 2007). A study involving exposure of six healthy male volunteers to NMP in an exposure chamber for 8 h at concentrations of 10, 25 and 50 mg/m^3 showed rapid uptake following inhalation, with metabolism to the mono-hydroxy metabolite 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), which was further metabolised to N-methylsuccinimide (MSI) and then to 2-hydroxy-N-methylsuccinimide (2-HMSI) (Åkesson and Paulsson, 1997; Åkesson and Jönsson, 2000c, Jönsson and Åkesson, 2003). The half-lives of NMP, HNMP, MSI and 2-HMSI following inhalation of NMP were 4, 6, 8 and 16 h respectively, with 100% urinary excretion and the relative proportions of NMP and its metabolites in urine were 2% NMP, 60% 5-HNMP, 0.1% MSI and 37% 2-HMSI (Åkesson and Paulsson, 1997). More recently, Carnerup and co-workers have identified 2-pyrrolidone (2-P) as a minor metabolite in both humans and in rats (Carnerup et al., 2005, 2006). 2-P has been reported to be a developmental toxicant and may possibly be responsible for the reproductive effects seen in animal studies with NMP (Carnerup et al., 2005, 2006).

Bader and co-workers have confirmed 5-HNMP, 2-HMSI and free NMP as the major urinary metabolites following inhalation exposure of 16 male volunteers to concentrations of 10, 40, 80 and 25/160 mg/m^3 NMP (Bader et al., 2007). The relative proportions of these urinary metabolites were approximately 68:31:1at the exposure level of 40 mg/m^3 NMP under resting conditions. Half-lives of 3.9, 7.5 and 28 hours for NMP, 5-HNMP and 2-HMSI respectively at the exposure level of 40 mg/m^3 NMP under resting conditions were reported by these workers (Bader et al., 2007).

Xiaofei and co-workers studied the pharmacokinetics of NMP in four workers exposed to 0.46 – 2.84 mg/m^3 for 12 hours per day for a 5 day working week and five volunteers who observed the work processes for a single 8 hour day and were exposed to a mean concentration of 1.15 mg/m^3 (Xiaofei et al., 2000). NMP levels in plasma and urine were monitored in both workers and volunteers and the data used to derive a pharmacokinetic model for NMP. Metabolic saturation was not predicted at concentrations below approximately 40 mg/m^3. The predictability of the model was demonstrated by monitoring of NMP levels in plasma and urine in a second set of workers.

After oral administration of 100 mg NMP to three healthy male volunteers, 65% of the administered dose was recovered in urine, comprising 2% NMP, 67% 5-HNMP, 0.1% MSI and 31% 2-HMSI (Åkesson and Jönsson, 1997). One third of the oral dose of NMP was not found as any of these metabolites, the lack of mass balance possibly indicating incomplete absorption from the gastrointestinal tract or the presence of unidentified metabolites (Åkesson and Jönsson, 1997).

A 6-h topical study in male and female volunteers using a single dose of 300 mg undiluted NMP showed peak plasma concentrations of NMP three hours after application. 22 – 24% of the total dose was recovered in the urine for females and males respectively (Åkesson and Jönsson, 2000b). Mean peak plasma levels of 5-HNMP were observed after 4 hours in females and after 6 in males, while plasma MSI and 2-HMSI peaked after 8 hours and 24 hours respectively (Åkesson et al., 2004). These workers compared the pharmacokinetics of 50% aqueous dermally applied NMP in a group of 6 male volunteers with the results obtained with undiluted NMP and showed peak levels of NMP 8 hours after application, MSI after 12 hours and 2-HMSI after 24 – 30 hours (Åkesson et al., 2004). These results indicated delayed absorption of NMP in aqueous formulations, a result also found in animal studies. Bader and co-workers have also reported delayed elimination of NMP following dermal-only exposure

to NMP vapour, with peak times for free NMP, 5-HNMP and 2-HMSI being delayed by approximately 4 hours (Bader et al., 2007).
Akrill and co-workers examined the excretion of 5-HNMP in 2 volunteers exposed for 15 minutes to aqueous NMP solutions (5 – 25%: one hand) followed by urine collection for 48 hours (Akrill et al., 2002). They found that urinary levels of 5-HNMP were at a maximum after about 10 h and excretion continued for 48 h after exposure. The half-life of 5-HNMP was approximately 11 h, confirming the delayed absorption of NMP and prolonged half life of NMP and its metabolites observed by Åkesson and co-workers following dermal exposure compared with inhalation, particularly for aqueous solutions of NMP. It can be estimated from these data that 15 minutes exposure to 15% aqueous NMP is equivalent to inhalation of 10 mg/m^3 NMP with respect to absorption and elimination profile (Akrill et al., 2002).

A permeability rate through human skin of 171 + 59 g/m^3 has been derived for NMP (Ursin et al., 1995). Ligocka and co-workers demonstrated a mean 67.9% absorption of NMP through the skin in 12 human volunteers exposed to 300 mg NMP via a skin patch (Ligocka et al., 2003). 12.6% of the total dose was excreted as 5-HNMP, 6-12 hours after exposure, while 2-HMSI peaked at 2 time periods, 12-24 hours after exposure (3.3% of dose) and 36-48 hours after exposure (3.2% of dose). The authors demonstrated a significant relationship between CYP2E1 mRNA content on peripheral blood lymphocytes and levels of 5-HNMP and 2-HMSI excreted in urine within 24 hours and suggested that the activity of this enzyme in an individual should be taken into account when interpreting the results of biological monitoring of exposure to NMP (Ligocka et al., 2003).

Animal data

Toxicokinetic studies have been carried out in rats using the dermal, inhalation, oral or intravenous routes, with similar results. As in humans, NMP was rapidly absorbed and distributed, with 80 - 90% excretion in urine within 24 h (Wells and Digenis, 1988; RTI, 1990, cited in IPCS, 2001); Midgley et al., 1992; Ravn-Jonsen et al., 1992; Payan et al., 2003). Faecal excretion was 2 – 4% of total dose, and there was very limited excretion as CO_2 (0.9 – 1.7%) or volatile organic compounds. A distribution study following intravenous administration of radiolabelled NMP showed distribution to all tissues, with highest levels of radioactivity being observed in the liver, bile and small intestine, kidneys, stomach and testis (Wells and Digenis, 1988).
Following topical administration of NMP radiolabelled with ^{14}C on the C_2 atom to rat skin at doses of 0.2, 2 and 20 mg/cm^2, applied to an area of 12 cm^2, there was 50% absorption of the 2 lower doses while 75% of the 20 mg/cm^2 dose was absorbed, suggesting that NMP promotes its own absorption. Maximum blood levels were observed approximately 8 hours after application (RTI, 1990). A percutaneous absorption study applying NMP as the pure substance, as a 30% solution in water and as a 30% solution in limonene, showed uptakes of 31%, 3.5% and 72% respectively, suggesting that the use and nature of the vehicle also influences uptake (Huntingdon Life Sciences, 1998, cited in IPCS, 2001). Payan and co-workers demonstrated that percutaneous absorption flux in rats was proportional to the concentration of NMP applied and was dependent on skin thickness, suggesting a passive diffusion process (Payan et al., 2003). Maximum absorption fluxes of 10 and 20 mg/cm^2 NMP were determined for 20µl/cm^2 and 40µl/cm^2 respectively. Absorption decreased when neat NMP was diluted.
In a study in which rats were exposed to 618 mg/m^3 NMP (whole body) for 6 h, NMP crossed the placental barrier, with an equilibrium being reached between foetal and maternal blood. Elimination of NMP was slower in pregnant than non-pregnant rats (Ravn-Jonsen et al., 1992).
As also observed in the human volunteer studies reported by Åkesson and co-workers, the main urinary metabolite in rats was 5-HNMP, amounting to 70 – 75% of administered dose following intravenous administration (Wells et al., 1992) and 60% after an oral dose of 500 mg/kg (RTI, 1990). In the latter study, a further 10 – 15% was a metabolite of 5-HNMP and 5% was unchanged NMP. At the lower oral dose of 5 mg/kg, NMP was completely metabolised and at least 4 urinary metabolites were detected, indicating that metabolic pathways may become saturated at higher dose levels. The study did not identify the 2-HMSI metabolite demonstrated in humans (Åkesson and Paulsson, 1997).

Ligocka and co-workers also demonstrated that the main metabolite in rats following dermal administration of NMP was 5-HNMP (Ligocka et al. 2003). Excretion of this metabolite was significantly reduced in rats pre-treated with the CYP2E1 inhibitor diethylthiocarbamate, indicating that CYP2E1 is involved in the metabolism of NMP (Ligocka et al. 2003).

Biological monitoring

Studies in both humans and animals show that NMP is readily absorbed through the skin (RTI, 1990; Midgely et al, 1992; Åkesson and Jönsson, 2000b). Bader and co-workers have measured dermal absorption of NMP from the vapour phase, equivalent to approximately ~ 30 % of the total inhalation dose in an experimental study in human volunteers, the design of which included a phase in which inhalational uptake was prevented by face shields and absorption of NMP vapour was likely to have occurred mainly through the exposed skin areas of the hands, arms and lower neck (Bader et al., 2007). Dermal absorption can therefore contribute significantly to body burden. Measurement of non-metabolised NMP in plasma or urine has been proposed as a biological monitor, reflecting exposure from both the inhalatory and dermal routes (Åkesson and Paulsson, 1997; Xiaofei et al., 2000), but has several disadvantages, including the comparatively short half-life of NMP and the low concentrations in urine.

The metabolites of NMP are more appropriate biological indicators of exposure, and measurement of the major metabolite 5-HNMP, with a half life of 6-7 hours, in urine or plasma has been proposed as a suitable method for biological monitoring (Åkesson and Jönsson, 2000c; Jönsson and Åkesson, 2003). The same group has also suggested measurement of MSI, given the readily-available analytical method for this metabolite (Jönsson and Åkesson, 2001). However MSI has a somewhat larger volume of distribution and levels in urine are low.

In an 8-hour inhalation study in male volunteers exposed to NMP at concentrations of 10, 25 and 50 mg/m^3. Åkesson and Jönsson reported an excellent correlation between NMP air levels and plasma or urinary 5-HNMP (Åkesson and Jönsson, 2000c). An 8-hour exposure to an air concentration of 10 mg/m^3 NMP resulted in a level of 22 mmol 5-HNMP/mol creatinine in urinary samples collected during the last 2 hours of exposure, while exposure to 50 mg/m^3 NMP resulted in a level of 110 mmol/mol creatinine. The same group have also proposed 2-HMSI in urine as an appropriate biological marker for NMP exposure, sensitive analytical methodology for measurement in plasma and urine being available (Carnerup et al, 2001; Jönsson and Åkesson, 2003; Carnerup et al., 2005, 2006). Given the long half-life of 2-HMSI, this metabolite may have advantages over 5-HNMP as a biological marker, particularly in situations where workers may be exposed topically to aqueous solutions of NMP. In such situations, dermal absorption of NMP is delayed (Åkesson et al., 2004), but the impact of this on peak levels of 2-HMSI is much less marked than for 5-HNMP.

More recently, in a comprehensive study in 15 human volunteers (see section 4.5 for methodological details of this study), Bader and co-workers have confirmed that levels of urinary NMP, 5-HNMP and 2-HMSI show close correlations to airborne NMP. In this study, the authors considered that 5-HNMP and 2-HMSI were preferable biomarkers for workplace surveillance (Bader et al., 2007). The delayed peak maximum of 16-24 h post-exposure and the long biological half-life makes urinary HMSI especially suitable for the surveillance of accumulative effects during a working week (Bader et al. 2007). The results of Bader and co-workers indicate that the optimum sampling time for 5-HNMP is 2-4 h post-exposure, while in the case of 2-HMSI, a urine collection 16 h post-exposure (i.e. on the morning after an 8 h work-shift) is indicated. Although Åkesson and co-workers have proposed that results of biological monitoring should be expressed as absolute urinary concentrations of 2-HMSI or 5-HNMP, adjusted for urine density, rather than relative to urinary creatinine levels (Åkesson et al., 2004), Bader and co-workers corrected both parameters for urinary creatinine to compensate for diuretic variations (Bader et al., 2007).

Linear regression analysis of NMP in air and of post-exposure 5-HNMP in urine in the above study indicated an average concentration of approximately 60 mg/g creatinine (post exposure) for an exposure of 40 mg/m^3 NMP without workload and approximately 75 mg/g creatinine in a work scenario with moderate workload (six 10 minute periods of exercise during the 8-hour exposure period, on a bicycle ergometer (75 Watt)) (Bader et al., 2007). The regression curve reported by Åkesson and Jönsson (2000) for a series of 8 h exposures to 10, 25 and 50 mg/m^3 NMP was considerably steeper than the curve calculated in the Bader and co-workers study, and provided an estimate of 90 mg/g creatinine for urinary 5-HNMP after an 8 h exposure to 40 mg/m^3 NMP. Similarly, regression analyses between NMP in air and urinary 2-HMSI peak values (16 – 24 h post-exposure) in the Bader et al. study pointed to an average concentration of 16 mg/g creatinine (without workload) and to 22 mg/g creatinine (moderate workload) for a whole-body exposure at 20 mg/m3 NMP. Comparable to the situation with 5-HNMP, the results from an inhalation study by Jönsson and Åkesson (2003) point to higher urinary 2-HMSI concentrations than in the Bader and co-workers study, with an estimated level of 40 mg 2-HMSI/g creatinine at 40 mg/m^3 NMP. These differences may be due to methodological differences between the two studies although Bader and co-workers also suggest that differences in the dermal absorption of NMP are likely to have contributed to the discrepancies between the studies(Bader et al., 2007).

4.2 Acute toxicity

No information is available on the acute toxicity of NMP in humans, but the substance is of low acute toxicity in animals. Oral LD_{50} values in rats, mice, rabbits and guinea pigs are reported to lie in the range 3500 – 7900 mg/kg (Bartsch et al., 1976; Ansell & Fowler, 1988) while dermal LD_{50} in rats and rabbits are in the range 4000 – 10,000 mg/kg (Bartsch et al., 1976; Weisbrod, 1981; Clark, 1984). No deaths occurred in an acute inhalation study in rats exposed nose-only to a 5100 mg/m^3 vapour/ aerosol mixture with a mass median aerodynamic diameter (MMAD) of 4.6 µm (respirable fraction 87%), the LC_{50} was >5100 mg/m^3 (BASF, 1988, cited in IPCS, 2001). LC_{50}s in the range of 3100 – 8800 mg/m^3 were determined in another study also involving exposure to an aerosol (DuPont, 1988). Effects seen following oral or inhalational exposure included mucosal irritancy, narcosis and non-specific symptoms of toxicity (BASF, 1988, cited in IPCS, 2001; Ansell & Fowler, 1988).

4.3 Irritation and corrosivity

Human data

Leira and co-workers reported development of skin irritancy and contact dermatitis in 10/12 workers exposed to NMP 8 hours a day for 2 days (Leira et al., 1992). Åkesson & Jönsson observed redness, swelling and thickening and vesiculation of the skin in workers in the paint-stripping industry coming into contact with NMP (Åkesson & Jönsson, 2000a), while irritant contact dermatitis was seen in three workers newly exposed to NMP; this was attributed to a hygroscopic effect of the solvent on the stratum corneum (Jungbauer et al. 2001).

Animal data (1) Skin

Several skin irritation studies in rabbits involving a single application of 0.5 ml NMP under an occlusive dressing have shown a low potential for irritancy (Draize et al., 1944; Ansell & Fowler, 1988). In contrast, severe erythema and subsequent scaling at the application site was reported in a study in rabbits carried out by BASF (BASF, 1963). Repeated daily dermal administration of 450 mg/kg body weight to rabbits caused painful and severe haemorrhage and eschar formation after four doses; the reaction to a dose of 150 mg/kg body weight per day was less marked (BASF, 1993a, cited in IPCS (2001). Application of 20 daily doses of undiluted NMF at dose levels up to 1645 mg/kg/day to the intact or abraded skin of rabbits caused only mild irritation (GAF, 1990).

Animal data (2) Eyes

Following instillation of 0.1 ml NMP into the eyes of New Zealand White marked conjunctival irritancy including corneal opacity, iritis, and conjunctivitis was observed (Draize et al., 1944). The effects were reversible within the 21 day observation period of the study. Moderate to marked eye irritancy was also reported in studies carried out in rabbits by BASF (BASF, 1951, 1963; Ansell & Fowler (Ansell & Fowler, 1988) and GAF (GAF, 1990).

4.4 Sensitisation

There are no reports of sensitisation in workers following dermal contact with NMP. NMP produced no signs of contact sensitisation in a repeated-insult patch test in human subjects, although minor to moderate transient irritation was observed (Lee et al., 1987). In a modified Draize test in guinea pigs, repeated application of a 5% NMP solution did not produce any signs of sensitisation (Lee et al., 1987). No further details were provided. Similarly, no evidence of sensitisation was observed in an intradermal sensitisation potential test involving 4 intradermal injections of 0.1 ml of 1% NMP in saline given at weekly in guinea-pigs, followed by application of a 5% or a 50% aqueous solution of NMP and examination after 24 and 48 h (du Pont, 1976a). The 50% solution produced slight irritancy at the application site.

4.5 Repeated dose toxicity

Human data

In a human volunteer study, involving six subjects exposed to 10, 25, or 50 mg/m^3 NMP over an 8 hour period, there were no acute changes in the nasal cavity as assessed by continuous acoustic rhinometry, and no significant differences were observed in FEV_1 (forced expiratory volume in 1 s), vital capacity or forced expiratory capacity, measured by spirometry (Åkesson and Paulsson, 1997). Two volunteers reported detecting an odour at 50 mg/m^3. The subjects did not experience any symptoms of eye or respiratory tract irritation, or other symptoms such as headache, dizziness, and nausea. Workers exposed to up to 280 mg/m^3 NMP in working areas in the microelectronics fabrication industry where warm NMP (80 °C) was being handled reported severe eye irritation and headache (Beaulieu and Schmerber, 1991). Due to methodological deficiencies, an exposure – response relationship could not be established in this study.

In a study of 38 graffiti removers, working 8 hour shifts in the Stockholm underground system and exposed to a mixture of solvents including NMP, there was a significantly higher prevalence of tiredness, headaches and symptoms affecting airways, eyes and skin than population controls (Langworth et al., 2000). 8-hour exposures (TWA) were below 20% of the Swedish Permissible Exposure Limit for all solvents measured, but short-term exposures occasionally exceeded the short-term exposure limits. Mean short-term exposure to NMP over 15 minutes was 4.71 $\pm$ 6.17 mg/m^3 (AM), with a range of 0.01 – 24.61. The relationship between the different exposure measurements and reported symptoms were generally weak, and no specific relationship with NMP exposure could be identified.

More recently, a comprehensive study in 16 healthy young male volunteers has been undertaken, in order to investigate possible chemosensory effects of NMP under workplace conditions (Bader et al., 2007). One subject dropped out of the study at an early stage for reasons unrelated to NMP exposure. Exposure scenarios used in the study were 10 mg/m^3, 40 mg/m^3, 80 mg/m^3 and 25/160 mg/m^3, the latter including peak exposures up to 160 mg/m^3. The 10 mg/m^3 condition was defined as a non-irritating odorous control condition. The subjects were exposed for an 8-hour (typical shift) period once a week over an 8 week period, with an exposure-free period of 1 week between two subsequent sessions, i.e. a total of 4 exposures during the experimental period. All four inhalational conditions

were investigated with and without additional physical workload. The physical workload consisted of six 10 minute periods of exercise during the 8-hour exposure period on a bicycle ergometer (75 Watt).

Chemosensory effects of NMP were assessed using the following measures: (1) eye blink rates, based on EMG recordings, (2) nasal air flow, assessed by anterior rhinomanometry, (3) breathing rates, based on electrophysiological measurements, (4) neurobehavioral/psychological tests of attentional functions (chemosensory mediated distraction), (5) subjective acute symptoms (including acute symptoms of odor and trigeminally mediated health effects) according to the Swedish Performance Evaluation System (SPES), (6) intensity of chemosensory sensations (e.g. odor intensity, intensity of eye irritations) based on ratings assessed with the labeled magnitude scale (LMS) and (7) odor threshold shifts measured by flow-olfactometry (self- and cross-adaptation). The study included a dermal-only exposure phase in which inhalational uptake was prevented by face shields, in order to measure dermal uptake of NMP from the vapour phase (see sections 3. and 4.1 above), and urine samples were taken in order to investigate the absorption and elimination of NMP in humans under workplace-oriented conditions.

The results showed that NMP could be smelled by the subjects (odor intensity, showing some adaption over the 8 hour exposure period) and it was reported to be slightly annoying. However other symptomology indicative of an irritant potential, especially trigeminal sensations, were not elicited by NMP. Median intensity ratings of annoyance only reached “moderate” intensities. The odor intensity was rated slightly higher than annoyance, but the ratings exceeded “moderate” only during exposure peaks. The peak concentrations were mirrored by the ratings of odor intensity and annoyance. However, neither nasal flow values (AAR), nor eye blink rates, and breathing rates showed any dose-related response, even at the peak exposure of 160 mg/m^3. Behaviorally, none of the neuropsychological tests revealed any NMP-related effect with respect to cognitive abilities of the subjects during the exposures. The authors of the study concluded that NMP can be characterised as an odorous substance without irritant potency even during peak exposures of 160 mg/m^3 (Bader et al., 2007).

Animal data

4.5.1 Inhalation

A series of subacute, subchronic and chronic inhalation toxicity studies have been carried out in rats involving exposure to NMF as an aerosol or as a vapour. Of these, the studies of Lee and co-workers (Lee et al., 1987) and the 1992 – 1995 studies carried out by BASF are considered to be the most reliable for determining a No-Adverse-Effect-Level (NOAEL).

Lee and co-workers exposed rats to 100, 500, or 1000 mg/m^3 NMP for 6 h/day, 5 days/week, for 4 weeks, using whole-body exposure (Lee et al., 1987). Exposure was predominantly to an aerosol, with >95% of droplets <10 µm. Deaths were seen at 1000 mg/m^3 NMP, accompanied by bone marrow hypoplasia and evidence of toxicity in lymphoid tissue (thymus, spleen, and lymph nodes) Concentration-related lethargy and irregular respiration were observed at all dose levels, reversible within 30 – 45 min of exposure at the 100 and 500 mg/m^3 exposure levels. No treatment-related histopathological changes were reported at these dose levels.

Irritation of the nasal passages at levels of 1000 mg/m^3 and above was observed in inhalational toxicity studies carried out using exposure levels of between 10 and 10,000 mg/m^3 NMP as a liquid aerosol, head-only exposure, 6 h/day, 5 days/week for 2, 4 or 13 weeks (BASF, 1992, 1993b, 1993c, 1994). Deaths were seen at 7000 mg/m^3 NMP and above, with female rats being more sensitive than males, while exposure to 3000 mg/m^3 for 13 weeks or to 4000 mg/m^3 and above for 14 days caused respiratory tract and pulmonary irritation, decreased testis weights associated with histopathological changes including cell loss in the germinal cell epithelium and evidence of mild systemic toxicity, comprising body weight loss, mild hepatotoxicity and treatment-related changes in haematological

parameters. The NOAEL was 500 mg /m^3 for both male and female rats (BASF, 1994). Yellow discoloration of the urine noted at levels of 100 mg/m^3 and higher may be due to a coloured unidentified metabolite or to hepatic dysfunction (IPCS, 2001). In a series of whole-body inhalation studies in rats, comparing fine aerosols with coarse aerosols and varying humidity conditions, toxicity was more marked when exposure was to coarse droplets and high relative humidity (BASF, 1995a,b,c,d,e,f,g, cited in IPCS, 2001).

Exposure of rats to NMP vapour at a level of 1750 mg/m^3 for 6 h/day, 5 days/week for 6 weeks caused only slight irritation of nasal passages (BASF, 1983), while repeated exposure to 6600 mg/m^3 was lethal to mice but without effect on rats, guinea pigs, rabbits or cats (BASF, 1964a).

In a 2-year inhalation study, CD-1 rats (120 per sex per dose level) were exposed to NMP as a vapour at levels of 0, 40, or 400 mg/m^3 for 6 h/day, 5 days/week, whole body exposure (Lee et al., 1987). Ten rats per sex were subjected to haematology and blood and urine chemistry analysis after 1, 3, 6, 12, and 18 months of exposure and ten rats per sex were sacrificed after 3, 12, and 18 months. All surviving rats were killed at the end of 24 months of exposure. Minimal inflammation in the lung was observed at the highest exposure level of 400 mg/m^3. Male rats exposed to 400 mg/m^3 for 18 months showed slight body weight loss, higher haematocrit and higher alkaline phosphatase levels in serum than were observed in the control group. There was no such difference after 24 months of exposure. At the 400 mg/m^3 dose level, male rats excreted larger urine volumes, and both males and females excreted dark yellow urine.

4.5.2 Oral

Repeat-dose oral toxicity studies have been carried out in rats, mice, rabbits, guinea pigs, dogs, and cats (BASF 1964b, 1978a; Meleschtschenko, 1970; Becci et al., 1983; GAF, 1990; Malek et al. (1997); Malley et al. (1999); Malley et al. (2001). Of these, only the BASF (1978a) gavage study in rats and the 28-day, 90-day and 18-month/2 year dietary studies of Malek, Malley and co-workers provide adequate detail about methodology and results obtained.

In a 28-day study in rats administered 0, 257, 514, 1028, or 2060 mg/kg bw/day NMP for 5 days/week by oral gavage (BASF, 1978a), dose-related changes included tremor, restlessness, ruffled fur, and defensive reactions, decreases in body weight gain and increases in relative liver and kidney weights. Relative and absolute testis weights were decreased in males receiving 2060 mg/kg bw/day, accompanied by histological changes in the testis. The NOAEL in this study was 514 mg NMP/kg bw (BASF, 1978a).

The feeding studies in rats carried out more recently by the NMP Producers Group (Malek et al.,1997; Malley et al., 1999; Malley et al., 2001) at dietary dose levels up to 30,000 mg NMP/kg for 28 days, 18,000 mg NMP/kg for 90 days and 15,000 mg NMP/kg for 2 years showed sedative effects and consistent decreases in body weight and body weight gain at higher dose levels, accompanied by lower food consumption. Centrilobular hypertrophy accompanied by increases in liver weight was reported in high dose females in the 90-day study, while increases in kidney weight in both sexes were not associated with histopathological changes. At 2 years, male rats at the highest dose level showed a significantly increased incidence of severe progressive nephropathy, accompanied by decreased survival. In addition, top dose males showed increased incidences of polyarteritis in the caecum, mesenteric lymph node and testis and accumulation of pigment-containing macrophages in the spleen. While the kidney was concluded to be the main target organ in male rats, testicular degeneration and atrophy in top dose males was also a consistent finding. Top dose females showed lymphoid depletion of the mesenteric lymph node and accumulation of pigment-containing macrophages in the spleen at 2 years. Although there were dose-related trends in the incidence of a number of these changes in lower dose groups, none were statistically significant. The NOEL in the 90-day study was 3000 mg/kg diet, equivalent to 169 mg/kg bw in male rats and 217 mg/kg bw in female rats, and in the 2-year study was reported to be 5000 mg/kg, equivalent to 207 mg/kg bw in male rats and 283 mg/kg bw in female rats (Malley et al., 1999; Malley et al., 2001).

In 28-day, 90-day and 18-month studies in mice (Malek et al., 1997; Malley et al., 1999; Malley et al., 2001), effects on body weight body weight change and food consumption were less marked than those seen in rats. Centrilobular hypertrophy accompanied by increases in liver weight were seen in male and female B6C3F1 mice administered NMP at levels of 7500 mg/kg in the diet for 90 days or 2 years (equivalent to 1931 mg/kg bw) and at 1200 mg/kg in the diet (males only) for 2 years, while histological changes were reported in the kidneys of mice receiving 2030 mg/kg diet and above for 28 days. The NOEL in the 90-day study was 1000 mg/kg diet, equivalent to 277 mg/kg bw, and in the 18-month study was 600 mg/kg in male mice (equivalent to 89 mg/kg bw) and 1200 mg/kg in female mice (equivalent to 115 mg/kg bw) (Malley et al., 1999; Malley et al., 2001).

4.5.3 Dermal

Summary details only of a repeat-dose dermal toxicity study in rabbits are provided in the 1990 report of GAF (GAF, 1990). Undiluted NMF applied to the intact or abraded skin of rabbits at levels of 0, 411, 822 or 1645 mg/kg/day for 20 days produced local irritancy but there were no signs of systemic toxicity although one animal treated with 1645 mg/kg/day (abraded skin) died.

4.6 Genotoxicity

4.6.1 Mutagenicity in vitro

A number of bacterial mutagenicity studies have been carried out, using tester strains TA 97, 98, 100, 102, 104, 1535, 1537 and NMP dose levels in the range of 0.01–1000 µmol/plate, equivalent to 0.99 µg/plate to 99 mg/plate, with cytotoxicity being evident at the highest dose levels (BASF, 1978b; Maron et al., 1981; Mortelmans et al., 1986; Wells et al., 1988). All tests gave negative results with and without metabolic activation. Negative results were also obtained in mammalian cells, in the L5178Y mouse lymphoma test (du Pont, 1976b), in the HPRT (hypoxanthine guanine phosphoribosyl transferase) test in CHO cells and in the UDS (unscheduled DNA synthesis) assay in rat primary hepatocyte cultures (GAF, 1990). NMP at high concentrations of 77–230 mmol/litre, equivalent to7.6–23 g/litre, has however been reported to induce aneuploidy in *Saccharomyces cerevisiae* strain D61 (Mayer et al., 1986, 1988; Mayer and Goin, 1988; Zimmermann et al., 1988).

4.6.2 Mutagenicity in vivo

Animal data

No evidence of clastogenicity or aneugenicity was seen in a micronucleus test in which male and female NMRI mice were administered single oral doses (by gavage) of 950, 1900, or 3800 mg NMP/kg body weight. The animals showed signs of systemic toxicity as evidenced by irregular respiration, colored urine, and general poor health (Engelhardt and Fleig, 1993). Similarly, no evidence of clastogenicity or aneugenicity was seen in a bone marrow chromosomal aberration study in which male and female Chinese hamsters were administered single oral doses of 1900 or 3800 mg NMP/kg body weight, dose levels which produced signs of systemic toxicity (Engelhardt and Fleig, 1993).

Significantly increased postimplantation losses were observed compared with controls in a dominant lethal test in male NMRI mice given 391 mg NMP/kg body weight intraperitoneally once per week for 8 consecutive weeks (BASF, 1976a). In a micronucleus test in male and female Chinese hamsters exposed for 6 weeks (6 h/day, 5 days/week) to 3300 mg NMP/m^3 a slight but non-significant increase in structural chromosomal aberrations in the bone marrow was reported (BASF, 1976b). Neither study was performed according to current regulatory standards, and they are not adequate for the purposes of evaluation of the mutagenicity of NMP.

4.7 Carcinogenicity

In a 2-year inhalation study, groups of 120 male and 120 female Charles River CD rats were exposed to NMP as a vapour at levels of 0, 40, or 400 mg/m^3 for 6 h/day, 5 days/week, whole body exposure, as already detailed in section 4.5.1 (Lee et al., 1987). Male rats exposed to 400 mg/m^3 showed slight body weight loss, higher haematocrit and higher alkaline phosphatase levels in serum and they excreted larger urine volumes than did the control group. Both males and females excreted dark yellow urine. Minimal inflammation in the lung was observed at the highest exposure level of 400 mg/m^3. There were no significant differences in morbidity or mortality and no evidence of carcinogenic effects of NMP in this study.

The recent carcinogenicity studies in rats and mice commissioned by the NMP Producers Group (and reported in Section 4.5.2 above) showed no evidence of a carcinogenic effect in rats at dietary concentrations of 15000 mg/kg and below (Malley et al. (2001). Male B6C3F1 mice receiving NMP at levels of 7200 mg/kg in the diet (equivalent to 1089 mg/kg bw) showed a significant increase in hepatocellular carcinoma (13/50 compared with 4/50 in the control group) (Malley et al. (2001). Female mice in this dose group also showed a significant increase in hepatocellular carcinoma (3/50 compared with 0/50 in the control group), however the incidence fell within the historical control. Hepatocellular adenomas were also increased in both male and female mice. The authors considered that these tumours were produced by a non-genotoxic mechanism, due to enhanced cell proliferation in the liver (Parod et al., 2001).

No epidemiological studies in humans have been published.

4.8 Reproductive toxicity

Human data

A 23-year-old laboratory technician was occupationally exposed to NMP during her first 20 weeks of pregnancy, in particular due to an NMP spill in week 16 of pregnancy. She experienced malaise, headache, and nausea during the 4 days following the spill, and at week 25, signs of delayed foetal development were observed. A stillborn foetus was delivered at week 31. No information on the mother's level of exposure to NMP is available, and it was concluded that it was impossible to establish if exposure to NMP was the causative factor (Solomon et al., 1996; Bower, 1997).

Animal data

Fertility

The repeat dose toxicity studies summarised in section 4.5 indicate that exposure of male rats to high levels of NMP is associated with decreased testicular weight and histopathological changes including cell loss in the germinal cell epithelium (BASF, 1978a, 1992, 1993b, 1993c, 1994; Malek et al., 1997). A toxicokinetic study in rats with radiolabelled NMP showed highest levels of radioactivity in the testis, liver, bile and small intestine, kidneys and stomach, with 0.9% of the administered dose being recovered in the testis (Wells and Digenis, 1988).

However, Fries and co-workers reported no effect on testis or sperm morphology and sperm count in 24 male Wistar rats exposed to NMP vapour at a level of 618 mg/m^3 (150 ml/ m^3) for 90 days (Fries et al. 1992).

Multi-generation studies

NMP has been shown to cross the placental barrier, with an equilibrium being reached between foetal and maternal blood (Ravn-Jonsen et al., 1992). In a two-generation reproduction study in rats 10 males

and 20 females per dose level were exposed whole body to 0, 41, 206, or 478 mg/m^3 of NMP vapour (relative humidity 40–60%) for 6 h/day, 7 days/week, for a minimum of 14 weeks (Solomon et al., 1995). Animals were mated after a 12 week exposure period and both parents and offspring were examined for adverse effects on reproduction. No effects on reproductive ability were recorded. However, reduced body weight gain was evident in the F_1 pups whose parents had been exposed to 478 mg/m^3 and mild foetotoxicity was seen in F_2 pups. P_0 dams showed reduced sensitivity to noise. The NOAEL for both reproductive and maternal toxicity was reported as 206 mg/m^3 (Solomon et al., 1995).

In a multi-generation reproduction study by the oral route, in which rats were exposed in the diet to NMP at doses of 50, 160, or 500 mg/kg bw. per day, the highest dose level caused an increased incidence of stillbirths, decreased parental body weight and food consumption, slightly lower male fertility and female fecundity (Exxon, 1991, cited in IPCS, 2001). Because of pup toxicity at the 500 mg/kg bw. level, the dose was decreased to 350 mg/kg bw. for the remainder of the study. There was a concomitant reduction in survival and growth rates in the F_1 generation and testis weights were reduced in the male pups. No effect was seen in the 50 and 160 mg/kg bw. per day groups. When the dose was reduced to 350 mg/kg bw./day, NMP did not cause maternal toxicity or reduced pup survival. The NOEL for parental and reproductive effects was 350 mg/kg bw./day and that for growth and development of the offspring was 160 mg/kg bw./day

Developmental toxicity

The developmental toxicity of NMP has been investigated in a number of studies conducted in accordance with current test protocols and with exposure by the inhalation, oral and dermal routes.

Inhalation

NMP was reported to have no embryotoxic, foetotoxic or teratogenic effects in pregnant rats exposed whole body to 0, 100, or 360 mg /m^3 for 6 h/day on days 6–15 of gestation (Lee et al., 1987). Maternal toxicity was not observed. Whole body exposure of pregnant rats to 680 mg/m^3 NMP vapour for 6 h/day on days 4–20 of gestation resulted in increased preimplantation loss compared with the control group, there was no significant effect on the number of implantations per dam or on number of live foetuses (Fries et al., 1992; Hass et al., 1995). Delayed ossification of the skull, cervical vertebrae, sternebrae, and metatarsal and digital bones was also observed, in the absence of clinical signs of maternal toxicity, malformations were not increased.

A developmental toxicity study by the inhalation route, at exposure levels of 0, 41, 206, or 478 mg/m^3 of NMP vapour, was carried out in rats by Solomon and co-workers as part of the two-generation reproductive toxicity study reported above (Solomon et al., 1995). No effects on pregnancy rate, numbers of viable litters, corpora lutea, implantations, foetal deaths, resorptions, litter size, or incidence of foetal malformations or variations were reported, although mean foetal weight in the exposed groups was slightly decreased.

In a study conducted by Saillenfait and co-workers, pregnant rats were exposed whole body to NMP vapour at concentrations of 0, 30, 60 and 120 ppm (0, 125, 250, 500 mg/m^3) for 6 h/day, on days 6 – 20 of gestation (Saillenfait et al., 2003). Significant decreases in maternal body weight gain and food consumption were seen at 120 ppm, with some decrease in body weight gain also being evident at 60 ppm. There were no adverse effects on embryo/foetal viability or evidence of teratogenicity at any concentration tested. Foetal toxicity indicated by reduced foetal weight was observed at 120 ppm. The no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 30 and 60 ppm (125 and 250 mg/m^3) respectively

In a neurobehavioural teratology study in pregnant rats exposed whole body to 622 mg/m^3 NMP vapour for 6 h/day on days 7–20 of gestation, most of the behavioural tests gave similar results for the exposed and control animals (Hass et al., 1994). An occasionally increased latency in Morris

swimming maze and a statistically borderline impairment in operant behaviour with delayed spatial alternation were however noted among the exposed offspring. Pups had a somewhat lower body weight and slight delay in achieving some developmental milestones in the preweaning period.

Pregnant rabbits were exposed head only for 6 h/day to 0, 200, 500, or 1000 mg/m^3 NMP (vapour/aerosol; MMAD 2.7–3.5 µm) on days 7–19 post-insemination. Slight foetotoxicity in the absence of maternal toxicity was manifest as an increased occurrence of supernumerary 13th ribs in the 1000 mg/m^3 group (BASF, 1993d, cited in IPCS, 2001). The NOAEL for developmental and maternal toxicity was 500 mg/m^3.

Oral

Pregnant rats were given daily NMP doses of 0, 40, 125, or 400 mg/kg body weight by oral gavage on days 6–15 of gestation. Maternal and foetotoxicity were observed at the highest dose level compared with control, as evidenced by decreases in maternal body weight gain decrement, reduced foetal body weights and increased incidence of foetal stunting (EXXON, 1992, cited in IPCS, 2001). Oral gavage administration of 997 mg/kg bw./day to rats on days 6–15 of gestation caused increased resorptions (95%) and malformations in 8 out of 15 surviving foetuses, accompanied by foetal mortality, reduced placental and foetal weights, and reduced foetal lengths (BASF, 1971). Insufficient detail was provided on maternal toxicity in this study. In an oral developmental toxicity study in Sprague Dawley rats, using dose levels of 0, 125, 250, 500 and 750 mg/kg body weight by gavage, significant impairments in maternal body weight gain and food consumption were noted at doses of 500 mg/kg body weight and above (Saillenfait et al. 2002). At the 250 mg/kg dose, effects on body weight gain (day 6 – 21) and absolute weight gain were about 10% below control and associated with a reduction in foetal weight at this dose level. Only the latter gained statistical significance. Foetal body weight was dose dependently decreased at 250 mg/kg (10%) and 500 (30%) or 750 mg/kg (47% less than control) as was maternal body weight gain. A significant increase (p 0.01 or 0.05) in malformations was observed at 500 and 750 mg/kg and consisted of external (anasarca, anal atresia, the latter considered not to be dose-related), soft tissue (persistent truncus arteriosus) and skeletal findings (fusion or absence of cervical arches were most prominent).

In rabbits, gavage administration of 55, 175 or 540 mg/kg bw./day NMP on days 6–18 of gestation caused developmental toxicity as evidenced by post-implantation loss, altered foetal morphology, and increased incidences of cardiovascular and skull malformations at 540 mg/kg body weight per day (GAF, 1991). The NOAEL for developmental toxicity was 175 mg/kg bw./day. Maternal toxicity as evidenced by decreased body weight gain was apparent at 175 and 540 mg/kg bw./day.

In mice, oral doses of 0, 1055, or 2637 mg/kg bw./day on days 11–15 of gestation in mice caused an increase in resorptions, increased incidence of runts, diminished foetal weight and length, and an increased rate of malformations including as cleft palate at the highest dose level (BASF, 1970). The lower dose level caused no apparent foetotoxicity, however insufficient detail was provided on maternal toxicity in this study.

Dermal

Pregnant rats were administered daily dermal doses of 0, 75, 237, or 750 mg/kg bw./day NMP on days 6-15 of gestation (Becci et al., 1982). Maternal and developmental toxicity were evident at the highest dose level, evidenced by decreased maternal body weight gain, increased resorptions and decreased foetal body weight, skeletal abnormalities including missing sternebrae and fused/split/extra ribs, incomplete closing of the skull, incomplete ossification of vertebrae, fused atlas and occipital bones, and reduced or incomplete hyoid bone on day 20 of gestation. There was no increase in the incidence of soft tissue anomalies. The NOAEL for maternal and developmental toxicity was 237 mg/kg body weight per day.

In rabbits dermally exposed to 0, 100, 300, or 1000 mg/kg bw./day NMP as a 40% aqueous solution for 6 h/day on days 7–19 post-insemination, slight foetal toxicity as evidenced by an increased occurrence of supernumerary 13th ribs was apparent at 1000 mg/kg bw./day (BASF, 1993a). There were no signs of maternal toxicity. The NOAEL for maternal and developmental toxicity was 300 mg/kg body weight per day.

Other routes

Intraperitoneal studies in mice have shown evidence of developmental toxicity of NMP, evidenced by exencephaly, open eyelids, microphthalmia, cleft palate, oligodactyly, shortened or kinked tails, fusions and curvature of neck and chest vertebrae, and fusion of sternebrae and ribs (BASF, 1970; Schmidt, 1976). Conclusions cannot be drawn from these studies, due to the inappropriate method of exposure and lack of detail on maternal toxicity.

Recommendation

In deriving a health-based OEL (8-hour TWA) for NMP, the following effects have been considered: (a) the potential of the substance to produce respiratory irritation and chemosensory effects, both in humans and animals, and also narcosis and related findings at higher exposure levels, (b) the systemic toxicity of NMP, in particular reproductive toxicity in studies in experimental animals.

There were no indications of respiratory irritation or other health effects of NMP in a study involving exposure of human volunteers to 10, 25 or 50 mg/m^3 over an 8 hour period (Åkesson & Paulsson, 1997). Workers exposed to levels of up to 280 mg/m^3 reported severe eye irritation and headache, but no dose-response relationship could be established (Beaulieu & Schmerber (1991). In a recent comprehensive study in 16 healthy young male volunteers exposed to 10 mg/m^3, 40 mg/m^3, 80 mg/m^3 and 25/160 mg/m^3, the latter including peak exposures up to 160 mg/m^3, NMP could be smelled by the subjects and it was reported to be slightly annoying. However, other symptomology indicative of an irritant potential, especially trigeminal sensations, were not elicited by NMP. The authors of the study concluded that NMP can be characterised as an odorous substance without irritant potency even during peak exposures of 160 mg/m^3 (Bader et al., 2007).

Developmental toxicity and some effects on fertility have been reported in reproductive toxicity studies in rats, rabbits and mice, following exposure to NMP by the inhalation or the oral route at maternally toxic doses. NOAELs for reproductive effects range from 206 - 500 mg /m^3 in inhalation studies. In a 2-year chronic toxicity/carcinogenicity study by the inhalation route in rats minimal inflammation of the lung and slight systemic toxicity was reported in male rats at 18 months, but not at 24 months, at the highest exposure level of 400 mg/m^3 (Lee et al., 1987). The dose level of 400 mg/m^3 in this study can be considered a borderline LOAEL/NOAEL. The increased incidence of hepatocellular adenomas and carcinomas seen at the top dose of 7200 ppm NMP in the diet in an 18-month feeding study in mice (Malley et al., 2001) is not regarded as relevant for the establishment of an OEL in humans, given the absence of genotoxic activity of NMP in vitro or in vivo and the recognised sensitivity of the B6C3F1 mouse and other mouse strains to development of hepatocellular tumours.

Taking into consideration the potential of NMP to produce respiratory irritation and chemosensory effects, both in humans and animals, and systemic toxicity, in particular reproductive toxicity in studies in experimental animals, a health-based OEL (8-hour TWA) of 10 ppm (40 mg/m^3) is recommended. A STEL (15 mins) of 20 ppm (80 mg/m^3) is proposed, in order to limit peaks of exposure which could result in irritation. This recommendation is supported by the results of inhalation studies in animals.

While the human volunteer study of Bader and co-workers (Bader et al., 2007) could support an OEL of 20 ppm (80 mg/m^3), given the absence of effects other than odour detection and slight perception of annoyance following exposure to up to 160 mg/m^3 NMP in this study, the lower value of 10 ppm (40 mg/m^3) is recommended in order to provide an adequate margin of safety for possible reproductive effects in exposed workers. In relation to the reproductive toxicity seen in studies with NMP in rats, rabbits and mice, changes seen at exposure levels of 250 - 500 mg/m^3 by the inhalation route were minor (decreased pup weight and pup weight gain in the presence of maternal toxicity). NOAELs lay in the range 206 - 500 mg /m^3. Application of an Uncertainty Factor (UF) of 5 to the lowest figure in this range provides an OEL of 40 mg/m^3.

NMP is well-absorbed through the skin, both in humans and in animal studies and some systemic toxicity (including developmental toxicity) is seen following dermal uptake. A “skin” notation is therefore considered necessary.

Due to the significant dermal uptake of NMP, biological monitoring is also recommended. 5-HNMP and 2-HMSI, two key metabolites of NMP, are appropriate biological indicators of exposure, and monitoring of either of these metabolites can be undertaken. The optimum sampling time for 5-HNMP is the first 2-4 h post-exposure, while in the case of the longer half-life metabolite 2-HMSI a urine collection 16 h post-exposure (i.e. on the morning after an 8 h work-shift) is advised. Both parameters should be corrected for urinary creatinine to compensate for diuretic variations. The delayed peak maximum of 16-24 h post-exposure and the long biological half-life makes urinary HMSI especially suitable for the surveillance of accumulative effects during a working week (Bader et al. 2007). However either parameter may be chosen, depending on the available analytical methodology and the conditions pertaining in the particular workplace

For the longer half-life metabolite 2-HMSI, an 8-h TWA of 10 ppm (40 mg/m^3) corresponds to a biological value of approximately 16 mg/g creatinine, 16 h post exposure for a work scenario without workload and approximately 22 mg/g creatinine for a work scenario with moderate workload (75 Watt). A Biological Limit Value (BLV) of 20 mg/g creatinine is recommended for 2-HMSI, measured on the morning after an 8 h work-shift. This value is intermediate between the work scenario without workload and the work scenario with moderate workload, as assessed by Bader and co-workers, and is likely to be representative of a typical work scenario involving some physical activity.

For 5-HNMP, an 8-h TWA of 10 ppm (40 mg/m^3) corresponds to a biological value of approximately 60 mg/g creatinine, 2-4 h post exposure for a work scenario without workload and approximately 75 mg/g creatinine for a work scenario with moderate workload (75 Watt). A Biological Limit Value (BLV) of 70 mg/g creatinine is recommended for5-HNMP, measured 2-4 hours after the end of exposure. This value is intermediate between the work scenario without workload and the work scenario with moderate workload, as assessed by Bader and co-workers, and is likely to be representative of a typical work scenario involving some physical activity.

At the levels recommended, no measurement difficulties are foreseen, either with the measurement of NMP in air or 5-HNMP or 2-HMSI in urine.

5. References

Åkesson, B. (1994). Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals, No. 115. N-methyl-2-pyrrolidone (NMP). Arbetsmiljöinstitutet & Författarna 1994, Arbetsmiljöinstitutet, 171 84 Solna, Sweden.

Åkesson, B. and Jönsson, B. (1997). Major metabolic pathway for N-methyl-2-pyrrolidone in humans. Drug metabolism and disposition, 25, 267-269.

Åkesson, B. and Paulsson, K. (1997). Experimental exposure of male volunteers to N-methyl-2-pyrrolidone (NMP): acute effects and pharmacokinetics of NMP in plasma and urine. Occupational and environmental medicine, 54, 236-240.
Åkesson, B. and Jönsson, B. (2000a) Occupational study in paint stripping workers. Lund, University Hospital, Department of Occupational & Environmental Health. Unpublished report, cited in IPCS (2001).

Åkesson, B. and Jönsson, B (2000b). Dermal absorption study on N-methyl-2-pyrrolidine in male and female volunteers. In: *26th International Congress on Occupational Health, Singapore* (Abstract OT1390).

Åkesson, B. and Jönsson, B. (2000c). Biological monitoring of N-methyl-2-pyrrolidine using 5-hydroxy-N-methyl-2-pyrrolidine in plasma and urine as the biomarker. *Scan. J. Work.Env.Health*, 26, 213-218.

Åkesson, B., Carnerup, M.A and Jönsson, B.A.G. (2004). Evaluation of exposure markers from percutaneous absorption of N-methyl-2-pyrrolidine. *Scan. J. Work.Env.Health*, 30, 306-312.

Akrill, P., Cocker, J., Dixon, S. (2002). Dermal exposure to aqueous solutions of *N*-methyl pyrrolidone. Toxicol Lett., 13, 265-269.
Andersson, B., Andersson, K. (1991). Determination of heterocyclic tertiary amines in air. *Appl.Occup.Env.Hyg.*, 6, 40–43.

Ansell JM, Fowler JA (1988) The acute oral toxicity and primary ocular and dermal irritation of selected *N*-alkyl-2-pyrrolidones. *Food chem.toxicol.*, 26, 475–479.

Anundi, H., Lind, M-L., Friis, L., Itkes, N., Langworth, S., Edling, C. (1993). High exposures to organic solvents among graffiti removers. *Int.Arch.Occup.Env.Health*, 65, 47–251.

Anundi, H., Langworth, S., Johanson, G., Lind, M-L., Åkesson, B., Friis, L., Itkes, N., Söderman, E., Jonsson, B.A., Edling, C. (2000). Air and biological monitoring of solvent exposure during graffiti removal. *Int.Arch.Occup.Env.Health*, 73, 561–569.

Bader, M., Wrbitzky, R., Blaszkewicz, M. and van Thriel, C. (2007). Human experimental exposure study on the uptake and urinary elimination of *N* -methyl-2-pyrrolidone (NMP) during simulated workplace conditions. Archives of Toxicology, 81(5), 335-346

Bader et al. 2007 . Personal communication to SCOEL reflecting a Final Report on a human volunteer study on chemosensory effects and evaluation of a threshold limit value in biological material of N-methyl-2-pyrrolidone (NMP) after inhalational and dermal exposure). Report to the NMP Producers Group, c/o Bergeson & Campbell, P.C., 1203 Nineteenth Street, NW, Suite 300, Washington, DC, USA
Bartsch W, Sponer G, Dietmann K, Fuchs G (1976) Acute toxicity of various solvents in the mouse and rat. *Arzneimittel-Forschung*, 26:1581–1583.

BASF (1951). Vorläufiger Bericht über die biologische Prüfung von Methylpyrrolidon. Ludwigshafen, BASF Aktiengesellschaft. Unpublished report, cited in DFG (1998).

BASF (1963). Bericht über die acute Toxizität von N-methylpyrrolidon dest. Ludwigshafen, BASF Aktiengesellschaft., Unpublished report, cited in DFG (1998).

BASF (1964a). Bericht über die Prüfung der akuten inhalationstoxizität von N-methylpyrrolidon., BASF Aktiengesellschaft. Unpublished report, cited in DFG (1998).

BASF (1964b). Bericht über die acute und subakute Toxizität von NMP für Meerschweinchen, Kaninchen und Katzen. Unpublished report, cited in DFG (1998).

BASF (1970) Bericht über die Prüfung von N-methylpyrrolidon auf etwaige teratogene Wirkung an der Maus. Unpublished report, cited in DFG (1998).

BASF (1971) Bericht über die Prüfung von N-methylpyrrolidon auf etwaige teratogene Wirkung an der ratte bei peroraler Applikation. Unpublished report, cited in DFG (1998).

BASF (1976a) Bericht über die Prüfung von N-methylpyrrolidon auf mutagene Wirkung an der männlichen Maus nach einmaliger intraperitonealer Applikation. Ludwigshafen, BASF Aktiengesellschaft. Unpublished report, cited in DFG (1998).

BASF (1976b) Bericht über die Prüfung von N-methylpyrrolidon auf mutagene Wirkung am chinesischen Streifenhamster nach 6wöchiger Inhalation. Ludwigshafen, BASF Aktiengesellschaft (Report No. 1581-5). Unpublished report, cited in DFG (1998).

BASF (1978a). Evaluation of the toxicity of N-methylpyrrolidone in the rat by the 4 week oral intubation test. Ludwigshafen, BASF Aktiengesellschaft (XXV/436). Unpublished report, cited in DFG (1998).

BASF (1978b). Ames test for N-methylpyrrolidone. Ludwigshafen, BASF Aktiengesellschaft (Report No. 77/585). Unpublished report, cited in DFG (1998).

BASF (1983). Bericht über die orientierende *Prüfung der subakuten Inhalationstoxizität von N-methylpyrrolidon fürSprague-Dawlwy-Ratten.* Ludwigshafen, BASF Aktiengesellschaft. Unpublished report, cited in DFG (1998).

BASF(1988). *Prüfung der akuten inhalationstoxizität LC50 von N-methylpyrrolidone als Flüssigkeitsaerosol an Ratten. Exposition über 4 Stunden.* Ludwigshafen, BASF Aktiengesellschaft (Project No. 13I0548/877054). Unpublished report, cited in IPCS (2001).

BASF (1992). Brief Report. Study on the inhalation toxicity of N-methylpyrrolidone in rats. 14-day study. Head-nose exposure to a liquid aerosol. Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0794/87088). Unpublished report, cited in IPCS (2001).

BASF (1993a). Study of the prenatal toxicity of N-methylpyrrolidone in rabbits after dermal application. Ludwigshafen, BASF Aktiengesellschaft (Project No. 44R0544/90078). Unpublished report, cited in IPCS (2001).

BASF (1993b). Brief Report. Study on the inhalation toxicity of an aqueous solution of N-methylpyrrolidone as a liquid aerosol in rats (14-day study). Ludwigshafen, BASF Aktiengesellschaft (Project No. 50I0544/90061). Unpublished report, cited in DFG (1998).

BASF (1993c). Brief Report. Study on the inhalation toxicity of an aqueous solution of N-methylpyrrolidone as a liquid aerosol in rats (28-day study). Ludwigshafen, BASF Aktiengesellschaft (Project No. 50I0544/90058). Unpublished report, cited in DFG (1998).

BASF (1993d). Study of the prenatal toxicity of N-methylpyrrolidone in rabbits after inhalation of vapor–aerosol mixtures. Ludwigshafen, BASF Aktiengesellschaft (Project No. 41R0544/90100). Unpublished report, cited in IPCS (2001).

BASF (1994). Study on the inhalation toxicity of an aqueous solution of N-methylpyrrolidone as a liquid aerosol in rats (90-day study). Ludwigshafen, BASF Aktiengesellschaft (Project No. 50I0544/90067). Unpublished report, cited in DFG (1998).

BASF (1995a). Respiration measurement during 2-week inhalation of N-methylpyrrolidone as a liquid aerosol/vapour in rats. Whole body exposure (fine/generation mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89054). Unpublished report, cited in IPCS (2001).

BASF (1995b). Study on the inhalation toxicity of N-methylpyrrolidone as a liquid/aerosol/vapour in rats. 4 week test whole body exposure (coarse/dry mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89023). Unpublished report, cited in IPCS (2001).

BASF (1995c). Study on the inhalation toxicity of N-methylpyrrolidone as a liquid/aerosol/vapour in rats. 2 week test whole body exposure (fine/dry generation mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89042). Unpublished report, cited in IPCS (2001).

BASF (1995d). Study on the inhalation toxicity of N-methylpyrrolidone as a liquid/aerosol/vapour in rats. 2 week test whole body exposure (coarse/wet mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89044). Unpublished report, cited in IPCS (2001).

BASF (1995e). Study on the inhalation toxicity of N-methylpyrrolidone as a liquid/aerosol/vapour in rats. 2 week test whole body exposure (fine/wet generation mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89045). Unpublished report, cited in IPCS (2001).

BASF (1995f). Study on the inhalation toxicity of N-methylpyrrolidone (25% aqueous solution) as a liquid/aerosol/vapour in rats. 2 week test whole body exposure (coarse/wet generation mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89070). Unpublished report, cited in IPCS (2001).

BASF (1995g). Study on the inhalation toxicity of N-methylpyrrolidone as a liquid aerosol vapour in rats. 2 week test. Comparison between whole-body and head-nose exposure (coarse/wet generation mode). Ludwigshafen, BASF Aktiengesellschaft (Project No. 36I0587/89069). Unpublished report, cited in IPCS (2001).

Beaulieu, H.J., Schmerber, K.R.(1991). M-pyrol (NMP) use in the microelectronics industry. Applied occupational and environmental hygiene, 6:874–880.

Becci, P.J., Knickerbocker, M.J., Reagan, E.L., Parent, R.A., Llewellyn, W.B. (1982). Teratogenicity study of *N*-methylpyrrolidone after dermal application to Sprague-Dawley rats. *Fund.Appl.Toxicol.*, 2, 73–76.

Becci, P.J., Gephart, L.A., Koschier, F.J., Johnson, W.D., Burnette, L.W. (1983). Subchronic feeding study in beagle dogs on *N*-methylpyrrolidone. *J.Appl.Toxicol.*, 3,83–86.

Blome, H., Hennig, M. (1984). Messung ausgewählter aliphatischer und aromatischer Amine in der Luft von Arbeitsbereichen. *Staub-Reinhaltung der Luft*, 44, 27–32.

Bower, D.B. (1997). Letters to the editor: Stillbirth after occupational exposure to *N*-methyl-2-pyrrolidone. *J. Occup. Env. Med.*, 39, 393–394.

Carnerup, M.A., Åkesson, B., Jönsson, B.A.G. (2001). Determination of 5-hydroxy-*N*-methyl-2-pyrrolidone and 2-hydroxy-*N*-methylsuccinimide in human plasma and urine using liquid chromatography electrospray tandem mass spectrometry. J. Chromat. B. Biomed. Sci. Appl., 761, 107 – 113.

Carnerup M.A., Saillenfait, A.M., Jönsson, B.A.G. (2005). Concentrations of *N*-methylpyrrolidone (NMP) and its metabolites in plasma and urine following oral administration of NMP to rats. Food. Chem. Toxicol. 43, 1441–1447

Carnerup M.A., Spanne, M., Jönsson, B.A.G. (2006). Levels of *N*-methylpyrrolidone (NMP) and its metabolites in plasma and urine from volunteers after experimental exposure to NMP in dry and humid air. Toxicology Letters 162, 139-145.

Clark, B., Furlong, JW, Ladner A, Slovak AJM (1984) Dermal toxicity of dimethyl acetylene dicarboxylate, *N*-methyl pyrrolidone, triethylene glycol dimethyl ether, dioxane and tetralin in the rat. Deutsche Forschungsgemeinschaft (DFG) (1998), Ed. H. Greim. Occupational Toxicants, Critical Data Evaluation for MAK Values and Classification of Carcinogens, Volume 10, N-Methyl-2-Pyrrolidone (vapour). Wiley-VCH, Weinheim, New York, Chichester, Brisbane, Singapore, Toronto

Draize, J.H., Woodward. G., Calvery. H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of pharmacology and experimental therapeutics*, 82, 377–390.

du Pont (1976a). Primary skin irritation and sensitization test on guinea pigs (Haskell Laboratory Report No. 307-776). Unpublished report, cited in IPCS (2001).

du Pont (1976b). Mutagenicity in the mouse lymphoma L5178Y cell line (Haskell Laboratory Report No. 677-76). Unpublished report, cited in IPCS (2001).

du Pont (1988). Four-hour inhalation approximate lethal concentration (ALC) in rats exposed to N-Methyl-2-Pyrrolidone supplied by GAF and BASF. Haskell Laboratory Report N0. 772-88. Unpublished report, cited in DFG (1998).

Engelhardt, G., Fleig, H. (1993). 1-Methyl-2-pyrrolidone (NMP) does not induce structural and numerical chromosomal aberrations in vivo. *Mutat. Res.*, 298,149–155.

EXXON (1991) Multigeneration rat reproduction study with N-methylpyrrolidone. Biomedical Science, Inc.; Study for GAF Corp. (USA), Project No. 236535. Unpublished report, cited in IPCS (2001).

EXXON (1992) Developmental toxicity study in rats with N-methylpyrrolidone. EXXON Biomedical Science, Inc.; Study for GAF Corp. (USA), Project No. 136534. Unpublished report, cited in IPCS (2001).

Fries, A.S., Hass, U., Jakobsen, B.M., Jelnes, J.E., Lund, S.P., Simonsen, L. (1992). Toxic effects of N-methylpyrrolidone on foetal development, the central nervous system, testes and semen in rats. Copenhagen, Arbeijdsmiljøfondet (Report 790037). Cited in DFG (1998).

GAF (1990). M-Pyrol® (N-Methylpyrrolidone). Summary of toxicity information. GAF Chemical Corporation, Wayne, USA. Unpublished report, cited in DFG (1998).

GAF (1991). Developmental toxicity study in New Zealand White rabbits. Prepared by GAF Chemicals Corporation, Wayne, NJ, for the International Research and Development Corporation. Unpublished report, cited in DFG (1998).

Hass, U., Jakobsen, B.M., Lund, S.P. (1994). Effects of prenatal exposure to *N*-methylpyrrolidone on postnatal developmental in rat. *Pharmacol. Toxicol.*, 76,406–409.

Hass. U., Lund, S.P., Elsner. J. (1995). Developmental toxicity of inhaled *N*-methylpyrrolidone in the rat. *Neurotoxicol.teratol.*, 16,241–249.

HSE (1997): N-Methyl-2-Pyrrolidone: Risk assessment document EH72/10, HSE Books, Sudbury, Suffolk.

Huntingdon Life Sciences (1998). [^{14}C]-N-methylpyrrolidone: Topical application: dermal absorption study in the rat. Huntingdon, Cambridgeshire, Huntingdon Life Sciences. Unpublished report, cited in IPCS (2001).

IPCS (2001) Concise International Chemical Assessment Document No. 35, N-Methyl-2-Pyrrolidone. Inter-Organization Programme for the Sound Managment of Chemicals (IOMC), World Health Organization, Geneva.

Jonsson, B.A., Åkesson, B. (2001). N-methylsuccinimide in plasma and urine as a biomarker of exposure to N-methyl-2-pyrrolidone. *Int.Arch. Occup. Environ. Health*., 74, 289-294.

Jonsson, B.A., Åkesson, B. (2003). Human experimental exposure to N-methyl-2-pyrrolidone (NMP): toxicokinetics of NMP, 5-hydroxy- N-methyl-2-pyrrolidone, N-methylsuccinimide and 2-hydroxy- N-methylsuccinimide (2-HMSI), and biological monitoring using 2-HMSI as a biomarker. *Int.Arch. Occup. Environ. Health*., 76, 267-74.

Jungbauer, F.H., Coenraads, P.J., Kardaun, S.H. (2001). Toxic hygroscopic contact reaction to N-methyl-2-pyrrolidone. *Contact Dermatitis*, 45, 303-304.

Langworth, S., Anundi, H., Friis, L., Johanson, G., Söderman, E. and Åkesson, B. (2001). Acute health effects common during graffiti removal. *Int.Arch. Occup. Environ. Health*., 74, 213-218.

Lee, K.P., Chromey, N.C., Culik, R., Barnes, J.R., Schneider, P.W. (1987). Toxicity of *N*-methyl-2-pyrrolidone (NMP): teratogenic, subchronic and two-year inhalation studies. *Fund.Appl.Toxicol.*, 9,222–235.

Leira, H.L., Tilitnes, A., Svendsen, K., Vetlesen. L. (1992). Irritant cutaneous reactions to *N*-methyl-2-pyrrolidone (NMP). *Contact dermatitis*, 27, 148–150.

Ligocka, D., Lison, D., Haufroid, V. (2003). Contribution of CYP2E1 to N-methyl-2-pyrrolidone metabolism. *Arch Toxicol*. 77, 261-266.

Malek, D.E., Malley, L.A., Slone, T.W., Elliot, G.S., Kennedy, G.L., Mellert, W., Deckardt, K., Gembardt, C., Hildebrand, B., Murphy, S.R., Bower, D.B., Wright, G.A. (1997). Repeated dose toxicity study (28 days) in rats and mice with *N*-methylpyrrolidone (NMP). *Drug Chem.Tox*., 20, 63–67.

Malley, L.A., Kennedy, G.L., Elliot, G.S., Slone, T.W., Mellert, W., Deckardt, K., Gembardt, C., Hildebrand, B., Parod, R. J., McCarthy, T.J., Griffiths, J.C. (1999). 90-Day subchronic toxicity study in rats and mice fed *N*-methylpyrrolidone (NMP) including neurotoxicity evaluation in rats. *Drug Chem.Tox*., 22, 455-480.

Malley, L.A., Kennedy, G.L., Elliot, G.S., Slone, T.W., Mellert, W., Deckardt, K., Kuttler, K., Hildebrand, B., Banton, M. I., Parod, R. J., Griffiths, J.C. (2001). Chronic toxicity and oncogenicity of *N*-methylpyrrolidone (NMP) in rats and mice by dietary administration. *Drug Chem.Tox.*, 24, 315-338.

Maron, D., Katzenellenbogen, J., Ames, B.N. (1981). Compatibility of organic solvents with the *Salmonella*/microsome test. *Mutat.Res.*, 88, 343–350.

Mayer, V.W., Goin, C.J., Taylor-Mayer, R.E. (1986). 2-pyrrolidinone and 1-methyl-2-pyrrolidinone induce aneuploidy induction in *Saccharomyces cerevisiae. Environ.Mutagen.*, 8, Suppl. 6, 53.

Mayer, V.W., Goin, C.J., Taylor-Mayer, R.E. (1988). Aneuploidy induction in *Saccharomyces cerevisiae* by two solvent compounds, 1-methyl-2-pyrrolidinone and 2-pyrrolidinone. *Environ.Mol.Mutagenesis*, 11, 31–40.

Mayer, V.W., Goin, C.J. (1988). Investigations of aneuploidy-inducing chemical combination in *Saccharomyces cerevisiae. Mutat.Res.*, 201, 413-421..

Meleschtschenko, K.F. (1970). The hygienic properties of methylpyrrolidone as a pollutant of water reservoirs (in Russian). Gig.i.Sanit., 35, 84-85.

Midgley, I., Hood, A.J., Chasseud, L.F., Brindley, C.J., Baughman, S., Allan. G. (1992). Percutaneous absorption of co-administered *N*-methyl-2-[^{14}C]pyrrolidone and 2-[^{14}C]pyrrolidone for rats. *Food Chem.Toxicol.*, 30,57–64.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., Zeiger, E. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environmental mutagenesis*, 8, 1–119.

Parod, R. J., Kaufmann, W., Deckardt, K., Mellert, W., Banton, M. I., Griffiths, J.C., Bahnemann, R. (2001). Liver tumours in mice - *N*-methylpyrrolidone (NMP) acts via enhanced cell proliferation. The Toxicologist, 60, 1360 – 1365.

Payan, J.P., Boudry, I., Beydon, D., Fabry, J.P., Grandclaude, M.C., Ferrari, E., Andre, J.C. (2003). Toxicokinetics and metabolism of N-[(14)C]N-methyl-2-pyrrolidone in male Sprague-Dawley rats: in vivo and in vitro percutaneous absorption. *Drug Metab.Dispos*., 31, 659-669.

Ravn-Jonsen, A., Edelflors, S., Hass, U., Lund, S.P. (1992). The kinetics of *N*-methyl-2-pyrrolidone in pregnant rats and their foetuses compared with non-pregnant rats. *Toxicol. Let. (suppl. 136)*, Abstract P5/P8.

RTI (1990) Absorption, distribution, metabolism and elimination of N-methyl-2-pyrrolidone in rats after oral and dermal administration. Research Triangle Park, NC, Research Triangle Institute (Report RTI/3662/00-13P). Unpublished report, cited in DFS (1998)

Saillenfait, A. M., F. Gallissot, I. Langonne, J. P. Sabate and G. Morel (2002): Developmental toxicity of N-Methyl-2-Pyrrolidone administered orally to rats Food and Chemical Toxicology 40, 1705-1712

Saillenfait, A. M., Gallissot, F., Morel, G. (2003). Developmental toxicity of *N*-methyl-2-pyrrolidone in rats following inhalation exposure. *Food. Chem. Toxicol.*, 42, 583 – 588.

Schmidt, R. (1976). Tierexperimentelle Untersuchungen zur embryotoxischen und teratogenen Wirkung von *N*-Methyl-Pyrrolidon (NMP). *Biologische Rundschau*, 14, 38–41.

Solomon, H.M., Burgess, B.A., Kennedy, G.L. Jr, Staples, R.E. (1995). 1-Methyl-2-pyrrolidone (NMP): Reproductive and developmental toxicity study by inhalation in the rat. *Drug Chem.Toxicol.*, 18, 271–293.

Solomon, G.M., Morse, E.P., Garbo, M.J., Milton, D.K. (1996). Stillbirth after occupational exposure to *N*-methyl-2-pyrrolidone. *J. Occup.Env.Med.*, 38, 705–713.

Ursin, C., Hansen, C.M., Van Dyk, J.W., Jensen, P.O., Christensen, I.J., Ebbehoej, J. (1995). Permeability of commercial solvents through living human skin. *Am. Ind. Hyg. Assoc.Journ.*, 56, 651–660.

Wells, D., Thomas, H., Digenis, G.A. (1988). Mutagenicity and cytotoxicity of *N*-methyl-2-pyrrolidone and 4-(methylamino) butanoic acid in the *Salmonella* microsome assay. *J.Appl.Toxicol.*, 8,135–139.

Wells, D., Digenis, G.A. (1988). Disposition and metabolism of double-labelled [^{3}H and ^{14}C] *N*-methyl-2-pyrrolidone in the rat. *Drug Metab.Disp.*, 16:243–249.

Wells, D., Hawi, A.A., Digenis, G.A. (1992). Isolation and identification of the major urinary metabolite of *N*-methylpyrrolidone in the rat. *Drug Metabol.Dispos.*, 20,124–126.

Weisbrod, D., (1981). Praktische Erfahrungen bei der Bestimmung der acute Toxizität(LD_{50}). Akad. Landwirtsch.Wiss DDR 1987, 213-217.

Xiaofei, E., Wada, Y., Nozaki, J., Miyauchi, H., Tanaka, S., Seki, Y., Koizumi, A. (2000). A Linear Pharmacokinetic Model Predicts Usefulness of N-Methyl-2-Pyrrolidone (NMP) in Plasma or Urine as a Biomarker for Biological Monitoring for NMP Exposure. *J. Occup.Health*, 42, 321-327.

Zimmermann, F.K., Holzwarth, U.L.I., Scheel, I., Resnick, M.A. (1988) Aprotoic polar solvents that affect brain tubulin aggregation *in vitro* induce aneuploidy in yeast cells growing at low temperatures. *Mutat.Res.*, 201, 431–442.