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Experimental exposure of male volunteers to N-methyl-2-pyrrolidone (NMP): acute effects and pharmacokinetics of NMP in plasma and urine

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Abstract

***Objectives*—To study the acute effects of exposure to the increasingly used solvent, N-methyl-2-pyrrolidone (NMP) in male volunteers. Further, to determine the NMP concentration in plasma and urine during and after the exposure.**

***Methods*—Six male volunteers were exposed for eight hours on four different days to 0, 10, 25, and 50 mg/m³ NMP. Plasma was collected and urine was sampled during and after the exposure. Changes in nasal volume were measured by acoustic rhinometry and in airway resistance by spirometry.**

***Results*—The eight-hour experimental exposure to 10, 25, and 50 mg/m³ did not induce discomfort to eyes or upper airways. Acute changes in nasal volume were not found, and no changes in the spirometric data could be registered. The elimination curves suggested a non-linear pattern and at the end of exposure showed mean (range) half lifes of NMP in plasma of about 4·0 (2·9–5·8) hours and in urine 4·5 (3·5–6·6) hours. The unmetabolised NMP found in urine samples collected during exposure and at the subsequent 44 hours corresponded to about 2% of the calculated absorbed dose. At the end of the exposure there was a close correlation between exposures and the plasma concentration and urinary excretion of NMP.**

***Conclusions*—NMP was absorbed through the respiratory tract and readily eliminated from the body, mainly by biotransformation to other compounds. Exposure to 10, 25, or 50 mg/m³ NMP did not cause nose, eye, or airway irritation. Thus, NMP is a mild irritant.**

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N-methyl-2-pyrrolidone (NMP; structural formula C_5H_9NO; CAS number 872-50-4; boiling point 202°C at 101·3 kPa) is a widely and increasingly used solvent. The many applications of its commercial uses are due to its strong and selective solvent power. It dissolves most monomers and polymers and catalyses many polymerisation reactions. One of the main uses of NMP is in the petrochemical industry as an extraction agent, and it is also used in the microelectronics fabrication industry, for manufacturing electrolytic capacitors and batteries, as well as in the production of insecticides, herbicides, and fungicides. Furthermore, NMP is used as a penetration enhancer for topically applied drugs. An increasing use of NMP may be as a substitute for other solvents of higher inherent toxicity in occupational and environmental settings—for example, for methylene chloride in paint strippers. The use of NMP as a remover of graffiti has rapidly increased.[1]

In the rat, NMP is readily absorbed through the skin[2] and the respiratory[3] and gastrointestinal[2] tracts, distributed to all major organs,[4] and biotransformed to polar metabolites which are excreted in urine, mainly as 5-hydroxy-N-methyl-2-pyrrolidone.[5] The urinary excretion, studied after application of NMP to the skin, suggests a percutaneous uptake of about 70%.[2]

Animal studies also show that exposure to NMP may cause degenerative changes in the respiratory system and the haematopoietic and the lymphoid tissues.[5] Effects such as lethargy and irregular respiration found after inhalation and oral administration may be due to a neurotoxic effect. The studies on reproductive toxicity show that NMP may cause developmental toxicity at doses causing mild or no maternal toxicity.[6]

The toxicity of NMP in humans is not well known.[1] The irritating effect found in the occupational setting on skin and eyes predicts that NMP, in accordance with animal studies,[7] may be a moderate to severe irritant. Workers exposed to concentrations of NMP in air ranging from 3–6 mg/m³, for even short periods (30 min), reported severe eye irritation and headache.[8] Reversible dermatitis is reported in workers after a few days of work with NMP,[9] and experimental skin exposure to NMP in humans caused transient irritation.[1]

These findings of adverse effects show that exposure to NMP may present a risk of injury to human health. Knowledge of the effect of exposure to NMP in humans is lacking. Human studies involving experimental inhalation are needed for evaluation of the effect of short term exposure. The metabolism in humans needs to be evaluated for assessing the risk of exposure and for studies of the possibility of biological monitoring.

Materials and methods

SUBJECTS AND STUDY DESIGN

Six healthy male volunteers (subjects 1, 2, 3, 4, 5, and 6; age 28, 29, 35, 39, 39, and 41

years; weight 62, 70, 80, 72, 80, and 79 kg; height 170, 183, 178, 176, 186, and 178 cm) were studied. All participants were subject to a general health examination, with special attention to liver, kidney, and haematology. They had no history of nasal problems, no midfacial abnormalities, no notable septal deformities, and no turbinate hypertrophy. They were also examined with allergen skin tests. None of the subjects were using any kind of drug at the time of the experiment, and none had consumed alcohol within the 24 hours before the experiment or as long as urine samples were collected or blood samples were sampled. There were no restrictions on the diet before or after the exposure. During the exposure all subjects at all four exposures had the same diet (after two hours coffee and two slices of bread and cheese; four hours 200 g pizza; six hours coffee and one piece of cake; water as desired). The study was run during February and March, a period when the pollen count in air is low. Two of the subjects responded slightly positively to prick test for histamine (3 mg/ml).

The study design was approved by the ethics committee of Lund University (LU 451–94, Medical Faculty, Lund University, Sweden), and all six subjects gave their written informed consent to participate in the study.

EXPOSURE

The volunteers were exposed in an exposure chamber (1·5 m × 1·5 m; height 2·5 m) with an air turn over rate of 20 per hour. The subjects were exposed two at a time. There was an exposure free period of about five minutes after two, four, and six hours of exposure for examination and biological sample collection. The concentration of NMP in air was measured by continuously adding NMP on a heated surface (190°C) placed in the air inlet to the chamber. The concentration of NMP in air was levelled by dilution of the NMP with water. The concentration of NMP in air in the chamber was continuously measured with an infrared spectrophotometer (Miran-1A; wavelength 7·75 μm; path length 20·5 m). The exposure was assessed by four consecutive two-hour sampling periods in the personal breathing zone of each subject. The sampling was performed on solid sorbent tubes (Amberlite XAD 7; SKC pumps; 0·2 l/min).

ANALYSIS OF SAMPLES OF NMP IN AIR

The air samples, after collection, were kept in darkness at 4°C and analysed within two days. The NMP was desorbed from the solid adsorbent (main layer and control layer separately) with 2 ml ethyl acetate with 5% ethanol.[10] After shaking for two hours, the organic phase was transferred into glass vials with polyfluorotetraethylene (PFTE) screw caps. The analyses of NMP were performed by gas liquid chromatography (GLC) (Varian 3700; autosampler 8035) with a nitrogen phosphorus detector (NPD; Varian TSD). A 30 m × 0·25 mm internal diameter fused silica column with 0·25 μm DB-5MS (J&W Scientific (Fisons), Folsom, CA, USA) was used. The carrier gas was nitrogen (20 ml/min). The temperature of the split/splitless injector was 220°C, the column 125°C, and the detector 250°C. The peaks were evaluated by integration (Shimadzu C-R3A Chromatopac integrator). Stock NMP solution was dissolved in ethyl acetate with 5% ethanol and stored at 4°C. The calculations of the desorbed concentrations of NMP were made by comparison with diluted stock solutions with added solid adsorbent.

BLOOD ANALYSIS

Blood samples were taken, before the start of exposure, immediately after the exposure, and 16 hours after the exposure for the analysis of the number of leucocytes, neutrophils, eosinophils, lymphocytes, basophils, monocytes, and thrombocytes and the concentrations in serum of IgE, bilirubin (conjugated as well as total), alkaline phosphatase (S-ALP), glutamyltransferase (S-GT), aspartate aminotransferase (S-ASAT), and alanine aminotransferase (S-ALAT).

RHINOMETRY

The geometry of the nasal cavity was assessed by continuous acoustic rhinometry (Rhin 2000 with software program 1. 19; SR Electronic, Lynge, Denmark) before and 2, 4, 6, and 8 hours after the start of exposure. At the end of exposure the volunteers were given two puffs of nasal spray (oxymetazolin hydrochloride 0·5 mg/ml) in each nostril (into the inferior and middle nasal meatus) and the decongestion were assessed after 30 minutes. The nasal cavity, both left and right sides, was evaluated by the minimal cross sectional areas found in two distances (d_1) between 10 to 32 mm and (d_2) between 32 to 64 mm from the nostril, and by the volumes (V_1 and V_2) of the nasal space in these two distances. Due to the nasal cycle[11] all calculations were made on the summed minimal cross sectional areas and volumes of left and right nasal cavities.

PULMONARY FUNCTION

Pulmonary function was performed by spirometry according to the guidelines of the American Thoracic Society.[12] The forced expiratory volume in one second (FEV_1), vital capacity (VC), and the highest forced expiratory capacity (FVC) were measured with a vitalograph (Vitalograph, Buckingham, England) before and after the exposure.

QUESTIONNAIRE

The volunteers filled in a questionnaire before the start of exposure and then every two hours for 16 hours. The following symptoms were registered on a scale rated from 0–10 (0 = no symptoms, 5 = moderate symptoms, and 10 = not tolerated): hacking cough, nose secretion, or blockage, sneezing, itching, or dryness in the mouth and throat, or other symptoms in the upper airways; itching, secretion, smarting pain, visual disturbances, or other symptoms in the eyes; symptoms such as headache, dizziness, and nausea; and other symptoms.

Table 1 Change in variables of lung ventilation in six volunteers before and after eight hours of exposure to 0, 10, 25, and 50 mg/m³ NMP

	Exposure to (mg NMP/m³)											
	0			*10*			*25*			*50*		
Subject	*FVC_I*	*VC*	*FVC*	*FVC_I*	*VC*	*FVC*	*FVC_I*	*VC*	*FVC*	*FVC_I*	*VC*	*FVC*
1:												
0 h	4·20	4·80	4·85	3·90	4·55	4·60	4·10	4·85	4·80	4·00	4·55	4·70
8 h	0	0	+0·05	+0·10	−0·10	0	0	−0·20	+0·05	0	0	0
2:												
0 h	4·60	5·15	5·15	4·65	5·10	5·25	4·40	5·10	4·95	4·40	5·05	5·10
8 h	−0·10	+0·05	−0·05	−0·05	+0·05	−0·10	+0·05	−0·05	+0·05	+0·10	0	−0·05
3:												
0 h	4·80	5·75	5·70	4·75	5·95	5·90	4·65	5·90	5·85	4·60	5·90	5·80
8 h	0	−0·05	+0·20	0	−0·05	0	0	0	−0·05	0	−0·20	−0·10
4:												
0 h	4·35	5·75	5·75	4·45	5·80	5·80	4·35	5·65	5·75	4·40	5·70	5·80
8 h	+0·10	0	+0·05	0	−0·05	0	+0·05	−0·05	−0·05	0·10	+0·05	+0·10
5:												
0 h	4·05	4·75	4·85	4·00	4·85	4·85	4·05	5·00	4·90	4·10	4·90	4·90
8 h	−0·10	0	−0·10	−0·05	+0·05	−0·15	0·05	−0·15	−0·05	−0·15	−0·15	−0·10
6:												
0 h	4·25	5·40	5·30	4·30	5·40	5·30	4·10	5·35	5·25	4·10	5·30	5·25
8 h	+0·10	0	+0·15	−0·05	−0·05	0	0	+0·05	+0·10	+0·15	+0·20	+0·15

0 h = Before start of exposure; 8 h = the change after end of exposure.

BLOOD AND URINE SAMPLES FOR THE PHARMACOKINETIC STUDY

Blood samples (20 ml) were collected by venepuncture in evacuated heparinised tubes (Venoject, Teruma Europe NV, Leuven, Belgien) before, and at 4, 8 (end of exposure), 9, 10, 12, 16, 24, 32, and 48 hours after the start of exposure. After 30 minutes at room temperature the blood samples were centrifuged (1500 *g* for 15 minutes) and the plasma was frozen and kept at −16°C until analysis. Urine was collected in polyethylene bottles before exposure, at two-hourly intervals up to 16 hours after the start of exposure, and then at three four-hourly and three eight-hourly intervals. The urine samples were frozen immediately and kept at −16°C until analysis.

ANALYSIS OF NMP IN PLASMA AND URINE SAMPLES

To 2 ml of plasma or urine, 2 ml toluene and 4 ml 12 M potassium hydroxide (KOH), containing 0·25% ammonia were added. After shaking for 10 minutes the toluene phase was transferred into glass vials with PFTE screw caps.

The analyses of NMP were performed by a modification of the same GLC procedure as for samples of NMP in air. The column temperature programming was 125°C for five minutes, 250°C (increasing by 20°C/min), and 250°C for three minutes. Standard samples of NMP in plasma and urine were stored at −16°C and the concentrations of NMP in plasma and urine were calculated by comparison with these plasma and urine standards. In both plasma and urine the detection limit was about 0·01 μg/l, and the relative error was 5%, based on 10 duplicate 1·0 μg/l samples.

Table 2 Summed nasal cavity volumes (right + left) in the distance between 32 to 64 mm from the nostril after eight hours of exposure to 10, 25, and 50 mg NMP/m³ expressed as percentage of the volumes found for zero exposure experiments in the six subjects (volumes 30 minutes after dosing with nasal spray are also presented)

	Nasal cavity volume (%)			
	After end of 8 h exposure		*After end of 8 h exposure and 30 min after dosing with xylometazolin*	
Exposure (mg NMP/m³)	*Median*	*Range*	*Median*	*Range*
10	94	71–136	95	79–131
25	100	80–141	98	94–161
50	111	94–161	95	86–131

Results

EXPOSURE

The mean (range) eight hour time weighted averages (TWAs) of the three exposures, from lowest to highest, were 10 (9–13) mg/m³, 24 (24–26) mg/m³, and 53 (48–58) mg/m³, respectively.

SUBJECTIVE JUDGEMENTS

Questionnaire

The records of subjective judgements of odour perception and nose, eye, and airway irritation showed no discomfort or any irritative effects. Two subjects reported an odour of acetone at 50 mg/m³. The odour was reported as "not uncomfortable".

OBJECTIVES

Blood analysis

The NMP exposure did not affect the studied blood variables. There was no systematic difference before and 16 hours after exposure in any variables measured. The results for the blood analysis may be obtained on request.

Pulmonary function

There were no significant differences in the spirometric data displayed by FEV_1, VC or FVC before or after any level of exposure. The largest difference in the spirometric data before and after exposure was 0·2 l (table 1).

Rhinometry

The geometry of the nasal cavity did not show an acute reaction to NMP in the series of the eight-hour exposure experiments. A comparison of the measurements of d_1, d_2, V_1, and V_2 (right plus left) before and after exposure showed no obvious differences (table 2). Neither did xylometazolin decongest differently between the experiments. However, the

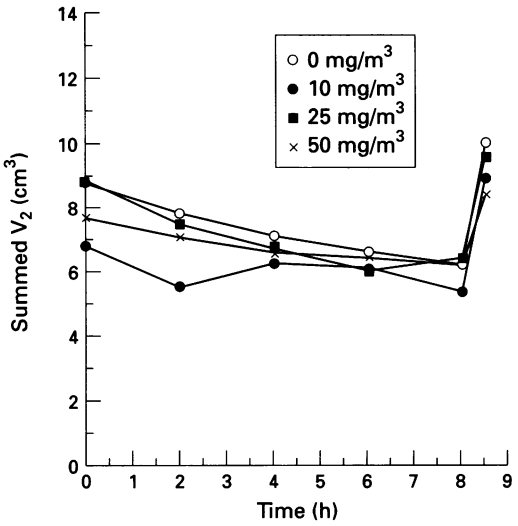

Figure 1 Nasal space, as the summed volume (V_2; left and right sides) found in the distances between 32 to 64 mm from the nostril, in subject 1 before, during, and after exposure to 0, 10, 20, and 50 mg/m³ NMP and after decongestion with two puffs of 0·5 mg/ml oxymetazolin hydrochloride to each nostril.

95% confidence interval (95% CI) was wide and thus, there may be an undetected change in the volume of the nasal cavity. Figure 1 shows typical nasal cavity volume-time curves in one subject (subject 1; before, during, and after exposure and after decongestion with nasal spray). The decreasing pattern during the exposure, typical in all subjects, was due to a decreasing activity of the subjects in the chamber.

PHARMACOKINETICS

No NMP was detected in plasma or urine samples obtained before the start of exposure. After the start of the exposure to NMP, the unchanged compound appeared in plasma and urine. The mean (range) concentrations of NMP in plasma (P-NMP; fig 2) at the end of exposure to 10, 25, and 50 mg/m³ were 0·33 (0·20–4·3) mg/l, 0·99 (0·44–2·2) mg/l, and 1·6 (1·2–2·4) mg/l, respectively. After the end of exposure the NMP in plasma decreased. The elimination curves suggested a non-linear pattern (fig 3). An evaluation of the elimination by linear regression of the semilogarithmic concentration-time curves between two and 24 hours after the end of exposure showed mean (range) half lifes of NMP in plasma of 4·0 (2·9–4·9) hours, 3·9 (2·9–5·8) hours, and 3·4 (2·9–3·8) hours for the exposures to 10, 25, and 50 mg/m³, respectively.

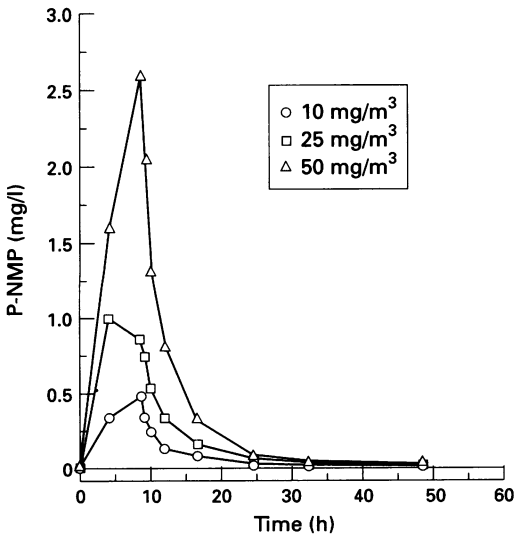

Figure 2 Mean NMP plasma concentration (P-NMP) before, during, and after NMP exposure at 10, 20, and 50 mg/m³ in six human volunteers.

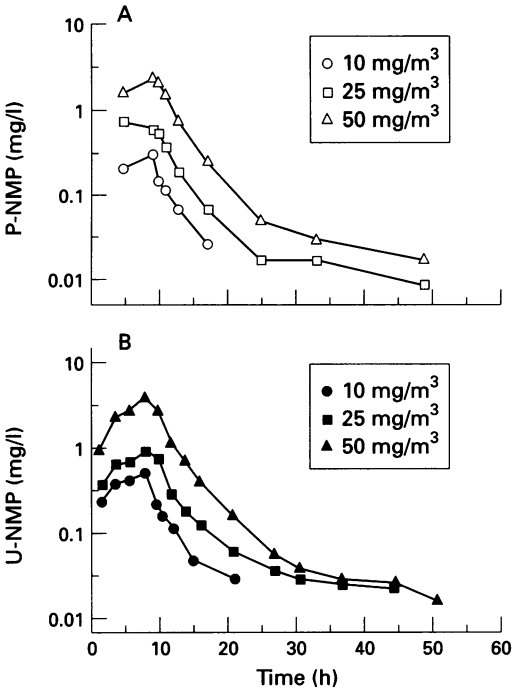

Figure 3 Concentrations of NMP (A) in plasma (P-NMP) and (B) in urine (U-NMP) before, during, and after NMP exposure at 10, 20, and 50 mg/m³ in subject 4.

There was a close correlation between the concentration of NMP in plasma and urine. The linear relation between NMP in plasma (mg/l) at one hour after exposure and NMP in urine (mg/l) zero to two hours after exposure showed individual slopes from 0·82 to 1·51 (median 1·18) and correlation coefficients > 0·95 ($P < 0·05$).

After the end of exposure the concentration of NMP in urine decreased in a similar non-linear pattern as in plasma (fig 3). The mean (range) half lives of NMP in urine, calculated by linear regression of the semilogarithmic concentration-time curves between two and 30 hours after the end of exposure were 4·5 (3·6–5·5) hours, 5·2 (4·5–6·6) hours, and 3·9 (3·5–4·3) hours for exposures 10, 25, and 50 mg/m³, respectively.

There was good correlation between exposure and excretion in urine. The mean (range) NMP excretion rates zero to two hours after exposure (six subjects; fig 4) were 10 mg/m³, 27 (16–43) μg/h; 25 mg/m³, 111 (75–153) μg/h; and 50 mg/m³, 275 (111–508) μg/h. There were high correlations zero to two hours after exposure, between the exposures and concentration of NMP in urine, calculated on an individual basis. The individual slopes varied from 0·025 to 0·062 (median 0·042) with correlation coefficients > 0·98.

The amount of NMP excreted in the urine collected during the exposure and 44 hours after the end of exposure corresponded to about 2% of the calculated absorbed dose (a pulmonary ventilation of 8 m³ and a retention factor of 0·65. At 10 mg/m³ the mean (range) absorbed dose was 1·6% (0·6%–1·9%), at 25 mg/m³ it was 1·8% (1·0%–2·1%), and at 50 mg/m³ it was 2·2% (0·9%–1·9%)).

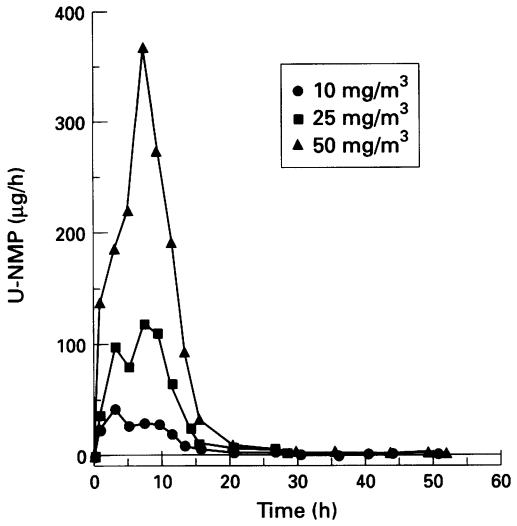

Figure 4 Mean NMP urine excretion (U-NMP) before, during, and after NMP exposure at 10, 20, and 50 mg/m³ in six human volunteers.

Discussion

The effect of eight hours of exposure to concentrations below the threshold limit value (TLV) for NMP has been studied. In the range of exposure to NMP of 10–50 mg/m³, there were no subjective self reported sensations of eye, nasal, or respiratory irritation. Neither were the pulmonary functions or the nasal cavities affected by the NMP exposure. Despite the limitation of the present study with only six subjects, the results are consistent and be reasonably well defined. Thus, the results indicate that NMP is a mild irritant. This does not accord with the reports of unacceptable eye irritation caused by exposure to 3–6 mg/m³ NMP (8 h TWA).[8] The discrepancy may be due to NMP work processes performed at temperatures above the boiling point of NMP (202°C) in that study. Thus, the 8 h TWA concentration may have contained periods of extremely high peak exposures or the warm NMP vapour may condense to an areosol vapour which is irritating to the eyes. However, it is expected that with normal hygiene standards work processes with volatile compounds which are heated up to their boiling points should be enclosed. The NMP work processes at room temperature result in air concentrations of only a few mg/m³.

The NMP was readily eliminated from the body with a half life in plasma of about four hours. The elimination was mainly by biotransformation to other compounds. Only a small part, 2%, was excreted in urine as NMP. These findings are in accordance with reported data from animal studies.

The correlation between exposure and the NMP concentrations in plasma and urine indicates that biological monitoring of exposure to NMP or risk from NMP is possible. The high percutaneous uptake of NMP[2] and the large number of women working with it—for example, in the microelectronics fabrication industry—indicate the urgent need for biological monitoring. However, due to the almost total biotransformation of NMP, the main pathways in humans need to be mapped before a suitable method could be adopted.

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