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Biological monitoring of N-methyl-2-pyrrolidone using 5-hydroxy-N-methyl-2-pyrrolidone in plasma and urine as the biomarker

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Objectives The aims were to study the toxicokinetics of 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) in blood and urine after exposure to N-methyl-2-pyrrolidone (NMP) and to study the suitability of 5-HNMP as a biomarker for assessing NMP exposure.

Methods Six male volunteers were exposed for 8 hours to NMP concentrations of 0, 10, 25, and 50 mg/m³. Blood and urine were sampled before, during, and up to 40 hours after exposure. Aliquots of urine and plasma were purified, derivatized, and analyzed for 5-HNMP on a gas chromatograph/mass spectrometer in the electron impact mode.

Results The mean plasma concentration [P-(5-HNMP)] after 8-hour NMP exposure to 10, 25, and 50 mg/m³ was 8.0, 19.6, and 44.4 µmol/l, respectively. The mean urinary concentration [U-(5-HNMP)] for the 2 last hours of exposure was 17.7, 57.3, and 117.3 mmol/mol creatinine, respectively. The maximal P-(5-HNMP) and U-(5-HNMP) concentrations occurred 1 hour and 0–2 hours, respectively, after the exposure. The half-times of P-(5-HNMP) and U-(5-HNMP) were 6.3 and 7.3 hours, respectively. The 5-HNMP urinary concentrations were 58% of the calculated retained dose. There was a close correlation (*r*) between P-(5-HNMP) (*r*=0.98) and U-(5-HNMP) (*r*=0.97) with NMP exposure.

Conclusions 5-HNMP is an excellent biomarker for assessing exposure to NMP. Its plasma and urinary half-times (6–7 hours), the minimal risk for contamination during sampling in occupational settings, and the close correlation of P-(5-HNMP) and U-(5-HNMP) with NMP exposure makes 5-HNMP suitable for monitoring exposure to NMP. 5-HNMP in plasma is recommended.

Key terms graffiti remover, paint stripper, reproductive toxicity, skin uptake, solvent.

N-Methyl-2-pyrrolidone (NMP; CAS number 872–50–4) is, due to its strong and selective solvent power, a widely used organic solvent. NMP is a water-miscible colorless liquid. It is hygroscopic with a mild amine odor. NMP is used in the petrochemical industry, in the microelectronics fabrication industry, and in the manufacturing of various compounds (eg, pigments, cosmetics, drugs, insecticides, herbicides, and fungicides). NMP is used as a substitute of solvents of higher inherent toxicity in occupational and environmental settings (eg, dichloromethane in paint strippers). The use of NMP as a remover of graffiti has strongly increased. NMP has been suggested as a skin penetration enhancer for use in transdermal therapy in humans (1).

Experimental exposure of human volunteers shows that NMP is readily absorbed through the respiratory tract and eliminated from the body (mainly) by biotransformation to other compounds. Only about 2% of the

inhaled dose is excreted in urine as NMP (2). In the rat, Wells and his co-workers (3) showed that NMP is mainly metabolized to 5-hydroxy-N-methylpyrrolidone (5-HNMP). A metabolic pathway in humans, where NMP is first hydroxylized to 5-HNMP and then further oxidized to N-methylsuccinimide (MSI), which in turn is hydrolyzed to 2-hydroxy-N-methylsuccinimide (2-HMSI), has been suggested (4). It has been shown that NMP possesses a high permeability through both human (5) and rat (6, 7) skin. In rat, about 70% of the radioactivity of skin-administered radiolabeled NMP is excreted in urine (7).

Animal studies show that exposure to NMP may cause degenerative changes in the respiratory system and in hemopoietic and lymphoid tissues. Lethargy and irregular respiration have also been recorded (8). Studies in female rats exposed to a concentration of 1000 mg/m³ showed only minor nasal irritation when exposed through

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the nose only, but massive increased mortality and severe effects on major organs occurred when whole-body exposure was employed (unpublished data).

Experimental NMP exposure of humans for 8 hours to 50 mg/m³ indicates that NMP is a mild irritant (2). However, in occupational settings, an irritative effect has been recorded on skin and eyes, and this finding indicates that NMP may be a moderate to severe irritant (9, 10).

Animal studies on reproductive toxicity show that NMP may cause developmental toxicity at doses causing mild or no maternal toxicity (11). Moreover, a still-birth after occupational exposure to NMP has recently been described in a case report (12).

The large number of women exposed to NMP in industry makes it important to develop methods for assessing exposure to NMP. Methods for analyzing NMP in air have been described (2). However, the extensive percutaneous absorption of NMP makes biological monitoring preferable for assessing the exposure. A method, NMP in plasma and urine, for the biological monitoring of NMP exposure has also been reported (2). However, the ready biotransformation of NMP to other compounds suggests that the metabolites of NMP, rather than NMP itself, may be the most suitable compounds for biological monitoring.

No methods for assessing exposure to NMP using a metabolite of NMP have been described so far. In our study, we report a method for the biological monitoring of NMP exposure using the concentration of 5-HNMP in plasma and the excretion of 5-HNMP in urine.

Subjects and methods

Subjects and study design

Six healthy male volunteers [mean age 35 (range 28–41) years, mean weight 74 (range 62–80) kg, and mean height 179 (range 170–186) cm] were studied. All the participants were subject to a general health examination, with special attention to liver, kidney, and hematological conditions. None of the subjects were using any kind of drug at the time of the experiment, and none had consumed alcohol within 24 hours of the experiment or as long as urine samples were collected or blood samples were sampled. There were no dietary restrictions before or after the exposure. During the exposure all the subjects at all 4 exposure levels had the same diet (after 2 hours, coffee and 2 slices of bread and cheese; after 4 hours, 200 grams of pizza; after 6 hours, coffee and 1 piece of cake; water as desired). The Ethics Committee of the Medical Faculty, Lund University, approved the study design, and all 6 subjects gave their written, informed consent to participate in the study.

Exposure

The volunteers were exposed in an exposure chamber with a turnover rate of 20 times per hour. The subjects were exposed 2 at a time. There was an exposure-free period of about 5 minutes after 2, 4, and 6 hours of exposure for the examination and biological sample collection. The exposure was determined by 4 consecutive 2-hour sampling periods in the personal breathing zone of each subject. The sampling was performed on Amberlite XAD-7 tubes (lot no 754; SKC pump, model 222). The 3 exposure levels (8-hour time-weighted average) were means of 10 (range 8–13) mg/m³, 24 (range 22–26) mg/m³, and 53 (range 44–60) mg/m³. The generation of NMP air concentration and the analysis of the NMP air samples have been described earlier (2). There was at least 2 full weeks between the experimental exposures.

Blood and urine samples for the toxicokinetics study

Blood samples (20 ml) were collected by venepuncture in evacuated heparinized tubes (Venoject, Teruma Europe NV, Leuven, Belgium) prior to, and at 4, 8 (end of exposure), 9, 10, 12, 16, 24, 32, and 48 hours after the start of the exposure. After 30 minutes at room temperature the blood samples were centrifuged (1500 grams, 15 minutes), and the plasma was frozen and kept at -16°C until the analysis. Urine was collected in polyethylene bottles before the exposure, at 2-hour intervals up to 16 hours after the start of the exposure, and then at 3 4-hour and 3 8-hour intervals up to 52 hours. The urine samples were frozen immediately and kept at -16°C until the analysis.

Analysis of 5-hydroxy-N-methyl-2-pyrrolidone in plasma and urine samples

The method for determining 5-HNMP in urine (13) and plasma (14) has been described earlier. 5-HNMP was purified from the urine and plasma by adsorption to a C₈ solid phase extraction column and then eluted by ethyl acetate:methanol (80:20). After evaporation, the samples were derivatized at 100°C for 1 hour by bis(trimethylsilyl)trifluoroacetamide. Ethyl acetate was then added, and the samples were analyzed on a gas chromatograph (GC model 8065, Carlo-Erba, Milan, Italy) connected with a mass spectrometer (VG Trio 1000 quadrupole, Fisons, Manchester, UK) in the electron impact mode. The detection limit was 2 µmol/l for urine and 0.1 µmol/l for plasma.

Results

After the onset of the exposure to NMP, the hydroxylized compound (5-HNMP) appeared in plasma and urine.

The mean 5-HNMP plasma concentrations [P-(5-HNMP)] (figure 1) at the end of the exposure to 10, 25, and 50 mg/m³ were 8.0 (range 6.9–10.6), 19.6 (range 16.0–23.6), and 44.4 (range 36.2–51.8) µmol/l, respectively. After the end of the exposure the P-(5-HNMP) still increased during the following 1-hour period and then decreased.

The 5-HNMP in the plasma elimination curves suggested a linear pattern (figure 2). An evaluation of the elimination by linear regression of the semilogarithmic concentration-time curves between 3 and 32 hours after the end of the exposure showed mean half-times of 5-HNMP in plasma of 6.2 (range 5.3–6.8), 6.5 (range 5.8–7.5), and 6.1 (range 5.4–7.1) hours for the exposure levels 10, 25, and 50 mg/m³, respectively.

P-5-HNMP (µmol/l)

Figure 1. Mean concentration of 5-hydroxy-N-methyl-2-pyrrolidone in plasma (P-5-HNMP) before, during, and after the exposure of 6 human volunteers to N-methyl-2-pyrrolidone (NMP) concentrations of 0, 10, 25, and 50 mg/m³.

P-5-HNMP (µmol/l)

Figure 2. Mean concentrations of 5-hydroxy-N-methyl-2-pyrrolidone in urine (U-5-HNMP, corrected for creatinine concentration) and in plasma (P-5-HNMP) during and after the exposure of 6 male subjects to N-methyl-2-pyrrolidone (NMP) concentrations of 10, 25, and 50 mg/m³.

The concentration of 5-HNMP in urine [U-(5-HNMP)] collected before the start of the NMP exposure and during the 0-exposure experiment was below the detection limit of 2 µg/l. The U-(5-HNMP) rose during the exposure and during the following 2-hour period after the end of the exposure. Two hours after the end of the exposure the U-(5-HNMP) decreased, displaying a slight irregular pattern (figure 3). The irregular pattern was not fully eliminated by correction for the concentration of creatinine (figure 4) or for density. There was a very close correlation between U-(5-HNMP) corrected for creatinine and the excretion rate (correlation coefficients of 1.0 for all 3 exposure levels).

The mean U-(5-HNMP) in urine sampled during the last 2-hour period of exposure was 190 (range 140–250,

U-5-HNMP (µmol/l)

Figure 3. Mean concentration of 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) in urine before, during, and after the exposure of 6 male subjects to N-methyl-2-pyrrolidone (NMP) concentrations of 0, 10, 25, and 50 mg/m³.

U-5-HNMP (mmol/mol creatinine)

Figure 4. Mean concentration of 5-hydroxy-N-methyl-2-pyrrolidone in urine (U-5-HNMP, corrected for creatinine concentration) before, during, and after the exposure of 6 male subjects to N-methyl-2-pyrrolidone (NMP) concentrations of 0, 10, 25, and 50 mg/m³.

460 (range 200–840), and 775 (range 490–1400) $\mu\text{g/l}$ for the exposure levels of 10, 25, and 50 mg/m^3 , respectively (figure 3). The corresponding creatinine-corrected means of the U-(5-HNMP) concentrations were 17.7 (range 8.0–29.3), 57.3 (range 43.9–77.8), and 117 (range 83.1–146) $\text{mmol/mol creatinine}$, respectively (figure 4). There was a close correlation between the concentration of 5-HNMP in plasma at the end of the exposure and the 5-HNMP excretion in urine during the last 2-hours of the sampling period [correlation coefficient (r) 0.97].

An evaluation of the mean excretion corrected for creatinine concentration by linear regression of the semi-logarithmic concentration-time curves between 4 and 40 hours after the end of the exposure showed half-times of 6.7, 8.2, and 6.9 hours for the exposure levels of 10, 25, and 50 mg/m^3 , respectively (figure 2).

The amount of U-(5-HNMP) excreted in the urine collected during the exposure and 44 hours after the end of the exposure corresponded to 52 (range 43–66)%, 62 (range 57–70)%, and 59 (range 41–74)% of the absorbed dose for the exposure levels of 10, 25, and 50 mg/m^3 , respectively. The mean absorbed dose was calculated at an estimated pulmonary ventilation rate of 6 m^3 , a retention factor determined at the 50 mg/m^3 exposure level of 0.90 (range 0.88–0.93), and the inhaled NMP air concentration.

There was a close correlation between the 5-HNMP concentration in plasma ($r=0.98$) (figure 5) and the amount of U-(5-HNMP) excreted in the urine ($r=0.95$) (figure 6) at the end of exposure versus the exposure to NMP. Individually, the linear relationship between 5-HNMP at the end of the exposure in plasma and urine and the NMP exposure (mg/m^3) was even closer. For plasma ($\mu\text{mol/l}$) the correlation coefficients were >0.99 with slopes from 0.67 to 1.00 (mean 0.84) and for urine

($\text{mmol/mol creatinine}$) the corresponding value was >0.98 with slopes from 1.5 to 2.9 (mean 2.3).

Discussion

The present study displayed close correlations between the NMP air concentration and the 5-HNMP in plasma and urine. 5-HNMP is a major NMP metabolite with a half-time of about 6 hours in plasma. Thus, for assessing NMP exposure, 5-HNMP seems to be an excellent biomarker. Both 5-HNMP in plasma and in urine can be used.

Blood samples were collected up to 40 hours after the end of exposure. The highest 5-HNMP concentration in plasma [P-(5-HNMP)] was found in samples obtained 1 hour after the end of the exposure (figure 1). At the end of the studied period the P-(5-HNMP) was well above the detection limit for P-(5-HNMP). When calculated with a half-time of 6.3 hours and 1-compartment elimination, the P-(5-HNMP) at end of the study (6 x half-time) were expected to be 0.13, 0.32, and 0.77 $\mu\text{mol/l}$, respectively, for the 3 exposure levels. P-(5-HNMP) levels twice as high were found, however. This result may have been caused by a second, slower, compartment in the elimination phase, not observed due to the low P-(5-HNMP) at the end of the studied period. In a study in which we administered NMP orally and collected urine during 9 days, we did not find a second phase of elimination (4). Moreover, the semilogarithmic P-(5-HNMP) concentration-time curves may display a slow uptake-distribution phase for 2 to 14 hours after exposure (figure 2). One explanation is the possibility of a concomitantly minor uptake through the skin, particularly if the absorption rate through the skin is low. Even though no aerosolization was observed during the exposure, based on the absence of condensation on cold surfaces, skin expo-

Figure 5. Concentration of 5-hydroxy-N-methyl-2-pyrrolidone in plasma (P-5-HNMP) collected at the end of the exposure of 6 male subjects to N-methyl-2-pyrrolidone (NMP) concentrations of 0, 10, 25, and 50 mg/m^3 .

Figure 6. Concentration of 5-hydroxy-N-methyl-2-pyrrolidone in urine (U-5-HNMP, corrected for creatinine concentration) sampled during the 2 last hours of the exposure of 6 male subjects to N-methyl-2-pyrrolidone (NMP) concentrations of 0, 10, 25, and 50 mg/m^3 .

sure to NMP aerosol cannot be excluded. It should be noted that NMP may exist in various proportions of vapor and aerosol depending on the concentration, temperature and atmospheric humidity. The maximum vapor phase is 1320 mg/m³ at room temperature and dry air (0% RH), 410 mg/m³ at normal humidity (60% RH), and 0 mg/m³ for wet air (100% RH) (unpublished data). There is, of course, also a possibility of uptake of gaseous NMP through the skin. Thus the calculated half-time in plasma may be somewhat different than the reported half-times of 6–7 hours. In a previous study with the oral administration of NMP in humans, a half-time of about 4 hours was roughly estimated (4). The recovered fraction of 5-HNMP in urine, 60%, may also be overestimated if there is additional skin uptake. The importance of skin uptake has to be further investigated.

The elimination curve of U-(5-HNMP) showed a slight irregular pattern after the end of exposure. The irregularity was observed for all the subjects and at all the exposure levels. It may be caused by sample instability or analysis error. However, the stability of samples during prolonged storage has been checked, and the analysis method has been shown to display high reproducibility (12, 13). The irregular pattern of the elimination curve may be a result of skin uptake showing a second peak in the elimination curves about 4 hours after the first peak. The irregularity was not seen for plasma, but the sampling of plasma was less frequent than the urine sampling. The irregularity was not eliminated with correction for creatinine, density, or excretion rate. However, for biological monitoring, the irregularity seems to be of minor importance.

The major metabolite of NMP was 5-HNMP. The intraindividual difference in the hydroxylation of NMP to 5-HNMP was minor at the 3 studied exposure levels. Interindividually, the difference varied by a factor of 2 for urine. However, currently we have no knowledge of the toxicity of metabolites and, thus, whether small or large formations of 5-HNMP are favorable.

The close correlation between the NMP exposure level and the 5-HNMP concentration in plasma and urine offers several possible methods for biological monitoring. The use of 5-HNMP as the biomarker is favorable, as no risk of contamination of blood or urine may occur during the sampling. Because of the close correlation ($r=0.98$) between the air level and the P-(5-HNMP) concentration at the end of exposure (figure 5) and the slight irregular elimination of U-(5-HNMP) (figure 4), we prefer plasma for biological monitoring. Exposure during 8 hours to an NMP air concentration of 10 mg/m³ during light work corresponds to a P-(5-HNMP) concentration of 8.2 µmol/l at the end of exposure. However, if non-vascular methods are preferable, the correlation between the NMP air level and U-(5-HNMP, µmol/mol creatinine) or U-(5-HNMP, µmol/hour) showed correlation factors

of 0.95 and 0.96, respectively, for urine samples collected during the 2 last hours of exposure. U-(5-HNMP) of 22 µmol/mol creatinine and U-(5-HNMP) of 15 µmol/hours corresponds to an airborne 8-hour NMP exposure of 10 mg/m³ during light work. Biological monitoring using the excretion rate for assessing the exposure is more difficult with urine sampling. Thus U-(5-HNMP) corrected for creatinine concentration is recommended for assessing the exposure to NMP when P-(5-HNMP) is not preferred.

The Swedish occupational exposure limit for NMP is 200 mg/m³. The corresponding limit in Germany is 90 mg/m³. With the recommended method for biological monitoring it is possible to assess exposures far below 1 mg/m³.

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