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ORIGINAL ARTICLE

Biological monitoring and health effects of low-level exposure to *N*-methyl-2-pyrrolidone: a cross-sectional study

Vincent Haufroid · Veronika K. Jaeger · Stefan Jeggli · Rolf Eisenegger · Alfred Bernard · Drita Friedli · Dominique Lison · Philipp Hotz

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Abstract
Purpose To examine the value of urinary 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) in a population of workers exposed to *N*-methyl-2-pyrrolidone (NMP) and to look for health effects of exposure to this organic solvent.
Methods Airborne NMP was determined according to the NIOSH method. Urinary 5-HNMP and 2-HMSI (after and before next shift) were determined by liquid chromatography with tandem mass spectrometry. Outcomes were effects on lung, kidney, skin and mucous membranes, nervous system, haematopoiesis and liver determined by clinical examination and laboratory measurements. Univariate statistical methods and multiple regressions were used to analyse results. Skin resorption, smoking and other potential confounders were taken into account.
Results Three hundred twenty-seven workers were eligible out of which 207 workers (63 %) participated. Ninety-one of these worked with NMP. Occupational exposure to NMP did often not occur daily and ranged from non-detectable to 25.8 mg/m^3 (median = 0.18). Urinary 2-HMSI (mg/l; before next shift) was the best biomarker of exposure to NMP, explaining about 70 % of the variance, but most likelihood ratios did not allow for ruling exposure in or out, at these low levels of exposure. Creatinine adjustment did not improve the results clearly. No clear and consistent health effects could be associated with NMP exposure. No indication for a bias due to non-participation was found.
Conclusions Biological monitoring, primarily urinary 2-HMSI (mg/l; before next shift), is of value to estimate exposure to NMP even when exposure is irregular and low. Likelihood ratios of urinary 5-HMNP or 2-HMSI are, however, not quite satisfactory at these low levels. No irritant or other health effects were found.

Keywords Biological monitoring · *N*-methyl-2-pyrrolidone · Skin uptake · Irritation

Introduction

N-methyl-2-pyrrolidone (NMP; CAS 872-50-4) is a solvent used in the petrochemical industry, as a reaction medium in the polymer industry and as a stripping and cleaning agent in the electronics industry. It is a formulating agent in inks, dyes and pesticides and is a constituent of paint stripper and products for graffiti removal.

Two main sources give information about NMP toxicity in humans. The first one is made of experimental exposures typically conducted in small groups of young, healthy volunteers, who neither smoked nor consumed alcohol. At the most, these studies support a very limited irritant effect at airborne concentrations up to 160 mg/m^3 (Vanthriel et al. 2007; Akesson and Paulsson 1997) or after dermal application of 300 mg NMP (undiluted or diluted) for 6 h in Finn chambers (Akesson et al. 2004). By contrast, workplace studies, which represent the

V. Haufroid · A. Bernard · D. Lison
Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, IREC/LTAP, Avenue E. Mounier 52 bte B1.52.12, 1200 Brussels, Belgium

V. K. Jaeger · S. Jeggli · R. Eisenegger · D. Friedli · P. Hotz (✉)
Unit of Occupational and Environmental Medicine, University Hospital and University of Zurich, Outpatients' department Rämistrasse 100, 8091 Zürich, Switzerland
e-mail: philipp.hotz@usz.ch

second main source of information, have suggested toxic skin reactions (Jungbauer et al. 2001; Leira et al. 1992) and irritant effects (Langworth et al. 2001) even at levels as low as 3 mg/m^3 (Beaulieu and Schmerber 1991). Nephrotoxicity has been suggested at low concentrations too (geometric mean of 8-h time-weighted average concentrations in breathing zone air of 0.66, range 0.03–4.52 mg/m^3) (Langworth et al. 2001). A limitation of this second source is that it is comprised mainly of case series. Therefore, a sound risk assessment is difficult (Poet et al. 2010) as exemplified by the 8-h limit values in Europe (20–200 mg/m^3) (Gestis Database, accessed December 2012). Other effects on nervous system, haematopoiesis and liver have been suggested, but the literature is scarce (Carnerup 2004).

Some divergences between the conclusions of the studies (particularly irritant effects) may be explained by higher peak and/or mixed exposures, significant dermal absorption of liquid and/or vapour NMP (Bader et al. 2008; Akesson et al. 2004) and workplace micro-environment (temperature and humidity) (Carnerup et al. 2006). However, careful scrutiny of the available literature (Vanthriel et al. 2007) and power calculations strongly suggest that methodological factors (power, exposure assessment, co-exposures, definition of outcomes and statistical analyses) are probably an important source of variation as well. A further issue is the correlation between exposure to NMP and urinary metabolites as biological monitoring may be less useful under field conditions than assumed on the basis of the volunteer studies (Bader et al. 2007; Jonsson and Akesson 2003; Akrill et al. 2002). Indeed, volunteer studies were carried out under well-standardised and well-controlled conditions, which did not reflect everyday working conditions (Anundi et al. 2000). Therefore, it had also to be checked whether correlation coefficients of 0.8–0.9, which were found in volunteer studies, could also be found under field conditions.

It has been suggested that cytochrome P450 2E1 (CYP2E1) activity (phenotype) should be taken into account for an accurate interpretation of biological monitoring of exposure to NMP on the basis of an experiment in 12 human volunteers (Ligocka et al. 2003), but the influence of CYP2E1 genetic polymorphism on the interpretation of biological monitoring of NMP exposure is still unknown.

To assess reliably (correlation and likelihood ratio) the value of biomarkers of exposure under field conditions and the possible local and systemic effects of exposure to NMP (lung, kidney, skin and mucous membranes, nervous system, haematopoiesis and liver), a cross-sectional study with suitable power and taking into account dermal absorption, micro-environment (temperature and humidity) and CYP2E1 genotypes was conducted.

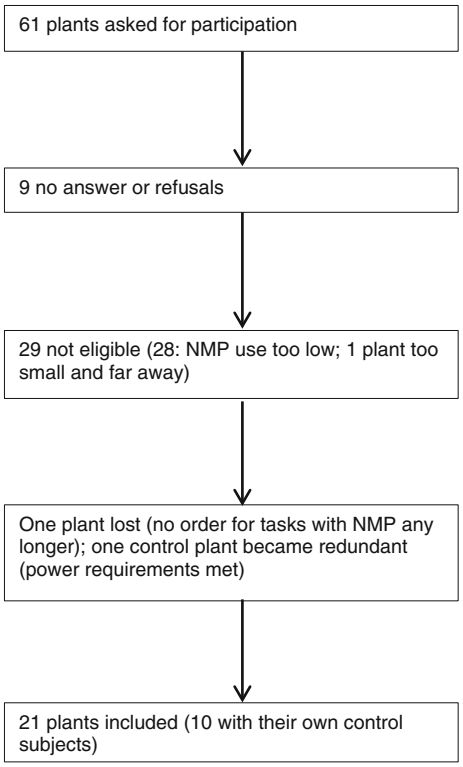

Fig. 1 Selection procedure and reasons for non-inclusion

Methods

The cross-sectional study was carried out between 2006 and 2011 in northeast Switzerland. As there is no list of plants using NMP, companies were identified with several methods (Internet searches, contact with NMP vendors or occupational health specialists or other sources of information) and asked for participation (Fig. 1). Controls were comparable subjects from the same or another plant.

Regarding power, assuming that irritation is an critical effect of NMP and that an exposure to about 1–3 mg/m^3 (0.25–0.75 ppm) is sufficient to increase the prevalence of respiratory symptoms 2–4 times (Langworth et al. 2001; Anundi et al. 2000; Beaulieu and Schmerber 1991), it is necessary to examine two groups with 60 subjects each to disclose a threefold increase in prevalence (10 and 30 % in the non-exposed and exposed group, respectively) with a probability of 80 % and at a significance level of 5 %. With respect to kidney effects (Langworth et al. 2001), a study population of at least 75 non-exposed and 75 exposed subjects are necessary to reach a power of about 85–90 %, given the following assumptions: urinary albumin in non-exposed subjects (geometric mean ± standard deviation) 5.3 ± 1.8 mg/g, significance level of 5 %, postulated true difference between groups of 0.9 mg/g.

Exposure was either due to graffiti removal or through other uses of NMP. The graffiti removers used several

NMP-containing products applying these with a cloth or a brush. An aerosol could arise when hot water was sprayed to complete the graffiti removal. The time necessary to remove the graffiti was very variable according to colour and surface, ambient temperature, etc. The other companies or firms used NMP as a solvent, in the production (cleaning agents, coatings, polymers, etc.) or in syntheses. Exposure to NMP did not always occur daily but depended on the orders the company had.

All persons gave their informed consent prior to inclusion into the study. The participants underwent a semi-structured clinical examination. The coding of the answers was reviewed by the study coordinator and divergences resolved by checking information and codes. Questions about respiratory symptoms and conditions were taken from the SAPALDIA study without changing the wording (Zemp et al. 1999; Leuenberger et al. 1998; Ackermann-Liebrich et al. 1997). Smoking was assessed with questions from the questionnaire of the European Community of Steel and Coal, revision 1967. Thirteen of the 16 questions were transposed from the Q16 questionnaire into German. The Q16 questionnaire was developed to monitor early effects of neurotoxic exposures in working populations (Lundberg et al. 1997). Two questions ("Do you sometimes feel an oppression of your chest" and "Do you often have a painful tingling in some part of your body") were already queried in other questions of the checklist and were, therefore, not included as well as the question "Do you have any problems with buttoning and unbuttoning". Current symptoms were defined as having occurred during the 4 weeks prior to the clinical examination if not defined otherwise (for example questions from the SAPALDIA study). Work-related symptoms were defined as those brought about by a specific task, occurring during a specific task in coworkers as well and without other cause.

With respect to metabolite concentration, "exposed to NMP" means current average monthly duration of exposure to NMP > 0 h. With respect to health effects, exposure was defined by a lifelong exposure score (Hotz et al. 1989) with additional questions about NMP use. The participants were categorised into five subgroups according to the results of the occupational exposure score: never exposed to NMP or organic solvents, formerly exposed only, current exposure to NMP only, current exposure to organic solvents but not to NMP and current exposure to NMP and organic solvents.

Suitable masks were defined as personal protective equipment suitable for removing NMP (based on an evaluation of the constituents of the mask according to a walk-through of the workplace), worn for at least 50 % of the working time and maintained correctly (according to occupational history). Suitable gloves were defined as made from butyl or latex and worn for at least 50 % of the working time. Both questions on masks and gloves were related to usual conditions. Furthermore, wearing gloves and contact with products (wet skin, splashes on skin and hand washing with organic solvents) on the day of the biomonitoring and NMP concentration (per cent) in the products mostly used on the day of the biomonitoring (middle of the percentage range) were also recorded.

Spirometry was carried out according to the ATS criteria (American Thoracic Society 1995) and results assessed independently by two physicians. Predicted values of forced expiratory volume in the 1st second (FEV1 per cent predicted) were calculated according to Quanjer et al. (1993). Non-Caucasians were excluded from these calculations.

Workplace temperature and humidity were measured at the beginning and at the end of the shift, and the mean was calculated.

5-hydroxy-NMP (5-HNMP) and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) were selected because they represent a large fraction of NMP metabolites found in urine and have a fairly long half-life (about 6 and 26 h, respectively) (Bader et al. 2008), which is more suitable with irregular exposure patterns, and since there is no risk of external contamination (Bader et al. 2007; Akesson et al. 2004; Akesson and Jonsson 1997). Both parameters were measured in urine at the end of the shift (ES) on the day of air sampling and before the next shift, i.e. after about 16 h off work and before any exposure due to the next shift (BNS) (Akrill et al. 2002). As clinical examination may decrease exposure, air sampling was carried out on a different day (within $\pm$ 1 week of the clinical examination).

5-HNMP and 5-HNMP-*d4* were synthesised by Synthelec (Lund, Sweden), 2-HMSI was purchased from Sigma Aldrich, and 2-HMSI-*d3* was synthesised by Ramidus AB (Lund, Sweden).

The samples were analysed in the laboratory of the Louvain centre for Toxicology and Applied Pharmacology (Université catholique de Louvain, Belgium) without knowledge of their exact provenance (blind analysis).

Airborne NMP was collected by personal sampling over a whole workday with solid sorbent tubes (Coconut shell charcoal, 100/50 mg; SKC 226-01) and pumps (150 ml/min) (SKC model 210-2002, SKC, Inc., Eighty-Four, PA) which were calibrated before and after personal sampling. NMP was determined according to the NIOSH method (NIOSH 1994). Limit of quantification (LOQ) was 0.3 µg. Among the 66 blanks, 63 (95 %) did not contain any NMP. In the three remaining tubes, 1.8, 0.5 and 0.4 µg were found.

5-HNMP and 2-HMSI were quantified in urine samples using a method previously described (Suzuki et al. 2009) with slight modifications. Briefly, urine samples were only diluted 10 times with an aqueous solution containing

deuterated compounds (5-HNMP-*d4* and 2-HMSI-*d3*) as internal standards, and chromatographic separation was performed on a Zorbax Eclipse Plus C18 analytical column, 100 mm long × 4.6 mm i.d., 3.5 µm particle size (Agilent). The mobile phase consisted of a gradient of 0.1 % formic acid in aqueous solution and acetonitrile. The liquid chromatography with tandem mass spectrometry (LC–MS/MS) system used was a 6460 Triple Quad LC/MS from Agilent.

Using this LC–MS/MS validated method, the LOQ was set as 0.2 mg/l for both NMP metabolites (5-HNMP and 2-HMSI) and the laboratory has obtained successful results (including a certificate for successful participation) in external quality assessment scheme organised by the Institute for Occupational, Environmental and Social Medicine of the University of Erlangen, Germany, during the period of samples analysis (G-EQUAS 49).

A concentration equal to half the LOQ was attributed to samples below this limit.

Methods for determining serum Clara cell protein (CC16), urinary albumin (U-alb) and retinol-binding protein (U-RBP) have been described elsewhere (Steiner et al. 2005; Bernard and Lauwerys 1983). Serum creatinine (S-creatinine) and gamma-glutamyltransferase (GGT) activity were determined with an Olympus AU2700 analyser (Olympus, Hamburg, Germany). Blood cells were counted automatically. After liquid–liquid extraction of alkalinised urine (NaOH 8 N), cotinine concentration was measured with high performance liquid chromatography-diode array detection. Limit of quantification was 0.05 mg/l and variation coefficient <10 % for values higher than 0.2 mg/l. Samples below the limit of quantification were attributed a concentration equal to half that limit.

CYP2E1 genotypes were determined in a limited subpopulation ($n = 93$). Genomic DNA was extracted from whole blood using QIAamp DNA Mini Kit (Qiagen), and *CYP2E1* genotyping was performed using methods previously described for *CYP2E1*1B* (A1/A2), *CYP2E1*5* (c1/c2) and *CYP2E1*6* (D/C) (Haufroid et al. 1998) and *CYP2E1*1D* (insertion) (Nomiyama et al. 2001), respectively.

Statistical analysis

Statistical analysis was performed using SAS 9.2 statistical software (SAS Institute, Cary, North Carolina, USA). Variable distributions were examined for normality, and non-parametric tests or logarithmic transformation used when appropriate. The efficacy of this logarithmic transformation was reconfirmed by examining the residuals of the linear regression models before and after transformation. Subjects with a missing value for one variable were excluded from the analysis using this variable. Results under the LOD (or LOQ) were included in the calculations and were not considered as missing. To exclude a bias due to these values, regression models including metabolite concentrations were applied both in exposed workers only and in the whole group. Indeed, as presented in the "Results" section, metabolites concentrations below the LOQ were found especially in non-exposed workers.

In the multiple linear regression models, the concentrations of the NMP metabolites in the urine were adjusted for urine dilution in two ways: via dividing the metabolite concentration by urine creatinine concentration and via including the urine creatinine concentration as a separate covariate in the regression model. Linear regression models were laid out before the beginning of the analysis and included the following covariates as a priori variables: age (years), smoking (pack years), ex-smoker (number of years since smoking cessation), GGT (IU/l), S-creatinine (mg/dl) and creatinine in urine (g/l). Urinary creatinine was not included in the model for those metabolites which were previously creatinine adjusted. Additional covariates considered for inclusion were NMP concentration in the air on the day of the biomonitoring (mg/m^3), current average monthly duration of exposure to NMP according to occupational history (hours), NMP concentration in the products mostly used on the day of the biomonitoring (per cent, middle of the range), suitable gloves usually worn at least 50 % of the time (yes/no), currently having a skin disease according to clinical history (no/yes), healthy hand skin (objectively ascertained at clinical examination) (yes/no), splashes of NMP solution onto the skin on the day of the biomonitoring (no/yes), gloves worn on the day of the biomonitoring (yes/no), did hands stay dry on the day of the biomonitoring (yes/no) and genetic factors (see above). Dichotomous variables had the code 0 for the most favourable situation (for example, no splashes) or the healthy state. To decide on which variables to retain in the final model, the Bayesian information criterion (BIC), Akaike's information criterion (AIC) and the explanatory value (adjusted r^2) were used. Variables were included in the final model if they substantially lowered the BIC and the AIC and increased the adjusted r^2 value, were statistically significant or were based on a priori knowledge. If the information gained by BIC, AIC and adjusted r^2 value did not indicate the same, all three were considered carefully and the BIC was given more weight than the AIC and adjusted r^2 value. Multiple linear regression models were built for only the exposed group as well as the whole sample. Each of the models was evaluated for the assumptions of linear regression and also assessed for collinearity by examination of variance inflation factors.

The screening accuracy of a biomarker is determined among other by the accuracy with which it can rule in or rule out exposure, which can be quantified by the

likelihood ratios (LRs). Therefore, LRs were calculated to characterise the screening accuracy of 5-HNMP and 2-HMSI for excluding or identifying exposure to NMP (defined as no hour of exposure to NMP compared to one or more hours of exposure to NMP). Thus, 5-HNMP and 2-HMSI concentrations were subdivided into 3 categories defined as low, medium and high and corresponding to <80, 80–120 and >120 % of the maximum metabolite concentration in the unexposed group. LRs >10 or <0.1 are considered to provide strong evidence for a biomarker being suitable for identifying or excluding exposure (Deeks and Altman 2004).

Results

Three hundred twenty-seven workers were eligible to participate out of which 207 (63 %) participated; 113 declined participation and 7 could not be examined due to organisational reasons. In these 327 subjects, neither age ($p = 0.9$) nor nationality ($p = 0.5$) (statistical analysis limited to the 230 subjects from 5 countries with >10 representatives) differed between participants and non-participants. Seven of the 8 eligible women declined participation, and hence, the only woman remaining was excluded leaving 206 male workers for statistical analysis. Firms differed widely with respect to participation (5–100 %), but no statistically significant difference in airborne NMP was found between plants on the basis of a participation cutoff of 20 % ($p = 0.2$; Wilcoxon two-sample test).

Information on demographics and main exposure characteristics are presented in Table 1. In the participants, the number of nationalities was very similar to that in all eligible subjects. Indeed, more than 14 nationalities were represented, but only 3 of them had more than 10 representatives ($n = 18$, 18 and 94 from Germany, Italy and Switzerland, respectively), limiting statistical analyses with this variable. Median (5th–95th percentile) lifetime duration of occupational exposure to NMP was 0 (0–0), 3.6 (1.7–6.10) and 2.05 (0.1–15.0) years in the subjects never occupationally exposed to NMP and/or organic solvents, currently occupationally exposed to NMP only, and currently exposed to NMP and other organic solvents. In the formerly exposed workers ($n = 22$), median time elapsed since cessation of exposure was 12.5 years (5th–95th percentile: 2.2–34.4). Only one worker had a previous exposure to NMP and other solvents, but no one had a former exposure to NMP only. The concentration of NMP in the preparations used by the workers was mainly between 20 and 25 % ($n = 28$) or 80 and 99 % ($n = 36$). In the NMP-exposed group and on the day of the biological monitoring, hands were seldom washed with solvent(s) ($n = 3$).

Table 1 Characteristics of the study population

	Unexposed workers ($n = 114$)	Exposed workers ($n = 91$)
Age (years)	44 (23–59)	45 (22–60)
Education level		
Low	26 (23)	36 (40)
Middle	80 (70)	53 (58)
High	8 (7)	2 (2)
Smoking		
Never smoker	38 (34)	24 (26)
Ex-smoker	25 (22)	17 (19)
Current smoker	50 (44)	50 (55)
Pack years (in smokers only)	15.9 (1.4–65.8)	18.9 (1.0–54.0)
Alcohol		
No/only socially	95 (83)	67 (74)
Daily	19 (17)	24 (26)
BMI (kg/m^2)		
18.5 to <25.0	31 (27)	34 (37)
25.0 to <30.0	57 (50)	39 (43)
≥30	26 (23)	18 (20)
S-creatinine (mg/dl)	0.90 (0.72–1.15)	0.89 (0.66–1.14)
GGT (IU/L)	24 (12–57)	26 (12–67)
Current average monthly duration of exposure to NMP (h)	0 (0–0)	10 (1–100)
Use suitable mask (>50 % of the time)		13 (15)
Use suitable gloves (>50 % of the time)		30 (33)
Any gloves today (yes)		62 (71)
Suitable gloves today (yes)		25 (29)
No wet hands today		50 (58)
NMP splash on the skin today (yes)		22 (29)

"Exposed to NMP" means current average monthly duration of exposure to NMP > 0 h. One worker with a missing value is excluded from the table

Figures are median (5th–95th percentile) or number (per cent). In this table, "today" refers to the day of the biomonitoring in NMP-exposed subjects only

Reference range for S-creatinine and GGT are 0.6–1.4 mg/dl and 7–50 IU/l, respectively

Median (5th and 95th percentile) duration of airborne sampling was 450 (310–525) min. Airborne NMP and urinary metabolite concentrations are presented in Table 2. Airborne NMP concentrations hardly correlated with workplace humidity and temperature (Spearman $\rho = -0.12$ and -0.01, respectively, $p > 0.09$). Many subjects did not work every day with NMP. Indeed, on the day of the clinical examination, only 37 (43 %) workers reported having worked with NMP on the previous day.

Table 2 Airborne NMP and urinary metabolites

	Non-exposed workers ($n = 114$)	Exposed workers ($n = 91$)
Airborne NMP (mg/m^3)[a]	0.01 (0.002–0.40)	0.18 (0.002–6.99)[b]
Airborne NMP not detectable (n; %)	38 (34)	8 (9)
Urinary metabolites (mg/l)		
End of shift		
5-HNMP	0.10 (0.10–0.80)	0.60 (0.10–29.00)
2-HMSI	0.30 (0.10–1.50)	0.80 (0.20–23.30)
Before shift		
5-HNMP	0.10 (0.10–0.60)	0.45 (0.10–22.90)
2-HMSI	0.30 (0.10–2.40)	1.55 (0.10–58.00)
Urinary metabolites (mg/g creatinine)		
End of shift		
5-HNMP	0.09 (0.04–0.60)	0.39 (0.05–41.41)
2-HMSI	0.23 (0.07–1.04)	0.56 (0.11–19.89)
Before shift		
5-HNMP	0.06 (0.04–0.32)	0.27 (0.04–10.51)
2-HMSI	0.20 (0.06–1.39)	1.06 (0.09–42.89)
Humidity (%)	50.2 (25.9–66.9)	51.0 (30.5–72.0)
Temperature (°C)	20.7 (10.2–26.4)	20.3 (1.0–24.3)

"Exposed to NMP" means current average monthly duration of exposure to NMP > 0 h

Figures are median and 5th–95th percentile

There were 3–8 missing values according to the measurement considered

Concentrations in exposed and non-exposed workers always differ at $p < 0.0001$ (Wilcoxon two-sample test)

[a] A concentration equal to half the limit of quantification was attributed to samples below this limit (see "Methods" section)

[b] 0.89, 2.77 and 25.83 mg/m^3 for the 75th and 90th percentile and the maximum, respectively

Urinary metabolites (mg/l) and creatinine concentrations were not significantly correlated ($p > 0.4$). Urinary metabolites correlated with airborne NMP concentrations ($0.6 <$ Spearman $\rho < 0.8$) with the highest correlation between airborne NMP and 2-HMSI concentration (mg/l) in the BNS sample (Spearman $\rho = 0.83$; $p < 0.0001$; $n = 193$). Correlations between age and metabolite concentrations were of borderline statistical significance. No statistically significant correlation was found between BMI, smoking (pack years, daily cigarettes and urinary cotinine) and metabolite concentration ($p > 0.6$ and 0.3, respectively). The number of workers with metabolite concentrations below the LOQ depended on exposure and metabolite. Concentrations below the LOQ occurred less often in exposed than non-exposed workers, and 2-HMSI was less often under the LOQ than 5-HNMP. 2-HMSI (ES sample) was non-detected in 3/87 exposed workers only, which represents the lowest percentage (3.4 %) of concentrations below the LOQ found in the study. In contrast, 5-HNMP (BNS sample) was under the LOQ in 89/106 non-exposed workers (84 %).

The main results of the multiple linear regressions for the exposed group are summarised in Table 3. As the 2-HMSI concentration (mg/l; BNS) showed the highest correlation with the NMP concentration in the air and as the results were relatively constant across the different metabolites, the multiple linear regression models for this biomarker will be illustrated. For the exposed workers, after adjusting for age, pack years, time since cessation of smoking, GGT, S-creatinine and urinary creatinine, the addition of the concentration of NMP in the air caused the biggest change in the BIC, AIC and adjusted r^2 value (adjusted $r^2 = 0.62$ compared to adjusted $r^2 = 0.1$ without air concentration). After addition of duration of exposure to NMP and further addition of the concentration of the NMP solution into the model, another increase in the adjusted r^2 value to 0.67 and 0.71, respectively, was achieved. Furthermore, having a skin disease increased the explanatory value of the model to 0.73. Wearing a mask had no statistically significant effect. The final multiple linear regression models for all the other biomarkers had substantially lower adjusted r^2 values, but were consistent with the finding that the air concentration was the most essential covariate. Using the sum of 5-HNMP and 2-HMSI did not improve the association with airborne NMP (Figs. 2, 3, 4, 5).

In the whole group, the main results were quite consistent with those found in the exposed group only (Table 4). However, the distribution of residuals was clearly less satisfactory than in the exposed group. Including all workers, after adjusting for age, pack years, time since smoking cessation, GGT, S-creatinine and creatinine in urine, similar to the exposed group adding the NMP concentration caused the biggest change in the BIC, the AIC and the adjusted r^2 value (adjusted $r^2 = 0.035$ compared to 0.715 after taking the air NMP concentration into account). After addition of the duration of exposure to NMP and further including having a skin disease into the model, the adjusted r^2 value increased to 0.746 and 0.750, respectively. Similar to the analysis of the exposed group, the final linear regression models of the other NMP metabolites gave considerably lower adjusted r^2 values.

Based on the relationship between ambient NMP and urinary 5-HNMP ES or 2-HMSI BNS and assuming linearity, biological indices corresponding to an airborne NMP concentration of 80 mg/m^3 could be approximated crudely by extrapolation. The tentative value obtained for 5-HNMP ES of 91 mg/l is similar to the one of 100 mg/l proposed by the American Conference of Industrial Hygienists. With respect to 2-HMSI (BNS), the tentative value would be

Table 3 2-HMSI and 5-HNMP (log transformed, in mg/l, before next shift) in the exposed group: multiple linear regression models

	Model 1		Model 2		Model 3	
	2-HMSI[a]	*P*	2-HMSI[a]	*p*	5-HNMP[a]	*p*
Sample size	66		66		66	
Intercept	−0.06147	0.9	0.06224	0.9	−0.14582	0.9
Age (years)	0.00221	0.7	0.00142	0.8	0.00863	0.2
Pack years (number)	−0.00071	0.9	−0.00024	0.9	0.00243	0.6
Time since smoking cessation (years)	0.00167	0.8	0.00296	0.7	0.00600	0.5
GGT (IU/L)	0.00201	0.5	0.00123	0.7	0.00100	0.8
S-creatinine (mg/dl)	−0.09306	0.8	−0.25110	0.5	−0.91202	0.04
Urine creatinine (g/l)	−0.03296	0.7	−0.02942	0.7	0.13740	0.2
NMP concentration in air[a] (mg/m^3)	0.56255	<0.0001	0.54045	<0.0001	0.63890	<0.0001
Exposure to NMP (h)[b]	0.00686	0.0002	0.00696	0.0004	0.00412	0.08
NMP concentration in solution[a, c] (%)	0.32231	0.02	0.38285	0.01	0.24652	0.2
History of any current skin disease (no/yes)[d]	0.39766	0.03	0.31719	0.09	NI	
Healthy hand skin (yes/no)[e]	NI		NI		0.28696	0.08
Gloves worn (yes/no)[f]	NI		−0.17750	0.2	−0.79036	0.003
Suitable gloves (>50 % of the time) (yes/no)[g]	NI		NI		0.82543	0.002
Splashes of NMP solution on skin[f] (no/yes)	NI		0.21346	0.1	NI	
Adjusted r^2	0.73		0.74		0.62	
Pr > F	<0.0001		<0.0001		<0.0001	

Figures indicate the partial regression coefficient with the corresponding significance level. Model 1 was found to have the lowest Bayesian Information Criterion; therefore, the variables identified in model 1 were also included in multiple linear regression models for the other metabolites. The following adjusted r^2 values were obtained: 2-HMSI in the first urine sample (uncorrected for creatinine)—0.5742, 2-HMSI in the first urine sample (corrected for creatinine)—0.6432, 2-HMSI in the second urine sample (corrected for creatinine)—0.6523, 5-HNMP in the in first urine sample (uncorrected for creatinine)—0.3805, 5-HNMP in the second urine sample (uncorrected for creatinine)—0.5528, 5-HNMP in the first urine sample (corrected for creatinine)—0.4110, 5-HNMP in the second urine sample (corrected for creatinine)—0.5189

NI variable not included in this run

[a] Log transformed

[b] Current average monthly duration of exposure to NMP according to occupational history (hours)

[c] NMP concentration in the products mostly used on the day of the biomonitoring (middle of the range)

[d] Question from clinical history

[e] Objectively ascertained at clinical examination

[f] On the day on the biomonitoring

[g] Suitable gloves usually worn at least 50 % of the time

about 257 mg/l. This value should be considered as tentative only because it is an evident approximation caused by a threefold extrapolation of the airborne concentration data.

With respect to LRs, in the category "low", the lowest LR was obtained for creatinine-adjusted 2-HMSI in the second urine sample and was well above 0.1 (0.293, 95 % CI 0.259–0.331), suggesting that 5-HNMP and 2-HMSI are not suitable for ruling out exposure. For the category "high", LR amounted to 4–8. The best result was obtained for the creatinine-adjusted 5-HNMP in the second urine sample (9.86, 95 % CI 6.30–15.44). In other words, if the prevalence (pre-test probability) of exposure is 50 %, a LR of 10 means that the post-test probability of being exposed amounts to 90 % when the 5-HNMP concentration (creatinine-adjusted 5-HNMP in the second urine) is in the high category, suggesting that this urinary metabolite is a fair (but not very good) biomarker for ruling in exposure.

In the genotyped subpopulation ($n = 93$), no influence of genetic factors was found on the NMP metabolite concentrations and the number of workers carrying *CYP2E1*1D* (insertion), the allele assumed to be the most important one (http://www.cypalleles.ki.se/cyp2e1.htm), was too small for meaningful statistical analyses ($n = 7$).

Respiratory and neuropsychological symptoms were compared among the 5 subgroups presented in Table 5 on the basis of 28 clinical questions (eye, nose or throat irritation, cough, phlegm, wheeze, memory, concentration,

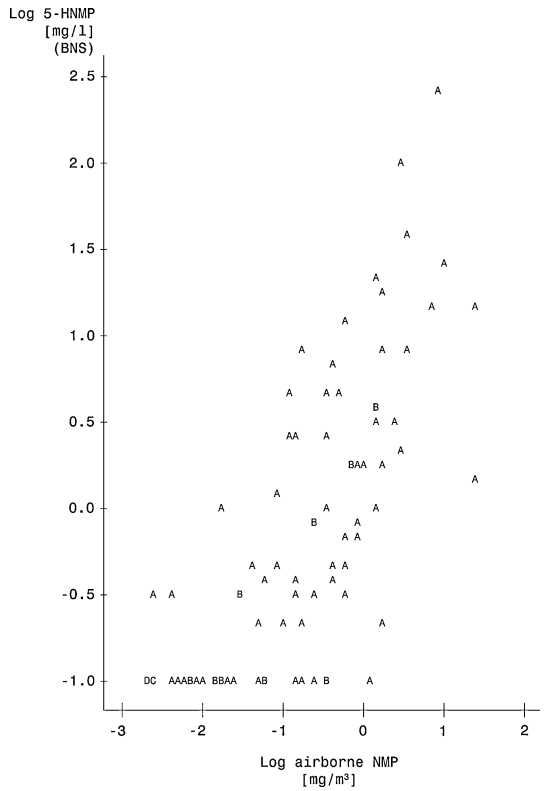

Fig. 2 Plot of the association between airborne NMP (mg/m^3) and concentration of 5-HNMP (BNS; mg/l) in 86 exposed workers (logarithmic transformation). $A = 1$, $B = 2$ observations, etc.). $r = 0.76$, $p \leq 0.0001$

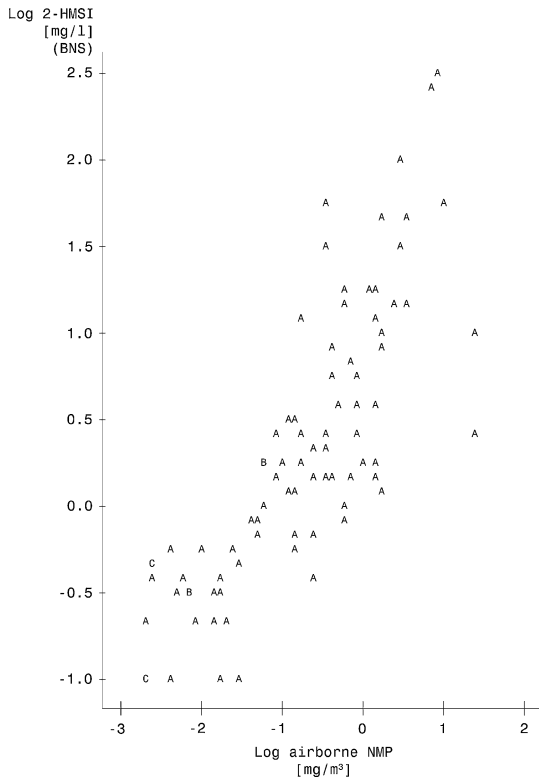

Fig. 3 Plot of the association between airborne NMP (mg/m^3) and concentration of 2-HMSI (BNS; mg/l) in 86 exposed workers (logarithmic transformation). $A = 1$, $B = 2$ observations, etc.). $r = 0.85$, $p \leq 0.0001$.

headache, dizziness, depressed without reason, fatigue, etc.). The number of symptomatic (work-related or not) cases was mostly low (below 10 or even 5 in each cell) making interpretation quite difficult and consideration of possible confounders impossible. However, a closer examination of the results for each variable to detect possible patterns did not disclose consistent and clear trends towards more symptoms in a specific subgroup. Alike, no clinically significant difference appeared regarding skin disease (history and status) or pallesthesia. Table 5 presents selected laboratory measures in these subgroups. No statistically significant effect of NMP exposure was observed for haematological variables either (haemoglobin, haematocrit, erythrocytes, leucocytes, granulocytes, lymphocytes, thrombocytes, details not shown). Grouping together all subjects non-exposed to NMP and comparing them to those exposed to this organic solvent did not alter these conclusions. Despite higher power, the significance level (Kruskal–Wallis test) of the difference in urinary albumin increased to 0.20 after excluding three obvious outliers ($67 <$ albumin < 343 mg/g) diagnosed with a 20-year diabetes or hypertension (systolic and diastolic pressure >150 and >104 mmHg) suggesting no occupational association either. In the subgroup of never smokers, symptoms of cough, phlegm or wheeze as well as FEV1 (per cent predicted) were not associated with exposure to NMP.

Overall, exposure to organic solvents was low. Indeed, by comparing the prevalence of acute solvent-induced work-related symptoms (Hotz et al. 1992) in all workers exposed to any organic solvents to that of all non-exposed subjects, only feeling drunk was related to work (13 exposed subjects, $p = 0.02$).

Discussion

This large study conducted in northeast Switzerland included 21 firms representing a large spectrum of activities and 91 workers exposed to NMP. The purpose was to examine the value of urinary 5-HNMP and 2-HMSI for assessing an occupational exposure to NMP and to look for health effects. A group of workers exposed only to organic solvents but not to NMP was included to increase the ability to detect possible differences, if any, in outcomes between NMP and other solvents.

Urinary metabolites correlated very well with airborne NMP concentrations, although these concentrations were

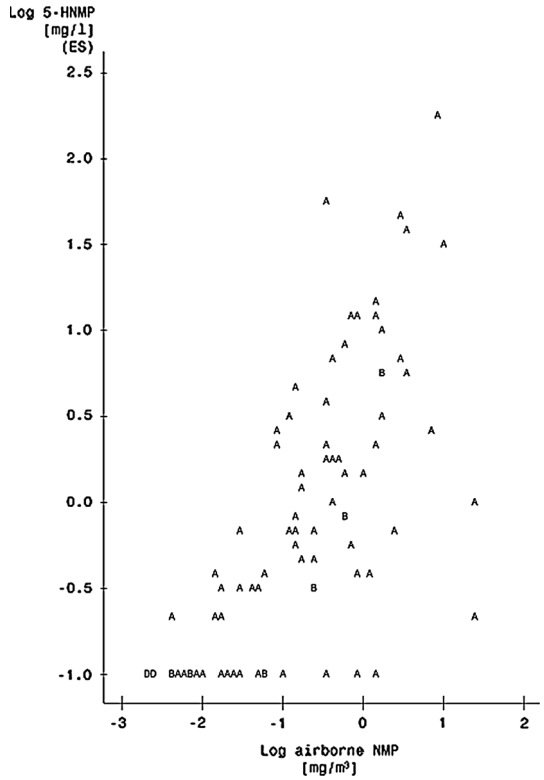

Fig. 4 Plot of the association between airborne NMP (mg/m^3) and concentration of 5-HNMP (ES; mg/l) in 87 exposed workers (logarithmic transformation). $A = 1$, $B = 2$ observations, etc.). $r = 0.74$, $p \leq 0.0001$

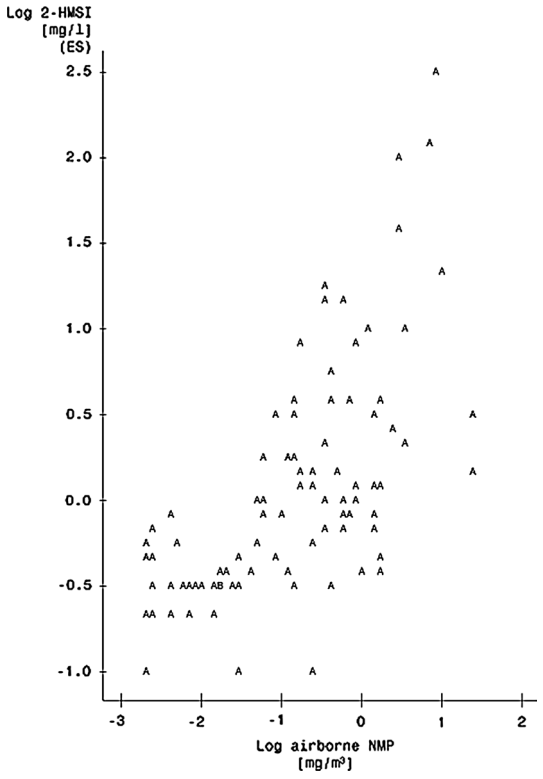

Fig. 5 Plot of the association between airborne NMP (mg/m^3) and concentration of 2-HMSI (ES; mg/l) in 87 exposed workers (logarithmic transformation). $A = 1$, $B = 2$ observations, etc.). $r = 0.69$, $p \leq 0.0001$

low. Indeed, r^2 reached values of about 60–70 %. The association was not confounded by age, BMI or smoking. However, at least at these exposure levels, a limitation was that the urinary metabolites had too high LRs to rule out exposure. By contrast, 5-HNMP (creatinine-adjusted, BNS) could be used to rule in exposure to this organic solvent. Interestingly, using the sum of 5-HNMP and 2-HMSI did not improve the association with airborne NMP, although the half-lives of these metabolites differ.

There were NMP metabolites in non-exposed subjects. Interestingly, in the control group ($n = 10$) examined by Anundi et al. (2000), mean 5-HNMP (range) was 0.37 mmol/mol creatinine (0.02–2.96), which agrees very well with the mean 5-HNMP (range) of 0.31 (0.03–7.6) found in the present study. Regarding 2-HMSI, the corresponding concentrations were 0.16 mmol/mol creatinine (0.04–0.56) and 0.36 (0.04–5.95), respectively. The occurrence of NMP metabolites in both control groups strongly suggests that there is some exposure of the control subjects. This exposure may be either occupational (contamination of the rooms the control subjects are working in) or non-occupational (household products such as cloth cleaning or stripping agents). In this population, skin resorption played a minor role. However, as the hands were not immersed constantly in NMP, changing gloves frequently may offer enough protection even if gloves are not quite impermeable.

Surprisingly, the creatinine adjustment did not work well, although only spot urine samples were collected. In this regard, findings are contradictory. Akesson et al. (2004) and Akesson and Jonsson (2000) reported that adjustment for creatinine or density did not always work, whereas according to Akrill et al. (2002) adjusting by creatinine concentration represents an improvement. According to Jonsson and Akesson (2003), creatinine adjustment should work better for 5-HNMP and 2-HMSI than for NMP and methylsuccinimide because of their higher renal clearance compared to that of creatinine. Actually, this was not confirmed in the present study.

On the day of the measurements, the upper range of airborne NMP concentrations included concentrations above the concentration of 0.4 mg/m^3 that was recommended to protect from irritant effects (Beaulieu and Schmerber 1991). Thus, some irritant effects could have been expected. Even if a lack of irritant effects due to too few subjects exposed to toxicologically significant concentrations cannot be excluded, a more consistent explanation is a genuine lack of effect. Indeed, well-controlled studies have not confirmed the irritant effect, and the

Table 4 2-HMSI and 5-HNMP (log transformed, mg/l) in the whole group: multiple linear regression models

	Model 1		Model 2		Model 3	
	2-HMSI (BNS)	*p*	5-HNMP (BNS)	*p*	5-HNMP (ES)	*p*
Sample size	183		183		182	
Intercept	0.53431	0.03	0.1486	0.6	0.53878	0.07
Age (years)	0.00399	0.2	0.00514	0.1	−0.00283	0.4
Pack years (number)	0.00031	0.9	−0.00067	0.7	0.00124	0.6
Time since smoking cessation (years)	−0.00051	0.9	0.00121	0.8	−0.00572	0.2
GGT (IU/L)	0.00028	0.9	0.00354	0.05	0.00116	0.6
S-creatinine (mg/dl)	−0.36334	0.09	−0.56055	0.02	−0.29202	0.2
Urine creatinine (g/l)	0.08971	0.04	0.00782	0.9	0.01426	0.8
Airborne NMP concentration (mg/m^3)	0.51423	<0.0001	0.42273	<0.0001	0.50339	<0.0001
Exposure to NMP (h)[a]	0.00558	<0.0001	0.00670	<0.0001	0.00163	0.3
History of any current skin disease (no/yes)[b]	0.16437	0.06	NI		NI	
Adjusted r^2	0.75		0.66		0.59	
Pr > F	<0.0001		<0.0001		<0.0001	

Figures indicate the partial regression coefficient with the corresponding significance level. The variables identified in model 1 were also included in multiple linear regression models for the other metabolites. The following adjusted r^2 values were obtained: 2-HMSI in the first urine sample (uncorrected for creatinine)—0.5791, 2-HMSI in the first urine sample (corrected for creatinine)—0.585, 2-HMSI in the second urine sample (corrected for creatinine)—0.7223, 5-HNMP in the first urine sample (uncorrected for creatinine)—0.5950, 5-HNMP in the second urine sample (uncorrected for creatinine)—0.6570, 5-HNMP in the first urine sample (corrected for creatinine)—0.5444, 5-HNMP in the second urine sample (corrected for creatinine)—0.6063

NI variable not included in this run, *ES* end of the shift on the day of air sampling, *BNS* before the next shift, i.e. after about 16 h off work and before any exposure due to the next shift

[a] Current average monthly duration of exposure to NMP according to occupational history (hours)

[b] Question from clinical history

Table 5 Selected outcomes in the different exposure subgroups

	Never exposed ($n = 30$)	Former exposure to solvents ($n = 22$)	Current exposure to NMP only ($n = 8$)	Current exposure to solvents (no NMP) ($n = 98$)	Current exposure (solvents and NMP) ($n = 38$)	*p* value
2-HMSI[a] (mg/l; BNS)	0.30 (0.10–1.60)	0.40 (0.10–10.70)	1.75 (1.10–50.20)	0.40 (0.10–8.80)	2.90 (0.10–239.0)	<0.0001
5-HNMP[a] (mg/g; BNS)	0.06 (0.04–0.19)	0.06 (0.04–0.83)	2.57 (0.05–8.67)	0.08 (0.04–1.79)	0.65 (0.06–89.55)	<0.0001
Lifetime occupational exposure to NMP (years)	0 (0–0)	0 (0–0)	3.60 (6.10–1.70)	0 (0–0)	2.05 (0.10–15.0)	<0.0001
GGT (IU/l)	21 (12–72)	25 (10.5–125)	25.5 (15–43)	23 (12–68)	27.5 (14–67)	0.3
Serum creatinine (mg/dl)	0.89 (0.73–1.16)	0.89 (0.76–1.12)	0.95 (0.70–1.04)	0.89 (0.66–1.15)	0.94 (0.58–1.32)	0.7
Urinary albumin[b] (mg/g)	2.72 (0.48–10.21)	3.92 (0.46–13.04)	0.54 (0.40–67.74)	3.62 (0.32–27.52)	4.65 (0.73–225.32)	0.06
Urinary RBP[b] (μg/g)	27.91 (0.62–107.81)	28.57 (0.73–276.81)	16.22 (2.28–318.42)	45.56 (0.79–133.78)	42.79 (1.08–163.89)	0.8
Serum CC16 (μg/l)[c]	15.40 (6.90–30.50)	13.95 (7.80–27.40)	12.10 (9.0–20.60)	14.00 (8.00–23.10)	15.70 (4.00–27.70)	0.3
FEV1 (per cent predicted)[d]	99.69 (70.23–108.52)	94.69 (65.02–119.35)	96.12 (54.50–119.85)	97.69 (75.28–118.26)	94.21 (74.97–112.32)	0.5

Definition of subgroups according to lifetime exposure to NMP and organic solvents (see "Methods" section)

Figures are median and 5th and 95th percentile. *n* subgroup size, *p* level of significance of the differences between different exposure subgroups (Kruskal–Wallis)

HMSI (mg/l; BNS) and HNMP (mg/g; BNS) had the highest r^2 and LR, respectively (see text)

[a] A concentration equal to half the limit of detection was attributed to samples below the limit of detection (see "Methods" section)

[b] 13 missing values. There were 5 and 25 concentrations <LOD for urinary albumin and RBP, respectively. The distribution of RBP concentrations <LOD did not differ significantly between groups

[c] 32 missing values

[d] Caucasian participants only ($n = 186$)

findings reported by Beaulieu and Schmerber (1991) are likely to be biased (Vanthriel et al. 2007). Moreover, the irritant effect described by Langworth et al. (2001) was likely due to esters rather than to NMP. As many workers did not work daily with NMP, habituation reducing the irritant effects is an unlikely explanation too.

Clear clues to neurological, renal, haematological or hepatic effects could not be found, which is in line with the scarce data from the literature (Carnerup 2004).

With respect to CYP2E1 genotypes, no effect appeared in the subpopulation examined. Determinations of the phenotype may give other results, as suggested by Ligocka et al. (2003), but this was not planned in this study because of financial constraints.

A limitation of the study is its cross-sectional design as a healthy worker effect could not be taken into account. No information was available on workers having previously left the plant.

In conclusion, biological monitoring, primarily urinary 2-HMSI (mg/l; BNS), is of value to estimate exposure to NMP even when exposure is irregular and low. However, 5-HMNP or 2-HMSI cannot be used to rule out such a low-level exposure to NMP. At the low levels found in this study, no irritant, renal, haematological or neurological effects could be found.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The protocol of the study was approved by the Ethics Committee of the medical faculty, University of Zürich (Switzerland).

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