The use of biomarkers of exposure of N,N-dimethylformamide in health risk assessment and occupational hygiene in the polyacrylic fibre industry

H U Käfferlein, C Ferstl, A Burkhart-Reichl, K Hennebrüder, H Drexler, T Brüning, J Angerer

Background: N,N-dimethylformamide (DFM) was recently prioritised for field studies by the National Toxicology Program based on the potency of its reproductive toxic effects.

Aims: To measure accurately exposure to DFM in occupational settings.

Methods: In 35 healthy workers employed in the polyacrylic fibre industry, N-methylformamide (NMF) and N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in urine, and N-methylcarbamoylated haemoglobin (NMHb) in blood were measured. Workplace documentation and questionnaire information were used to categorise workers in groups exposed to low, medium, and high concentrations of DFM.

Results: All three biomarkers can be used to identify occupational exposure to DFM. However, only the analysis of NMHb could accurately distinguish between workers exposed to different concentrations of DFM. The median concentrations were determined to be 55.1, 122.8, and 152.6 mmol/g globin in workers exposed to low, medium, and high concentrations of DFM, respectively. It was possible by the use of NMHb to identify all working tasks with increased exposure to DFM. While fibre crimpers were found to be least exposed to DFM, persons washing, dyeing, or towing the fibres were found to be highly exposed to DFM. In addition, NMHb measurements were capable of uncovering working tasks, which previously were not associated with increased exposure to DFM; for example, the person preparing the fibre forming solution.

Conclusions: Measurement of NMHb in blood is recommended rather than measurement of NMF and AMCC in urine to accurately assess exposure to DFM in health risk assessment. However, NMF and AMCC are useful biomarkers for occupational hygiene intervention. Further investigations regarding toxicity of DFM should focus on highly exposed persons in the polyacrylic fibre industry. Additional measurements in occupational settings other than the polyacrylic fibre industry are also recommended, since the population at risk and the production volume of DFM are high.
exposed and non-exposed persons. In addition, the biomarker should be related to DMF toxicity and sampling should be simple. Finally, the analytical results should be representative for a particular workplace situation.

In the study presented here, we analysed NMF and AMCC in urine, and NMHb in blood of 35 persons exposed to DMF in the polyacrylic fibre industry. The study aimed to investigate the suitability of NMF, AMCC, and NMHb for biological monitoring in occupational settings and to specifically identify working tasks and workplaces with increased exposure of DMF in the polyacrylic fibre industry. The main focus was also on the relation between expected exposure (from workplace descriptions and questionnaire information) and measured exposure to DMF (biological monitoring). Those relations are of importance for the development of job exposure matrices in epidemiological studies and retrospective risk assessment, where real measurements were not carried out and information on exposure relies on questionnaires.

**METHODS**

**Fibre production and exposure**

Polyacrylic fibres in the factory presented here are created by “extrusion” by forcing a thick, viscous liquid through a tiny hole of a spinneret to form continuous filaments of semi-solid polymer. In their initial state, the fibre forming polymers are solids and are first converted into a fluid state for extrusion. For this purpose, the fibre forming polymers are dissolved in various solvents (for example, DMF). The spinnerets themselves have from one to several hundred holes. The small openings are very sensitive to impurities and the liquid polymer feeding them is carefully filtered. Maintenance is critical and spinnerets in the factory are removed and cleaned on a regular basis to prevent clogging. As the filaments emerge from the holes in the spinneret, the liquid polymer is converted first to a rubbery state and then solidified to endless filaments (spinning process). Two methods of spinning filaments are carried out at the factory, dry and wet spinning. During wet spinning the spinnerets are submerged in a chemical bath and the fibre solution is extruded directly into a precipitating liquid, where the filaments precipitate from solution and solidify. Since DMF is soluble in both water and organic solvents, it is an ideal solvent to control and adjust the precipitation process. The fibres are finally transported via a system of baths (washing) and rollers (drawing and drying) to post-fibre treatment (fibre crimping). Finally the fibres are cut and baled. During dry spinning solidification of the filaments is achieved by evaporating the solvent in a stream of hot air instead of precipitating the polymer by dilution. The fibre tows are transported to large containers, where they are temporarily stored. Then, the fibres are drawn, washed, and dried. After post-fibre treatment the yarn is also cut and baled.

**Study subjects and categorisation of workers**

Thirty-five male workers exposed to DMF at different workplaces were studied with regard to their exposure to DMF. Standard questionnaire information was recorded about working tasks, working habits, exposure to chemicals other than DMF, alcohol consumption, smoking history, health, gender, and intake of any medications. The age range was 24–56 years. Eighteen persons were smokers, 16 non-smokers, and one person took snuff. The median duration of employment was 11.5 years (range 1–27 years). Despite their main working task and workplace during fibre production, all workers were able to carry out every working task during fibre production. Consequently, workers who are usually working in fibre crimping are also able to carry out the spinning process. However, a change of the workplace only occurs when a colleague is on vacation or sick leave. Therefore, workers were finally categorised in different groups based on the working tasks which they usually carry out most of their time, and data of ambient monitoring at the workplaces, which have already been assessed in previous studies of this cohort.29 30 Three major groups of exposure could be identified by workplace description and questionnaire information.

**Group 1 (n = 14)** consisted of persons employed in post-fibre treatment, the person working in the dissolving room, and the person in charge during the shifts. Workers in post-fibre treatment (n = 12) were responsible for fibre crimping. Fibre crimping is an automatic process and the last step in fibre production. The majority of DMF was already previously
removed by washing steps. The work predominantly consists of supervision of the crimping process. Therefore, the workers do not use any protective measures. The person working in the dissolving room is responsible for mixing the raw polyacrylic material in a dissolving lye containing DMF, present, either in the freshly prepared fibres or in the surrounding air. Therefore, the persons were required to wear impermeable gloves and a spill protector the entire working time to avoid direct contact of DMF to the skin or absorption via contaminated clothes. If necessary, workers were also encouraged to wear respiratory masks. However, for practical reasons it was impossible to use full protection during the shift primarily carries out administrative work and does not work directly at the spinning machines. However, he has to assist colleagues after spills, when he is also required to use full protection including impermeable clothing and breathing masks. All persons categorised in group 1 should not be exposed to DMF by inhalation or dermal absorption or inhalation due to uptake of DMF (fig 2). Once the spinning process has been started again, the lid to the spinnerets is closed and exposure to DMF is supposed to be lower. Then, protective clothing and breathing masks are no longer required. Overall, the potential of exposure to DMF for workers in group 2 is higher than that of workers from group 1.

Group 2 (n = 14) consisted of persons working directly at the spinning machines. The work consists of a variety of different working tasks, where exposure to DMF can be via both inhalation and dermal absorption. A critical step is the spinning process, which is carried out manually after the spinnerets have been clogged and then cleaned. During this process, breathing masks, a protection for spills, and impermeable gloves have to be used to protect the workers from uptake of DMF (fig 2). Once the spinning process has been started again, the lid to the spinnerets is closed and exposure to DMF is supposed to be lower. Then, protective clothing and breathing masks are no longer required. Overall, the potential of exposure to DMF for workers in group 2 is higher than that of workers from group 1.

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>Job description</th>
<th>NMF (mg/l)</th>
<th>NMF (mg/g)</th>
<th>AMCC (mg/l)</th>
<th>AMCC (mg/g)</th>
<th>NMHb (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure group 1</td>
<td>Fibre crimping (n = 11/12)</td>
<td>8.7</td>
<td>7.4</td>
<td>5.5</td>
<td>6.4</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>Person in charge (n = 1)</td>
<td>4.7</td>
<td>6.5</td>
<td>6.2</td>
<td>8.6</td>
<td>148.8</td>
</tr>
<tr>
<td></td>
<td>Washing machine (n = 1)</td>
<td>6.0</td>
<td>13.6</td>
<td>19.0</td>
<td>34.5</td>
<td>205.7</td>
</tr>
<tr>
<td></td>
<td>Colouring (n = 2)</td>
<td>21.4</td>
<td>18.9</td>
<td>17.0</td>
<td>12.3</td>
<td>143.0</td>
</tr>
<tr>
<td></td>
<td>Fibre towels (n = 3)</td>
<td>32.2</td>
<td>13.7</td>
<td>11.3</td>
<td>13.7</td>
<td>191.4</td>
</tr>
</tbody>
</table>

Volume and creatinine related concentrations are given for NMF and AMCC. EG, exposure group; S, smoker; NS, non-smoker; Wet, wet spinning; Dry, dry spinning; Min, minimum; Max, maximum; 95%, 95th centile; n, number of workers.
One worker was working next to the precipitating bath where the spinning mass is coagulated to the yarn. Three employees were working with cabled yarns to temporarily store them prior washing and drying. Two workers stirred up various dyes, which were used to colour the yarn. All fibre ropes still contained DMF. Finally, one worker was working at the washing machine, where DMF is removed from the fibre ropes using hot water and steam. It is a high temperature working place, where workers sweat (especially if they are wearing spill protection and breathing masks). Compared to the workplaces of groups 1 and 2, workers from group 3 were assumed to be exposed to the highest concentrations of DMF.

**Chemicals and reagents**

All chemicals which were used during sample preparation of blood and urine samples were of highest available analytical grade (>99.9%). AMCC was synthesised as previously described. All other chemicals and reagents were obtained from Merck KgA (Darmstadt, Germany). Calibration and internal standardisation for the determination of NMHb was carried out using dipeptide standards N-methyl- and N-ethylcarbamoyl-d/-leucine anilide.

**Analytical and statistical methods**

_Determination of NMF and AMCC_ was carried out in urine samples collected at the end of the shift (Friday). Thirty four of 35 workers provided urine samples. The sample containers were capped, coded, and frozen until analysis. Final analysis was carried out in a blinded fashion. Sample preparation of urine samples was performed as previously described. Analysis was carried out by means of gas chromatography and thermionic sensitive detection with mass spectrometric confirmation (GC-TSD, GC-MS). The limit of detection (LOD, S/N 3) was 0.1 mg/l for NMF and 0.03 mg/l for AMCC.

_Determination of NMHb_ was carried out in blood samples taken at the end of the shift (Friday) from all workers. Blood samples (2x10 ml) were collected using disposable syringes (Sarstedt) containing EDTA as anticoagulant and coded. For NMHb analysis, erythrocytes were isolated from the blood samples by centrifugation. Red blood cells were washed three times with 0.9% NaCl solution and lysed by the addition of distilled water. About 200–250 mg globin was immediately prepared from 2 ml lysed erythrocytes according to a previously published method. The isolated globin was stored at −28°C until analysis. Dried globin samples were processed using a modified N-alkyl Edman degradation and analysed by gas chromatography-mass spectrometry (GC-MS) as previously described. Analysis of NMHb was also carried out in a blinded fashion. The limit of detection (LOD, S/N 3) was 1 nmol NMHb/g globin.

**RESULTS AND DISCUSSION**

Exposure assessment can be carried out by the determination of urinary metabolites NMF and AMCC. However, less information is available using biomarkers on the molecular level, such as NMHb. Knowing that risk is linked to exposure, which in turn is dependent on particular working tasks, we analysed NMF, AMCC, and NMHb in workers exposed to DMF in the polyacrylic fibre industry at different workplaces. Main focus was on the suitability of the biomarkers in order to assess exposure, the identification of working tasks, which are associated with increased exposure to DMF, whether or not the expected exposure (questionnaire information) does reflect the true exposure to DMF determined by biological monitoring, and finally, which biomarkers are suitable for health risk assessment and occupational hygiene.

**NMF in urine samples**

Median concentrations of NMF in urine at all workplaces were 10.2 mg/l (1.6–59.7 mg/l). Currently valid threshold limit values and advisory concentrations for NMF in urine are between 15 and 35 mg/l depending on different national regulations. Six of 34 persons (17.7%) had NMHb concentrations >35 mg/l, while 15 workers (44.1%) exhibited NMF levels >15 mg/l. Median levels of NMF in urine increased from groups of low to high exposure. The concentrations were analysed to be 8.0, 18.6, and 27.6 mg/l in workers exposed to low (group 1), medium (group 2), and high (group 3) concentrations of DMF, respectively (table 1). However, because of the wide range of NMF levels in every group, no statistically significant differences could be determined between all three groups (p = 0.3 for volume related concentrations, see fig 3A; p = 0.4 for creatinine related concentrations). There were also no differences between smokers and non-smokers and between persons working in dry and wet spinning.

The results clearly show the advantages and disadvantages of measuring NMF in urine. NMF is specific for occupational exposure to DMF. Because no NMF can be found in urine samples of non-exposed individuals, analysis of NMF is clearly suitable to distinguish between exposed and non-exposed persons. However, NMF cannot be used to categorise workers in different exposure groups, to identify particular working tasks with high exposures to DMF, or in risk assessment. Predominant reasons are the short half-life of NMF in urine (4–5 h) and that the role of NMF in DMF toxicity is unclear. In particular the short half-life of NMF is of concern in field studies for health risk assessment. Measurements of NMF in urine are only snapshots and do not provide information on cumulative and long term exposure. However, cumulative exposure is of major importance in risk assessment studies or if toxic effects are observed in workers. Therefore, exposure profiles on an...
individual basis for every single worker are necessary. Biomarkers with short half-lives—such as NMF—require continuous measurements on a near daily basis in order to identify exposures which are related to spills or improper use of protection measures. Despite the costs such daily analyses would require, sampling has to be carried out at particular time points (post-shift urine). Overall, NMF measurements fit least with the information obtained from standard questionnaires. However, the use of NMF is ideal for occupational hygiene control to determine the nature of exposure, for example, poor ventilation control, the effectiveness of personal protection measures such as the use of impermeable gloves, or if the worker moves to a different job location.

**AMCC in urine samples**

In contrast to NMF, AMCC has a half-life of 24–34 h\(^{16}\) and represents an index of the average exposure to DMF during the preceding 2–3 working days.\(^{18}\) In the study presented here, median concentration of AMCC in urine of exposed workers at all workplaces was determined to be 11.3 mg/l (0.6–116.5 mg/l, table 1). Currently, the US ACGIH suggests an advisory threshold limit value of 40 mg AMCC per litre urine prior to last shift of the work week.\(^{27}\) No threshold limit values or advisory levels exist for AMCC in other countries. Four of 34 workers (11.76%) exhibited AMCC levels >40 mg/l in our study. Levels of AMCC in urine increased from groups of low to high exposure. The concentrations were determined to be 6.2, 17.7, and 29.1 mg/l in workers exposed to low, medium, and high concentrations of DMF, respectively (fig 3B). In contrast to NMF, the differences between all three groups now were of borderline statistical significance (p = 0.04 for both volume and creatinine related concentrations). A detailed comparison between groups 1 and 2 and between groups 1 and 3 revealed p values of 0.08 and 0.02 for volume related concentrations, while for creatinine related concentrations p values of 0.03 were calculated. No statistical differences between smokers and non-smokers were found. Surprisingly, the person preparing the fibre solution had the highest AMCC level in urine (38.6 mg/l), while the persons working in fibre crimping and the supervisor had levels <11.9 mg/l. According to the workplace description obtained from the questionnaire, the person preparing the fibre solution should not have been exposed to DMF. Because his corresponding NMF level in urine (8.0 mg/l) was more similar to the NMF levels of fibre crimpers, he must have been exposed to DMF one or two days before our measurements were carried out. However, no detailed information could be obtained from the worker about his working tasks, which he carried out on the days prior to our measurements.

In a previous study median concentrations of 0.04 mg/l (<LOD -0.08 mg/l, n = 43) were analysed in persons not exposed to DMF.\(^{26}\) Therefore, the formation of AMCC is not totally specific for occupational exposure to DMF. However, single measurements of AMCC in urine can still be used to distinguish between occupationally exposed and non-exposed individuals, because AMCC concentrations are about 100 times higher in exposed workers than non-exposed individuals. Compared to NMF in urine, AMCC is more suitable to categorise and assess exposure of workers exposed to DMF, and AMCC measurements represent DMF exposure more accurately, which is expected from workplace conditions and questionnaires. Moreover, the formation of AMCC is linked to the metabolic formation of methyl isocyanate (MIC) after exposure to DMF and more related to toxic effects caused by exposure to DMF.\(^{10}\) Therefore, the formation and measurement of AMCC is also more suitable from a toxicological point of view. However, measurements of AMCC—although preferable to those of NMF—are still limited to certain time points of urine sampling. Only Thursdays and Fridays are appropriate time points for urine sampling in occupational settings. Moreover, it is still necessary to create exposure profiles for every exposed worker by AMCC measurements over several months, because the half-life of AMCC is not long enough. In conclusion, AMCC cannot be recommended as a biomarker of exposure in health risk studies because of its short half-life. However, AMCC is—similar to NMF—an ideal biomarker of exposure for occupational hygiene control.

Figure 3 Differences for (A) N-methylformamide (NMF), (B) N-acetyl-S-[N-methylcarbamoyl]cysteine (AMCC), and (C) N-methylcarbamoylated haemoglobin (NMHb) between workers exposed to low (group 1), medium (group 2), and high (group 3) concentrations of N,N-dimethylformamide. n, number of persons; Min, minimum; Max, maximum; 25%, 25th centile; 75%, 75th centile; Med, median.
NMHb in blood samples

Hb adducts—if stable—are supposed to be biomarkers at the molecular level with a mean life span of about 120 days. MIC, the reactive intermediate in the metabolism of DMF, is capable of reacting with several nucleophilic sites of Hb. However, only NMHb (the product of the reaction between MIC and the N-terminal valine of Hb) is of relevance for exposure assessment so far. NMHb was previously identified in workers exposed to DMF and after experimental exposure to DMF in animal and inhalation studies.

In the study presented here, median concentration of NMHb in blood of exposed workers at all workplaces was determined to be 121.2 nmol/g globin (21.3–464.9 nmol/g, table 1). No threshold values or advisory levels for NMHb are yet available for regulatory purposes. However, Mráz et al suggested a reference value of 135 nmol/g, representing the steady state level of NMHb in humans after experimental exposure of human volunteers to 10 ppm DMF by inhalation for 8 h/day, 5 days/week, and >20 weeks. In our study, 15 of 35 workers (42.86%) exhibited NMHb concentrations >135 nmol/g globin. In contrast to urinary levels of NMF and AMCC, there was a clear statistically significant difference between all three groups of exposed workers for NMHb (p = 0.01). The concentrations were determined to be 85.1, 122.8, and 152.6 nmol/g globin in workers exposed to low, medium, and high concentrations of DMF, respectively (fig 3C). A comparison between groups 1 and 2 and between groups 1 and 3 revealed p values of 0.02 and 0.006. The difference in the mean adduct levels between group 1 and 2 is more pronounced than between groups 2 and 3. The result is based on the fact that workers of group 3 use protective measures more often (impermeable gloves, respiratory masks) than workers of group 2, because they belong to an exposure group with higher risk of exposure. The results are in accordance with what is expected from the workplace description and questionnaire information obtained from the workers. As can be seen in table 2, fibre crimpers are least exposed to DMF. However, two persons of group 1, the person in charge and the person preparing the fibre solution, had higher levels of NMHb than expected from questionnaire information. Either person can be exposed to DMF, for example, after spills or when the tank for the spinning solutions is cleaned. Although they should wear protective clothing and breathing masks during such events, the results of NMHb analysis clearly show that protective clothing was either not used or used in an improper way, or—if correctly used—protection was not suitable to completely avoid uptake of DMF. Instead of having an exposure situation similar to fibre crimpers, the person in charge and the person preparing the fibre solution have an exposure scenario which is more similar to those workers, who are directly working at the spinning machine, or even to workers of exposure group 3. The latter group showed the highest concentrations of NMHb. Six of seven workers (85.71%) had NMHb levels higher than 135 nmol/g globin, which was suggested as reference concentration for NMHb in workers exposed to DMF. The persons were responsible for the precipitation bath (wet spinning), the washing machine, colouring dyes, and storage of fibre tow after dry spinning. Dermal uptake of DMF (hot temperature workplace) and the refusal to use protection measures for the entire shift due to practical reasons (sweating, weight of protection devices) resulted in increased uptake of DMF and increased NMHb concentrations in blood compared to groups 1 and 2.

Summary

The analysis of NMF, AMCC, and NMHb is capable of identifying workers who are exposed to DMF. However, only NMHb could accurately distinguish between workers who were exposed to different concentrations of DMF. NMHb analysis is sensitive enough to identify particular working tasks which cause increased exposure to DMF. Despite the identification of working tasks, which were already suspected from questionnaires and workplace description to cause increased exposure to DMF, NMHb analysis also uncovered working tasks which previously were not associated with high exposures to DMF, for example, the person in charge or the person responsible for preparing the fibre solution. In future field studies NMHb should be used as a biomarker of exposure, and particular focus has to be on those persons who were identified to be exposed to higher concentrations of DMF. These workers are more likely to develop toxic effects due to exposure to DMF. Therefore, NMHb is the preferred biomarker of exposure in health risk assessment. Our results can also be used to better evaluate exposure in retrospective studies, where actual measurements are missing, and to establish job exposure matrices for DMF exposure in the polyacrylic fibre industry. In contrast, short half-life biomarkers such as NMF and AMCC reflect exposure over the past 1–2 days, and responses to an increased level are much easier to correct. Therefore, the real value of measuring NMF and AMCC in urine is the use in occupational hygiene intervention rather than risk assessment.

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