

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

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Background: *N,N*-dimethylformamide (DMF) 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 DMF 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 DMF.

Results: All three biomarkers can be used to identify occupational exposure to DMF. However, only the analysis of NMHb could accurately distinguish between workers exposed to different concentrations of DMF. The median concentrations were determined to be 55.1, 122.8, and 152.6 nmol/g globin in workers exposed to low, medium, and high concentrations of DMF, respectively. It was possible by the use of NMHb to identify all working tasks with increased exposure to DMF. While fibre crimpers were found to be least exposed to DMF, persons washing, dyeing, or towing the fibres were found to be highly exposed to DMF. In addition, NMHb measurements were capable of uncovering working tasks, which previously were not associated with increased exposure to DMF; 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 DMF in health risk assessment. However, NMF and AMCC are useful biomarkers for occupational hygiene intervention. Further investigations regarding toxicity of DMF 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 DMF are high.

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The powerful solvent *N,N*-dimethylformamide (DMF) has the ability to dissolve different organic, inorganic, and resin materials. DMF is used in a variety of applications including the production of electronic components, photoactive chemicals, pharmaceutical products, textile coatings, and synthetic fibres.¹ There, DMF is used as a solvent for the fibre forming polymers. Global production of synthetic fibres was about 34 million metric tons in 2002.² Compared to cellulose, synthetic fibres have outstanding wickability, quick drying capabilities to move moisture from the body surface, flexible aesthetics, and are resistant to moths, chemicals, and sunlight degradation. Major uses are sportswear (sweaters, fleece wear), home furnishings (blankets, upholstery), and asbestos replacement in industrial settings.

During production of synthetic fibres, DMF is primarily taken up by absorption through the skin.^{3–5} DMF can cause severe liver damage.⁶ To minimise or avoid toxic effects in the liver, DMF is regulated for occupational exposure at 10 ppm in many countries.^{7–8} In addition to liver toxicity, DMF is also known to be embryotoxic and teratogenic in animal experiments at concentrations ≥ 100 mg/kg/day⁹ or ≥ 150 ppm.¹⁰ DMF is categorised in "pregnancy group B" by the German MAK and BAT commission (human fetal toxicity cannot be ruled out even at a MAK level of 10 ppm or lower at the workplace, exposure of pregnant women to DMF should be avoided). Case-control studies in humans also suggested a relation between exposure to DMF and the formation of germinal cell testicular cancer.^{11–12} Because the potency of reproductive toxic effects, the population at risk, and the

production volume of DMF is high, DMF was recently prioritised for field studies by the National Toxicology Program (NTP).¹³

The outstanding question in risk assessment is to identify whether or not a certain toxic effect is caused by exposure to a particular substance. Therefore, the reliability and accuracy of exposure assessment is of crucial importance. Exposure assessment in workers can be carried out by questionnaires (description of the workplace, working tasks, etc), the measurement of chemicals in the air at the workplace (ambient monitoring), or the determination of chemicals or their metabolites in blood and urine (biological monitoring). In the particular case of DMF, biological monitoring is accepted to be the ultimate measure of exposure assessment, because DMF is readily absorbed through the skin and workers have to carry out multiple and different working tasks. Currently, there are three biomarkers available (*N*-methylformamide, NMF; *N*-acetyl-*S*-(*N*-methylcarbamoyl)-cysteine, AMCC, and *N*-methylcarbamoylated haemoglobin, NMHb, Hb-adduct) to assess exposure to DMF in occupational settings (fig 1).^{14–16} However, the question remains which biomarker of exposure is the most suitable one for use in health risk assessment. The ideal biomarker has to be specific or at least sensitive enough to distinguish between

Abbreviations: AMCC, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine; DMF, *N,N*-dimethylformamide; Hb, haemoglobin; HMMF, *N*-hydroxy-*N*-methylformamide; MIC, methyl isocyanate; NMF, *N*-methylformamide; NMHb, *N*-methylcarbamoylated haemoglobin

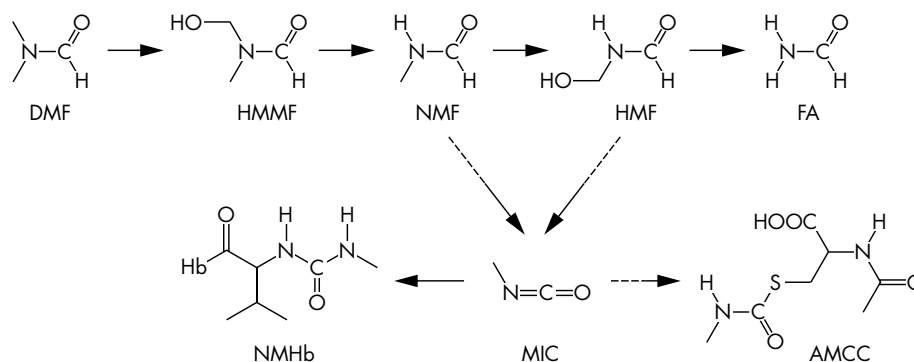


Figure 1 Metabolism of *N,N*-dimethylformamide (DMF). The toxifying pathway is supposed to run via the metabolic formation of methyl isocyanate (MIC), which can react with glutathione or haemoglobin.²⁹ So far, *N*-hydroxymethyl-*N*-methylformamide (HMMF), *N*-methylformamide (NMF), *N*-hydroxymethylformamide (HMF), formamide (FA), *N*-methylcarbamoylated haemoglobin (NMHb), and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) were identified as metabolites of DMF. NMF, NMHb, and AMCC were determined in the study presented here.

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.

Ambient air measurements by stationary and personal air sampling at the factory were carried out on a regular basis since 1994 using a method recommended by the US NIOSH¹⁷ with a limit of detection (LOD) of 0.1 ppm (1 ppm = 3 mg DMF/m³). The median of the air measurements was relatively constant throughout the years and was determined to be between 1 and 2 ppm. However, high single exposures up to approximately 160 ppm could be detected at workplaces where spills occurred.¹⁸ One of the major results in previous studies in this factory was that dermal absorption significantly contributes to the total internal dose of DMF.^{19, 20} Consequently, ambient monitoring on its own was not able to accurately identify exposure to DMF and biomonitoring turned out to be the only suitable measure for exposure assessment.

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.^{18–20} 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

Table 1 Descriptive statistical analysis of *N*-methylformamide (NMF), *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) in urine, and *N*-methylcarbamoylated haemoglobin (NMHb) in blood of workers exposed to *N,N*-dimethylformamide (DMF)

	Median	Min	Max	95%	Median	Min	Max	95%
	NMF (mg/l)				NMF (mg/g)			
All (n = 34/35)	10.2	1.6	59.7	48.1	9.6	2.5	72.8	33.1
EG 1 (n = 13/14)	8.0	1.6	47.7	38.4	6.5	2.5	26.9	26.8
EG 2 (n = 14)	18.6	4.7	49.0	47.4	11.3	3.1	33.5	33.1
EG 3 (n = 7)	27.6	1.8	59.7	54.1	12.5	5.1	72.8	58.5
S (n = 17/18)	9.7	1.6	45.0	34.8	9.1	2.5	29.8	27.5
NS (n = 16)	16.1	1.6	59.7	51.7	13.1	3.2	72.8	43.3
Wet (n = 10)	8.4	1.8	45.0	38.1	10.6	2.5	29.8	28.5
Dry (n = 14)	13.3	1.6	59.7	52.7	7.8	3.1	72.8	46.9
	AMCC (mg/l)				AMCC (mg/g)			
All (n = 34/35)	11.3	0.6	116.5	64.4	8.5	1.3	56.2	37.5
EG 1 (n = 13/14)	6.2	1.5	38.6	22.6	6.4	1.3	14.9	11.3
EG 2 (n = 14)	17.7	4.4	83.7	64.4	14.6	3.2	56.2	39.4
EG 3 (n = 7)	29.1	0.6	116.5	91.9	14.7	4.0	50.6	41.6
S (n = 17/18)	12.6	2.2	116.5	66.5	13.3	3.8	50.6	34.4
NS (n = 16)	9.4	0.6	83.7	46.9	7.2	1.3	56.2	29.4
Wet (n = 10)	17.5	0.6	116.5	83.2	17.6	3.8	50.6	40.5
Dry (n = 14)	11.7	2.2	83.7	64.4	8.1	4.6	56.2	39.4
	NMHb (nmol/g globin)							
All (n = 34/35)	121.2	21.3	464.9	221.1				
EG 1 (n = 13/14)	55.1	21.3	169.6	156.1				
EG 2 (n = 14)	122.8	53.0	464.9	297.1				
EG 3 (n = 7)	152.6	133.3	254.5	239.9				
S (n = 17/18)	129.7	22.1	464.9	244.6				
NS (n = 16)	117.7	21.3	254.5	218.7				
Wet (n = 10)	110.8	26.7	464.9	348.3				
Dry (n = 14)	129.7	41.8	254.5	223.5				

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.

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 before it is pressed through the nozzles of the spinneret. The process is carried out in closed cycles and no direct contact with DMF occurs during the work. When the tanks are cleaned, the worker is required to wear impermeable clothing and a breathing mask. The person in charge 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.

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 starting 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.

Group 3 consisted of seven persons who were working at open systems nearly all the time and could have been exposed to higher concentrations of DMF. DMF is always present, either in the freshly prepared fibres or in the surrounding air. Therefore, the persons were required to wear 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, including breathing masks for the entire shift (eight hours) every day.

Table 2 Median concentrations of *N*-methylformamide (NMF) and *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) in urine, and *N*-methylcarbamoylated haemoglobin (NMHb) in blood of workers exposed to *N,N*-dimethylformamide (DMF) in dependence on different job tasks

	No.	Job description	NMF (mg/l)	NMF (mg/g)	AMCC (mg/l)	AMCC (mg/g)	NMHb (nmol/g)
Exposure group 1	1	Fibre crimping (n = 11/12)	8.7	7.4	5.5	6.4	49.0
	2	Person in charge (n = 1)	4.7	6.5	6.2	8.6	148.8
	3	Spinning solution (n = 1)	8.0	3.1	38.6	14.9	169.6
Exposure group 2	1	Spinning machine (n = 14)	18.6	11.3	17.7	14.6	122.8
Exposure group 3	1	Precipitation bath (n = 1)	9.6	5.1	29.1	15.5	135.6
	2	Washing machine (n = 1)	27.6	12.0	116.5	50.6	205.7
	3	Colouring (n = 2)	21.4	18.9	17.0	12.3	143.0
	4	Fibre tows (n = 3)	32.2	13.7	11.3	13.7	191.4

Volume and creatinine related concentrations are given for NMF and AMCC. n, number of workers.



Figure 2 Manually starting the spinning process. Chemical resistant clothing, breathing masks, and non-permeable gloves are required.

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.²² All other chemicals and reagents were obtained from Merck KgaA (Darmstadt, Germany). Calibration and internal standardisation for the determination of NMHb was carried out using dipeptide standards *N*-methyl- and *N*-ethylcarbamoyl-*d*-*l*-valyl-*d*-*l*-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.²² 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 (2×10 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.²³ 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.¹⁵ 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.

Descriptive statistical analysis, *F*-tests, paired *t* tests and box plot presentations were carried out by SAS Software (SAS Inc., Cary, NC, USA) in order to study the differences between groups of exposed workers regarding the excretion of NMF and AMCC in urine and the formation of NMHb in blood. Differences between two or more groups were considered to be of statistical significance at $p \leq 0.05$. For this purpose mean concentrations (adjusted for smoking status) were used for evaluation. When levels for NMF, AMCC, or NMHb were below the LOD, half the LOD was the value used for all statistical calculations. All statistical analyses were carried out using volume and creatinine related concentrations of NMF and AMCC.

RESULTS AND DISCUSSION

Exposure assessment can be carried out by the determination of urinary metabolites NMF and AMCC.^{18–24–25} However, less information is available using biomarkers on the molecular level, such as NMHb.^{15–26} 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.^{7–27} Six of 34 persons (17.7%) had NMF 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,²² 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),^{18–28} and that the role of NMF in DMF toxicity is unclear.²⁹ 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

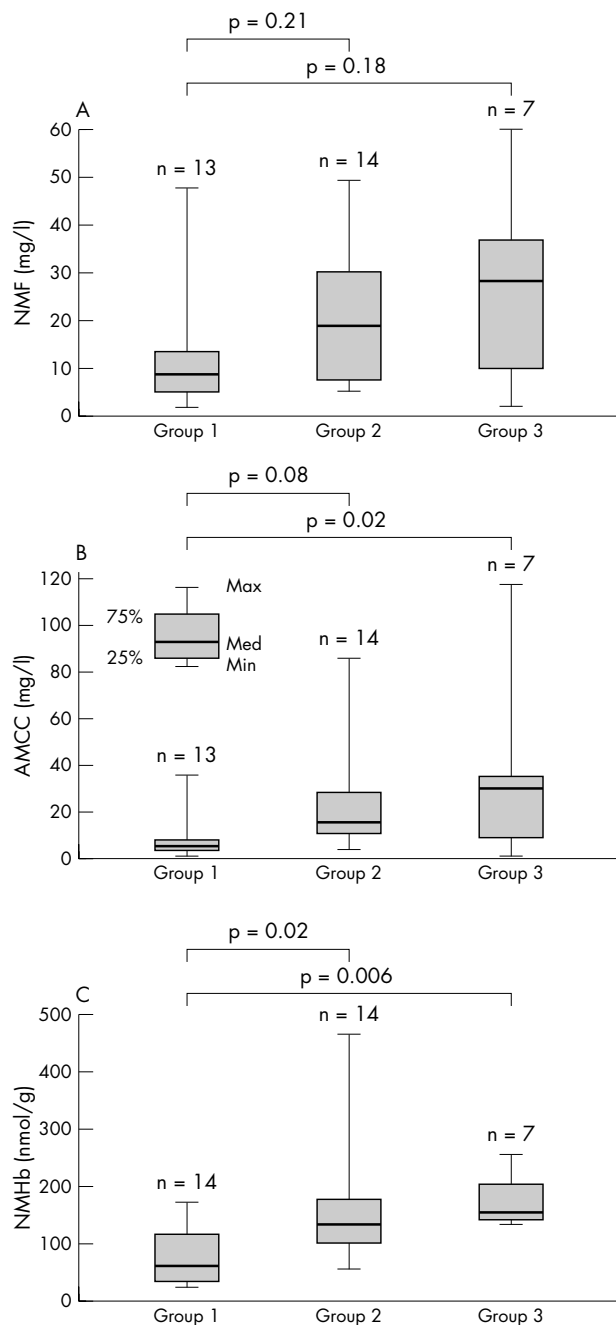


Figure 3 Differences for (A) *N*-methylformamide (NMF), (B) *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC), and (C) *N*-methylcarbamoylated haemoglobin (NMHb) between workers exposed to low (group 1), medium (group 2), and high (group 3) concentrations of *N,N*-dimethylformamide. n, number of persons;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

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 h²⁸ 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.³⁰ 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.¹⁵ 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.

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.³¹ 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^{15, 32} and after experimental exposure to DMF in animal and inhalation studies.^{26, 33} 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 limit values or advisory levels for NMhb are yet available for regulatory purposes. However, Mráz *et al* suggested a reference value of 135 nmol/g,²⁶ 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 55.1, 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 show that the provided and applied protective measures—if used—are capable of decreasing the uptake of DMF at the workplace.

In a previous study, median concentrations of 1.8 nmol NMhb/g globin (0.4–16.59 nmol/g, $n = 42$) were analysed in persons who were not exposed to DMF.¹⁵ Therefore, the formation of NMhb is—similar to AMCC—not totally specific for occupational exposure to DMF. However, individual measurements of NMhb still can be used for exposure assessment, because NMhb concentrations are about 50–100 times higher in exposed workers than in non-exposed persons. Moreover, NMhb is a biomarker of exposure on the molecular level and related to DMF toxicity. In contrast to NMF and AMCC, sampling for NMhb measurements is completely independent of specific time points. Exposure assessment can be carried out randomly 2–3 times per year, because the measurement does represent the mean exposure to DMF during the previous four months.

In the study presented here, NMhb was shown to be the best biomarker of exposure for use in future risk assessment studies. Therefore, we suggest NMhb as biomarker of exposure on the molecular level in occupational settings and field studies in order to link exposure and health effects due to DMF. Exposed workers can be clearly identified, even if exposure occurred several weeks prior sampling. NMhb concentrations in blood do reflect the exposure situation at the workplace far better than NMF or AMCC in urine, and 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 acrylic spinning solution, showed 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 tows 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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REFERENCES

- 1 **International Programme on Chemical Safety (IPCS).** *Dimethylformamide*, Environmental Health Criteria 114. Geneva: WHO Publications, 1991.
- 2 **Fibre Economics Bureau Inc.** Worldwide Manufactured Fiber Survey: 1996–2002. *Fiber Organon* 2001;**6**:95–112.
- 3 **Lauwerys RR, Kivits A, Lhoir M, et al.** Biological surveillance of workers exposed to dimethylformamide and the influence of skin protection on its percutaneous absorption. *Int Arch Occup Environ Health* 1980;**45**:189–203.
- 4 **Mráz J, Nohová H.** Percutaneous absorption of *N,N*-dimethylformamide in humans. *Int Arch Occup Environ Health* 1992;**64**:79–83.
- 5 **Chang HY, Tsai CY, Lin YQ, et al.** Urinary biomarkers of occupational *N,N*-dimethylformamide (DMF) exposure attributed to the dermal exposure. *J Expo Anal Environ Epidemiol* 2004;**14**:214–21.
- 6 **Kennedy GL Jr.** Biological effects of acetamide, formamide, and their monoethyl and dimethyl derivatives: an update. *Crit Rev Toxicol* 2001;**31**:139–222.
- 7 **Deutsche Forschungsgemeinschaft.** *List of MAK and BAT values 2004. Report Number 40 of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area.* Weinheim: WILEY-VCH, 2004.
- 8 **US Department of Labor, Occupational Safety and Health Administration (OSHA),** Washington, DC, USA (<http://www.osha.gov>).
- 9 **Saillenfait AM, Payan JP, Beydon D, et al.** Assessment of the developmental toxicity, metabolism, and placental transfer of *N,N*-dimethylformamide administered to pregnant rats. *Fundam Appl Toxicol* 1997;**39**:33–43.
- 10 **Hellwig J, Merkle J, Klimisch HJ, et al.** Studies on the prenatal toxicity of *N,N*-dimethylformamide in mice, rats and rabbits. *Food Chem Toxicol* 1991;**29**:193–201.
- 11 **Ducatman AM, Conwill DE, Crawl J.** Germ cell tumors of the testicle among aircraft repairmen. *J Urol* 1986;**136**:834–6.
- 12 **Levin SM, Baker DB, Landrigan PJ, et al.** Testicular cancer in leather tanners exposed to dimethylformamide. *Lancet* 1987;**8568**:1154.
- 13 **Moorman WJ, Ahlers HW, Chapin RE, et al.** Prioritization of NTP reproductive toxicants for field studies. *Reprod Toxicol* 2000;**14**:293–301.
- 14 **Mráz J, Tureček F.** Identification of *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine, a human metabolite of *N,N*-dimethylformamide and *N*-methylformamide. *J Chromatogr* 1987;**414**:399–404.
- 15 **Käfferlein HU, Angerer J.** *N*-methylcarbamoylated valine of hemoglobin in humans after exposure to *N,N*-dimethylformamide: evidence for the formation of methyl isocyanate? *Chem Res Toxicol* 2001;**14**:833–40.
- 16 **Mráz J, Dusková S, Gálová E, et al.** Improved gas chromatographic-mass spectrometric determination of *N*-methylcarbamoyl adduct at the *N*-terminal valine of globin, a metabolic product of the solvent *N,N*-dimethylformamide. *J Chromatogr B Biomed Sci Appl* 2002;**778**:357–65.
- 17 **Cassinelli ME, O'Connor PF, eds.** *NIOSH manual of analytical methods, no. 2004, 4th edn.* Publication 94-113. Department of Health and Human Services, National Institute of Occupational Safety and Health, 1994 (see also, <http://www.cdc.gov/niosh/nmam/nmampub.html>).
- 18 **Käfferlein HU, Göen T, Müller J, et al.** Biological monitoring of workers exposed to *N,N*-dimethylformamide in the synthetic fibre industry. *Int Arch Occup Environ Health* 2000;**73**:113–20.
- 19 **Wrbitzky R, Angerer J, Lehnert G.** External and internal monitoring in workers exposed to *N,N*-dimethylformamide. *Int Arch Occup Environ Health* 1996;**68**:508–10.
- 20 **Wrbitzky R, Angerer J.** *N,N*-dimethylformamide—influence of working conditions and skin penetration on the internal exposure of workers in synthetic textile production. *Int Arch Occup Environ Health* 1998;**71**:309–16.
- 21 **Slater JG, Rashed MS, Pearson PG, et al.** Biotransformation of methyl isocyanate in the rat. Evidence for glutathione conjugation as a major pathway of metabolism and implications for isocyanate-mediated toxicities. *Chem Res Toxicol* 1991;**4**:157–61.
- 22 **Käfferlein HU, Angerer J.** Simultaneous determination of two human urinary metabolites of *N,N*-dimethylformamide using gas chromatography-thermionic sensitive detection with mass spectrometric confirmation. *J Chromatogr B Biomed Sci Appl* 1999;**734**:285–98.
- 23 **Mowrer J, Törnqvist M, Jensen S, et al.** Modified Edman degradation applied to hemoglobin for monitoring occupational exposure to alkylating agents. *Toxicol Environ Chem* 1986;**11**:215–31.
- 24 **Sakai T, Kageyama H, Araki T, et al.** Biological monitoring of workers exposed to *N,N*-dimethylformamide by determination of the urinary metabolites, *N*-methylformamide and *N*-acetyl-*S*-(*N*-methylcarbamoyl) cysteine. *Int Arch Occup Environ Health* 1995;**67**:125–9.
- 25 **Lareo AC, Perbellini L.** Biological monitoring of workers exposed to *N,N*-dimethylformamide. II. Dimethylformamide and its metabolites in urine of exposed workers. *Int Arch Occup Environ Health* 1995;**67**:47–52.
- 26 **Mráz J, Dusková S, Gálová E, et al.** Biological monitoring of *N,N*-dimethylformamide. Reference value for *N*-methylcarbamoyl adduct at the *N*-terminal valine of globin as a biomarker of chronic occupational exposure. *Int Arch Occup Environ Health* 2002;**75**:S93–6.
- 27 **US-ACGIH, TLVs and BEIs.** *Threshold limit values for chemical substances and physical agents & biological exposure indices.* Cincinnati, OH, 2004.
- 28 **Mráz J, Nohová H.** Absorption, metabolism and elimination of *N,N*-dimethylformamide in humans. *Int Arch Occup Environ Health* 1992;**64**:85–92.
- 29 **Gescher A.** Metabolism of *N,N*-dimethylformamide: key to the understanding of its toxicity. *Chem Res Toxicol* 1993;**6**:245–51.
- 30 **Käfferlein HU, Angerer J.** Determination of *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine (AMCC) in the general population using gas chromatography-mass spectrometry. *J Environ Monit* 1999;**1**:465–9.
- 31 **Mráz J, Šimek P, Chvalová D, et al.** Studies on the methyl isocyanate adducts with globin. *Chem Biol Interact* 2004;**148**:1–10.
- 32 **Angerer J, Göen T, Krämer A, et al.** *N*-methylcarbamoyl adducts at the *N*-terminal valine of globin in workers exposed to *N,N*-dimethylformamide. *Arch Toxicol* 1998;**72**:309–13.
- 33 **Mráz J, Gálová E, Nohová H, et al.** Formation of *N*-methylcarbamoyl adduct with *N*-terminal valine of globin following exposure to *N,N*-dimethylformamide (DMF) and *N*-methylformamide (MF) in rats. In: *Proceedings of the 4th International Symposium on Biological Monitoring in Occupational and Environmental Health*, Seoul, Korea, 23–25 September, 1998:145–6.