The Significance of Using the Biomarkers of N, N-Dimethylformamide for Improvement of Health Risk Management in the Artificial Leather Industry

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To evaluate the usability of biological monitoring of levels of exposure to N,N-dimethylformamide (DMF) vapor by urinary N-methylformamide (NMF), we conducted a study in a factory in which DMF is used in the production of artificial leather. Severe liver damage in the worker exposed to 3 liters of DMF liquid was evidenced by raised levels of AST, ALT, γ-GTP and urinary NMF (327 IU/l, 158 IU/l, 233 IU/l and 854 mg/l, respectively). A laparoscopy revealed the liver to be markedly deformed by extensive scarring. After this accident, we investigated the handling and management of DMF in this factory, and it revealed that, of workers engaged in the polymer coating section, 44% had over 40 mg/l of urinary NMF, and 32% had 10 to 40 mg/l, despite DMF concentrations in the air of less than 2.2 ppm. After changes of operational procedures by our intervention, the workers had over 40 mg/l and between 10 to 40 mg/l of urinary NMF decreased to 25% and 52%, respectively. Our occupational intervention revealed that urinary NMF concentration was a useful representative index of daily exposure to DMF in the factory.

Key words: N,N-dimethylformamide, urinary N-methylformamide, biological monitoring, biological exposure index, liver damage

INTRODUCTION

N, N-Dimethylformamide (DMF) has been widely used in synthetic leather and polyacrylonitrile fiber industries as a washing solvent. Because of its high miscibility with both water and organic solvents, DMF has been important in these industries in washing off impurities, despite its toxicity. DMF exposure has been reported to increase the possibility of liver dysfunction, pancreatic disorders, testicular carcinoma, and etc. [1-3]. Notably, clinical case-reports on DMF toxicities have a confirmed liver damage with elevated serum aspartate and alanine aminotransferase (AST, ALT) levels [4-5] as well as histopathology [6-8]. In occupational medicine, liver dysfunction among polymer coating workers exposed to DMF has been noted in many papers [9-10]. Due to the toxicity of DMF, the Japan Society of Occupational Health (JSOH 2007) [11], the American Conference of Governmental Industrial Hygienists (ACGIH 2004) [12] recommend an occupational exposure limit to DMF of 10 ppm in the air.

DMF absorption occurs from dermal contacts, such as submerging hands in DMF fluid, and DMF vapor is also absorbed through both sinopulmonary and percutaneous exposure. Therefore, ambient air monitoring alone does not effectively reflect the
The major metabolites of DMF are N-methylformamide (NMF) and N-hydroxymethyl-N-methylformamide (HMMF) [10, 17] the latter being primary. HMMF is formed after hydroxylation of one of its methyl groups by CYP2E1 [18-19]. Smaller amounts of NMF are also found in urine [17, 20]. During the process of gas chromatography (GC), HMMF undergoes thermolytic degradation to form formaldehyde and NMF [21-22]. Consequently in GC analysis, quantities of HMMF and NMF are determined by the level of NMF. Biological monitoring of urinary NMF is recommended by both the ACGIH, DFG (Deutsche Forschungsgemeinschaft) for workers exposed in DMF at the workplace; the biological exposure index (BEI) for DMF proposed by ACGIH and DFG, recommends less than 15 mg/dl and 10mg/dl of urinary NMF, respectively, for evaluation of internal exposure at the end of work time.

This paper presents two report, a case of acute exposure for DMF by occupational accident at factory, and investigation and intervention of liquid handling of DMF in the190 factory workers. The results of the biological monitoring of 190 factory workers exposed to DMF by measurement of urinary NMF. The main focus of our investigation was on whether urinary NMF had useful diagnostic reliability for the exposure to the DMF and could be used in health management in a factory.

MATERIALS AND METHODS

Subjects and Factory

The chemical factory in Japan in which we conducted our study in September 2007 produces mainly artificial leather. A total of 190 workers were employed by the factory, 128 of which handled DMF; of the remaining workers, 46 who did not handle DMF also participated in this study. The DMF handling workers were engaged in the wet forming process, including polymer coating section (101 workers, 95 male and 6 female) and quality control engineering section (27 workers, 22 male and 5 female). These processes were being operated in an open area with no physical barriers and without local ventilation equipment. The 46 non-DMF handling workers worked in the inspection section (29 workers, 6 male and 23 female) and other sections (17 male workers). These 46 non-DMF handling workers worked in different buildings from that of the wet forming processes. The remaining 16 workers worked in offices and did not participate in this study. The Ethics Committee of Shimane University School of Medicine approved all study protocols (approval no. 1633).

Sampling of Diffusive DMF vapors

Air samples for measuring of diffusive DMF vapor were collected at eighteen loci in the factory, and analyzed by GC, as described previously [23-24].

Collection of urine samples and analysis

Urine samples from all subject workers were collected at the end of daily work, because timely collection is necessary as DMF has a short biological half-life [22, 25-27]. The samples were kept frozen in plastic containers and thawed immediately before analysis. A previously published method was used for the simultaneous determination of urinary NMF concentrations, based on HPLC with UV detection [28-29]. Workers having urinary NMF concentrations were categorized as under 10 mg/l, 10 to 40 mg/l, and over 40 mg/l, according to Japanese regulations on health checks for organic solvents.

Statistical analyses

Statistical analyses of data were done with SPSS software version 16.0J (SPSS Inc., Tokyo, Japan). In this study, results are expressed as means ± S.D. Comparisons of urinary NMF concentrations obtained pre- and post-improvement of process regulations were performed by student-t test, and P-value of less than 0.05 was used to assess significance.

RESULTS

Acute exposure by accident

On June 10, 2007, a 23 year old male worker, engaged in the process of polymers coating sec-
tion, was exposed to 3 liters of DMF fluid over his clothes. The work clothes were immediately removed except for underwear, and the worker continued to work for another 6 hours after the exposure. The following day, a periodical medical check showed the elevation of serum hepatic enzyme and urinary NMF levels (AST 158 IU/l, ALT 327 IU/l, γ-GTP 233 IU/l, Urinary NMF 854 mg/l). On June 29, 2007, nineteen days after the accident, the worker was admitted to his neighborhood hospital, with a confirmed diagnosis of acute severe liver damage. Laboratory data on admission was as follows: AST 249 IU/L, ALT 343 IU/L, γ-GTP 315 IU/L, alkaline phosphatase (ALP) 273 IU/L. No history of habitual alcohol consumption, no blood transfusions, and no drug-use were remarked, and the markers for viral hepatitis were negative. A physical examination showed no remarkable findings and he had no subjective symptoms. Except for abnormality of serum hepatic marker, there were no remarkable findings.

The temporal changes of hepatic markers and urinary NMF for him are shown in Fig.1. A laparosco-

![Fig.1. Clinical-course changes in serum liver markers and urinary NMF. The occupational accident occurred on June 10, 2007 and the laparoscopy was performed on July 9, 2007](image)

![Fig.2. Laparoscopic findings of left (a) and right (b) lobe of liver. The shapes of both lobes were markedly atrophied. Geographic depressions were seen on the superior surface of the left lobe (a) and lateral side of the right lobe (b)](image)
copy and biopsy were performed on July 9, 2007, twenty-nine days after the accident. A laparoscopy showed that both lobes of the liver were markedly atrophic; geographic depressions were observed on the superior surface of the left lobe and lateral side of the right lobe (Figure 2a and b). These findings suggested massive hepatic cell necrosis. Histological findings of the biopsy specimen revealed significant inflammatory cell infiltrations in the intralobular and portal area of the liver (Figure 3a), and ballooning and multinucleation of the hepatocytes (Figure 3b). These findings were compatible with the recovery stage of acute liver damage. Accordingly, the diagnosis of acute liver damage due to DMF exposure was confirmed. The patient was treated with rest in the hospital for 17 days, and the serum hepatic marker levels became normal, accompanied by negative urine NMF levels.

**DMF vapor in the factory**

In September 2007, we intervened in the factory to perform the working environment measurement (WEM) for DMF vapor in the factory air, and to investigate the handling of DMF liquid of workers. The DMF concentrations in the air at the eighteen loci in the factory were determined to be between under 0.1 ppm and 2.2 ppm, with a median concentration of 0.21 ppm. Ten of the eighteen loci had concentrations of under 0.1 ppm, with DMF concentrations at all loci under 10 ppm, as recommended by JSOH and ACGIH [10-11].

**Counter measures to DMF exposure**

To investigate the daily exposure to DMF, we collected urine samples from the workers at the end of daily work, and urinary NMF concentrations were measured by HPLC. Table 1 shows the results for NMF concentrations in urine of workers engaged in polymer coating section and quality control engineering section, both part of the wet forming process. Results for Aug. 2007, for the polymer coating section showed the highest levels of urinary

![Fig.3. Microscopic findings of liver histology. The extensively scarred connective tissue (a) was containing the remains of degenerative hepatocyte ballooning (small arrow) and forming rosette-like structure (arrow). Multinucleated and mitotic cells were observed in the same specimen (b) | Yamasaki et al.](image)

<table>
<thead>
<tr>
<th>Sections</th>
<th>Pre-improvement</th>
<th>Post-improvement</th>
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<tr>
<td>Polymer coating (n=101)</td>
<td>50.8 ± 87.1</td>
<td>64.3 ± 116.9</td>
</tr>
<tr>
<td>Quality control engineering (n=27)</td>
<td>18.3 ± 39.6</td>
<td>11.8 ± 10.3</td>
</tr>
<tr>
<td>Inspection (n=29)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Others (n=17)</td>
<td>8.3 ± 8.1</td>
<td>9.6 ± 12.8</td>
</tr>
</tbody>
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Values indicate mg/l and mean ± SD. Student t-test was performed and \(a, b, c\) indicates a significant difference for Sep. 2006, Jun. 2007, Aug. 2007, respectively. \(P < 0.05\)
NMF concentrations, reaching 81.6 ± 63.6 mg/l. In the quality control engineering section showed relatively high concentrations of urinary NMF as well, the greatest being 19.3 ± 26.8 mg/l in Aug. 2007.

Figure 4a shows the distribution for polymer coating workers with urinary NMF concentrations. In Aug. 2007, just prior to our investigation, 75% (44/59) had over 40 mg/l and 22% (13/59) had 10 to 40 mg/l of urinary NMF. Only 3% (2/59) had under 10 mg/l of urinary NMF. For the quality control engineering section, 16% (3/19) had over 40 mg/l and 37% (7/19) had 10 to 40 mg/l of urinary NMF (Fig.4b).

Countermeasure to DMF exposure in the factory

We determined that cause for the large amount of daily exposure to DMF in the wet forming process worker was due mainly to percutaneous absorption of DMF vapor during the worker handling of DMF liquid, because the WEM in any points of the factory were low levels. Therefore, to minimize such exposure to DMF, we had the workers make the following change in operations: 1) Upgrades in efficiency local ventilation equipment (Fig.5a); 2) Mechanization of the material feeding and ejection system of the coating solution which includes large amounts of DMF fluid (changing from manual to automated operation, Fig.5b); 3) Automatization of the movement of DMF fluid by pump.

After our intervention for the factory

Table 1 shows the results after regulatory improvements in Dec. 2007. The urinary NMF became significantly lower than that of the three measurement times prior to process changes in the polymer coating section (17.8 ± 15.8 mg/l vs 50.8 ± 87.1 mg/l, 64.3 ± 116.9 mg/l and 81.6 ± 63.6 mg/l). Though there were no significant differences between the urinary NMF levels after changes, the quality control engineering section levels were lower than for the three measurement times.

In the polymer coating section, the distribution for workers having over 40 mg/l of urinary NMF decreased to 12% in post-improvement (Dec. 2007), down from the pre-improvement (32% in Sep. 2006, 44% in Jun. 2007 and 75% in Aug. 2007). Further, the number of workers having less than 10 mg/l of urinary NMF increased to 46% in post-im-
Fig. 5. Instance of counter measures. (a) was an upgrading in efficiency local ventilation equipment; (b) was a mechanization of the ejection system of the coating solution.
DISCUSSION

The subject worker suffered severe acute liver damage from percutaneous exposure to large amounts of DMF fluid. In this case, the diagnosis was established by elevation of level of deviation enzymes (AST, ALT, ALP and γ-GTP) and urinary NMF compared to data for 6 month ago, the result of liver biopsy specimens, clinical history and negative hepatitis viral serology. The toxic properties of DMF are well reported [17, 30-31]. The primary organ for DMF toxicity is the liver, and numerous studies on humans and animals have shown that DMF is hepatotoxic in several species [32-33].

However, there has been little morphological investigation about DMF intoxication of humans. Kakio et al. reported a case of liver damage induced by DMF exposure, and liver laparoscopic and histological examination showed massive cell necrosis and scarring [8]. The laparoscopic and histological findings in our case were similar. Our microscopic findings revealed diffuse degenerative hepatocytes and a small amount of regenerative change, indicating an early convalescence stage. These findings were consistent with the Kakio et al. report [8], which indicated that extensive ranges of geographic depression, massive cell necrosis and scar were common features of liver damage induced by DMF exposure. Nomiyama et al. stated in their case report that the patient had no significant subjective symptoms, although they had acute severe liver damage from DMF exposure [16]. In our case, the patient showed severe liver damage without subjective symptoms as well. From these data, it would appear that a common feature in patients with DMF-related liver disease shows no or only mild significant subjective symptoms concomitant to the evident morphological changes in the liver and the markedly elevated blood markers.

Thus the importance of accurate assessment of absorbed DMF levels in the bodies of worker engaged in handling DMF.

The highlight of our intervention study was that urinary NMF, a DMF metabolite, is greater worth as a daily index for exposure levels of DMF, as compared to levels of DMF concentrations in the air. Thus, measurement of urinary NMF could useful to occupational management of workers engaged in DMF handling. Based on our results, airborne DMF concentration do not appear to be an accurate index of daily DMF exposure, as a large number of workers indicated high levels of urinary NMF, although the DMF concentrations in the air by the WEM were less than 10 ppm in all areas of the factory, as recommended by JSOH (2007) [10] and ACGIH (2004) [11]. In September 2007, just prior to our intervention, 60% of the subject workers had over 40 mg/l of urinary NMF, and 26% had 10mg/l to 40mg/l, despite airborne DMF concentrations under 2.2 ppm in all loci of the factory. Other studies have reported similar wide-ranging urinary NMF values, ascribing such results to various possible influencing factors of BEI score, including percutaneous absorption, alcohol intake, collection period of urine samples and individual metabolic pathway [13, 16]. Of those factors, it was considered that percutaneous absorption was the main influencing factor of urinary NMF for the end of work in a factory. Wrbitzky and Angerer indicated that the wide range of individual urinary NMF levels might be due to percutaneous absorption in workers with or without protective devices [14]. It was therefore surmised that the results for the wide range covered by the urinary NMF levels were mainly caused by dermal absorption of DMF vapor or direct contact with DMF fluid, due to improper use of protective systems, machines or clothing. Our investigation succeeded in reducing DMF exposure of the workers by improvement of five operational instructions with emphasis on avoiding the absorption of DMF vapor through the skin. After intervention, averages of urinary NMF decreased significantly to 17.8 ± 15.8 mg/l as compared with any point before. Further, the distribution for workers with over 40 mg/l of urinary NMF decreased to 12% from 75% (Aug. 2007), and the number of workers with less than
10 mg/l of urinary NMF increased from 3% to 46%. By the same manner, in the quality control engineering section, the distribution for workers having over 40 mg/l of urinary NMF decreased to 4%, and those with less than 10 mg/l of urinary NMF increased to 76%. In fact, the success of our intervention strongly indicates that the cause of daily DMF exposure for the factory worker was mainly the percutaneous absorption of DMF vapor, and the measuring urinary NMF was useful for control and prevention of DMF exposure.

We conclude that large amounts of DMF are absorbed as vapor through the skin by handling of DMF liquid, and not mainly through respiration, and that of prevention of such exposure served to significantly decrease DMF exposure. Therefore, the biological monitoring of urinary NMF as BEI of DMF is a very significant, even if WEM is kept low, and useful in the health managements for the worker handling DMF liquid in a factory.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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