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.

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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 disfunction 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

amount of real absorption. In fact, some papers have indicated that air monitoring is no longer sufficient to evaluate worker exposure to DMF, and that biological monitoring of workers is necessary [13-16]

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 laparos-

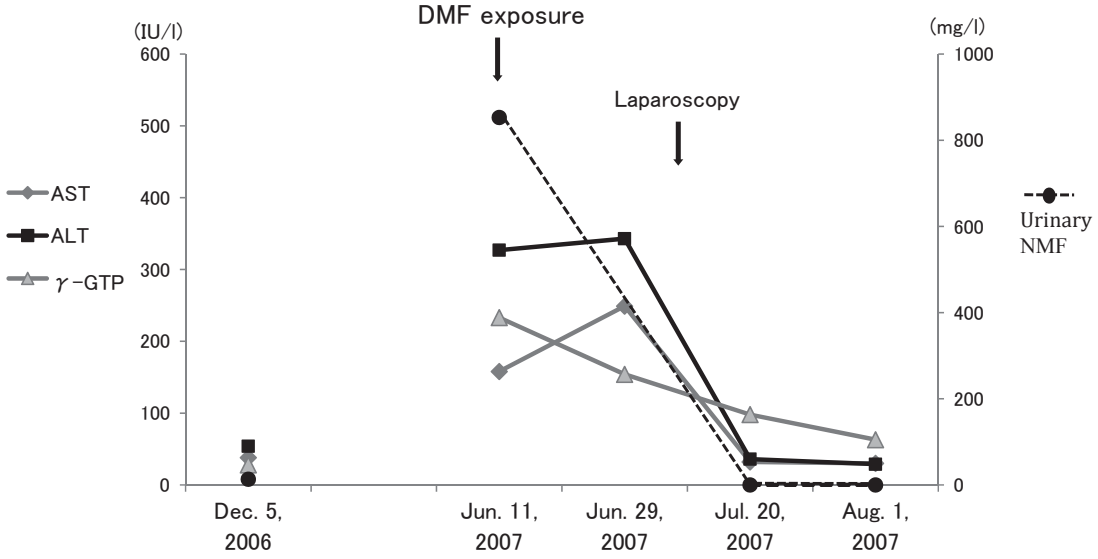

Fig.1. Clinical-course changes in serum liver markers and urinary NMF. The occupational accident occurred on June 10, 2007 and the laparoscopy was performed on July 9, 2007

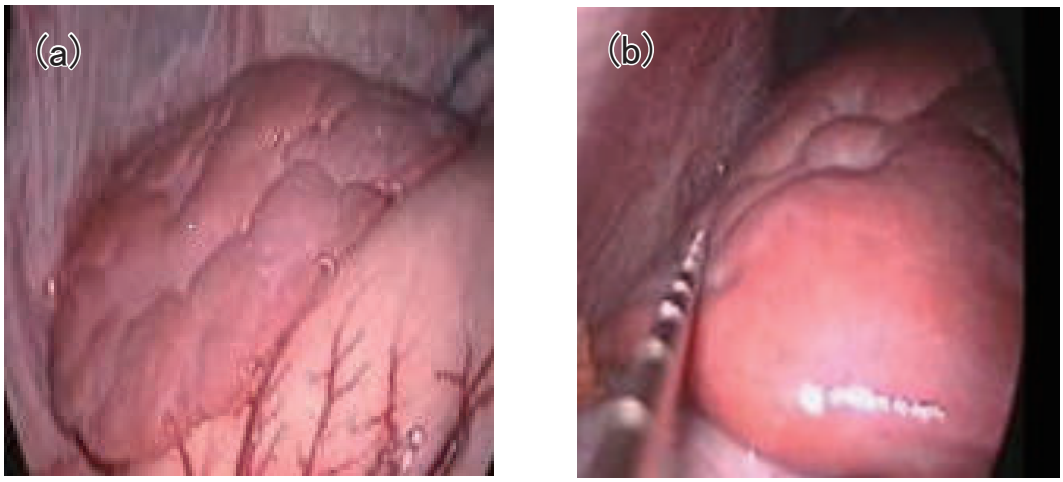

Fig.2. Laparoscopic findings of left (a) and right (b) lobe of liver. The shapes of both lobes were markedly atrophied. Geographic depressions were seen on the superior surface of the left lobe (a) and lateral side of the right lobe (b)

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

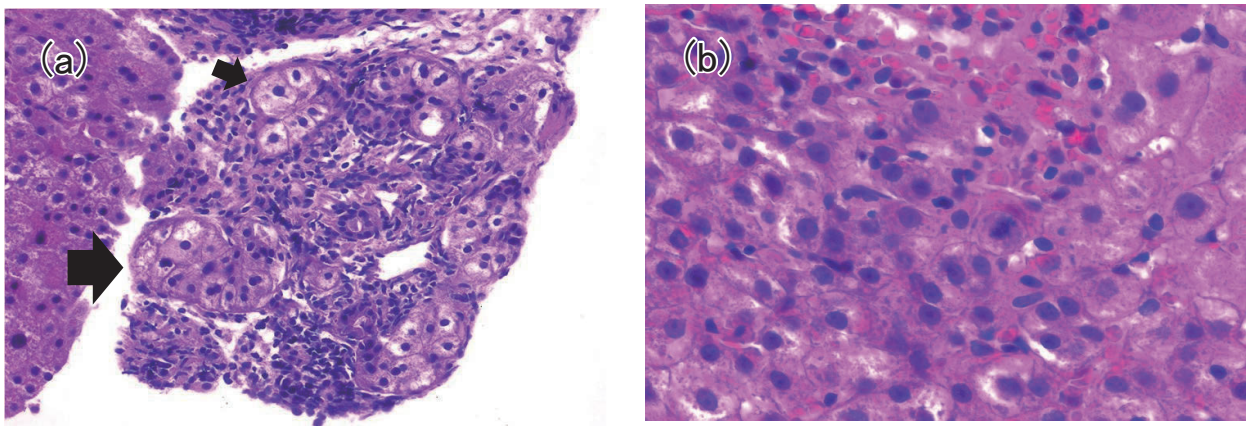

Fig.3. Microscopic findings of liver histology. The extensively scarred connective tissue (a) was containing the remains of degenerative hepatocyte ballooning (small arrow) and forming rosette-like structure (arrow). Multinucleated and mitotic cells were observed in the same specimen (b)

Table 1. Urinary NMF concentrations for Pre- and Post-improvement of operational regulations

	Pre-improvement			Post-improvement
Sections	Sep. 2006	Jun. 2007	Aug. 2007	Dec. 2007
Polymer coating (n=101)	50.8 ± 87.1	64.3 ± 116.9	81.6 ± 63.6	17.8 ± 15.8 [a, b, c]
Quality control engineering (n=27)	18.3 ± 39.6	11.8 ± 10.3	19.3 ± 26.8	9.7 ± 10.7
Inspection (n=29)	–	–	–	43.2 ± 45.4
Others (n=17)	8.3 ± 8.1	9.6 ± 12.8	36.1 ± 30.0	4.1 ± 8.6 [c]

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. $P < 0.05$

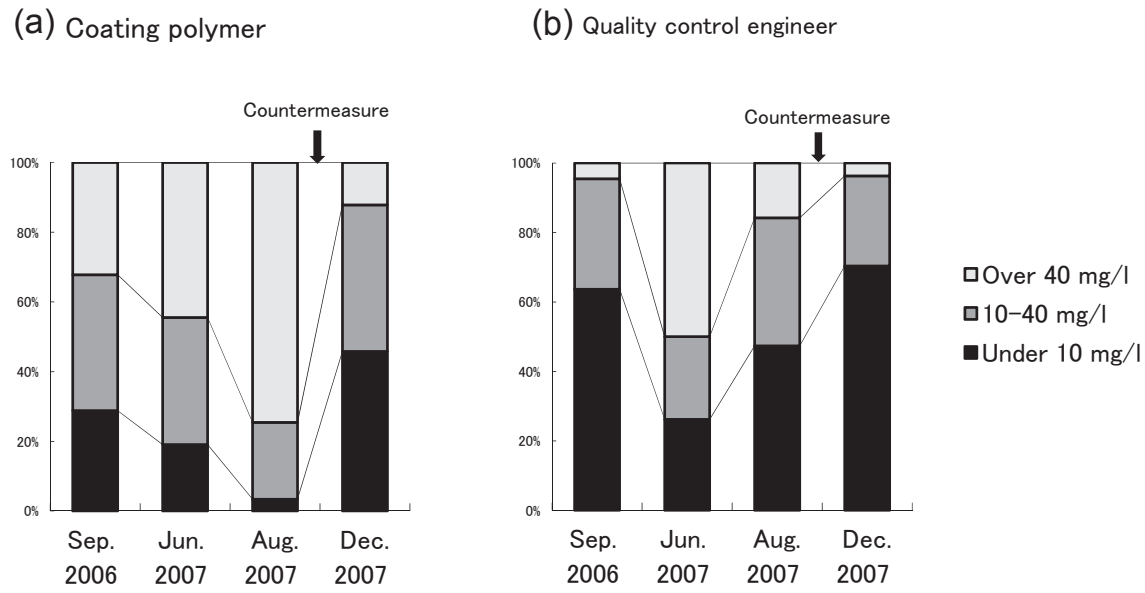

Fig.4. Distribution of urinary NMF concentrations in polymer coating section (a) and quality control engineering section (b). September 2006, June 2007 and August 2007 were before improvements of handling of DMF, and December 2007 was after improvements

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-

(a)

(b)

Fig.5. Instance of counter measures. (a) was an upgrades in efficiency local ventilation equipment; (b) was a mechanization of the ejection system of the coating solution

provement, up from pre-improvement (29% in Sep. 2006, 19% in Jun. 2007 and 3% in Aug. 2007, Fig. 4a). In similar fashion, in the quality control engineering section, the distribution for workers with over 40 mg/l of urinary NMF decreased to 4%, and those with less than 10 mg/l of urinary NMF increased to 70% with the procedural changes (Fig.4b).

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 compare 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 intervention 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.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

1) Yang JS, Kim EA, Lee MY, Park IJ and Kang SK (2000) Biological monitoring of occupational exposure to *N,N*-dimethylformamide-the effects of co-exposure to toluene or dermal exposure. *Int Arch Occup Environ Health* 73: 463-470.

2) Scailteur V, de Hoffmann E, Buchet JP and Lauwerys R (1984) Study on in vivo and in vitro metabolism of dimethylformamide in male and female rats. *Toxicology* 29: 221-234.

3) Redlich CA, West AB, Fleming L, True LD, Cullen MR and Riely CA (1990) Clinical and pathological characteristics of hepatotoxicity associated with occupational exposure to dimethylformamide. *Gastroenterology* 99: 748-757.

4) Potter HP (1974) Toxicity of dimethylformamide. *Lancet* 2 (7888): 1084.

5) Weiss LR and Orzel RA (1967) Some comparative toxicologic and pharmacologic effects of dimethyl sulfoxide as a pesticide solvent. *Toxicol Appl Pharmacol* 11: 546-557.

6) Tolot F, Arcadio F, Lenglet JP and Roche L (1968) Dimethylformamide intoxication. *Arch Mal Prof* 29: 714-717.

7) Fleming LE, Shalat SL and Redlich CA (1990) Liver injury in workers exposed to dimethylformamide. *Scand J Work Environ Health* 16: 289-292.

8) Kakio T, Ukida M, Ito T, *et al*. (1992) A case of funnel liver induced by dimethylformamide. *Dig Endosc* 4: 76-81.

9) Redlich CA, Beckett WS, Sparer J, *et al*. (1988) Liver disease associated with occupational exposure to the solvent imethylformamide. *Ann Intern Med* 108: 680-686.

10) Kafferlein HU, Ferstl C, Burkhart-Reichl A, *et al*. (2005) The use of biomarkers of exposure of *N,N*-dimethylformamide in health risk assessment and occupational hygiene in the polyacrylic fibre industry. *Occup Environ Med* 62: 330-336.

11) Japan Society Occupational Health (JSOH) (2007) Recommendation of occupational exposure limit (2007-2008). *J Occup Health* 49: 328-344.

12) American Conference of Governmental Industrial Hygienists (ACGIH) (2002) Threshold limit values for chemical substances and physical agents and biological exposure indices. ACGIH , Cincinnati.

13) Fiorito A, Larese F, Molinari S and Zanin T (1997) Liver function alterations in synthetic leather workers exposed to dimethylformamide. *Am J Ind Med* 32: 255-260.

14) Wrbitzky R and Angerer J (1998) *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* 71: 309-316.

15) Yonemoto J and Suzuki S (1980) Relation of exposure to dimethylformamide vapor and the metabolite, methylformamide, in urine of workers.

Int Arch Occup Environ Health 46: 159-165.

16) Nomiyama T, Uehara M, Miyauchi H, Imamiya S, Tanaka S and Seki Y (2001) Causal relationship between a case of severe hepatic dysfunction and low exposure concentrations of *N,N*-dimethylformamide in the synthetics industry. *Ind Health* 39: 33-36.

17) Gescher A (1993) Metabolism of *N,N*-dimethylformamide: key to the understanding of its toxicity. *Chem Res Toxicol* 6: 245-251.

18) Mraz J, Jheeta P, Gescher A, Hyland R, Thummel K and Threadgill MD (1993) Investigation of the mechanistic basis of *N,N*-dimethylformamide toxicity. Metabolism of *N,N*-dimethylformamide and its deuterated isotopomers by cytochrome P450 2E1. *Chem Res Toxicol* 6: 197-207.

19) Nomiyama T, Haufroid V, Buchet JP, *et al*. (2001) Insertion polymorphism of CYP2E1 and urinary N-methylformamide after *N,N*-dimethylformamide exposure in Japanese workers. *Int Arch Occup Environ Health* 74: 519-522.

20) Kestell P, Gill MH, Threadgill MD, Gescher A, Howarth OW and Curzon EH (1986) Identification by proton NMR of *N*-(hydroxymethyl)-*N*-methylformamide as the major urinary metabolite of *N,N*-dimethylformamide in mice. *Life Sci* 38: 719-724.

21) Scailteur V and Lauwerys R (1984) In vivo and in vitro oxidative biotransformation of dimethylformamide in rat. *Chem Biol Interact* 50: 327-337.

22) Brindley C, Gescher A and Ross D (1983) Studies of the metabolism of dimethylformamide in mice. *Chem Biol Interact* 45: 387-392.

23) Nomiyama T, Nakashima H, Chen LL, *et al*. (2001) *N,N*-dimethylformamide: significance of dermal absorption and adjustment method for urinary *N*-methylformamide concentration as a biological exposure item. *Int Arch Occup Environ Health* 74: 224-228.

24) Tanaka S, Nomiyama T, Miyauchi H, *et al*. (2002) Monitoring for *N,N*-dimethylformamide and *N,N*-dimethylacetamide with a diffusive sampler using distilled water as an absorbent. *AIHA J (Fairfax, Va)* 63: 726-731.

25) Maxfield ME, Barnes JR, Azar A and Trochimowicz HT (1975) Urinary excretion of metabolite following experimental human exposures to DMF or to DMAC. *J Occup Med* 17: 506-511.

26) Krivanek ND, McLaughlin M and Fayweather WE (1978) Monomethylformamide levels in human urine after repetitive exposure to dimethylformamide vapor. *J Occup Med* 20: 179-182.

27) Kafferlein HU, Goen T, Muller J, Wrbitzky R and Angerer J (2000) Biological monitoring of workers exposed to *N,N*-dimethylformamide in the synthetic fibre industry. *Int Arch Occup Environ Health* 73: 113-120.

28) Kawai T, Yasugi T, Mizunuma K, *et al*. (1992) Occupational dimethylformamide exposure. 2. Monomethylformamide excretion in urine after occupational dimethylformamide exposure. *Int Arch Occup Environ Health* 63: 455-460.

29) Imbriani M, Maestri L, Marraccini P, *et al*. (2002) Urinary determination of *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine and *N*-methylformamide in workers exposed to *N,N*-dimethylformamide. *Int Arch Occup Environ Health* 75: 445-452.

30) Tanaka K (1971) Toxicity of dimethylformamide (DMF) to the young female rat. *Int Arch Arbeitsmed* 28: 95-105.

31) Itoh H, Uchikoshi T and Oikawa K (1987) Histopathological investigation of DMF-induced hepatotoxicity. *Acta Pathol Jpn* 37: 1879-1889.

32) Massmann W (1956) Toxicological investigations on dimethylformamide. *Br J Ind Med* 13: 51-54.

33) Senoh H, Aiso S, Arito H, *et al*. (2004) Carcinogenicity and chronic toxicity after inhalation exposure of rats and mice to *N,N*-dimethylformamide. *J Occup Health* 46: 429-439.