Dimethylformamide-Induced Liver Damage Among Synthetic Leather Workers

JUNG-DER WANG, M.D., Sc.D.
MING-YANG LAI, M.D., Ph.D.
Center for the Research of Environmental and Occupational Disease
Graduate Institute of Public Health and Department of Internal Medicine
National Taiwan University College of Medicine
Taiwan, Republic of China
JUI-SAN CHEN, M.D.
Department of Clinical Pathology
National Taiwan University Hospital
Taiwan, Republic of China
JAI-MING LIN, Ph.D.
JEN-RON CHIANG, M.S.
SHWU-JEN SHIAU, M.S.
WU-SHIU CHANG, M.D.
Center for the Research of Environmental and Occupational Disease
Graduate Institute of Public Health and Department of Internal Medicine
National Taiwan University College of Medicine
Taiwan, Republic of China

ABSTRACT. Prevalence of liver injury associated with dimethylformamide (DMF) exposure was determined. Medical examinations, liver function tests, and creatine phosphokinase (CPK) determinations were performed on 183 of 204 (76%) employees of a synthetic leather factory. Air concentrations of solvents were measured with personal samplers and gas chromatography. The concentration of DMF in air to which each worker was exposed was categorized. High exposure concentrations of DMF (i.e., 25–60 ppm) were significantly associated with elevated alanine aminotransferase (ALT) levels (ALT $\geq$ 35 IU/l), a result that did not change even after stratification by hepatitis B carrier status. Modeling by logistic regression demonstrated that exposure to high concentrations of DMF was associated with an elevated ALT (p = .01), whereas hepatitis B surface antigen (HBsAg) was slightly but independently associated with an elevated ALT (p = .07). In those workers who had normal ALT values, there occurred still significantly higher mean ALT and aspartate aminotransferase (AST) activities, especially among those who were not HBsAg carriers. A significant association existed between elevated CPK levels and exposure to DMF. However, an analysis of the CPK isoenzyme among 143 workers did not reveal any specific damage to muscles. This outbreak of liver injury among synthetic leather workers is ascribed to DMF. It is recommended that the occupational standard for DMF and its toxicity among HBsAg carriers be evaluated further.

IN NOVEMBER 1986, a 26-y-old male synthetic leather worker was hospitalized at the National Taiwan University Hospital. During the 2 mo that had preceded his admission, he experienced recurrent epigastric pain, nausea, fatigue and abnormal liver function. His serum tested negative for (a) hepatitis B surface antigen (HBsAg), (b) IgM antibody against hepatitis B core antigen, and (c) IgM antibody against hepatitis A virus. A liver needle biopsy revealed multiple zonal necrosis, which was present mainly in the central zones, accompanied by scarce cell infiltrates. After 2 wk in the hospital, his alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels dropped from 901 U/l and 390 U/l to 61 U/l and 32 U/l, respectively. After it was determined he did not abuse alcohol and drugs and that his job involved exposure to dimethylformamide (DMF)—a well-known hepatotoxic agent[1-5]—DMF-induced hepatitis was highly suspected. The authors attempted to confirm this diagnosis and to determine the prevalence of liver damage in synthetic leather workers by conducting a study of his co-workers.

With the exception of alcohol,[6,7] very little has been reported about synergism between exposure to hepatotoxic solvents and hepatitis B carrier status. Because the prevalence of hepatitis B carriers can be 15-20% in Taiwan,[8,9] we had the opportunity to evaluate any possible interactive effects that occurred in the liver.

Materials and methods

Workplace evaluation. The factory at which the patient had been employed for 2 mo produces mostly synthetic leather and select polyurethane-coated fabrics. Two processes, one wet and one dry, are used to prepare the materials. The wet process entails using a large amount of DMF; lesser quantities of other solvents, e.g., ethyl acetate, methylethyl ketone (MEK), butanone, acetone, methylene dichloride, and toluene, are used. Solvents and various coloring agents are mixed with polyurethane components or with other resins in a mixer, after which they are pumped onto fabric, which is then dipped in water. The dry process involves more MEK and less DMF and toluene. After a complete mixing, the solvent-resin mixture is pumped and hand-ladled onto the moving fabric. The finished product—rolls of synthetic leather and polyurethane-coated fabric—are used to make handbags and other items.

After an initial walk-through survey, we decided to measure air concentrations of DMF, ethyl acetate, and MEK—the three solvents consumed in the greatest quantity during the two processes. Even though toluene and methylene dichloride had not been used during the preceding 3 wk, we nonetheless measured their concentration in air.

A total of 19 random samples of organic solvents were conducted: 8 samples were obtained with charcoal tubes, and 11 were obtained with silica gel tubes. With the exception of 1 measurement, all levels of ethyl acetate were within the threshold limit values (TLVs) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).[14] Toluene and methylene dichloride were undetectable. All acetone and butanone levels were within TLVs.

Factory areas were designated by exposure categories, which were determined by air concentration of DMF and potential dermal contact with solvents. Random, 10-min samples obtained with silica gel tubes were analyzed, and air concentrations of DMF ranged from 9 to 60 ppm. Administrative and maintenance positions and other jobs in which individuals did not experience direct exposure to solvents, and where the air concentration of DMF was less than 10 ppm, were assigned an exposure index of 0. Jobs that entailed dry process material mixing and separation and a DMF concentration of 10–40 ppm (i.e., DMF was not used as the major solvent) were assigned an exposure index of 1. Exposure index 2 included wet processing and mixing jobs in which DMF was used as the major solvent, and direct skin contact with the solvent occurred more frequently than during the two job categories described above. Air concentrations of DMF in exposure index 2 ranged from 25 to 60 ppm.

Charcoal and silica gel tubes connected to personal samplers were used for 10-min random air samples, which were analyzed by gas chromatography according to the method recommended by the National Institute of Occupational Safe and Health (NIOSH).[10] The factory's operations continued uninterrupted throughout the day; therefore, we assumed that the random samples were representative of the air typically inhaled by workers.

Health evaluations. We asked all 240 employees to participate in the study. Complete physical examinations, liver function tests, and serum creatinine phosphokinase (CPK) and HBsAg assays were conducted. Each worker provided a detailed history of (a) work, including potential skin contact with solvents, (b) alcohol consumption, (c) cigarette smoking, and (d) blood transfusions. Symptoms of headache, dizziness, anorexia, nausea, epigastric pain, or general weakness, if present, were also noted for each worker. Physical examinations were performed by two doctors who were instructed to take particular note of chloracne or neurological symptoms.

Statistical analysis. Data were analyzed using the Mantel-Haenszel summary procedure[11] and the Mantel extension for the test of trends.[12] Logistic regression analyses (EGRET statistical package[13]) were conducted to estimate the independent and interactive effects of hepatitis B carrier status and exposure to DMF.

Results

Of the 183 workers examined, none showed abnormal neurological signs or chloracne. There was a high turnover rate at the factory, and most of the workers were young (Table 1). Most workers who were exposed directly to DMF did not drink much alcohol, a result most likely influenced by the fact that alcohol use was a precursor to nausea and flushing of their faces and hands. Two workers had a history of blood transfusions; therefore, they were excluded from the liver

Table 1.—Age, Sex, Duration of Employment, and Smoking and Alcohol Consumption of Workers Exposed to Various Concentrations of Dimethylformamide (DMF)

Factors	DMF exposure index* 0	1	2
Air concentration of DMF (ppm	< 10	10–40	25–60
Workers examined (*n*)	76	83	24
Active workers examined (%)	72.9	86.2	60.0
Male (%)	67.9	90.1	100.0
Age (y)	28.8 ± 6.6	28.4 ± 4.3	27.3 ± 2.7†
Duration of employment (mo)	38.4 ± 25.0	33.5 ± 21.8	32.2 ± 29.3†
Smokers (%)	39.7	55.6	58.3
Alcohol consumption > 24 g/d (%)	6.4	3.7	0
No. who had ever had blood transfusion(s)	1	1	0

*Factory areas were designated by exposure categories, which were determined by air concentration of DMF and potental dermal contact with solvents (see "materials and methods").
†$p > .25$ (ANOVA).

function test analysis so that confounding by non-A and non-B hepatitis would be prevented.

There was a significant association between a higher prevalence of abnormal liver function and a higher index of exposure to DMF (Table 2). Logistic regression analysis revealed that high concentrations of DMF were associated with elevated ALTs ($p = .01$), whereas HBsAG carrier was slightly but independently associated with an elevated ALT ($p = .07$). Some workers had normal values of ALT (i.e., < 35 IU/l); however, the exposed individuals in this group had higher mean values of ALT, and among the non-HbsAg carriers, a higher mean value of AST (Table 3).

Mean values of transaminases were generally higher among HbsAg carriers. There was, however, no similar trend for serum amylase, albumin-globulin ratio, gammaglutamyl transferase (GGTR), and alkaline phosphatase. As well, no similar trend was noted for indicators of renal damage, i.e., serum creatinine and urine protein.

Fatigability, anorexia, nausea, and epigastric pain occurred more frequently among workers who were exposed to high concentrations of DMF, but the associations were not statistically significant (Table 4). Workers exposed to a higher concentration of DMF were more likely to have an elevated CPK, but HBsAG carriers did not have a higher frequency of abnormal CPK levels (Table 5). Only 143 of the 183 workers examined were available for further analysis of CPK isoenzyme. There appeared to be no association between higher exposure levels of DMF and elevated MB function of CPK isoenzyme (Table 6). An analysis of variance demonstrated that there was no difference in mean level of MB fraction among the different exposure groups.

Discussion

Even though we found 1 case of suspicious chemical hepatitis, and 15 additional workers had abnormal liver functions, it does not necessarily follow that these effects were caused by DMF. The authors, however, pro-

Table 2.—Number of Workers Who Had Abnormal Liver Function,* Stratified by Index of Exposure to Dimethylformamide (DMF) and Hepatitis B Surface Antigen (HBsAg)

Status	Liver function (ALT level)	DMF exposure index 0	1	2	1–2
HBsAg(+)	⩾ 35	2	2	3	5
	< 35	19	17	4	21
HBsAg(−)	⩾ 35	2	3	3	6
	< 35	52	60	14	74
Total	⩾ 35	4	5	6	11
	< 35	71	77	18	95
SRR		1.0	1.2	6.2	2.5
χ^2 for odds ratio = 1					9.71 ($p = .008$)
$\chi^2(1)$ (Mantel-Extension for trend)					5.15 ($p = .02$)

	Modeling by logistic regression: Odds ratio	95% CI	*p* value
HBsAg	2.81	0.92– 8.59	.07
DMF			
Exposure index 1	1.23	0.31– 4.81	.77
Exposure index 2	6.16	1.53–24.79	.01

*Defined by an alanine aminotransferase (ALT) level > 35 IU/l.

Table 3.—Comparison of Serum Enzyme Activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Gammaglutamyl Transferase (GGT) among Workers Who Have Normal Values of ALT* Stratified by Index of Exposure and Hepatitis B Carrier Status (HBsAg)

	DMF exposure index		
	0	1	2
HBsAg(+)			
No. of workers	19	17	4
ALT (IU/l)	14±6	18±7†	25±6‡
AST (IU/l)	18±6	17±5	22±4
GGT (U/l)	19±30	13±7	25±18
HBsAg(−)			
No. of workers	52	60	14
ALT (IU/l)	12±7	16±8§	18±8‡
AST (IU/l)	15±4	16±4§	18±5§
GGT (U/l)	15±17	18±12	22±17

*Normal value of ALT = < 35 IU/l.
†p = .06 (Student's t test).
‡p < .01 (Student's t test).
§.01 < p < .05 (Student's t test).

Table 4.—Prevalence of Symptoms among Workers Exposed to Different Levels of Dimethylformamide (DMF)

	DMF exposure index			
	0	1	2	p value*
Workers examined (n)	75	82	24	
Fatigability (%)	10.3	25.9	16.7	.11
Dizziness (%)	1.3	9.9	12.5	.032
Anorexia (%)	3.8	7.4	14.3	.12
Nausea (%)	1.3	2.5	4.3	.38
Epigastric pain (%)	5.4	5.2	20	.12

*Mantel-Extension for trend.

Table 6.—Percentage of MB Fraction of Creatine Phosphokinase Isoenzyme, Stratified by Index of Exposure to Dimethylformamide (DMF)

	DMF exposure index		
	0	1	2
Percentage of MB fraction ($\bar{x} \pm SD$)	2.8±3.0	2.7±3.0	1.5±2.2
No. workers with:			
0%	24	29	13
1–5%	18	21	6
> 5%	14	16	2
Total	56	66	21

vide the following arguments in support of an association between DMF and the effects noted.

First, the possibility that non-A/non-B hepatitis accounted for the effects is almost nonexistent because workers who had histories of blood transfusions were excluded (Table 2).

None of the workers who experienced considerable DMF exposure were alcoholics (Table 1). Conversely, alcohol consumption (> 24 g/d) was inversely related to the DMF exposure index and to abnormal liver function. Most of the workers who had abnormal liver function had higher ALTS than ASTs, which differs from alcohol-induced liver function abnormality.[15] We conclude that alcohol was not responsible for liver damage. At the time of study, none of the workers ex-

Table 5.—Number of Workers with Muscle Damage,* Stratified by Index of Exposure to Dimethylformamide (DMF) and Hepatitis B Surface Antigen (HBsAg)

Status	Liver function (CPK)	DMF exposure index 0	1	2	1–2
HBsAg(+)	⩾ 161	3	5	2	7
	< 161	18	14	5	19
HBsAg(−)	⩾ 161	4	10	5	15
	< 161	50	53	12	65
Total	⩾ 161	7	15	7	22
	< 161	68	67	17	84
SRR		1.0	2.4	4.2	2.7
$\chi^2(1)$ (Mantel-Haenszel)					5.87 (p = .05)
$\chi^2(1)$ (Mantel-Extension for trend)					5.18 (p = .02)

	Modeling by logistic regression	
	Odds ratio	95% CI
HBsAg	1.68	0.70–4.00
DMF	2.02	1.13–3.60

*Muscle damage was assumed if creatinine phosphokinase (CPK) levels exceeded 161.

amined were taking any specific drugs other than vitamins and antacids prescribed by their physicians for treatment of liver problems and epigastric pain; therefore, hepatotoxic drugs provide an unlikely explanation for liver damage.

Despite the use of many solvents at the factory, air concentrations of solvents—ethyl acetate excepted—were all below TLVs. Ethyl acetate is rarely hepatotoxic, and it probably was not associated with a high prevalence of abnormal liver function in our study. Furthermore, both toluene and methylene dichloride were not in use and were not detected at the workplace.

Whereas chronically active HBsAg carriers are numerous in Taiwan,[7] noncarrier workers had abnormal liver functions that correlated with exposure to higher levels of DMF (Table 2). Exposed workers who had a normal value of ALT still had increased activities of ALT and AST, especially in those who were not HBsAg carriers (Table 3). Therefore, hepatitis B is not singly responsible for liver damage among the factory workers. We checked the e antigen for stronger viral activity among all HbsAg carriers and found that 9 of 43 (21%) were positive; only 3 of 9 had abnormal elevations of ALT (1 was found in each DMF exposure index of 0, 1, and 2, respectively).

We conclude that DMF was responsible for the high prevalence of abnormal ALT and elevated mean aminotransferase activities among synthetic leather workers.

Perhaps HBsAg carriers, who are more susceptible to liver damage by alcohol,[6,7] are more susceptible to hepatotoxic solvents, e.g., DMF. Logistic regression demonstrated an independent effect between HBsAg carrier status and effect of DMF exposure on prevalence of abnormal liver function (Table 2). HBsAg carriers had an increased mixed-function oxidase enzyme function.[16] Whereas DMF is metabolized in the liver, it probably requires this enzyme during demethylation to monomethyl formamide (MMF).[1] HBsAg carriers probably generate MMF more rapidly and in larger quantities. No study has been conducted in which the hepatotoxicity of DMF and MMF in humans has been compared, but animal data reported by Lundberg et al.[17] suggest that MMF is more toxic to the liver than is DMF. The higher susceptibility of HBsAg carriers to DMF might be explained by a more rapid conversion of DMF to MMF that results from an elevated cytochrome p-450 enzyme function. This prompts speculation that other organic solvents or chemicals might have a similar enhanced hepatotoxic effect on HBsAg carriers via this pathway. Our study was relatively small, and the effect of HBsAg only showed a borderline statistical significance; therefore, no strong inference could be drawn. More epidemiological studies and animal studies on duck HBsAg carriers and noncarriers are needed to elucidate the mechanism whereby infection by the hepatitis B virus enhances the hepatotoxic effect of some organic solvents.

Epigastric pain, nausea, and loss of appetite have occurred after exposure to 20 ppm of DMF.[1,18,19] A few studies have reported suspicious liver or lung damage at DMF levels less than 50 ppm.[1,2,17] We found similar symptoms and liver injury at DMF levels of 10–60 ppm (especially at levels greater than 25 ppm) (Tables 2 and 4). Moreover, exposed workers who had normal ALT values had significantly increase transaminase activity (Table 3).

Even though only 11 random, 10-min air samples were obtained, they are representative of the air normally present in the factory. Our on-site observations of work practices and occupational settings confirmed this. Whereas workers who are exposed to higher concentrations of DMF in air are also more likely to have direct skin contact, we are unable to determine what portion of exposure is attributed to inhalation. Nevertheless, we recommend that the 10 ppm TLV for DMF be re-evaluated in the future to insure a proper margin of safety. Biological monitoring and assessment of skin exposure should also be performed and considered in future standard-setting activities.

To date, the detrimental effects of DMF on human muscle tissue have not been reported. Animal studies suggest that DMF damages the myocardium in animals,[18] and that the final metabolite, formamide, may be toxic to muscle fibers in rats and frogs.[1,20] Although our study revealed a clear, linear trend that associated an abnormal level of CPK with degree of DMF exposure (Table 5), further analysis of the isoenzyme of CPK did not show any specific elevation of MB or BB fractions (Table 6). Perhaps damage to muscles is nonspecific. Because CPK is an indicator of local muscle strain in occupational settings,[21] and exposure categories 0, 1, and 2 were associated with a concomitant increase in muscle effort, we tentatively conclude that our findings may indicate that muscle strain occurred more frequently in individuals who were in these exposure categories.

* * * * * * * * * *

This study was supported by a special grant from the Department of Health, Executive Yuan, Republic of China Project No. DOH77-24.

Submitted for publication March 19, 1990; revised; accepted for publication September 10, 1990.

Requests for reprints should be sent to: Dr. Jung-Der Wang, Graduate Institute of Public Health, National Taiwan University, College of Medicine No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan, ROC (10018).

* * * * * * * * * *

References

1. Kennedy GL Jr. Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives. CRC Crit Rev Toxicol 1986; 17:129–82.
2. Scailteur V, Lauwerys RR. Dimethlyformamicle hepatoxicity. Toxicol 1987; 43:231–38.
3. Redlich CA, Beckett WS, Sparer J et al. Liver disease associated with occupational exposure to the solvent dimethylformamide. Ann Intern Med 1988; 108:680–86.
4. Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. New York: Appleton-Century-Crofts, 1978; pp. 303–15.
5. Potter HP. Dimethylformamide-induced abdominal pain and liver injury. Arch Environ Health 1973; 27:340–41.
6. Villa E, Rubbiani L, Barchi T et al. Susceptibility of chronic symptomless HBsAg carriers to ethanol-induced hepatic damage. Lancet 1982; 2:1243–44.

7. Chevillottee G, Durbec JP, Gerolami A et al. Interaction between hepatitis B virus and alcohol consumption in liver cirrhosis: an epidemiologic study. Gastroenterology 1983; 85:141–45.
8. Chen DS, Sung JL. Hepatitis B virus infection and chronic liver diseases in Taiwan. Acta Hepatogastroenterol 1978; 25:423–30.
9. Sung JL, Chen DS, Lai MY et al. Epidemiological study on hepatitis B virus infection in Taiwan. Chinese J Gastroenterol 1984; 1:1–9.
10. National Institute of Occupational Safety and Health (NIOSH). NIOSH manual of analytic methods, 2nd ed. S255, S 49, S343, S329, S1, S3. Cincinnati, OH: NIOSH, U.S. Department of Health, Education and Welfare, 1977.
11. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959; 22:719–48.
12. Mantel N. Chi-square tests with one degree of freedom: extension of Mantel-Haenszel procedure. J Am Stat Assoc 1963; 58: 690–700.
13. Statistics and Epidemiology Research Corporation. EGRET users' manual. Seattle, WA: Statistics and Epidemiology Research Corp., 1988.
14. American Conference of Governmental Industrial Hygienists (ACGIH). Threshold limit values for chemical substances in the work environment adopted by ACGIH. Cincinnati, OH: ACGIH Inc., 1986.
15. Cohen JA, Kaplan MM. The SGOT/SGPT ratio—an indicator of alcoholic liver disease. Dig Dis Sci 1979; 24:835–38.
16. Guebel AP, Pauwels S, Buchet JP, Dumont E, Dive C. Increased cyt P-450 dependent function in healthy HBsAg carriers. Pharmac Ther 1987; 33:193–96.
17. Lundberg I, Lundberg S, Kronevi T. Some observations on dimethlyformamide hepatotoxicity. Toxicol 1981; 22:1–7.
18. Clayton JW, Barnes JR, Hood DB, Schepers GWH. The inhalation toxicity of dimethlyformamide. Am Ind Hyg Assoc J 1963; 24: 144–54.
19. Jacob JS, Rosen ES, Young E. Report on the presence of a toxic substance, dimethylformamide, in sodium fluorescein used for fluorescein angiography. Br J Ophthalmol 1982; 66:567–68.
20. Califano F, Eusetsi F, Marconi PL, Nisini R. Formamide blocks excitation-contraction coupling in mammalian muscles. Cell Mol Biol 1982; 28:593–94.
21. Hagberg M, Michaelson G, Ortelius A. Serum creatinine kinase as an indicator of local muscular strain in experimental and occupational work. Int Arch Occup Environ Health 1982; 50: 377–86.