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Skin penetrating abilities and reservoir effects of neat DMF and DMF/water mixtures

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ABSTRACT

This study was set out to determine the skin permeabilities of neat N, N-dimethylformamide (DMF, denoted as $DMF_{100\%}$) and DMF/water mixtures (including 50% DMF/50% water and 10% DMF/90% water mixtures (v/v), denoted as $DMF_{50\%}$ and $DMF_{10\%}$, respectively) and to assess their skin reservoir effects on the systemic absorption. The penetration fluxes for $DMF_{10\%}$ and $DMF_{50\%}$ (= 0.015 and 0.126 mg/cm^2/h, respectively) were only ~1.1%and 15% in magnitude as that of $DMF_{100\%}$ (= 0.872 ± 0.231 mg/cm^2/h), respectively. The above results could be because the perturbation effect of the DMF content was much more significant than the rehydration effect of the water content on skin permeability. We found that 85.9%, 96.6% and 98.7% of applied doses were still remaining on the skin surface, 4.98%, 0.838% and 0.181% were still remaining in the skin layer, and 9.09%, 2.61% and 1.17% penetrated through the skin layer after the 24-h exposure for $DMF_{100\%}$, $DMF_{50\%}$ and $DMF_{10\%}$, respectively. We found that the half-life ($T_{1/2}$) of DMF retaining in the skin layer were 12.3, 4.07 and 1.24 h for $DMF_{100\%}$, $DMF_{50\%}$ and $DMF_{10\%}$, respectively. The estimated reservoir effect for $DMF_{100\%}$ (= 34.1%) was higher than that of $DMF_{50\%}$ and $DMF_{10\%}$ (= 27.1% and 14.1%, respectively). The above results suggest that the impact associated with the internal burden of DMF could be prolonged even the external exposure of DMF is terminated, particularly for those dermal contact with DMF/water mixtures with high DMF contents.

1. Introduction

N, N-dimethylformamide (DMF) is extensively used in various industries because of its excellent solubility in water and many other organic solvents. The estimated worldwide production rate of DMF is ~250,000 tons per year (Käfferlein et al., 2000). Major adverse health effects associated with DMF exposures include alcohol intolerance (Chivers 1978; Lyle et al., 1979; Cox and Mustchin 1991), hepatotoxicity (Fiorito et al., 1997; Wrbitzky, 1999), male reproductive cancers, possible embryotoxicity, teratogenicity in human and animals (Hansen and Meyer, 1990; Anonymous, 1997; Fail et al., 1998), and sperm mortality perturbation in humans (Chang et al., 2004a). In 2000, DMF was selected as one of four chemicals needed for conducting human field study (Moorman et al., 2000).

Both inhalation and dermal absorption are important routes for human DMF exposures (Mraz and Nohova 1992; Nomiyama et al., 2001; Chang et al., 2004b; Wang et al., 2007). However, when workers are exposed to DMF via both inhalation and dermal absorption routes simultaneously, the latter might play more important role than the former on the resultant body burdens. For example, volunteers with one palm exposed to neat DMF (denoted as $DMF_{100\%}$) for 15 min would result in a similar urinary metabolite (ie., N-methylformamide) concentrations to those exposed to DMF vapor via inhalatory route continuously for 8 h at the level of 60 mg/m^3 (i.e., two times of PEL-TWA) (Mraz and Nohova, 1992). Our previous study found that skin exposure accounted for ~70% of the total internal burdens when workers were exposed to DMF vapor simultaneously via both dermal absorption and inhalation routes (Wang et al., 2007). Here, it should be noted that the above results were all obtained from $DMF_{100\%}$ exposures. But it also should be noted that DMF/water mixtures are also widely used many industrial processes (e.g., synthetic leather manufacturing industries). It is known that the skin permeability of a given chemical depends on its disruption effect on the properties of the skin barrier (i.e., the stratum corneum (SC)). For example, the neat glycol ethers are known to have a dehydration effect on the skin barrier and hence result in the decrease of their skin permeability. On the other hand, the presence of water (i.e., glycol ethers/water mixtures) would result in a rehydrate effect on the skin barrier and lead to the increase of the permeability of glycol ethers (Van der Merwe and Riviere, 2005; Traynor et al., 2007). Therefore, it is

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expected that the skin permeability of $DMF_{100\%}$ and DMF/water mixtures could be very different. In other words, to assess the skin penetrating abilities of DMF/water mixtures could be an important issue for assessing workers' exposures, particularly for those workers directly contacting with DMF/water mixtures.

It is known that the biological monitoring is the best method for assessing skin DMF exposures of workers. Nevertheless, it should be noted that the biological monitoring results could be affected by the skin reservoir effect. For example, an *in vitro* diffusion cell study, focusing on estimating the fate of chemicals after dermal application, has found that the skin reservoir effect had a significant contribution on the amount of material absorbed by the human body (Yourick et al., 2004). But to the best of our knowledge, skin reservoir effects for both $DMF_{100\%}$ and DMF/water mixtures have never been investigated.

The present study was set out first to determine the skin permeability of $DMF_{100\%}$ and DMF/water mixtures. Then, their skin reservoir effects on the systemic absorption were also examined. The results obtained from this study will provide helpful information to assess skin DMF exposures not only for those exposed to $DMF_{100\%}$ but also DMF/water mixtures.

2. Methods

2.1. Preparation of skin sample

The porcine skin (2–4 months old) used in this study was obtained from Taiwan Sugar Corporation (TSC). After the lateral-abdomen skin was removed, its hair was cut and the subcutaneous fat was trimmed. A split-thickness layer of the pretreated skin was prepared by using a dermatome (Padgett Instruments, Kansas City Assemblage Co., Kansas City, MO) set at a thickness of 650 μm. The skin integrity was determined by measuring the electrical impedance across the skin. In the present study, all test skins were found with impedance value less than 4-kΩ (Davies et al., 2004). All skin samples were stored in a −20 °C freezer prior to the experiment being conducted.

2.2. In vitro skin absorption study

Skin penetration experiments were conducted by using an *in vitro* flow-through diffusion cell (Laboratory Glass Apparatus, Berkeley, CA, USA). The cell was equipped with an exposure area of 1 cm^2, a receptor chamber of 3 ml in volume, and operated at a constant temperature (i.e., 37 °C) controlled by a circulating water bath. The receptor chamber was filled with phosphate buffered saline (PBS) and was continuously stirred at 700 rpm by a Teflon-coated magnet. The skin was mounted onto the diffusion cell with the epidermal side up, and the other side of skin was in contact with the receptor fluid (flowrate = ~5.7 ml/h).

In the present study, DMF (HPLC grade, 99.5%) was directly obtained from Tedia Company Inc. (Fairfield, OH, USA). In addition to $DMF_{100\%}$, 50% DMF/50% water and 10% DMF/90% water mixtures (v/v; denoted as $DMF_{50\%}$ and $DMF_{10\%}$, respectively) were prepared. For each experiment, 0.2 ml of $DMF_{100\%}$ (or DMF/water mixture) was applied to the epidermal side of the prepared skin (occluded by the Parafilm) for 24 h. The receptor fluid was collected per 0.5, 1.0, and 2.0 h respectively during the periods of 0–4, 5–12, and 13–24 h to examine its penetration rates during the 24-h exposure period. Immediately after the above 24-h exposure, the skin sample was rinsed by 10 ml de-ion water for ten times to remove the DMF residual remaining on the surface of the skin sample. After the above rinsing procedure, 100 μl of de-ion water was applied on the epidermal side of the skin sample to maintain its moisture content. Then a 48-h extending skin penetration experiment was conducted on each skin sample to examine its post penetration rate after the above 24-h exposure. Here, the receptor fluid was collected per 0.5, 1.0, 6.0, 8.0 and 12.0 h respectively during the post exposure periods of 0–1, 1–2, 2–8, 8–24 and 24–48 h. In the present study, the amount of DMF containing in the rinsed de-ion water was regarded as the DMF remaining on the skin surface after the 24-exposure (denoted as the "unabsorbed"). The total amount of DMF containing in both the receptor fluid collected during the 48-h extending skin penetration experiment and the test skin after the 48-h extending experiment was regarded as the total amount of DMF retaining in the skin layer after the 24-h exposure (denoted as the "skin retention"). Finally, the amount of DMF containing in the receptor fluid collected during the 24-h exposure period was regarded as the amount of DMF penetrated through the skin layer during the 24-h exposure period (denoted as the "skin penetration"). The above three amounts were calculated and were used to check the recovery of DMF from the mass balance aspect.

2.3. Analysis method

For each collected sample (including the "unabsorbed", "skin retention" and "skin penetration"), 0.5 ml sample fluid was first mixed with 0.5 ml methanol (HPLC grade; Tedia), then its DMF content was analyzed by using a gas chromatography equipped with thermionic sensitive detector (Varian 3600 CX GC/TSD; GenTech Scientific, Inc., Arcade, NY, USA) coupled to an auto-sampler (Varian 8200 CX; GenTech). The limit of detection was 0.53 mg/ml. Detailed analytical procedures can be found in our previous publication (Chang et al., 2005).

2.4. Data analysis

The cumulative amount of chemical in the receptor fluid (M) can be described using the following equation (Bronaugh and Maibach, 1985):

$$M = \frac{Dct}{h} - \frac{-hc}{6}\frac{2hc}{\Pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}exp\left(\frac{-Dn^2\Pi^2 t}{h^2}\right) \quad (1)$$

where,

D	diffusion coefficient of chemical in the skin
h	thickness of the skin
c	concentration of chemical in donor compartment
t	elapsed time of the experiment

The steady-state penetration flux (J_{ss}; mg cm^{-2} h^{-1}) of DMF was determined as the slope of the linear part of the curve of the cumulative DMF (i.e., M) by using the linear regression analysis. Lag time (T_{lag}) was also determined from the linear regression as y-axis equal to zero. The permeability coefficient (Kp), representing a chemical's capacity to penetrate the skin, was calculated using the following two equations (Bronaugh and Maibach, 1999):

$$Q = Kp*A*C_v*(T_e - T_{lag}) \quad (2)$$

$$J_{ss} = \frac{Q}{A(T_e - T_{lag})} = Kp*C_v \quad (3)$$

where,

Q	amount of solute absorbed
Kp	permeability coefficient
A	exposure area
C_v	exposure concentration
T_e	exposure time
T_{lag}	lag time
J_{ss}	steady-state penetration flux

The test skin used in the present study consisted of the SC, viable epidermis, and part of dermis with a thickness of 650 μm in total. In