Skin Absorption of Solvent Mixtures
— Effect of Vehicles on Skin Absorption of Toluene —

Hiroshi TSURUTA

National Institute of Industrial Health,
21-1, Nagao 6-chome, Tama-ku, Kawasaki 214, Japan

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Abstract: The skin absorption rates of toluene in various solvent mixtures were investigated in mice. The skin absorption rate of toluene in toluene/methanol mixtures exhibited a parabolic relationship to the mixed ratio. The maximum rate was obtained at a mixed ratio of 50% (V/V). The skin absorption rate of toluene at this point (50%) was about 4.7 times higher than that of pure toluene. The permeability coefficient (Kp) of toluene increased as the mixed ratio of methanol increased. Methanol enhanced skin absorption of toluene. The skin absorption rate of toluene in a toluene/benzene mixture was inversely proportional to the concentration of benzene. The Kp of toluene is kept constant through the mixed ratios of benzene, and benzene does not have an enhancing effect on the skin absorption of toluene contained in the toluene/benzene mixture. We examined the effects of various vehicles on the skin absorption rates of toluene in mixtures containing 50% (v/v) of toluene. Methanol was a good penetration enhancer for toluene, and its effect is similar to the effect of well-known skin penetration enhancers like DMSO, N,N-Dimethylacetamide, and N,N-Dimethylformamide. Therefore, it is necessary to take special precautions against the skin absorption of toluene when handling thinners that contain methanol.

Key words: Skin absorption — Solvent mixtures — Toluene — Methanol — Benzene — Mouse — Penetration enhancer

INTRODUCTION

Toluene is commonly used as a solvent and a thinner component for paints, coatings, adhesives, and inks. Thinners are usually composed of toluene, methanol, ethylacetate, xylene, and methylethylketone. A combination of toluene and methanol is one of the most common paint thinners in Japan[1]. In certain work environments, skin exposure might be a primary route of absorption into the body.

Address correspondence to: Hiroshi Tsuruta, National Institute of Industrial Health, 21-1, Nagao 6-chome, Tama-ku, Kawasaki 214, Japan
In this case, the quantitative evaluation of the skin absorption of organic solvents in work environments is important for managing workers’ health. Moreover, it is important to investigate the skin absorption of toluene from a mixture of methanol and other solvents. The skin absorption of pure toluene in the liquid phase has been investigated in humans\textsuperscript{2-6} and animals\textsuperscript{7-11}, while in the vapor phase, it has been investigated in humans\textsuperscript{12} and animals\textsuperscript{13-15}. However, there have been few reported studies on the skin absorption of solvents in mixtures\textsuperscript{16-19}. The present study used mice to examine the effect of the vehicle on the skin absorption rate of toluene in various solvent mixtures.

**Materials and Methods**

**Materials**

The present study used special-grade solvents obtained from Wako Pure Chem. Co., Tokyo. The purity of all the solvents was examined by gas chromatography using a flame ionization detector, and was found to be higher than 98%. An adhesive agent made of α-cyanoacrylate glue, “Samedain 3000”, was obtained from Semedain Co., Tokyo. A dental cement made of polycarboxylate, “Livicenera”, was obtained from G-C Dental Industrial Co., Tokyo.

**Animals and Treatment**

Male mice (ICR) weighing 30 to 40 g were used in the present study. Each mouse was anesthetized with a subcutaneous injection of 2% pentobarbital solution at a dose of 3 ml/kg, then tracheotomized and connected with a “Y” tracheal cannula. The surgically treated part was sealed using the above-mentioned adhesive agent and dental cement. The cannula was then attached to a rodent respirator (Model 683, Harvard, USA) via a 1/16-inch Teflon tube. The respirator was used to prevent any intake of solvent by respiration, and to keep respiration constant. The skin of the abdominal region was clipped with an electric clipper. A small glass funnel (I.D.: 2 cm) was glued to the abdominal skin using “Samedain 3000”. In the present experiment, the surface area of the skin to be exposed to the test solvent mixture was fixed at 3.14 cm\(^2\). Five hundred microliters of a test solvent mixture was applied to this area for a period of 15, 30, or 60 min. As soon as the test solvent was applied, the top of the glass funnel was closed with a plastic adhesive tape to avoid evaporation. Figure 1 illustrates the entire system used to measure skin absorption in mice.

**Analysis of toluene in the whole body**

At the end of exposure, the mouse was sacrificed with carbon monoxide gas. The skin that had been immersed in the test solvent mixture was removed from the mouse body, together with the glass funnel. The whole body of the mouse was then frozen in liquid nitrogen and further homogenized in liquid nitrogen with
a polytron homogenizer (Kinematica, Switzerland). Then approximately 5 g of the homogenate mixed with liquid nitrogen was placed in a preweighed 50 ml Brown-Erlenmeyer flask with a stopper. After evaporation of the liquid nitrogen, the flask was reweighed to obtain the exact weight of the homogenate. Twenty milliliters of carbon disulfide (CS$_2$) containing an internal standard of ethylbenzene were added immediately thereafter. The flask was shaken vigorously for 1 min and allowed to stand still until the CS$_2$ layer was clearly separated. One microliter of the CS$_2$ layer was injected into a gas chromatograph equipped with a flame ionization detector (Type 5880A, Hewlett-Packard, USA). The toluene was then analyzed by gas chromatography under the following conditions. The chromatograph column was a 4-foot glass tube (I.D. 2 mm, O.D. 6 mm) packed with 60 to 80-mesh Chromosorb 101. The column temperature was 190°C. The carrier gas was nitrogen and its flow rate was 30 ml/min. The injection temperature was 240°C, and the detector temperature was 300°C. The procedure has already been described in detail in the previous paper$^{70}$.

**Determination of skin absorption**

The amount of toluene absorbed through the skin during a given interval was determined by multiplying the skin absorption rate (Ks) by the exposure time. The skin absorption rate (Ks) was calculated based on the amount of toluene remaining in the whole body according to the single-compartment model$^{21}$, and was expressed by the following rate equation. At any time during skin absorption, the rate of change in the amount of toluene remaining in the body (dM/dt) equals the difference between the rate of skin absorption (Ks) and the rate of elimination ($k_e$ x M).

$$dM/dt = Ks - k_e x M$$  \(\text{(1)}\)
where $M$ is the amount remaining in the whole body at time $t$, $K_s$ is the skin absorption rate at time $t$, and $k_e$ is the elimination rate constant from the whole body via expiration and metabolism. Equation (1) can be transformed as follows by integration:

$$K_s = k_e \times \frac{M}{\left[1 - \exp\left(-k_e \times t\right)\right]} \quad (2)$$

Therefore, the skin absorption rate at time $t$ can be determined from the amount remaining at time $t$ and the elimination rate constant. The elimination rate constant is determined by the dose loss from a whole body injected with a single dose. The elimination rate constant (1/h) for toluene was 0.11. The method used has already been described in detail in the previous paper. The permeability coefficient ($K_p$) of toluene in a solvent mixture was determined by dividing the skin absorption rate by the concentration of toluene in the solvent mixture.

**RESULTS AND DISCUSSION**

Figure 2 shows the skin absorption curves of toluene when the content of methanol in a toluene/methanol mixture was 0, 25, 50, and 75% (V/V). Irrespective of the mixture ratio, the amount of toluene absorbed increased linearly with an increase in the exposure time after an initial lag phase, indicating that the skin absorption rate reached a steady state. The skin absorption rate was calculated from a linear regression slope obtained at the steady state, as indicated in Figure 2. The skin absorption rate of pure toluene thus obtained in vivo was 87 µg/hr/cm$^2$ of skin, which agreed with the reported rates obtained in vitro with rat skin (47 µg/hr/cm$^2$) and with human skin (80 µg/hr/cm$^2$). However, a large difference in the skin absorption rate was found between the present in vivo value of 87 µg/hr/cm$^2$ and the reported in vivo values of 14–23 mg toluene/hr/cm$^2$ of skin in humans and 2.95 mg toluene/hr/cm$^2$ of skin in hairless mice. This large difference may be attributable to the fact that different methods were employed for the in vivo measurement of the skin absorption rate of toluene. Skin that had been immersed in the test solvent was excluded for analysis of toluene in the present study, whereas the reported skin absorption rates were determined by a decrease in the weight of toluene on the skin surface of humans and hairless mice. The latter method would tend to overestimate the skin absorption rate because it would not consider the loss of toluene by evaporation.

Figure 3 shows the parabolic relationship between the skin absorption rates of toluene and the ratios of methanol in the toluene/methanol mixtures. The maximum skin absorption rate of toluene was found when the content of methanol in the mixture was 50% (V/V). The skin absorption rate of toluene in this case was about 4.7 times higher than that of pure toluene.

According to Fick's law, the skin absorption rate of a penetrant in a mixture
is proportional to the penetrant concentration, while the permeability coefficient (Kp) is independent of the penetrant concentrations. The independence of Kp from the penetrant concentration in Fick’s law does not hold true, if the vehicle has any concentration-dependent effect on the skin absorption rate of the penetrant in a penetrant/vehicle mixture. Figure 4 shows the relationship between the Kp of toluene and the ratio of methanol in the toluene/methanol mixture. We observed that Kp increased as the ratio of methanol in the mixture increased, indicating that methanol enhances the skin absorption of toluene.

Figure 5 shows the skin absorption curves of toluene when the content of benzene in a toluene/benzene mixture was 0, 25, 50, and 75% (v/v). In sharp contrast to the results obtained with the toluene/methanol mixtures, the amount of toluene absorbed in toluene/benzene mixtures was inversely proportional to the concentration of benzene in the mixture. Irrespective of the mixture ratio, the amount of toluene absorbed increased linearly with an increase in the exposure time after an initial lag phase, indicating that the skin absorption rate reached a steady state. The skin absorption rate was calculated from the linear regression slope obtained at the steady state, as indicated in Figure 5.
Fig. 3. Relationship between skin absorption rate and ratio of methanol in toluene/methanol mixtures.

Fig. 4. Relationship between the permeability coefficient of toluene and the ratio of methanol in toluene/methanol mixtures.
Figure 6 shows the relationship between the skin absorption rates of toluene and the ratios of benzene in a toluene/benzene mixtures. The skin absorption rate was found to decrease as the concentration of benzene in the mixture increased.

Figure 7 shows the relationship between the Kp values of toluene and the ratios of benzene in the toluene/benzene mixtures. The Kp values of toluene remained constant for all mixtures, indicating that benzene in a toluene/benzene mixture does not enhance the skin absorption of toluene.

We examined the effect of various vehicles on the skin absorption of toluene in mixtures containing toluene at a mixture ratio of 50% (V/V) and an exposure time of 30 min. The results are summarized in Table 1. The relative rate of toluene absorption in a solvent mixture compared to toluene alone was calculated using the permeability constants. In the case of methanol, the relative rate was 7.5. Table 1 indicates that well-known skin penetration enhancers such as DMSO, N,N-Dimethylformamide, and N,N-Dimethylacetamide produce high relative rates of 9.0, 6.5, and 4.1, respectively. It can therefore be concluded that methanol is a high penetration enhancer for toluene. The findings in the present study indicate that the enhancing effect of methanol on the skin absorption of toluene is attributable to the facilitation of a lipid-soluble pathway and the fluidization of skin lipids by methanol, which is similar to the reported effect of well-known skin penetration enhancers.
Fig. 6. Relationship between the skin absorption rate and the ratio of benzene in toluene/benzene mixtures.

Fig. 7. Relationship between the permeability coefficient of toluene and the ratio of benzene in toluene/benzene mixtures.
penetration enhancers like DMSO, N,N-Dimethylformamide, and N,N-Dimethylacetamide\(^2\).

The present study has clarified that methanol, the most frequently used solvent in industry, is a strong penetration enhancer, making the dermally absorbed amounts of a toluene/methanol mixture eight times higher than the amounts absorbed when no methanol is present. Therefore, special precautions should be taken when workers handle solvents containing both toluene and methanol, since the latter greatly enhances the skin absorption of the former.

REFERENCES

1) Inoue T, Takeuchi Y, Hisanaga N, Ono Y, Iwata M, Ogata M, Saito K, Sakurai H, Hara I,


