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Non-polar Solvents (Toluene and Styrene) Enhance Methanol Skin Absorption

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ABSTRACT *: The quantitative assessment of the penetration of organic solvents through skin is necessary for the evaluation of health hazards in occupational environments. We investigated the rate of dermal penetration when mixed or single forms of organic solvents were placed into a diffusion cell in vitro or into an experimental animal in vivo. The diffusion rates of methanol, toluene, and styrene were 6.07, 0.129, and 0.046 mg/cm^2/h, respectively. When skin was exposed to the mixed solvent of methanol and toluene, the penetration rate of toluene did not change significantly (0.110 mg/cm^2/h). However, the rate of methanol penetration increased to 43.90 mg/cm^2/h. The penetration rate of methanol also increased significantly to 54.69 mg/cm^2/h by mixing it with styrene. The concentration of methanol in the blood was monitored during the epicutaneous exposure in rats. The blood concentration of methanol was increased by mixing methanol with toluene as seen in the in vitro experiments. These results showed that the penetration rate of organic solvents would be enhanced by mixing them with other solvents.*

Key Words *: Skin absorption, Penetration rate, Diffusion cell, Organic solvents, Rat*

I. INTRODUCTION

The assessment of the percutaneous absorption of organic solvents has been studied by using a diffusion cell *in vitro* (Franz, 1975; Tsuruta, 1977; 1982; Loftsson, 1982; Dugard, 1984; Tojo, 1987; Mathias, 1983), or an experimental animal *in vivo* (Tsuruta, 1975, 1977, 1984, 1987, 1989). Many of these studies have been investigated by exposing a single solvent to the test system. Workers are, however, more likely to be exposed to a mixture of organic solvents in actual occupational settings. In this study, we investigated the percutaneous absorption of mixed organic solvents using the *in vitro* diffusion cell method. The absorption pattern was then compared with the *in vivo* percutaneous absorption.

II. MATERIALS AND METHODS

1. Chemicals

Methanol was purchased from Merck (USA), and toluene and styrene were purchased form Junsei Chemicals (Japan).

2. Experimental animals

Male Sprague Dawley rats were purchased from the Daehan Experimental Center (Korea). The animals were maintained under naturally and ventilated conditions. Filtered water and pelletted food (Purina rodent chow, Korea) were supplied ad libitum.

3. The measurement of *in vitro* skin penetration rate

A 10 week old Sprague Dawley rat was anesthetized with pentobarbital (60 mg/kg, Sigma, USA) and its abdominal hair was shaved by an electrical hair clipper. The abdominal skin was then excised, and the blood vessels and muscle were removed. The excised abdominal skin was glued to the teflon O-ring of the diffusion cell (Tsuruta, 1982) using instant glue (alpha-cyanoacrylate). The teflon O-ring on which the excised abdominal skin was attached was placed between the chambers of the diffusion cell. The skin area was

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2.55 cm^2. The lower chamber of the diffusion cell was filled to the top with 0.9% NaCl solution to prevent bubbling. The upper chamber contained 500 μl of experimental solvents. The diffusion cell was shaken and incubated at 25°C. 500 μl samples were taken at designated time points and analyzed with a gas chromatograph equipped with a head space sampler and FID detector (Hewkett Packard 5890). The lost volume due to the sampling was refilled immediately with fresh NaCl solution. The rate of skin penetration was calculated by using the slope of the steady-state linear region of the graph which is determined by the plots along the cumulative amount of penetration of the solvent versus the application time by using the least-squares method as described by Tsuruta (1996).

4. The measurement of *in vivo* skin penetration rate

A 10 week old Sprague Dawley rat was anesthetized with pentobarbital (60 mg/kg, Sigma, USA) and its abdominal hair was shaven by an electrical hair cutter. The glass skin absorption ring was glued to the shaven abdominal skin by using instant glue (alpha-cyanoacrylate). 750 μl of test solvents were then added into the ring, and the top was sealed with a cover glass to prevent evaporation of the solvent. To sample the blood at designated time points, a heparin contained PE-50 tube was inserted into the carotid artery of the experimental animal. 250 μl blood sample were taken at the designated time points, and mixed with 250 μl each of citric acid (4.8 g/l), sodium citrate (3.2 g/l), and dextrose (14.7 g/l). The solvent concentrations in the blood were determined by gas chromatograph equipped with a head space sampler and FID.

III. RESULTS

1. The rates of skin penetration *in vitro*

The rates of skin penetration for methanol, toluene and styrene were obtained by using the diffusion cell. The rates of skin penetration for methanol, toluene and styrene were 6.07 mg/cm^2/h, 0.129 mg/cm^2/h and 0.046 mg/cm^2/h, respectively (Fig. 1, closed circles; Fig. 2A, closed circles; Fig. 2B, closed squares). When methanol and toluene were mixed 1 : 1 together, the rate was 43.90 mg/cm^2/h for methanol (Fig. 1, closed squares) and 0.110 mg/cm^2/h for toluene (Fig. 2A, open circles). When comparing the mixed solvent to the single solvent, there was virtually no change in the rate of skin penetration for toluene. However, there was major difference regarding methanol; the rate of skin penetration increased approximately 6 times in the mixed solvent than that of the single solvent. Again, when methanol and styrene were mixed 1 : 1 together, the rates for methanol and styrene were 54.69 mg/cm^2/h (Fig. 1, closed triangles) and 0.056 mg/cm^2/h (Fig. 2B, open squares), respectively. The rate of skin penetration for methanol increased nearly 9 fold while styrene remained at a constant rate. All the methanol penetrated the skin within 2 hours (data

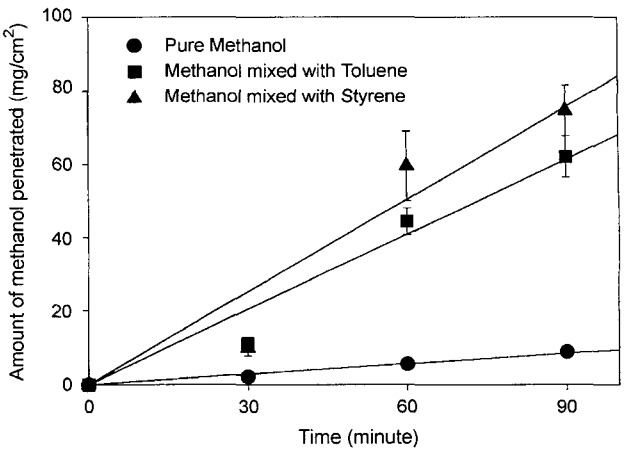

Fig. 1. Skin Absorption curves of methanol and its mixtures with styrene or toluene. Each regression line was calculated by the least squares method. Vertical bars represent a mean and standard error of 4 different experiments.

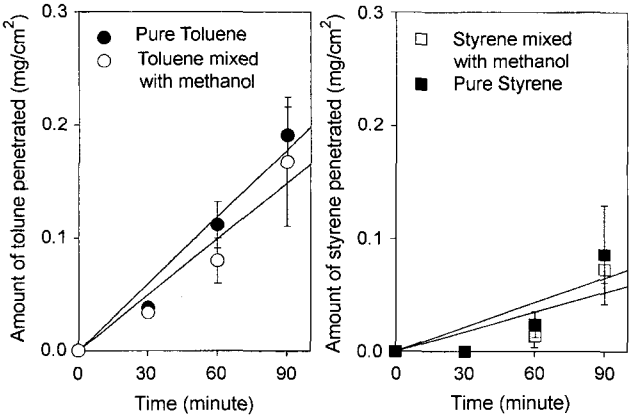

Fig. 2. Skin absorption curves of toluene and styrene. A, toluene and its mixture with methanol; B, styrene and its mixture with methanol. Vertical bars represent a mean and standard error of 4 different experiments.

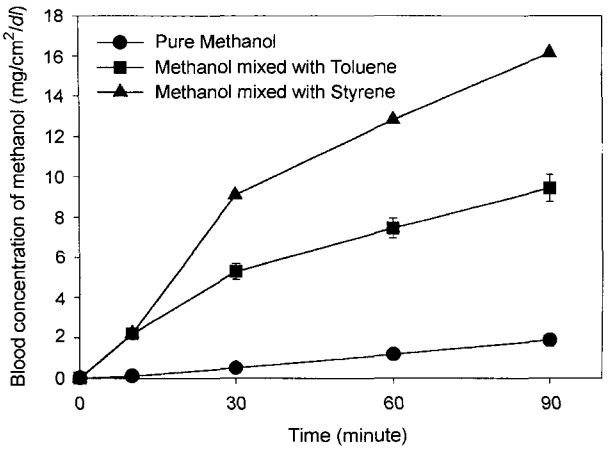

Fig. 3. Blood concentration of methanol absorbed through skin *in vivo*. Vertical bars represent a mean and standard error of 3 different experiments.

not shown).

2. The rate of skin penetration *in vivo*

The rate of skin penetration for methanol increased significantly in the mixed form of methanol and toluene than methanol alone. The rates of penetration *in vivo* monitored from blood samples were very similar to those obtained from the *in vitro* experiments (Fig. 3).

IV. DISCUSSION

Several factors play an important role in determining the rate of skin absorption (Philippe Grandjean, 1990). The vehicle is one of important factors of skin absorption. In occupational settings, chemicals are usually used as mixtures, and the majority of chemicals encountered in industry are rarely used in pure form. The barrier function of skin depends on hydration, age and damage, etc. In our experimental results, when the skin was exposed to the mixed solvents of methanol with styrene or toluene, the rate of skin penetration for styrene or toluene did not change, but the rate of skin penetration for methanol increased nearly 10 fold in the mixed solvents in comparison to methanol alone. In our experiment, non-polar solvents, toluene and styrene might extract or dissolve non-polar components of the epidermis, thus facilitating the diffusion of polar solvents such as methanol.

There is a report that methanol may cause severe local damage to the skin (NIOSH, 1976). Tsuruta (1996) reported that methanol enhanced the absorption of toluene and the skin absorption rate of toluene in toluene/methanol mixtures exhibited a parabolic relationship to the mixed ratio. The report further suggested that methanol was a good penetration enhancer such as DMSO, N,N-dimethylacetamide and N,N-dimethylformamide. Our results, however, showed methanol did not enhance the absorption of toluene *in vitro*. More detailed studies concerning the effect of alcohols on the rate of skin penetration should be done.

REFERENCES

Dugard, P.H., Walker, M., Maudsley, S.J. and Scott, R.C. (1984): Absorption of some glycol ehters through human skin *in vitro* Environ. *Health Persp.*, **57**, 193.

Franz, T.J. (1975): Percutaneous Absorption: On the relevance of *in vitro* data. *J. Incest Dermatol.*, **64**, 190.

Loffsson, T. (1982): Experimental and theoretical model for studying simultaneous transport and metabolism of drugs in excised skin. *Arch. Pharm. Chem. Sci. Ed.*, **10**, 17.

NIOSH (1976): Criteria for a recommended standard, Occupational exposure to methanol. (DHEW publication No. (NIOSH) 76-148.

Mathias, C.G.T. (1983): Percutaneous absorption: an automated technique for *in vitro* measurements using continuous perfusion duffusion cells, *Clin. Res.*, **31**, 586A

Philippe Grandjean (1990): Percutaneous absorption, Skin Penetration: Hazardous Chemicals at Work. 3-34.

Tojo, K. and Chien, Y.W. (1987): Drug permeation across the skin: effect of penetration hydrophilicity, *J. Pharm Sci.*, **76**, 123.

Tsuruta, H. (1975): Percutaneous absorption of organic solvents, *Industrial Health*, **13**, 227-236.

Tsuruta, H. (1977): Percutaneous absorption of organic solvents, *Industrial Health*, **15**, 131-139.

Tsuruta, H. (1982): Percutaneous absorption of organic solvents, *Industrial Health*, **20**, 335-345.

Tsuruta, H. and Iwasaki, K. (1984): A procedure for determining volatile solvents in mouse whole body, **22**, 219-222.

Tsuruta, H., Iwasaki, K. and Kanno, S. (1987): A method for calculating the skin absorption rate from the amount retained in the whole body of skin-absorbed toluene in mice, *Industrial Health*, **25**, 215-220.

Tsuruta, H. (1989): Skin absorption of organic solvent vapors in nude mice *in vivo*, *Industrial Health*, **27**, 37-47.