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IN VIVO PERCUTANEOUS ABSORPTION OF FRAGRANCE INGREDIENTS IN RHESUS MONKEYS AND HUMANS

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Abstract—The percutaneous absorption of the fragrance diethyl maleate was measured *in vivo* in human and monkey studies. With the application sites occluded, 54% of the applied dose of the volatile fragrance penetrated human skin in 24 hr compared with 69% absorption in the monkey skin. It was concluded that the monkey is a good model for human skin with regard to the penetration of this fragrance material since no significant difference in the absorption of diethyl maleate was observed. The percutaneous absorption of the fragrances benzyl acetate and five other benzyl derivatives (benzyl alcohol, benzyl benzoate, benzamide, benzoin and benzophenone) was determined *in vivo* in monkeys. Absorption through occluded skin was high for all compounds (approximately 70% of the applied dose in 24 hr) and no significant differences between the values for the different compounds were observed. No correlations were seen between skin penetration of these compounds and their octanol–water partition coefficients. Under unoccluded conditions skin penetration of the fragrances was reduced and there was great variability between compounds, presumably because of variations in the rates of evaporation from the site of application. The data suggest that humans may have significant systemic exposure to these fragrance materials.

INTRODUCTION

Fragrance ingredients used in cosmetic and other products are lipophilic compounds and therefore have the potential to be readily absorbed through skin. We previously demonstrated (Bronaugh *et al.*, 1985) that cinnamyl anthranilate and safrole, fragrances found to be carcinogenic in chronic animal feeding studies, are absorbed through human and monkey skin. For the highly volatile compound safrole, 4% of the applied dose was absorbed through monkey skin *in vivo* after an unoccluded application. For the less volatile cinnamyl anthranilate, 26% of the applied dose was absorbed.

In those studies (Bronaugh *et al.*, 1985), we also showed a similarity in absorption values for monkeys (*in vivo*) and humans (*in vitro*) for the fragrance ingredients safrole, cinnamyl anthranilate, cinnamic alcohol and cinnamic acid. Best agreement was obtained when application sites were occluded to prevent evaporation. We have now further examined the usefulness of the monkey as an animal model for human skin absorption studies by comparing the absorption of the fragrance diethyl maleate *in vivo* in humans and in monkeys. This compound has previously been demonstrated to cause skin sensitization in humans (Marzulli and Maibach, 1980).

Recently, the toxicity of benzyl acetate was evaluated in a chronic animal gavage study (National Toxicology Program, 1986). It was concluded that some evidence of carcinogenicity was found in mice for this fragrance. Benzyl acetate is a widely used fragrance ingredient with annual usage in the USA alone of 1 million kg (Opdyke, 1973). Many other benzyl derivatives are used as fragrance ingredients in cosmetics, and it seemed important to us to investigate the percutaneous absorption of some of these compounds by using *in vivo* techniques in the monkey. Benzyl acetate and five other related fragrances (benzyl alcohol, benzyl benzoate, benzamide, benzoin and benzophenone) were selected for study. Compounds with differing solubility properties were chosen to investigate the possibility of correlations between oil and water partitioning, chemical structure and skin permeation.

Abbreviations: ANOVA = analysis of variance; Ko/w = octanol–water partition coefficient.

MATERIALS AND METHODS

Radiolabelled compounds were used to facilitate detection in the biological samples. [1-^{14}C] Diethyl maleate (specific activity 17.56 mCi/mmol; purity 99%) was obtained from NEN Products (Boston, MA, USA). Amersham Corporation (Arlington Heights, IL, USA) supplied [7-^{14}C]-benzyl acetate (53 mCi/mmol; 96%), [7-^{14}C]benzyl alcohol (14.8 mCi/mmol; 97%) and [7-^{14}C]benzyl benzoate (7.2 mCi/mmol; 97%). Pathfinder Laboratories (St Louis, MO, USA) was the source of [*ring*-U-^{14}C]benzamide (5.29 mCi/mmol; 98.8%), [*carbonyl*-^{14}C]benzoin (9.2 mCi/mmol; 98%) and [*carbonyl*-^{14}C]benzophenone (5.4 mCi/mmol; 99.9%).

The human *in vivo* skin absorption studies were performed according to the procedure of Bucks *et al.* (1988). Six adult human volunteers each received a single 20-μl dose (0.91 μCi) of [^{14}C]diethyl maleate in acetone by topical application to 2.5 cm^2 of forearm skin (chemical dose = 4 μg/cm^2). The site was then occluded by using a polypropylene chamber (Hill Top Laboratories, Cincinnati, OH, USA) after the

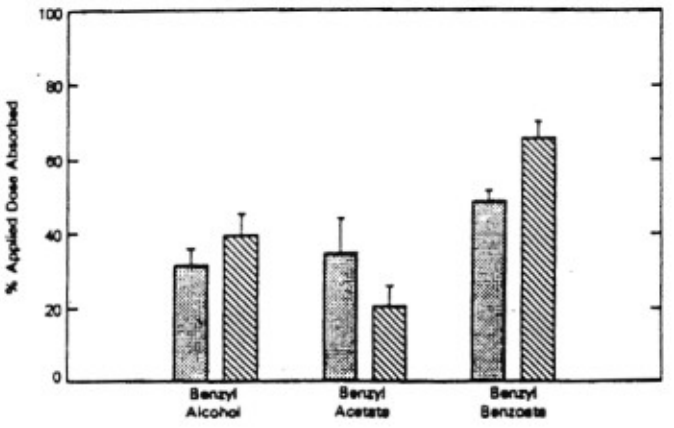

Fig. 2. Effect of vehicle on percutaneous absorption of fragrances. Application sites were unoccluded. Values are means ± SEM for four monkeys. The same monkeys were used for each compound. Benzyl alcohol and benzyl benzoate were applied to skin in 10 mg lotion/cm² skin. Benzyl acetate was applied in 30 mg lotion/cm². ■ = acetone vehicle; ■ = moisturizing lotion vehicle.

when compared with absorption after application in an acetone vehicle (t-test, $P > 0.05$).

DISCUSSION

While this work was in progress, studies measuring the percutaneous absorption of benzyl acetate *in vivo* in the rat were reported (Chidgey *et al.*, 1987). Similarities in methodology were that a volatile vehicle, ethanol, was utilized, and the sites of application were occluded. However, important differences in the technique used were that unabsorbed material was removed after only 6 hr of contact with the skin instead of the 24 hr permitted in our study, and that the absorption results of Chidgey *et al.* give only 24-hr urinary excretion values uncorrected for elimination by other routes. Also, the topical dose applied was approximately 1000-fold higher than that used in the present study; we used a concentration of benzyl acetate expected to be applied to skin during use of cosmetic products (Opdyke, 1973). The lowest concentration used by Chidgey *et al.* (1987; 3.2 mg/cm²) was chosen in order to duplicate the oral dose administered in the carcinogenesis bioassay conducted by the National Toxicology Program (1986). The absorption values for benzyl acetate in the two studies are therefore difficult to compare. The almost two-fold higher values in our study for the percentage of the applied dose absorbed (78.7% *v.* 39.7%) may be partially explained on the basis of the lower dose, longer application time and more complete accounting of the absorbed material in the present study—all factors that would enhance percutaneous absorption. However, rat skin would be expected to be more permeable to benzyl acetate than monkey skin, based on the limited previous studies (Franklin *et al.*, 1986).

Comparison of the percutaneous absorption of the fragrance ingredients with their octanol–water partition coefficients was informative. Since similar absorption values were obtained under occluded conditions, no correlation was obtained between skin permeation and the Ko/w values. The inability of the Ko/w values to predict skin permeation may be due to the failure of a partition coefficient to reflect absolute water and oil solubility. As the Ko/w increased from 4.4 (benzamide) to 9333 (benzyl benzoate), the water solubility of these fragrances decreased. Therefore the actual lipid solubility (g/litre) of the compounds with the high Ko/w values may, in fact, not differ substantially from that of the compounds with the lower Ko/w values. The high lipid solubility of these latter compounds was likely to be responsible for the fact that their skin penetration, observed under occluded conditions, was rapid and similar to that of the compounds with high Ko/w values.

In the measurement of absorption of volatile compounds, two competing factors are involved: absorption and evaporation. As evaporation is reduced, for example by occlusion of the skin, absorption may be increased. The effect of vehicles on the evaporation of a test compound will also influence the absorption results. In these experiments, the moisturizing lotion, at low doses (10 mg/cm²), may have decreased evaporation when compared with the volatile acetone vehicle. At high doses (30 mg/cm²), however, the lotion appears to have other effects as well, since no increase in the absorption of benzyl acetate was observed.

The metabolism of the test compounds was not examined in this study. The radioactivity determined in urine samples may differ in chemical identity from that of the parent compound but is useful as a measure of skin absorption. Benzyl acetate is known to be metabolized substantially by esterases found in the skin, blood and other organs.

The skin of the rhesus monkey was again shown to resemble human skin with regard to the percutaneous absorption of fragrance ingredients. No significant difference was observed in the absorption of diethyl maleate applied to human and monkey skin. The absorption of benzyl acetate and other benzyl derivatives measured *in vivo* in the monkey may be predictive of the ability of these compounds to be absorbed when applied to human skin under the conditions used in this study.

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