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In Vivo and *In Vitro* Dermal Penetration of Lipophilic and Hydrophilic Pesticides in Mice

R. E. Grissom, Jr., C. Brownie, and F. E. Guthrie

Toxicology Program, Box 7633, North Carolina State University, Raleigh, North Carolina 27695–7633

Dermal absorption is a major portal of entry for a wide variety of potentially toxic substances. In vivo and in vitro investigations assessing penetration of topically applied xenobiotics using both human and other animals have been conducted (Feldmann and Maibach, 1970; Franz, 1975; Grissom et al., 1985; Kao et al., 1985). Current ethical considerations have drastically curtailed the testing of xenobiotics in human volunteers; consequently, dermal penetration in humans is usually estimated from in vivo tests in animals and in vitro tests using either human or animal skin.

The relationship of dermal penetration between humans and other species of animals is often variable. A number of studies have attempted to show a relationship between dermal penetration in various species of animals and humans, but no single animal species can be consistently used to predict dermal penetration in humans (Montagna, 1971; Wester and Maibach, 1983).

In vitro methods using diffusion cells for both human and animal skins have been used to assess dermal penetration (Bronaugh and Maibach, 1983; Franz, 1975; Kao et al., 1985). Diffusion cell procedures have the advantage of being rapid, inexpensive, and easy to perform. Accurate results can be obtained since both the bathing medium and the skin patch can be assayed for radioactivity. Finally, diffusion cell procedures are an ethically acceptable method for assessing dermal penetration of toxic compounds through human skin.

In order for in vitro penetration results to be meaningful, there needs to be a close relationship with in vivo data. In vitro studies conducted using human skin (Franz, 1975) and rat skin (Bronaugh et al., 1982) suggest that there is a relationship between in vivo and in vitro dermal penetration of hydrophilic compounds; however, a correlation between in vivo and in vitro penetration of lipophilic compound continues to be problematic (Franz, 1975; Bronaugh et al., 1982). The objective of the

Send reprint requests to F. E. Guthrie at the above address.

present study was to investigate the relationship between *in vivo* and *in vitro* penetration of both hydrophilic and lipophilic compounds in mice.

MATERIALS AND METHODS

Fenvalerate (^{14}C-phenoxyphenyl labeled; sp act, 19.6 mCi/mmol) was obtained from Shell Development Corporation (Houston, TX). DDT (^{14}C-ring labeled; sp act, 29.7 mCi/mmol), and 2,4-D(^{14}C-ring labeled; sp act, 1.2 mCi/mmol) were obtained from Amersham Company (Arlington Heights, IL). Vamidothion (^{14}C-1,2-ethyl-thioethyl labeled; sp act, 27.0 mCi/mmol) was obtained from Rohne-Poulenc, France via Rohne-Poulenc Japan, Ltd. and Shionogi and Co., Ltd. Permethrin (^{14}C alcohol-labeled; sp act, 55.9 mCi/mmol) was obtained from FMC Corporation (Princeton, NJ). The purity of all compounds was 98% or greater. Partition coefficients, $CHCl_3$:water, were 87, 532, 6.9×10^{-3}, 2.5×10^{-4}, and 269, respectively.

The procedures were similar to those previously described (Grissom *et al.*, 1985). Twenty-six to twenty-eight gram female ICR mice were obtained from Dominion Laboratories (Dublin, VA) and given 48 hr to acclimate to laboratory conditions (photoperiod, 12:12; Purina rat chow (Purina, St. Louis, MO) and water *ad libium*)). The neck and upper back was clipped with electric clippers (Oster, Milwaukee, WI) 24 hr prior to treatment. A polyethylene ring (1.4 cm^2) containing ventilation holes was attached to the upper back with a cyanoacrylate adhesive. One hundred µl of acetone containing approximately 1 µCi of radiolabeled pesticide was mixed with non-radioactive pesticide to equal 1 mg/kg and applied to the surface of the skin inside the ring (20 $\mu g/cm^2$).

The dose was applied in approximately 3 µl aliquots with gentle evaporation of the solvent until the entire dose was applied. A cover was attached to the ring to protect the site of application. Three mice, chosen at random, for each compound tested at each time interval were placed individually in mouse metabolism cages.

The mice were killed at 1, 6, 24, and 48 hr of exposure ($CHCl_3$). The protective cap was removed, and any compound remaining on the cap was extracted with acetone. The dose available for penetration consisted of the applied dose minus the amount of compound found on the cap. The skin at the site of application was removed. The remainder of the mouse after the skin patch was removed was termed carcass. The urine and feces were removed from the metabolism cages, and the cages were rinsed with water followed by acetone and methanol. Aliquots of urine were placed in Insta Gel (Packard Instrument Co., Downers Grove, IL) and counted in a Packard Tricarb Scintillation Counter (Packard

Instrument Co., Downers Grove, IL). The carcass was homogenized in liquid nitrogen. The feces, aliquots of the carcass homogenate, and skin patches were combusted in a $^{14}CO_2$ trapping tissue oxidizer (Oxi-One, Radioanalytic Instrument and Chemical Co., Tampa, FL) which dispensed approximately 7.4 ml of an organic amine (Oxi-amin) and 12 ml of scintillation fluid (Oxi-scint) per sample. Total penetration was calculated as disappearance of radioactivity from the site of application and also as appearance of radioactivity in the carcass and excreta. All counts were converted to dpm. Quenching was corrected by internal standardization.

A static diffusion cell system containing eight individual cells (Crown Glass Co., Somerville, NJ) was used in the *in vitro* studies. New born calf serum (NBCS) containing 0.02 ml of gentamicin (Gibco, Grand Island, NY) per ml of NBCS was the bathing medium. A water circulator (HAAKE, Berlin, West Germany) kept the temperature of the diffusion cell system at 37°C. Female ICR mice with litters were obtained from Dominion Laboratories (Dublin, VA). The mice were maintained as described for the *in vivo* studies. Since it was not possible to utilize skin from 26 to 28 gram mice in the *in vitro* study due to technical problems involving epidermal-dermal separation, the skins from 8 to 9 gram mice were prepared for use on diffusion cells. Previous work investigating *in vitro* penetration has shown that animals of the ages used in this study appear to have similar penetration of topically applied compounds (Tregear, 1966). When the mice were 8 to 9 grams in weight, they were killed, and the hair on the back and neck was clipped. Care was taken not to damage the skin during clipping. The skin was removed and immediately mounted on the diffusion cells or was placed dermal side down in a petri dish containing 10 mg of collagenase (Worthington Diagnostics, Freehold, NJ) dissolved in 10 ml of phosphate buffered saline. Eight skins were individually incubated in the collagenase solution for approximately 3 hr.

After incubation, the epidermis was separated from the dermis with running water. The skins were inspected for dermal removal and damage under a disecting scope (Bausch & Lomb, Rochester, NY) and placed epidermal side up on the diffusion cell. Air bubbles were removed from beneath the skins. Caps were placed on the side arms of the diffusion cells to prevent evaporation, and the skins were held overnight. Evaporation, as well as visual inspection, was used as an indication of damage. Cells exhibiting evaporation greater than that observed for full thickness skins were removed from the study. The dose used in the *in vitro* studies was the same as that used in the *in vivo* studies except that the radioactivity was reduced to 0.045 µCi and the volume of the dose was reduced to 10 µl. The dose was applied to split thickness skin (1.13 cm^2) for all compounds and on full thickness skins being treated with DDT, fenvalerate and

permethrin. Approximately 3 µl of the dose were applied and the solvent was evaporated, as explained previously. The volume of the dose was reduced to 10 µl in the in vitro studies to reduce the amount of time required to apply the dose. Preliminary tests comparing in vitro penetration of each chemical disolved in 100 µl and 10 µl of acetone indicated that the volume of the solvent did not affect penetration.

Two hundred µl aliquots of medium were collected from each cell at 1, 6, 24, and 48 hr for each pesticide. At each sampling time, the amount of medium removed was immediately replaced with an equal volume of fresh medium. At the 48 hr sample time, the volume of the medium in each cell was determined, and the skins were removed, combusted, and radioactivity determined. Penetration at 1, 6, and 24 hr was based on radioactivity in the bathing medium. At 48 hr, penetration was based on both disappearance of radioactivity from the skin and appearance of radioactivity in the bathing medium. All counts were converted to dpm to facilitate analysis of the data.

Mean penetration for the in vitro and in vivo methods was compared for each time point. Approximate t-tests (Snedecor and Cochran, 1980) were used to make these comparisons because variability was not the same across methods nor for all chemicals within a method. A significance level of .05 was used for each comparison.

RESULTS AND DISCUSSION

In order for in vitro penetration to be similar to in vivo penetration, the barriers to in vitro penetration of topically applied compounds should be similar to those found in vivo. Normally, topically applied compounds penetrate through the epidermis and into the dermis where they enter the microcirculation of the papillary layer and do not penetrate into the lower dermis to an appreciable extent (Scheuplein and Blank, 1971). When skin is excised and mounted on a diffusion cell, the microcirculation is destroyed. Compounds applied to excised skin not only have to penetrate through the epidermis but must also traverse additional tissues before reaching an underlying bathing medium (Bronaugh and Maibach, 1983).

There does not appear to be consistent agreement on the medium that is most appropriate for use in diffusion cells. There appears to be a consensus, however, that failure to include appropriate receptors in the bathing medium may adversely influence penetration of lipophilic compounds. Previous work has shown that lipoproteins may be important for transport (Maliwal and Guthrie, 1982) and cellular uptake (Kaminiski, el al., 1985) of lipophilic compounds; therefore, NBCS was chosen for the bathing medium because it supplies an aqueous phase for hydro-

philic compounds and appropriate receptors for lipophilic compounds.

In *in vitro* studies, grooming cannot occur, and diffusion cells do not permit loss of epidermal cells caused by normal exfoliation of the epidermis. This is considered to be a potential source of error since any radioactivity attached to the exfoliated cells will be trapped at the site of application. The non-occlusive protective devise used in the *in vivo* studies prevented the mice from grooming the site of application of the dose as well as preventing the shedding of epidermal cells.

Figs. 1-3 show the comparison between *in vivo* and *in vitro* penetration of the compounds tested. The *in vivo* data at 1, 6, and 24 hr of treatment were taken from a previous study (Grissom *et al.*, 1985). Greater than 94% of the radioactivity was recovered in all cases. Results are expressed as percent of recovered dose.

The results of this study indicate that *in vivo* penetration of both hydrophilic and lipophilic compounds compares favorably with *in vitro* penetration when the hypodermis and deep dermis is removed as indicated in Figs. 1-3. With full thickness skin, very little *in vitro* penetration of lipophilic compounds occurred. This suggests that the lipophilic compounds are probably binding in the adipose or muscle layers of the hypodermis. Preliminary studies with 2,4-D indicated that *in vitro* penetration of compounds through full thickness skins was similar to *in vivo* penetration at 24 hr of exposure (unpublished results) suggesting that the adipose and muscle layers do not appear to bind hydrophilic compounds.

Previous work has found a reasonable agreement between *in vivo* and *in vitro* penetration for hydrophilic compounds (Bronaugh *et al.*, 1982). Hawkins and Reifenrath (1984) demonstrated that *in vitro* penetration of benzoic acid was increased when the epidermis was removed; however, penetration of DDT did not change appreciably. This suggests that the epidermis is a primary barrier to hydrophilic compounds while it is not the primary barrier to *in vitro* penetration of lipophilic compounds.

The goal of penetration studies is to be able to predict *in vivo* dermal penetration of topically applied xenobiotics from *in vitro* penetration data. The *in vitro* penetration data for vamidothion and DDT were collected prior to examination of *in vivo* penetration. The predicted penetration was similar as shown in Figs. 1a and 2b. Further work investigating *in vivo* and *in vitro* barriers to penetration may help reduce the variation observed between *in vivo* and *in vitro* studies and make extrapolations from *in vitro* to *in vivo* penetration possible in other species of animals including humans.

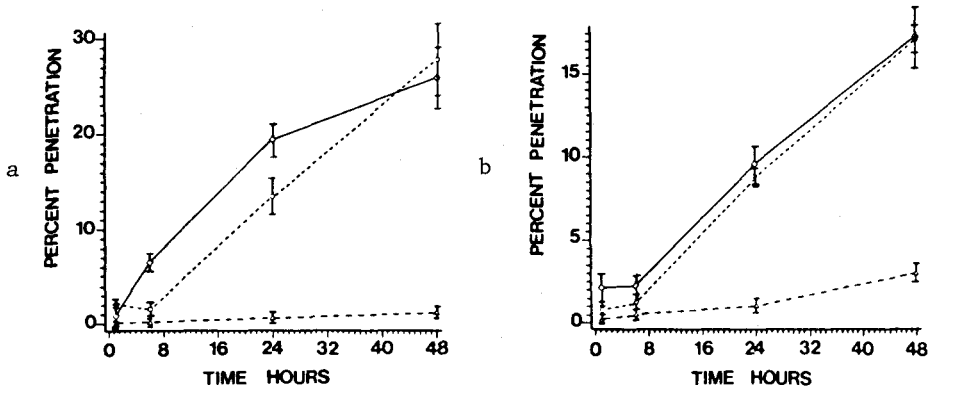

Figure 1. a. Dermal penetration of DDT. In vivo (—◇—), in vitro split thickness (---0---), and in vitro full thickness (--△--) cutaneous penetration of DDT. Values are X±SE for three mice in vivo and eight mice in vitro. b. Dermal penetration of fenvalerate. In vivo (◇), in vitro split thickness (---0---), and in vitro full thickness (--△--) cutaneous penetration of fenvalerate. Values are X±SE for three mice in vivo and eight mice in vitro.

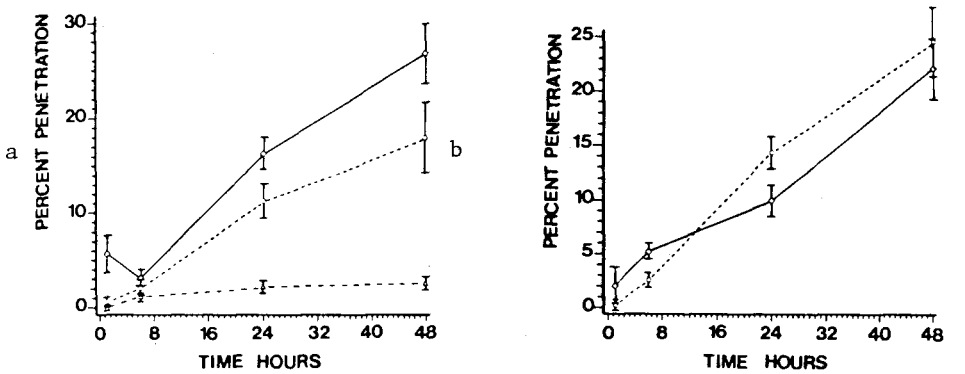

Figure 2. a. Dermal penetration of permethrin. In vivo (—◇—), in vitro split thickness (---0---), and in vitro full thickness (--△--) cutaneous penetration of permethrin. Values are X±SE for three mice in vivo and eight mice in vitro. b. Dermal penetration of vamidothion. In vivo (—◇—), in vitro split thickness (---0---) cutaneous penetration of vamidothion. Values are X±SE for three mice in vivo and eight mice in vitro.

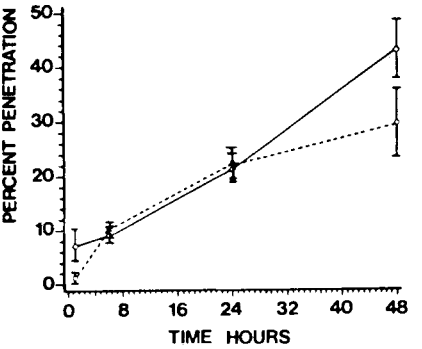

Figure 3. Dermal penetration of 2,4-D. *In vivo* (—◇—) and *in vitro* split thickness (---0---) cutaneous penetration of 2,4-D. Values are X±SE for three mice *in vivo* and eight mice *in vitro*.

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