

# Appendix E

## Percutaneous Absorption<sup>1</sup>

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### **IN VITRO PASSIVE DIFFUSION**

Most *in vitro* techniques entail placing excised skin in a diffusion chamber, applying a chemical compound to its surface, and assaying the skin for the presence of the compound in the collection vessel on the other side. Excised human skin, animal skin, or artificial membranes can be used, and the skin may be intact or separated into epidermis and dermis (Wester and Maibach, 1993; Bronaugh, 1997; and Roberts et al., 1999). *In vitro* systems can be used to test the percutaneous absorption of chemicals that are too toxic to test in humans.

*In vivo*, the penetrating compound may not pass completely through the dermis but may be removed by metabolic mechanisms, such as through capillaries, and enter the blood stream causing systemic effects. With *in vitro* systems, skin metabolism can be studied in viable skin without interference from systemic metabolic processes. Absorption measurements can be obtained more easily from diffusion cells than from analyses of biological specimens from clinical studies. *In vitro* techniques are easy to use, and the results can be obtained rapidly. A disadvantage, however, is that the collection bath is saline, thus compatible with hydrophilic but not hydrophobic compounds.

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<sup>1</sup>The following material was prepared for the use of the principal investigators of this study. The opinions and conclusions herein are the authors' and not necessarily those of the National Research Council.

## COMPARTMENTAL MODELS

Compartmental models are alternatives to diffusion models of percutaneous absorption. Absorption of solute through the skin is generally assumed to follow first-order kinetics. Much of the data analyzed with compartmental models is characterized by "flip-flop" kinetics (i.e., the absorption half-time is much longer than the elimination half-time) (Roberts et al., 1999).

## STRIPPING MODELS

Stripping models can be used to determine the concentration of chemicals in the stratum corneum at the end of a short application period (e.g., 30 minutes). First, the chemical is applied to skin of animals or humans. After 30 minutes, the stratum corneum is removed by successive applications of tape (Rougier et al., 1999; Surber et al., 1999). By linear extrapolation, stripping models can predict the percutaneous absorption of that chemical for longer periods. Rougier and coworkers (1986) established a linear relationship between the stratum corneum, reservoir content, and percutaneous absorption using the standard urinary excretion method (Feldmann and Maibach, 1967). The major advantages of the stripping method are: (1) absorption can be determined independent of urinary (and fecal) excretion; and (2) nonradiolabeled percutaneous absorption can be determined because the stripped skin samples contain enough chemical for modern chemical assay methods (Wester and Maibach, 1999a).

## RADIOISOTOPIC TRACER METHODS

Radiolabeled compounds are widely used as tracers in both *in vitro* and *in vivo* studies. Many radiochemicals are commercially available; others may be synthesized to order. Radiochemicals are usually used to determine the amount of radioactivity in the "dermal" compartment (receiver fluid) or in the skin compartment (epidermis, dermis).

Radiochemicals are also used to determine percutaneous absorption *in vivo* by the indirect method of measuring radioactivity in excreta (urine and feces) after topical application. Plasma radioactivity can be measured and the percutaneous absorption determined by the ratio of the areas under the plasma concentration to time curves following topical and intravenous administration (Wester and Maibach, 1999a). This method can detect low levels of chemical absorption.

## ACCELERATOR MASS SPECTROMETER

Accelerator mass spectrometry (AMS) uses mass selection and energy gain to separate the isotope of carbon (and other elements) so that ions of the radioisotope can be counted. Tissue samples can be analyzed to quantify radioisotopes regardless of their decay times (Keating et al., 1999). AMS has distinct advantages over other methods of measuring percutaneous penetration. First, its analytic sensitivity is a thousand times greater than liquid scintillation counting (LSC). Therefore, flux determinations can be made using test chemicals at low enough concentrations to conduct human *in vivo* studies. AMS can also be used with other methods to quantify chemical absorption on tape strips after *in vivo* human dermal exposure (Keating et al., 1999).

Gilman et al. (1998) used AMS to detect  $^{14}\text{C}$ -labeled urinary metabolites of atrazine (a triazine herbicide) and compared the analytical performance of AMS with LSC. Human subjects were given a dermal dose of  $^{14}\text{C}$ -labeled atrazine over a 24-hour period. Urine samples from the subjects were collected over a seven-day period. The concentrations of  $^{14}\text{C}$  in the samples determined by AMS and LSC ranged from 1.8 fmol/mL to 4.3 pmol/mL. The data from these two methods have a correlation coefficient of 0.998 for a linear plot of the entire sample set. AMS provides concentration (2.2 vs. 27 fmol/mL) and mass (5.5 vs. 54,000 amol) detection limits superior to those of LSC for these samples. The precision of the data provided by AMS for low-level samples is 1.7 percent; the day-to-day reproducibility of the AMS measurements is 3.9 percent.

## REAL-TIME *IN VIVO* BIOAVAILABILITY

Wester and Maibach (1999a) used a real-time *in vivo* method to determine the bioavailability of organic solvents following dermal exposure. Breath analysis was used to obtain real-time measurements of volatile organic compounds in expired air following exposure. Human volunteers and animals breathed fresh air via a breath inlet system for continuous real-time analysis of undiluted exhaled air. The air supply system was self-contained and separated from the exposure solvent-laden environment. The system used an ion-trap mass spectrometer equipped with an atmospheric sampling glow discharge ionization source. The ion-trap mass spectrometer system was used to measure individual chemical components in the breath stream in the single-digit parts per billion detectable range for each of the compounds proposed for study, while maintaining linearity of response over a wide dynamic range.

## OCCLUSION

Occlusion is covering the applied dose, either intentionally (e.g., bandaging) or unintentionally (e.g., putting on clothing) after applying a topical agent. A vehicle such as an ointment can also have occlusive properties. Occlusion results in a combination of many physical factors that affect the skin and the applied compound by enhancing hydration and sometimes increasing skin temperature. Occlusion also prevents the accidental wiping or evaporation of the applied compound, in essence ensuring a higher applied dose. Occlusion increases flux and is synergistic with skin damage (Wester and Maibach, 1983). Occlusion is a practical clinical method of enhancing percutaneous absorption, which suggests that its use in chemical defense should be studied further.

The relationship between occlusion and rate of penetration depends on the solubility of the penetrant. Furthermore, the extent of penetration may depend on the method of occlusion (Bucks and Maibach, 1999).

## REGIONAL VARIATION

Feldmann and Maibach (1967) systematically investigated regional variations in percutaneous absorption and found that the absorption of hydrocortisone differed at different anatomical sites. The scrotum was the highest absorbing skin site and the sole of the foot the lowest. Other studies have also focused on the influence of anatomical site on the absorption of various drugs and chemicals in humans and in animals (Wester and Maibach, 1999b). For example, scopolamine transdermal systems are placed in the postauricular area because an effective amount of the drug is absorbed at this site.

Mathematical models are used to estimate human health hazards of environmental contaminants even though data may be available for only one anatomic site. With toxicants on the exposed areas of the skin (head, face, and neck), flux will be greater than on glabrous skin. Estimates of skin absorption rates are integral to estimates of potential hazards (Wester and Maibach, 1999b).

## ANIMAL VERSUS HUMAN STUDIES

Human skin is unique, and the structural differences in various animal species may or may not affect the penetrability of a specific compound. Numerous *in vivo* and *in vitro* studies have been conducted comparing percutaneous absorption in animal and human skin. In general,

the skin of monkeys (rhesus and squirrel) and weanling pig most resembles human skin. The skin of rats and rabbits is more permeable than human skin. Animals can be used to generate kinetic data; but no one animal skin can simulate the percutaneous penetration in humans for all compounds. Therefore, the best estimates of human percutaneous absorption are based on *in vivo* experiments on humans (Zhai and Maibach, 1996).

### IN VITRO VERSUS IN VIVO STUDIES

Methods of *in vitro* percutaneous absorption are widely used to measure the absorption of topically applied compounds. A major advantage of *in vitro* systems is that they can be used to test compounds that are too toxic to test in humans. Metabolism can be measured if viable skin is obtained and the viability is maintained in the diffusion cells (Wester et al., 1998). Skin metabolism can be studied in viable skin without interference from systemic metabolic processes (Bronaugh et al., 1999). Finally, absorption measurements can be made more easily from diffusion cells than from analyses of biological specimens from clinical studies.

*In vitro* methods are simple, rapid, and safe and are recommended as a first step in defining percutaneous absorption. The major disadvantages of *in vitro* tests are: (1) excised (and usually stored) skin may not retain full enzymatic activity; (2) drug metabolism probably does not affect the amount of compound entering the stratum corneum, but it may affect the metabolic profile emerging from the skin; (3) the collection bath is saline, which is compatible with hydrophilic compounds but not with hydrophobic compounds; and (4) *in vivo*, the penetrating compound does not pass completely through the dermis but is removed by dermal capillaries (Wester and Maibach, 1983). Because of notable differences between *in vivo* and *in vitro* skin, the *in vitro* method alone is not always a reliable or accurate predictor of *in vivo* percutaneous absorption.

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