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*Technical Note*

# Mass Balance and Dose Accountability in Percutaneous Absorption Studies: Development of a Nonocclusive Application System

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## INTRODUCTION

For the assessment of human percutaneous absorption, we have developed a new *in vivo* method to account for the topically applied dose. The procedure requires a protective, but not occlusive, covering device which satisfies the following criteria. The system must (a) allow free passage of passive (insensible) transepidermal water loss, (b) retain exfoliated corneocytes on which a fraction of the applied compound may have been sequestered, and (c) constrain the administered compound to the site of application and prevent spreading over the skin surface. (d) The patch system must not irritate the skin. (e) The adhesive material used to retain the device on the skin surface must resist normal human activities such as showering and nonstrenuous movement.

In this note, the construction and performance of a modified plastic chamber, which satisfies all of the above requirements, are described.

## MATERIALS AND METHODS

The 2.5-cm-diameter polypropylene chambers used were obtained from Hilltop Research, Inc. (Cincinnati, Ohio). The semipermeable polytetrafluroethylene (0.2-μm-pore size) membrane (Gore-tex) was a generous gift from W. L. Gore and Associates, Inc. (Elkton, Md.). Adhesive transparent dressing (1625/Tegaderm) was obtained from 3M (St. Paul, Minn.).

The nonocclusive devices were assembled manually as follows. Six, equally spaced, 4.0-mm-diameter holes were punched through the plastic chamber using a No. 1 cork borer. Next, both the waxed paper backing and the cotton pad, with which the chambers were provided, were removed. Three reference lines were marked across the plastic chamber and the manufacturer-supplied adhesive tape. The latter was then removed and an intact 2.35-cm-diameter disk of Gore-tex membrane was placed on the adhesive side of the original adhesive tape. The plastic chamber was properly aligned on top of the Gore-tex membrane (on the original adhesive tape) using the reference marks on the chamber and the tape to align the six holes through the plastic chamber with the six holes through the adhesive support tape. Thus, the holes through the plastic chamber and adhesive tape are "plugged" by the "sandwiched" Gore-tex membrane. Finally, the reassembled device was returned to the original waxed paper backing of the plastic chamber. Compared to the original adhesive tape provided by the chamber supplier, Tegaderm was found to be more elastic, nonirritating, secure, and moisture permeable; consequently, its use led to improved subject compliance. The Tegaderm modification involved the following steps: in the center of a 7 × 6-cm sheet of Tegaderm, a 2.35-cm-diameter hole was punched.

Prior to application of the modified plastic chamber, the edges of the original adhesive tape were trimmed so as to leave a 3.3-cm-diameter circle, i.e., such that the tape overlapped the chamber by 0.6 cm around the circumference. The remaining waxed paper backing was removed, and the chamber placed on the ventral forearm of the subject. Finally, the chamber was secured to the arm by centering the modified Tegaderm over the chamber (Figs. 1 and 2).

## RESULTS

A nonocclusive patch system and, as a positive control for occlusion, an unmodified plastic chamber were applied to the ventral forearms of 10 normal healthy human volunteers. Transepidermal water loss (TEWL) was measured above the chambers, on the skin below the chambers, and on skin adjacent to the chambers 24 hr postapplication using a Servomed AB (Stockholm, Sweden) EP1 Evaporimeter. Measurements above the chambers were made immediately before their removal. Recordings from skin below the devices were obtained within 20 sec of system removal. Readings from "normal" skin adjacent to the protected site were randomly taken before or after system removal. At a closely adjacent site, skin surface temperature was evaluated with a Tele-Thermometer YSI Model 44 thermometer (Yellow Springs Instrument Co. Inc., Ohio). All TEWL

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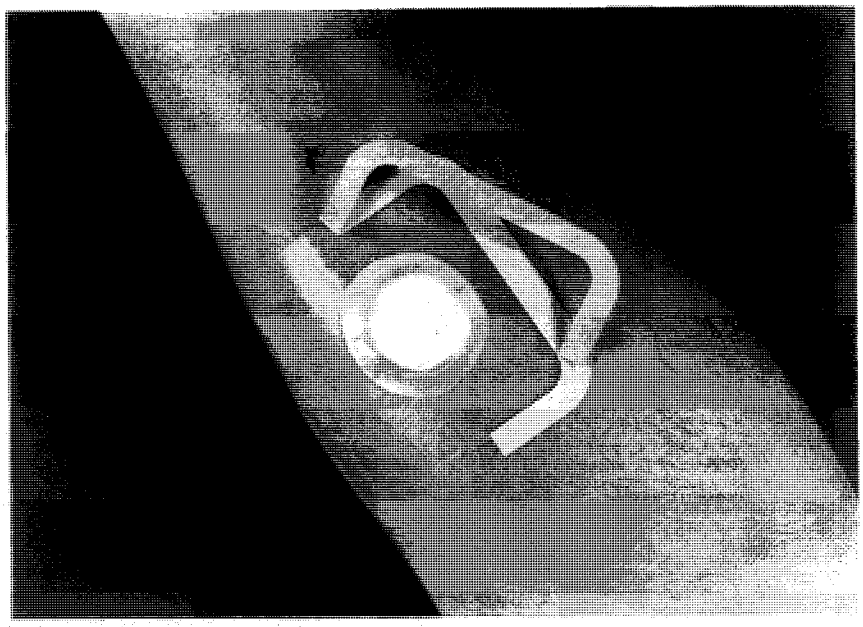

Fig. 1. Nonocclusive application system being applied to the ventral forearm of a volunteer.

values reported were corrected to a reference temperature of 30°C using a published procedure (1). The occlusivity of the unmodified chambers and the ability of the protective devices to allow transepidermal water loss are indicated by the data in Table I. A one-way analysis of variance indicated a significant difference ($P < 0.001$) between the sites of measurements. Results from the Newman–Keuls multiple-range test show (i) that the TEWL values from skin occluded for 24 hr with an intact Hilltop chamber are significantly greater ($a < 0.001$) than the TEWL values from the other sites of measurement and (ii) that there were no other significant differences ($a > 0.05$) between other TEWL measurements.

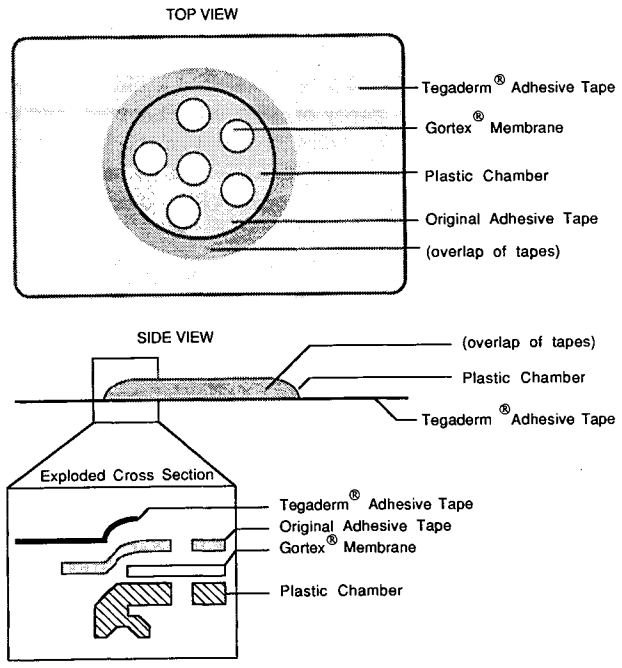


Fig. 2. Schematic diagram of the nonocclusive application system.

## DISCUSSION

The experimental methodology employed in recent human *in vivo* mass balance percutaneous absorption studies is described elsewhere (2). The guiding principle of this measurement is that the fraction of the dose absorbed plus the fraction recovered from both the protective device and the skin surface should equal the applied dose. Table II shows that this criterion is met for a series of para-substituted phenols, the skin penetration of which was determined in experiments that used the nonocclusive protective system described in this note.

In achieving the desired goal, the patch system must retain exfoliated skin squames, on which applied penetrant may be adsorbed, and prevent their loss to the environment. Clearly, this is not a problem for a fully occlusive, intact, plastic chamber, but the constraint is not so easily accommodated with a nonocclusive device. The use of Gore-tex as described above provides a membrane that has (i) a pore size of 0.2 μm, which is much smaller than the dimensions of a corneocyte, and (ii) a negligible barrier to the escape of transepidermal water loss from beneath the device (Table I). Thus, retention of desquamated material and nonocclusivity of the chemical delivery site are achieved. Furthermore, the Tegaderm adhesive tape securely affixes the chamber to the skin surface without inducing subject discomfort or irritation.

In summary, therefore, a nonocclusive patch system for percutaneous absorption and topical bioavailability studies has been developed. Although the device has been used exclusively in humans so far, it should also be suitable for animal studies when testing in humans is considered inappropriate (e.g., for evaluating dermal exposure to toxic chemicals). Additionally, the system will enable irritation and

Table I. Transepidermal Water Loss[a,b] 24 hr Postapplication

| Adjacent skin sites | Above intact HTC[c] | Skin below intact HTC | Above patch system | Skin below patch system |
|---|---|---|---|---|
| 5.4, 8.7 | 0.0 | 51. | 7.8 | 7.6 |
| 5.0, 6.2 | 0.0 | 51. | 5.8 | 7.2 |
| 4.4, 4.4 | 0.0 | 44. | 2.4 | 3.5 |
| 3.5, 4.0 | 0.0 | 23. | 1.8 | 3.1 |
| 3.1, 2.7 | 0.0 | 58. | 2.3 | 3.2 |
| 2.9, 5.8 | 0.0 | 35. | 1.8 | 3.9 |
| 3.7, 3.5 | 0.0 | 38. | 0.0 | 6.5 |
| 3.5, 5.1 | 0.0 | 63. | 3.0 | 3.3 |
| 4.0, 7.2 | 0.0 | 45. | 2.7 | 10.[d] |
| 4.2, 4.0 | 0.0 | 59. | 4.0 | 5.1 |

[a] Measurements were made in a single environment of relatively constant temperature (22–25°C) and humidity (34%).
[b] Measured as g/m²/hr and normalized to a skin temperature of 30°C.
[c] Occlusive Hilltop chamber.
[d] Skin temperature of this subject was 33.2°C at the time of measurement. This figure was at least 1°C higher than that of all other subjects.

Table II. Mass Balance[a] for a Series of Para-Substituted Phenols Under Protected Conditions (2)

| Phenol | % dose absorbed | % dose removed from skin surface | Accountable % dose |
|---|---|---|---|
| Acetaminophen | 4 (3) | 93 (5) | 97 (4) |
| Aminophenol | 6 (3) | 85 (4) | 91 (2) |
| Proprionylamidophenol | 11 (8) | 84 (7) | 95 (2) |
| Pentyloxyphenol | 13 (4) | 85 (3) | 98 (2) |
| Iodophenol | 24 (6) | 73 (7) | 97 (2) |
| Phenol | 26 (7) | 76 (18) | 102 (20) |
| Cyanophenol | 31 (16) | 70 (12) | 101 (5) |

[a] Reported as mean (SD).

sensitization assays to be performed under reproducible, but nonocclusive (and potentially more relevant), conditions.

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