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[^{14}C]-Testosterone: A Marker Assay For Human Skin Absorption

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Stratum Corneum/Skin

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Introduction

For *in vitro* skin absorption studies, OECD Test Guideline No. 428[1] recommends demonstrating the performance and reliability of the test system in the performing laboratory using Testosterone with reference to historical data. Van de Sandt *et al*. (2004)[2] previously demonstrated a wide variation in results between laboratories for this chemical when applied to human skin at a concentration of *ca* 4 mg/mL ethanol:water (1:1, v/v).

This study was designed to assess the extent of absorption of radiolabelled Testosterone following topical application of the same formulations used by Van de Sandt *et al*. (2004)[2]. However, the standard Charles River skin penetration method, following the recommendations set down in the OECD Test Guideline No. 428[1] and Guidance Document No. 28[3], was followed.

Method

Split-thickness human skin membranes (340-400 μm) were mounted into flow-through diffusion cells (Figures 1 and 2). Receptor fluid was pumped underneath the skin at a flow rate of *ca* 1.5 mL/h. The skin surface temperature was maintained at *ca* 32 ±1°C throughout. A predose tritiated water barrier integrity test was performed as described by Runciman *et al*. (2009)[4]. Samples exhibiting absorption >0.6% applied dose were excluded. The receptor fluid was tissue culture medium (TCM) containing bovine serum albumin (*ca* 5%, w/v), glucose (*ca* 1%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL) with the pH maintained with 5% CO_2 in air. The solubility of Testosterone in the receptor fluid was demonstrated to be ≥18.67 mg/L. For an application of 10 μL/cm^2 of test preparation, 25.6 μg of Testosterone was applied over the skin area of 0.64 cm^2. If 100% was absorbed in 1 h (1.5 mL of receptor fluid), then 25.6 μg/1.5 mL is 17 mg/L of Testosterone in receptor fluid. Therefore, this receptor fluid was not rate limiting for solubility and was accepted for use.

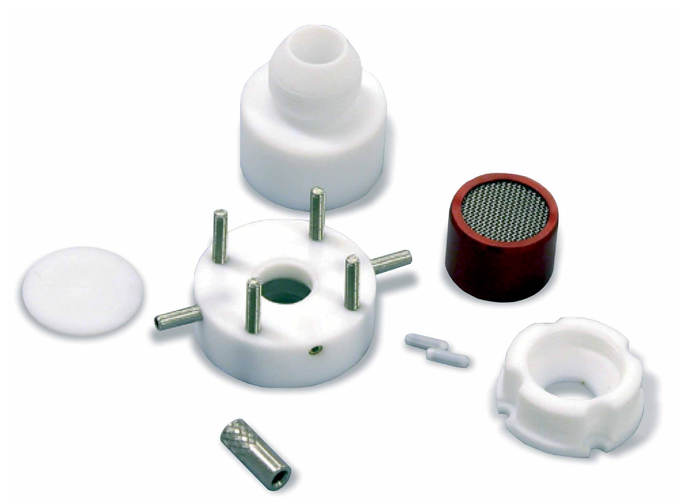

Figure 1. Flow-through diffusion cell.

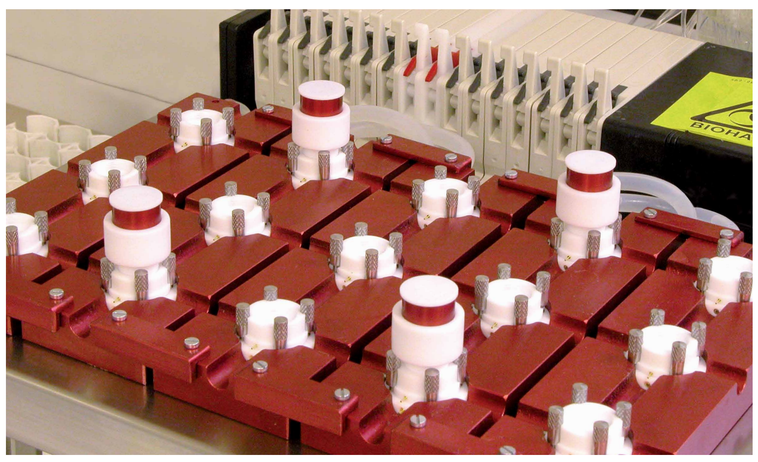

Figure 2. Flow-through diffusion cell manifold.

[^{14}C]-Testosterone was prepared in ethanol:water (1:1, v/v) test preparations at a concentration of 4.0 mg/mL. This was applied evenly over the stratum corneum surface of twelve skin membranes at an application level of 10 μL/cm^2.

Receptor fluid was collected in hourly fractions from 0 to 6 h post dose and then 2-hourly from 6 to 24 h post dose. At 24 h post dose, the exposure was terminated by washing the skin with commercial hand wash soap diluted in water (2%, v/v) and dried. The underside of the skin was rinsed with receptor fluid (receptor rinse). The stratum corneum was removed by 20 successive tapes. Each tape strip was analysed separately. The unexposed skin was separated from the exposed skin. All samples were analysed by liquid scintillation counting.

Results and Discussion

A summary of the distribution of [^{14}C]-Testosterone at 24 h post dose is provided in Table 1. For [^{14}C]-Testosterone applied to human skin, the test item was effectively washed off the skin (80.47%). The absorbed dose and dermal delivery were 2.25% (0.91 μg equiv./cm^2) and 7.19% (2.90 μg equiv./cm^2), respectively. The mass balance was 100.74% of the applied dose.

Distribution	% Applied Dose		μg equiv./cm^2	
	Mean	SD	Mean	SD
Total Dislodgeable Dose	80.47	10.07	32.50	4.07
Stratum Corneum 1-5	6.66	3.30	2.69	1.33
Stratum Corneum 6-10	2.97	2.02	1.20	0.81
Stratum Corneum 11-15	1.66	1.13	0.67	0.46
Stratum Corneum 16-20	1.32	1.07	0.53	0.43
Total Stratum Corneum	12.61	6.86	5.10	2.77
Unabsorbed Dose	93.55	4.96	37.78	2.01
Total Absorbed Dose	2.25	1.50	0.91	0.61
Dermal Delivery	7.19	5.05	2.90	2.04
Mass Balance	100.74	1.56	40.68	0.63

Table 1. Distribution of radioactivity.

Steady state flux was observed from 4 h to 24 h post dose (34.41 ng equiv./cm^2/h). Using linear regression of the cumulative absorption (time points 4 h to 24 h), the lag time for Testosterone was extrapolated to be 1 h 40 min. Cumulative and flux absorption profiles are provided in Figures 3 and 4, respectively. The distribution of Testosterone through the stratum corneum is provided in Figure 5.

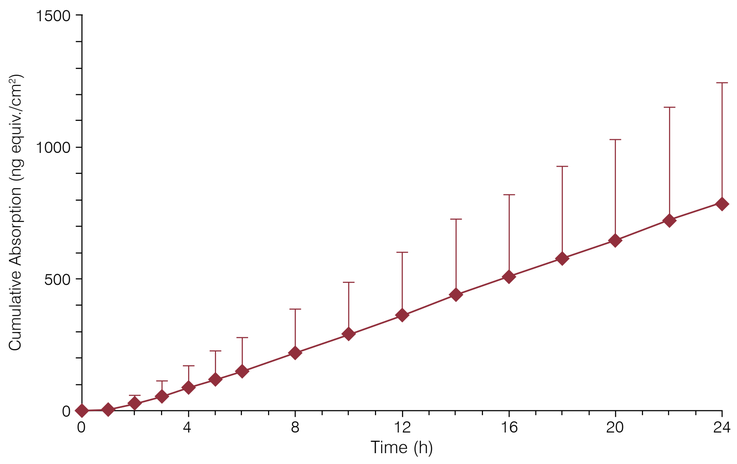

Figure 3. Absorption Profile for [^{14}C]-Testosterone (ng equiv./cm^2) in Receptor Fluid Following Topical Application of [^{14}C]-Testosterone in Test Preparation (4 mg/mL) to Human Split-Thickness Skin (Mean + SD, n = 12)

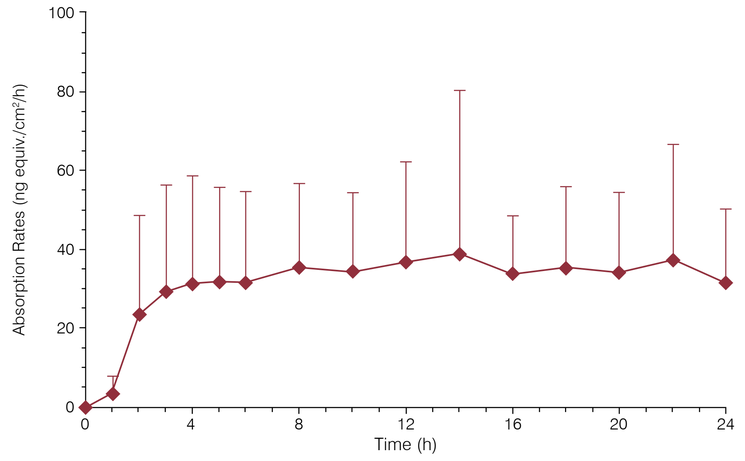

Figure 4. Flux Profile for [^{14}C]-Testosterone (ng equiv./cm^2/h) in Receptor Fluid Following Topical Application of [^{14}C]-Testosterone in Test Preparation (4 mg/mL) to Human Split-Thickness Skin (Mean + SD, n = 12)

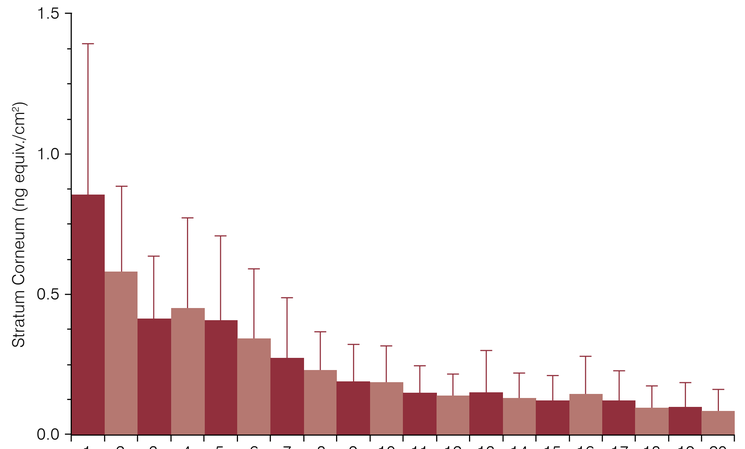

Figure 5. Distribution of [^{14}C]-Testosterone (µg equiv./cm^2) in the Stratum Corneum at 24 h Post Dose Following Topical Application of [^{14}C]-Testosterone in Test Preparation (4 mg/mL) to Human Split-Thickness Skin (Mean + SD, n = 12)

Conclusion

In conclusion, the standard design used at Charles River Laboratories has been successfully validated against data in the literature in compliance with the OECD Guidance Document No. 28[3].

References

1. OECD Guideline for Testing of Chemicals No 428: Skin Absorption: *In Vitro* Method (2004).
2. Van de Sandt JJM *et al*. (2004). *In vitro* predictions of skin absorption of caffeine, testosterone and benzoic acid: a multi-centre comparison. Regulatory Toxicology and Pharmacology 39 271-281.
3. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004).
4. Runciman J, Roper CS and Madden S (2009). Evaluation of Rapid Tritiated Water Skin Barrier Integrity Method for Use in Regulatory Toxicology Testing *In Vitro*. OEESC 2009, Edinburgh, UK.