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Effect of Receptor Fluid and Area on Testosterone Absorption Across Human Skin

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Introduction

For *in vitro* skin absorption studies, OECD Test Guideline No. 428 recommends demonstrating the performance and reliability of the test system in the performing laboratory using reference substances (e.g. Testosterone)[1,2]. In this study, two different exposure area static diffusion cell systems were compared using Testosterone as reference test item. The effects of two receptor fluids on Testosterone absorption were also investigated. The experimental design was based upon Van de Sandt *et al* 2004[3].

Split-thickness human skin membranes (*ca* 400 μm) were mounted into static diffusion cells (0.64 or 3.14 cm^2) and maintained at a temperature of 32 ± 1°C. Photographs of the static cell and cells in manifolds are provided in Figures 1 and 2.

Figure 1. Static diffusion cell.

Method

Tissue culture medium containing BSA (*ca* 5%, w/v), glucose (*ca* 1%, w/v), streptomycin (0.1 mg/mL) and penicillin G (100 units/mL) (RFA) or ethanol: phosphate buffered saline at 1:1, v/v (RFB) were used as receptor fluids. An electrical resistance barrier integrity assessment was performed and any 3.14 cm^2 skin sample exhibiting a resistance <4 kΩ was excluded from subsequent absorption measurements.

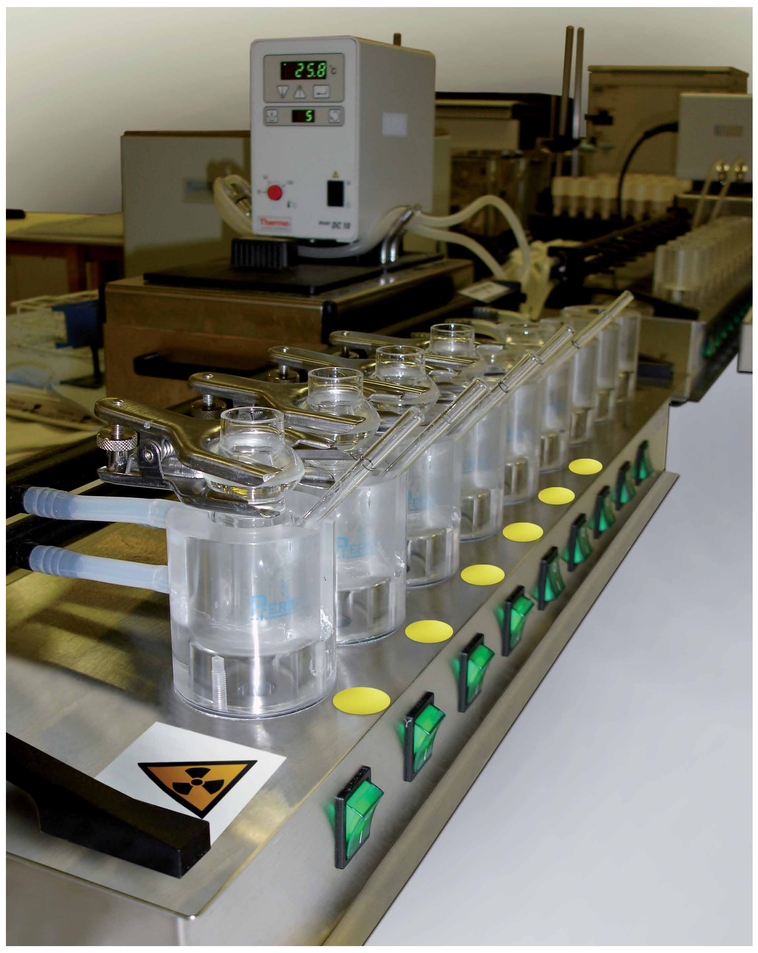

Figure 2. Static diffusion cells in manifold.

This laboratory does not have rejection criteria for static cells with an area of 0.64 cm^2, therefore, no samples were rejected from subsequent absorption measurements. The test preparation, [^{14}C]-Testosterone in ethanol: water (1:1, v/v) at 4 mg/mL, was applied at 10 μL/cm^2 to 8 samples of skin from 4 different donors in duplicate for each of 4 test groups.

Absorption was assessed by collecting receptor fluid fractions at 0, 0.5, 1, 2, 4, 8 and 24 h post dose.

At 24 h post dose, exposure was terminated by washing the skin surface with concentrate and dilute soap solution in water (2%, v/v). The skin was also dried with tissue swabs. The stratum corneum was removed with 20 successive tape strips and the exposed and unexposed skin separated. All samples were analysed by liquid scintillation counting.

Results and Discussion

The distribution of [^{14}C]-Testosterone at 24 h post dose is provided in Table 1.

	[^{14}C] Testosterone (μg equiv./cm^2)			
Test Group	**RFA, 0.64 cm^2**	**RFB, 0.64 cm^2**	**RFA, 3.14 cm^2**	**RFB, 3.14 cm^2**
Dislodgeable Dose	36.63	35.18	38.10	34.98
Stratum Corneum	1.32	1.13	1.32	1.12
Unabsorbed Dose	37.96	36.31	39.43	36.35
Total Absorbed Dose	0.77	2.38	0.60	2.61
Dermal Delivery	1.44	3.01	0.98	4.08
Mass Balance	39.40	39.32	40.41	40.43

Table 1. Comparison of distribution of testosterone at 24 h post dose.

A student's T-Test was performed to compare the effects of receptor fluid or area on dislodgeable dose and absorption of Testosterone. These are summarised in Table 2.

Test Group	**Testosterone Absorption**		**Testosterone Dislodgeable Dose**	
Statistics	**P-value**	**Significance**	**P-value**	**Significance**
0.64 v 3.14 cm^2 (RFA)	0.39500	NS	0.06854	NS
0.64 v 3.14 cm^2 (RFB)	0.36332	NS	0.84579	NS
RFA v RFB (0.64 cm^2)	0.00413	**	0.23492	NS
RFA v RFB (3.14 cm^2)	0.01002	*	0.04198	*

Table 2. Statistical comparison of testosterone absorption and dislodgeable dose by test group.

The absorption profiles for [^{14}C]-Testosterone from the 4 test groups are provided in Figures 3-5. Exposure area resulted in no visible differences in the absorption profile for Testosterone. This was confirmed by student's T-Test (Table 2). However the receptor fluid did visibly affect the absorption profile of [^{14}C]-Testosterone with RFB increasing absorption over RFA. This was confirmed to be statistically different (Table 2). Testosterone absorption at 0.64 cm^2 was significantly higher (P=0.004) for RFB than RFA. Testosterone absorption at 3.14 cm^2 was significantly higher (P=0.01) for RFB than RFA.

Dislodgeable dose of [^{14}C]-Testosterone was also compared by T-Test and was not found to be significantly different between any group except for RFB which was significantly lower (P=0.04) than RFB at 3.14 cm^2 only. This difference was attributed to the differences in dermal delivery which was higher in RFB than RFA.

Conclusions

In conclusion, exposure area did not significantly affect any parameter tested. Washing was effective for all test groups. Ethanolic receptor fluid significantly increased Testosterone absorption through human skin. The results confirmed the acceptability of these test methods.

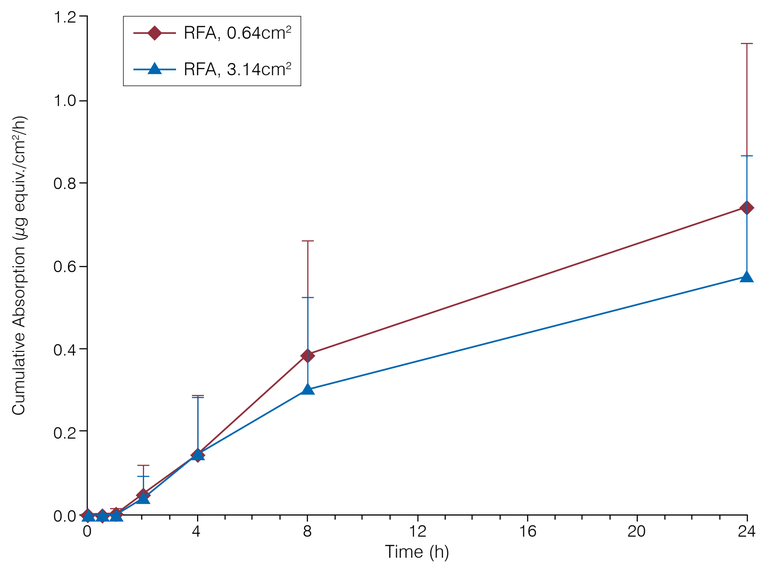

Figure 3. Comparison of exposure area on Testosterone absorption profile for RFA.

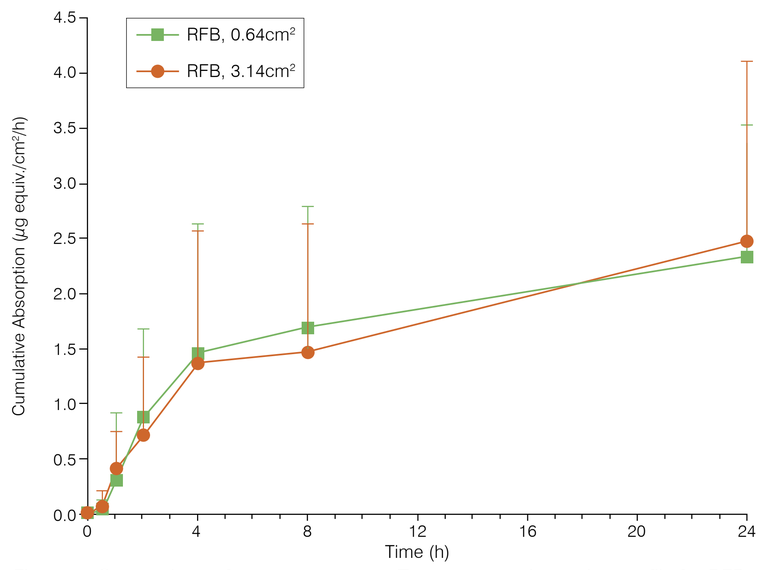

Figure 4. Comparison of exposure area on Testosterone absorption profile for RFB.

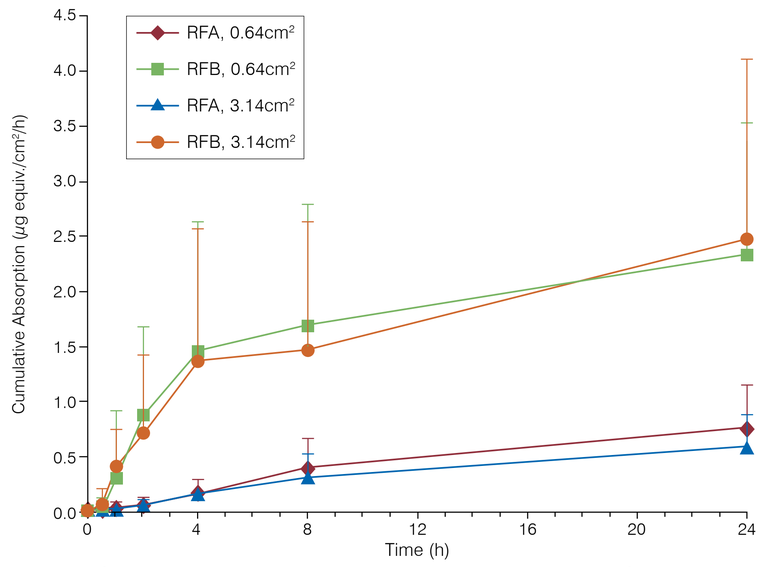

Figure 5. Comparison of Testosterone absorption profiles for all Test Groups.

References

1 OECD Guideline for Testing of Chemicals No 428: Skin Absorption: *In Vitro* Method (2004)

2 OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies (2004)

3 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

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