An Assessment of the Intra-Lab Variability of the *in vitro* Percutaneous Absorption of Testosterone through Human Skin

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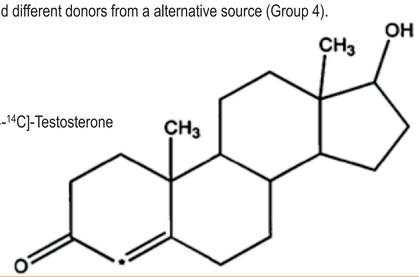
1 INTRODUCTION

OECD Test Guideline 428[a] describes the conduct of *in vitro* skin penetration studies. The accompanying OECD Guidance Document No. 28[b] states that laboratories performing *in vitro* skin absorption studies should demonstrate proficiency using the model penetrant, Testosterone. This laboratory performed 2 studies with the same study design on different days, but with minor methodological changes, to assess if these differences resulted in changes to the outcome of the overall results generated.

Study 1 examined two decontamination scenarios (Group 1 and Group 2) and Study 2 repeated Group 1 with different donors but from the same source (Group 3) and different donors from a alternative source (Group 4).

2 STRUCTURE

Figure 1. Structure and site of labelling (*) of [4-^{14}C]-Testosterone



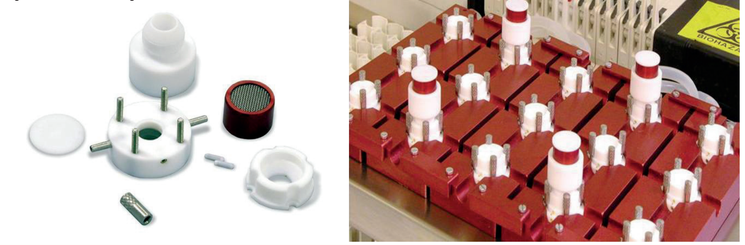
3 MATERIALS AND METHODS

Ethical approval for receipt and use of human skin was obtained from Lothian Local REC (Ref. No. 06/S1101/19) and Glasgow Royal Infirmary REC (Ref. No. 08/S0704/30). This lab regularly sources tissue from hospitals and tissue banks. Skin samples were obtained from elective plastic surgery carried out on a routine basis. Patients gave prior informed consent for the skin to be used for scientific research. On receipt to the laboratory, full thickness skin was cleaned, subcutaneous fat and muscle removed and the samples stored in a freezer set to maintain a temperature of -20°C, until required. The skin was cut to a depth of 200-400 µm with a Zimmer electric dermatome (Figure 2). The membrane thickness was confirmed using a micrometer. Membranes grossly damaged during the cutting procedure were discarded. For Group 4, skin was obtained from Alphenyx as ready prepared dermatomed discs.

Figure 2. Preparation of dermatomed split-thickness skin membranes

Split-thickness skin membranes were placed into flow-through diffusion cells (Figure 3) with an exposure area of 0.64 cm^2. The receptor fluid (physiological saline containing bovine serum albumin (*ca* 5%, w/v) and sodium azide (0.01%, w/v), pH 7.4) flow rate was 1.5 ± 0.15 mL/h and skin temperature was 32 ± 1°C. The barrier function was confirmed using a tritiated water barrier integrity test (acceptance criterion: absorption of water ≤0.6% of the applied dose in 1 h). The test preparation, [^{14}C]-Testosterone (1 mg/mL) in ethanol: water (40%, v/v), was applied at an application rate of 10 µL/cm^2 to human skin (n = 8 or n = 10). Absorption was assessed hourly (0-8 h) and 2-hourly (8-24 h). Exposure was terminated by washing at 8 h. Groups 1, 3 and 4 were washed with soapy water using natural sponge swabs. Group 2 was washed with soap concentrate and tissue paper swabs. Dose, skin wash, swabs, tapes, skin, receptor fluid and donor and receptor chamber extracts were collected and analysed by liquid scintillation counting.

Figure 3. Flow-through diffusion cells



4 RESULTS AND DISCUSSION

The results for the tritiated barrier integrity test (Table 1) showed no significant differences in the barrier function for Group 1, Group 2 and Group 3 skin samples. Group 4 was highly significantly higher than Group 3 and very highly significantly higher than Group 1 and Group 2. However, all samples used passed the historical barrier integrity test acceptance criterion as described in Meidan and Roper (2008)[c] and Runciman *et al* (2009)[d].

Table 1. Tritiated water barrier integrity results (% applied dose) and comparisons by T-Test

	Tritiated Water Absorption (% Applied Dose)			
Test Group	1	2	3	4
Min	0.08	0.09	0.05	0.21
Max	0.18	0.21	0.58	0.48
Mean	0.13	0.15	0.15	0.35
SD	0.03	0.04	0.15	0.08

Test Group Comparison	1v2	1v3	1v4	2v3	2v4	3v4
T-TEST	0.308	0.680	0.000	0.972	0.000	0.002
Significance	NS	NS	***	NS	***	**

Table 3. T-Test comparing test groups as P-Values (significance)

Test Group Comparison	1v2	1v3	1v4	2v3	2v4	3v4
Dislodgeable Dose 8 h	0.5695 (NS)	0.1712 (NS)	0.4116 (NS	0.4192 (NS)	0.1474 (NS)	0.0230 (*)
Stratum Corneum	0.9913 (NS)	0.9174 (NS)	0.0020 (**)	0.9119 (NS)	0.0026 (*)	0.0021 (**)
Total Absorbed	0.2509 (NS)	0.1326 (NS)	0.1951 (NS)	0.0189 (*)	0.8988 (NS)	0.0126 (*)
Dermal Delivery	0.2821 (NS)	0.7489 (NS)	0.1216 (NS)	0.1992 (NS)	0.6834 (NS)	0.0843 (NS)

P>0.05; not significant (NS), P<0.05; significant (*), P<0.01; highly significant (**), P<0.001; very highly significant (***)

Students T-Tests (Table 2) were performed comparing all 4 test groups with each other for key aspects of the distribution of Testosterone within these skin absorption studies *i.e.* dislodgeable dose (8 h), *stratum corneum*, total absorbed dose and dermal delivery at 24 h (µg equiv./cm^2). The distribution of Testosterone from the 4 test groups is provided in Table 3. The Testosterone mass balance was complete (within 100 ± 10%) for all test groups.

For dislodgeable dose (8 h) and total absorbed dose, there were no significant differences between Groups 1 and 2, 1 and 3, 1 and 4 and 2 and 4. The absorbed dose for Group 2 (0.48 µg equiv./cm^2) was significantly higher than for Group 3 (0.29 µg equiv./cm^2) which confirmed the expected donor differences (*i.e.* inter-donor variability). However, this did not result in any further significant differences between these groups. The main differences were observed when comparing data from skin samples obtained from different suppliers. Testosterone levels found in the *stratum corneum* were significantly higher in Group 4 (0.48 µg equiv./cm^2) than in Group 2 (0.20 µg equiv./cm^2) and highly significantly higher than in Groups 1 and 3 (both 0.20 µg equiv./cm^2) with no significant differences observed between Groups 1, 2 and 3 (all 0.20 µg equiv./cm^2). The dislodgeable dose of Testosterone at 8 h was significantly higher in Group 3 (9.37 µg equiv./cm^2) than in Group 4 (8.85 µg equiv./cm^2) with a correspondingly significantly lower absorbed dose (0.29 and 0.49 µg equiv./cm^2). This could be important for transdermal drug delivery and risk assessments. However, since there were no significant differences for dermal delivery for any comparison, despite the differences between barrier integrity between suppliers, the consistency of the test was confirmed.

5 CONCLUSIONS

This study confirmed that small changes in conditions did not affect the absorption and distribution of Testosterone and demonstrated that the study test is consistent when run on different occasions with only small differences attributable to the inter-donor variability.

Table 2. Distribution of Testosterone as % applied dose and amount (µg equiv./cm^2)

	% Applied Dose (Mean ± SD)				Amount in µg equiv./cm^2 (Mean + SD)			
Test Group	1	2	3	4	1	2	3	4
Dislodgeable Dose (8 h)	86.4 ± 5.46	87.9 ± 4.30	88.2 ± 2.54	83.3 ± 5.46	9.08 + 0.57	9.23 + 0.45	9.37 + 0.27	8.85 + 0.58
Total Dislodgeable Dose	91.3 ± 3.03	92.9 ± 2.07	91.4 ± 2.88	90.6 ± 3.28	9.60 + 0.32	9.76 + 0.22	9.71 + 0.31	9.63 + 0.35
Stratum Corneum	1.88 ± 0.80	1.88 ± 0.89	1.90 ± 0.67	4.56 ± 1.99	0.20 + 0.08	0.20 + 0.09	0.20 + 0.07	0.48 + 0.21
Unabsorbed Dose	93.3 ± 2.89	94.8 ± 1.76	93.7 ± 2.77	95.2 ± 2.21	9.80 + 0.30	9.96 + 0.19	9.95 + 0.29	10.1 + 0.23
Total Absorbed Dose	3.76 ± 1.15	4.54 ± 1.42	2.75 ± 1.39	4.58 ± 1.55	0.40 + 0.12	0.48 + 0.15	0.29 + 0.15	0.49 + 0.16
Dermal Delivery	4.67 ± 1.41	5.58 ± 1.82	4.36 ± 1.84	5.88 ± 1.89	0.49 + 0.15	0.59 + 0.19	0.46 + 0.20	0.62 + 0.20
Mass Balance	97.9 ± 2.37	100.4 ± 0.54	98.0 ± 1.35	101.0 ± 0.79	10.3 + 0.25	10.6 + 0.06	10.4 + 0.14	10.7 + 0.08

6 REFERENCES

[a] OECD Environmental health and safety publications series on testing and assessment No. 28 (2004). Guidance document for the conduct of skin absorption studies.
[b] OECD Test Guideline No. 428 (2004). Skin absorption: *in vitro* method.
[c] Meidan VM and Roper CS (2008). Inter- and intra-individual variability in human skin barrier function: a large scale retrospective study. Toxicology *In Vitro* 22; 1062-1069.
[d] Runciman J, Roper CS and Madden S (2009). Evaluation of a rapid tritiated water skin barrier integrity method for use in regulatory toxicology testing *in vitro*. OEESC 2009, Edinburgh, UK.