Inter-laboratory Comparison of the Penetration of Caffeine Through Silicone Membranes *in vitro*

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Introduction

Assessments of the between laboratory variability of absorption using artificial[1] and skin[2] membranes have been reported. Under the auspices of Skin Forum (www.skin-forum.eu), a new study was performed in which 12 laboratories (see Table 1) measured penetration of Caffeine through silicone membranes according to a well defined protocol written by each laboratory and approved by the study monitors.

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Table 1. Participating laboratories

Methods

Each laboratory used its own diffusion cell equipment (flow through and/or static design) and was responsible for measuring absorption by HPLC (using a simple validated method) or liquid scintillation counting. The membrane was supplied from a single batch of polymethyl siloxane (75 μm thickness) and the Caffeine was applied in an ethanol:water (1:1 v/v) vehicle at 1 mL/cm^2. Absorption was measured for 24 h post dose and, apart from a receptor chamber rinse, no terminal procedures were employed. Each laboratory produced a written report which was reviewed for compliance against the original protocol by the study monitors. Data from eight diffusion cells were requested with reasons for any samples rejected. 14 data sets were submitted from the 12 laboratories; 4 flow-through (2 academic, 2 industrial, area 0.64 cm^2) and 10 static (5 academic, 5 industrial, area range 0.2 to 5.41 cm^2). Example static and flow-through cells are provided in Figures 1 and 2, respectively.

Figure 1. Static diffusion cell

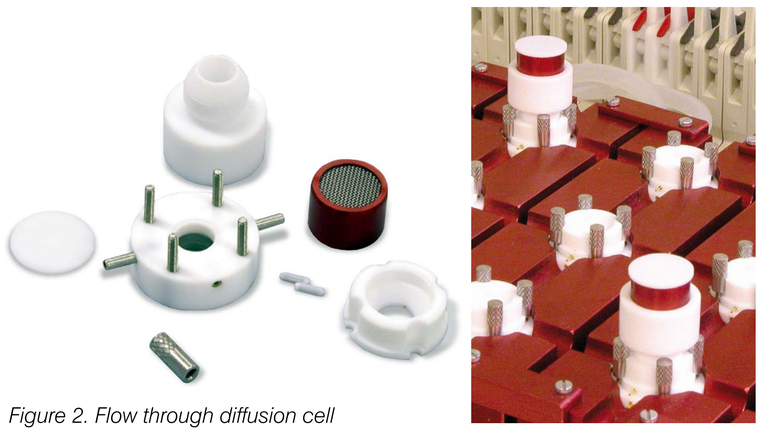

Figure 2. Flow through diffusion cell

Results

Mean cumulative absorption for all data sets is provided in Figure 3. As many of the data points after 6 h were not common for different laboratories, the mean flux at 6 h for all groups is provided in Figure 4. The time-curve demonstrated that steady state was not achieved and as such, the permeability coefficient (k_p) for Caffeine could not be calculated. This was confirmed by comparing total cumulative absorption (Figure 5) and flux (Figure 6) at 6 h and 24 h. Individual data sets are presented in Table 2.

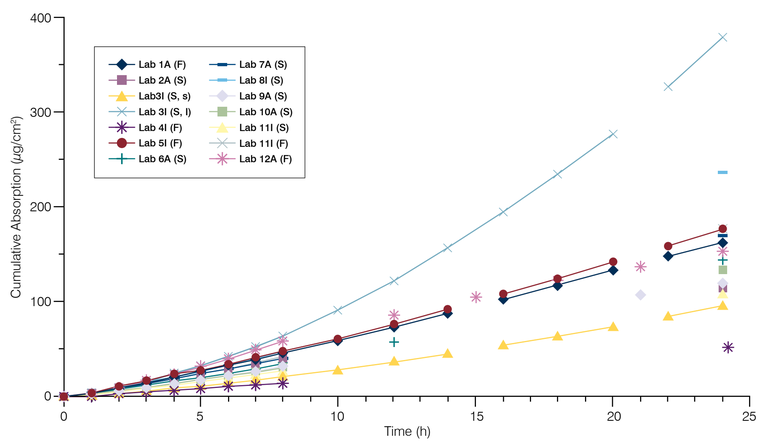

Figure 3. Mean cumulative absorption (µg/cm^2) of Caffeine through silicone membranes over 24 h: individual data sets.

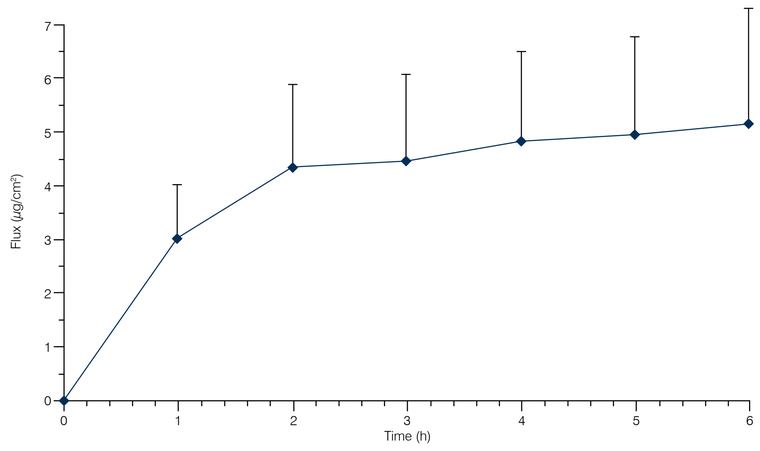

Figure 4. Flux (µg/cm^2/h) of Caffeine through silicone membranes to 6 h post dose (mean + SD, n=14).

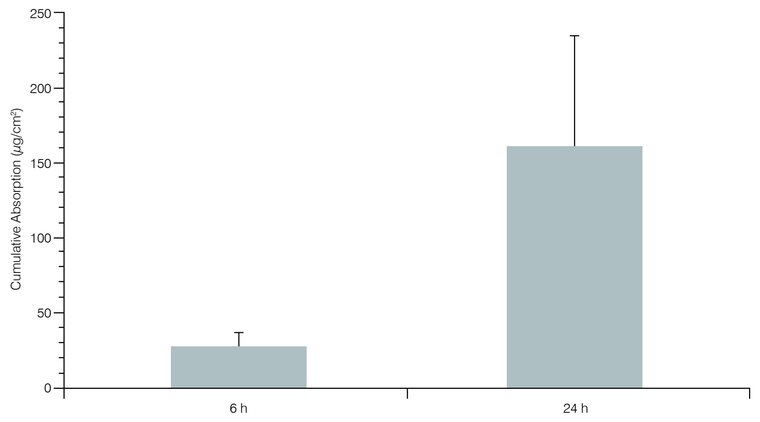

Figure 5. Comparison of mean cumulative absorption (µg/cm^2) of Caffeine through silicone membranes at 6 h and 24 h post dose (mean + SD, n=14).

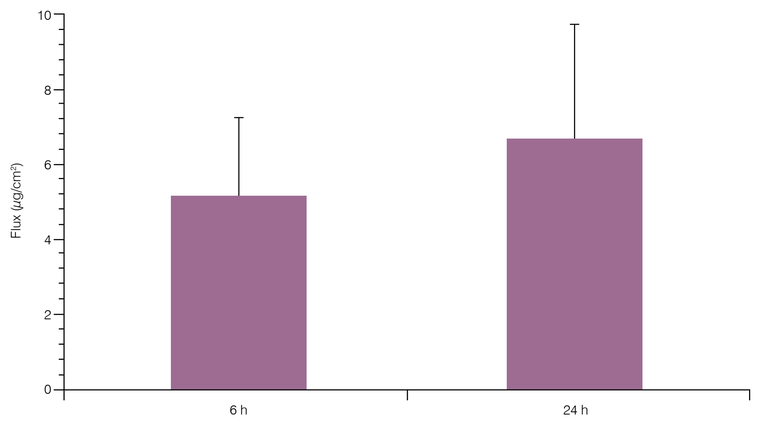

Figure 6. Comparison of mean flux (µg/cm^2/h) of Caffeine through silicone membranes at 6 h and 24 h post dose (mean + SD, n=14).

The data sets for flow-through and static diffusion cells were compared (Table 3). There was greater inter-laboratory variability in absorption measured at 6 h with static cells than with flow-through cells. The mean absorption at 6 h and maximum flux were significantly higher in flow-through cells. At 24 h, this trend continued but was not significantly different. There were no statistically relevant trends observed in absorption with cell surface area or chamber volume. The difference in absorption measured between static and flow-through cells could not be easily explained and previous studies of this type have not reported this finding with skin. However, it is postulated that there may have been an interaction between the membrane and test preparation excipients (ethanol?).

Lab ID	Cumulative Absorption (µg/cm^2)		Flux (µg/cm^2/h)	
	6 h Post Dose	24 h Post Dose	6 h Post Dose	24 h Post Dose
Lab 1A (F)	32.8	162	6.24	6.77
Lab 2A (S)	22.2	114	4.36	4.76
Lab 3I (S, 1.77)	14.6	95.6	2.90	3.98
Lab 3I (S, 4.52)	29.8	178	5.62	7.40
Lab 4I (S)	10.5	76.1	1.79	3.17
Lab 5I (F)	33.6	176	6.50	7.33
Lab 6A (S)	24.4	144	3.38	5.99
Lab 7A (S)	29.7	169	5.21	7.03
Lab 8I (S)	33.4	236	7.21	9.85
Lab 9A (S)	22.9	119	4.35	4.97
Lab 10A (S)	22.1	133	3.68	5.56
Lab 11I (S)	19.5	109	3.40	4.53
Lab 11I (F)	42.5	378	9.16	15.8
Lab 12A (F)	40.0	153*	8.37	6.39*
Mean ± SD	27.0 ± 9.18	160 ± 74.8	5.16 ± 2.15	6.68 ± 3.12

* air bubbles in line after 8 h, 6/8 samples should have been rejected

Table 2. Mean cumulative absorption (µg/cm^2) and flux (µg/cm^2/h) of Caffeine through silicone membranes at 6 h and 24 h post dose for individual data sets

		Static (n=10)	Flow-through (n=4)
Absorption 6 h (µg/cm^2)	Mean (range)	23.5 (10.5 to 35.1)	37.2* (33.6 to 42.5)
	CV%	35.7	12.7
Absorption 24 h (µg/cm^2)	Mean (range)	140.1 (76.1 to 236.3)	217.3 (153.3 to 378.2)
	CV%	33.8	49.5
Mx flux at 24 h (µg/cm^2)	Mean (range)	7.6 (3.9 to 14.8)	13.5* (9.0 to 25.7)
	CV%	40.8	60.1

*Table 3. Absorption of Caffeine through silicone membranes in ethanol: water vehicle (*P<0.05, Mann Whitney U test)*

Conclusions

There was inter-laboratory variability in absorption over the initial 6 h. However, this variability increased vastly to 24 h. This variability was considered to be due to a solvent/ membrane interaction that could not have been predicted prior to initiation of the test. The effect appeared to be greater in flow-through than static diffusion cells. This hypothesis will be further tested. The individual protocols and study conduct were well controlled.

The Future

Skin Forum is looking for companies and academic institutions to be involved in the next inter-laboratory test. This will involve human skin. For more details, please contact the authors or look out for more information on www.skin-forum.eu.

Acknowledgements

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References

1. Chilcott *et al* (2005). *Journal of Pharmaceutical Sciences* 94:632-638.
2. van de Sandt JJM *et al* (2004). *Regulatory Toxicology and Pharmacology* 39 (3) 271-281.

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