In Vitro – In Vivo Percutaneous Absorption Comparability

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Background. When chemicals come into contact with the skin surface, they may be absorbed into the skin, and may also penetrate through the skin to access the systemic circulation. It is, therefore, important to characterize their percutaneous absorption. In many cases, and particularly with toxic compounds, it is inappropriate to test them *in vivo* on human subjects, and alternate models must be utilized. One ideal approach to assess percutaneous absorption is the *in vitro* human cadaver skin model [1] using Franz diffusion cells [2]. This model has been utilized widely in research and drug development during the last several decades, has demonstrated its value through the years, and is widely regarded as the most valid *in vitro* model for evaluating the penetration of compounds into human skin. Nonetheless, fundamental questions re-emerge as subsequent generations of investigators enter the field. One such question is: "Can *in vitro* percutaneous absorption predict *in vivo* results?"

The published literature contains a significant body of work reporting on this *in vivo – in vitro* correlation of data, but as one might expect, the most compelling evidence is seen when appropriate and matching study designs are used. It became apparent in the early years of developing the *in vitro* human cadaver skin model that there was a correlation between percutaneous absorption monitored *in vitro* and *in vivo*, for a variety of significantly different compounds. The *in vitro* model has been repeatedly validated over the years, demonstrating it's ability to produce data that is consistent with *in vivo findings.* Some of the classic demonstrations include the works of Franz (1978), Bronaugh and Franz (1986), and Bronaugh and Collier (1993), which are collectively summarized in the Table 1. Another particularly meaningful body of work was compiled by Venkateshwaran (1997), who conducted an extensive *in vivo – in vitro* evaluation on two topical hormones. This work dramatically demonstrates the degree of correlation that can be achieved under proper study design and control, as can be seen in Figure 1.

Table 1. *In Vitro* and *In Vivo* Human Absorption Data from Different Vehicles (Percent of Dose Absorbed. [2,4,5]

Compound and Vehicle	***In Vivo***	***In Vitro***
Caffeine [4]		
Petrolatum	40.6	40.6
Ethylene glycol gel	55.6	32.3
Water gel	4.0	5.1
Testosterone [4]		
Petrolatum	49.5	39.4
Ethylene glycol gel	36.3	23.7
Water gel	49.2	41.4
Thiourea [2]	3.7	4.6
Hippuric acid [2]	1.0	1.25
Cortisone [5]	19.6	22.8
Benzoic acid [5]	37.0	35.5

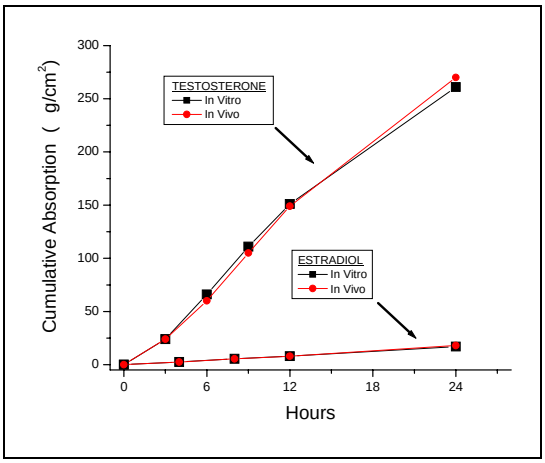

Figure 1. *In Vitro* and *In Vivo* Absorption Data for Transdermal Testosterone and Estradiol [6] (Cumulative Absorption as mg/cm^2)

While the literature contains many such demonstrations of the ability of the *in vitro* cadaver skin model to predict *in vivo* percutaneous penetration of compounds, there are also several reports indicating a lack of correlation, and this understandably raises a note of caution for many, regarding the validity of the model as a whole. Two common factors are seen in published works, which compromise the ability of the *in vitro* model to correlate with the *in vivo* situation: (1) unmatched *in vitro – in vivo* study designs, and/or (2) an unrefined implementation of the *in vitro* model when evaluating compounds that can be expected to display atypical penetration behavior.

In particular, special attention is warranted when utilizing this model to evaluate very lipophilic compounds, which may experience differential flux through the different compartments of ex *vivo* skin. Anatomically, the stratum corneum of the skin (the compact outer layer of the skin which is comprised of non-living cornified cells) functions as the primary barrier to absorbing chemicals. This non-living layer of the skin remains intact and functional whether *in vivo*, or excised for *in vitro* studies. However, ex *vivo* skin does lack dermal blood circulation, which is replaced *in vitro* with a reservoir solution to provide absorption sink conditions. When poor correlations are seen between *in vitro* and *in vivo* percutaneous absorption studies, this lack of active dermal vasculature *in vitro* is sometimes attributed as the probable cause..

Overall, comparing percutaneous absorption data generated *in vivo* and *in vitro* can be confounding. However, the authors' experience in conducting pre-clinical and clinical dermatology studies for industry on hundreds of compounds over many years, as well as experience from reviewing published literature over the years, suggests compellingly that the most common reasons for poor correlation between *in vitro* and *in vivo* percutaneous absorption studies are not due to a failure of the *in vitro* cadaver skin model, but rather, due to:

1) Inconsistency in study design between the *in vitro* and *in vivo* methods (e.g. different dose amounts applied, different formulations, different exposure durations, etc.)
2) Inexperience in the use of the Franz Cell and the Finite Dose Model.
3) Incomplete understanding of the penetrant's chemical characteristics in relation to skin physiology and the diffusion process.

As such, to receive valid *in vitro* data, it is essential to have appropriate implementation of the human cadaver skin model [1], and this is particularly critical when working with lipophilic compounds. The apparatus involved with the model is simple, but expertise and a thoughtful understanding of the principles underlying the skin model system are necessary. A case in point is described below, which demonstrates the importance of fully examining, and appropriately implementing, an *in vitro* study design. It illustrates the iterative series of studies conducted to 'tune' the *in vitro* model for studying a virtually water insoluble herbicide, Triallate; a thiocarbamate pre-emergent and post-emergent herbicide.

Methods. Human cadaveric skin sections were mounted in static Franz diffusion cells. Both split-thickness and epidermal preparations were evaluated with different reservoir solutions, stirred magnetically at ~600 rpm. Each chamber's temperature was controlled by a circulating water jacket to maintain a skin surface temperature of 32°C ± 1.0°C. Skin barrier integrity was confirmed prior to dosing using a standardized tritiated water penetration test. ^{14}C-Triallate was applied as a finite dose (5 µg/0.8 cm^2) in three formulations (acetone, Avadex BW EC and Avadex Spray). Collected samples were assayed for isotope content by liquid scintillation spectroscopy. Given the lipophilicity and volatility of Triallate, evaluation of its percutaneous absorption called for alternate study designs from the norm. Individual study phases included:

1) Alteration of the Reservoir Phase,
2) Assessment of the Dermal Barrier,
3) Assessment of Triallate Mass Balance (accountability of the applied dose),

Results. The data indicate that when using the classic unmodified Franz cell study approach (with phosphate buffer saline in the reservoir solution and with retention of the dermis during skin preparation) significantly underestimated the percutaneous absorption of Triallate. Following a variety of experiments, percutaneous absorption of Triallate could be better estimated using epidermal preparations (no dermal layer), and with the novel inclusion of a polyurethane plug in the saline to

serve as a sink to absorb and capture the Triallate [3]. Triallate absorption was found to range from 0.7 – 3.9% of the applied dose across the three formulations evaluated.

Study Conclusions. As a case study, this assessment of *in vitro* percutaneous absorption of Triallate demonstrates that optimization of the *in vitro* model is essential for poorly water soluble compounds. It is unfortunate that corresponding *in vivo* absorption data is not available for verification of the *in vitro* data for this compound. However, the intention of this particular case study is to offer a demonstration of the manner in which the principles underlying the operation of the cadaver skin model must be thoughtfully considered to generate meaningful data.

Overall Topic Discussion. Uncertainty regarding whether or not to accept the many reports of *in vivo – in vitro* correlation may arise from a difficulty for some to distinguish between inappropriate study design, and data indicating a meaningful lack of correlation. Additionally, acceptance of the correlative value of *in vitro* percutaneous absorption data may be tempered by caution in the face of uncertainty, because of the concern that *in vitro* data could grossly under-estimate the systemic levels resulting from a topical exposure to a potentially toxic compound. This is a responsible concern. However, the *in vitro* model can be implemented in a manner that provides meaningful, predictive, and essential data for compounds whose penetration through skin is extremely important to characterize. Indeed, the human cadaver skin model offers the unique and substantial advantage of providing a safe and inexpensive initial approach, to determine whether percutaneous absorption is rate-limited by the chemical characteristics of the molecule of interest. Thereafter, rational optimization of the parameters for utilizing the *in vitro* model with that molecule will provide a substantially better prediction of the *in vivo* exposure and thus more accurate assessment of safety prior to human exposure. A variety of guidances and books have been published which may prove to be useful in developing and assessing *in vitro* percutaneous absorption study designs [7,8,9,10,11, for example].

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