

Evaluation of the Safety and Skin Irritancy of PCCA VersaBase[®] Anhydrous HRT in Human Epidermis Model

SUMMARY: PCCA VersaBase Anhydrous HRT has been developed specifically for female hormone delivery. With water activity below 0.6 ($A_w < 0.6$), this anhydrous base is allowed extended default beyond-use dates (BUDs). The aim of this study was to evaluate the safety and skin irritancy potential of VersaBase Anhydrous HRT. An *in vitro* reconstructed human epidermis model (EpiDerm) that contains normal human-derived epidermal keratinocytes was used. The exposure time required for VersaBase Anhydrous HRT to reduce tissue viability by 50% (ET-50) was 24 hours. No sign of cell toxicity during 24-hour treatment has been observed. Based on the correlation between *in vivo* and *in vitro* irritancy response, VersaBase Anhydrous HRT is non-irritant, and is milder than baby shampoo.

Introduction:

VersaBase Anhydrous HRT is one of the proprietary bases in the PCCA Anhydrous system, and has been developed specifically for female bioidentical hormone delivery through the skin. With water activity below 0.6 ($A_w < 0.6$), an anhydrous base provides an unfavorable environment for microbial growth and therefore is allowed extended default beyond-use dates (BUDs) without compromising drug delivery capabilities.

Skin irritation is a local toxic response and is defined as reversible damage to skin following the application of a substance for up to 4 hours [1]. To ensure safety and predict toxicity, the potential of a base to induce skin irritation is an important consideration before using to deliver any active pharmaceutical ingredients (APIs). An *in vitro* reconstructed human epidermis model that contains normal human-derived epidermal keratinocytes has been validated by the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) for full replacement of the golden standard Draize test to differentiate skin irritants and non-irritants [2,3].

The aim of this study was to evaluate the safety and skin irritancy potential of VersaBase Anhydrous HRT using the EpiDerm tissue model.

Methodology:

Reconstructed Human Epidermis

The three-dimensional *in vitro* human EpiDerm system (EPI-200) was purchased from MatTek (Ashland, MA) (Figure 1). Cultures were maintained with supplied culture media according to the manufacturer's instructions. The EpiDerm tissues were exposed to 100 μ L of test base for 1, 4, 17 and 24 hours at 37°C. Each sample was duplicated. Triton X-100 1% solution was used as a positive control. Tissues left not dosed were to serve as a negative control. Following the exposure period, the dosing materials were removed and tissues were analyzed for viability.

Tissue Viability

Tissue viability was determined by measuring the reduction of 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT). The tissues were evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple). An MTT solution was prepared following the instruction of MatTek MTT-100 kit. At each time point, after the media and dosing solution were removed from all wells of EpiDerm tissue, the MTT solution was added to the basal side of each tissue and the tissues were incubated at 37°C for 3 hours. The purple formazan product was extracted using provided extractant applied to both the apical and basal side of the tissues. Optical density (OD) of samples were measured at 570 nm and reference OD at 650 nm with a plate reader. Tissue viability was calculated from the OD-Viability standard curve.

Results and Discussion:

VersaBase Anhydrous HRT was applied and spread on top of EpiDerm. Tissue viability was determined by MTT assay after 1, 4, 17 and 24 hours of application and results are shown in Figure 2.

Tissues exposed to VersaBase Anhydrous HRT were not affected by the base after 4 hours of exposure, followed by a

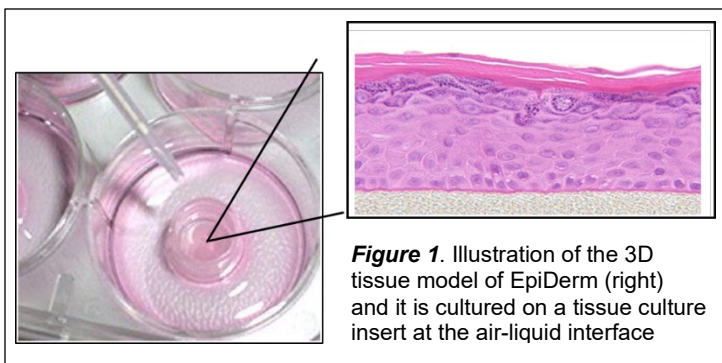


Figure 1. Illustration of the 3D tissue model of EpiDerm (right) and it is cultured on a tissue culture insert at the air-liquid interface

(Adapted and modified from MatTek Corporation, 2019)

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slow decline of tissue viability. After 24 hours, there were 51.66% tissue cells alive. In contrast, Triton X-100 at 1%, considered as a moderate-to-mild irritant, led to 22% tissue cell death in 4 hours, and only 3% cells survived Triton X-100 after 24 hours. Tissues treated by VersaBase Anhydrous HRT were above the irritation classification threshold of 50% viability at 24-hour time point, suggesting that VersaBase Anhydrous HRT is non-irritant. The exposure time required to reduce cell viability by 50% (ET-50) was 24 hours for VersaBase Anhydrous HRT, but only 9 hours for 1% Triton X-100 (Figure 2).

The correlation between *in vivo* and *in vitro* irritancy response is linked by ET-50 [4]. In our study, 1% Triton X-100 has shown ET-50 of 9 hours, which is consistent with the classification of moderate-to-mild irritancy and ET-50 range of 4 to 12 hours (Figure 3). A substance with ET-50 of 12 to 24 hours has the same irritancy as baby shampoo, which is very mild. If ET-50 is 24 hours, the irritancy is comparable to 10% Tween 20. Therefore, VersaBase Anhydrous HRT is milder than baby shampoo and as non-irritating as 10% Tween 20.

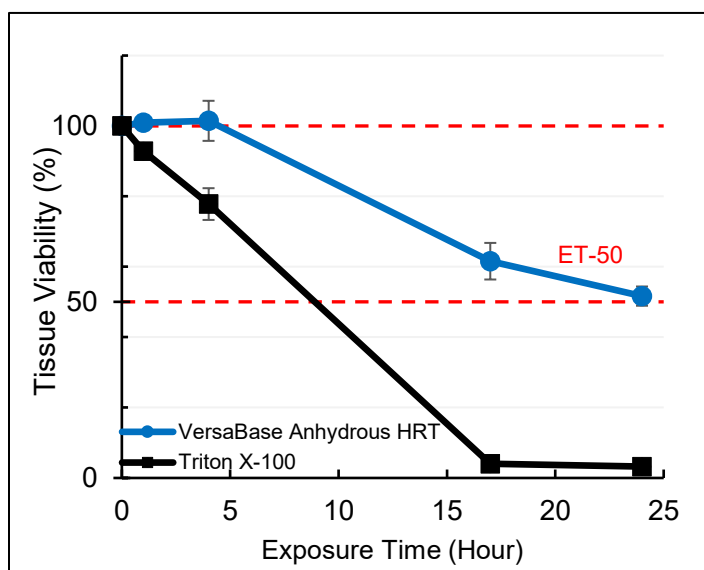


Figure 2. EpiDerm tissue viability profile after 24 hours exposure to VersaBase Anhydrous HRT and a positive control (1% Triton X-100).

Conclusions:

To ensure safety and predict potential toxicity, skin irritancy is an important consideration for any topical applied product. Due to the infeasibility of *in vivo* Draize test in most settings, EURL ECVAM has validated and recommended using the EpiDerm tissue model to define irritancy of a product.

Our study has shown no sign of skin toxicity and that VersaBase Anhydrous HRT is a non-irritating substance. It is milder than baby shampoo, and is as non-irritating as 10% Tween 20, which is a commonly used surfactant and emulsifier in personal care products. This study provides a safe option to physicians and compounding pharmacists when choosing bases for topical female hormone delivery with extended BUDs.

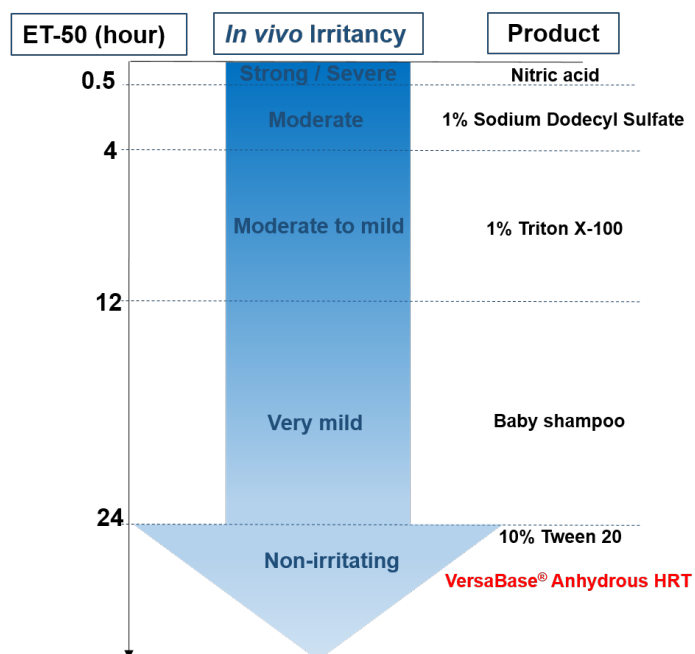


Figure 3. Correlation of *in vitro* and *in vivo* irritancy. (Modified based on MatTek protocol).

References:

- Globally harmonized system of classification and labelling of chemical (GHS), Fourth revised edition, United Nations, New York and Geneva, 2011.
- Faller C, Bracher M, Dami N, Roguet R. Predictive ability of reconstructed human epidermis equivalents for the assessment of skin irritation of cosmetics. *Toxicol In Vitro*. 2002;16(5):557-72. Bassani, A.S., Banov, D. and Phan, H. (2016) 'Characterization of the Percutaneous Absorption of Ketoprofen Using the Franz Skin Finite Dose Model', *Postgraduate Medicine*, 128 (2), p. 262-267. DOI:10.1080/00325481.2016.1144448.
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