Evaluation of the Safety and Skin Irritancy of PCCA PermE8™ Anhydrous Gel in Human Epidermis Model

SUMMARY: PCCA PermE8 Anhydrous Gel has been developed for a broad range of transdermal applications. With water activity below 0.6 (Aw<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 PermE8 Anhydrous Gel. An *in vitro* reconstructed human epidermis model (Epiderm) that contains normal human-derived epidermal keratinocytes was used. The exposure time required for PermE8 Anhydrous Gel to reduce tissue viability by 50% (ET-50) was longer than 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, PermE8 Anhydrous Gel is non-irritant, and is milder than baby shampoo.

Introduction:

PermE8 Anhydrous Gel is one of the proprietary bases in PCCA Anhydrous system, and has been developed for transdermal drug delivery [1]. With water activity below 0.6 (Aw<0.6), anhydrous base provides an unfavorable environment for microbial growth, is therefore 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 [2]. 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 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 [3,4].

The aim of this study was to evaluate the safety and skin irritancy potential of PermE8 Anhydrous Gel using EpiDerm tissue model.

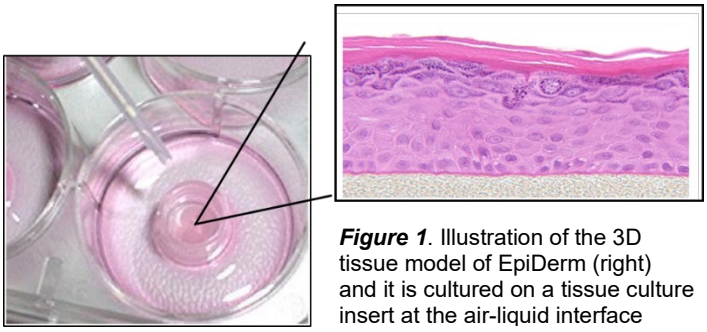

Figure 1. Illustration of the 3D tissue model of EpiDerm (right) and it is cultured on a tissue culture insert at the air-liquid interface

(Adapted and modified from MatTek Corporation, 2019)

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 µL 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:

PermE8 Anhydrous Gel 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 PermE8 Anhydrous Gel were not affected by the base after 4 hours exposure, and maintained 100% viability until 17 hours. After 24 hours, there were still

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94.78% 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 merely 3% cells survived Triton X-100 after 24 hours. Tissues treated by PermE8 Anhydrous Gel were above the irritation classification threshold of 50% viability at 24-hour time point, suggesting that PermE8 Anhydrous Gel is non-irritant. The exposure time required to reduce cell viability by 50% (ET-50) was significantly longer than 24 hours for PermE8 Anhydrous Gel, 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 [5]. 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, with ET-50 of longer than 24 hours, PermE8 Anhydrous Gel is milder than baby shampoo and as non-irritating as 10% Tween 20.

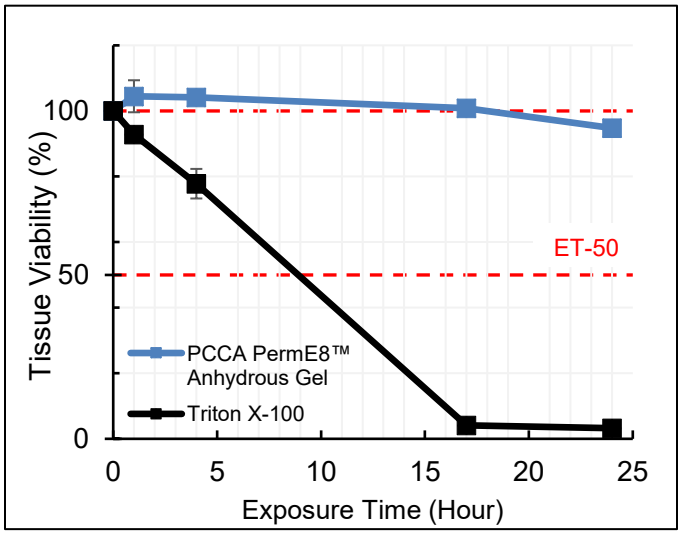

Figure 2. EpiDerm tissue viability profile after 24 hours exposure to PermE8 Anhydrous Gel and a positive control (1% Triton X-100).

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 PermE8 Anhydrous Gel 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 drug delivery with extended BUDs.

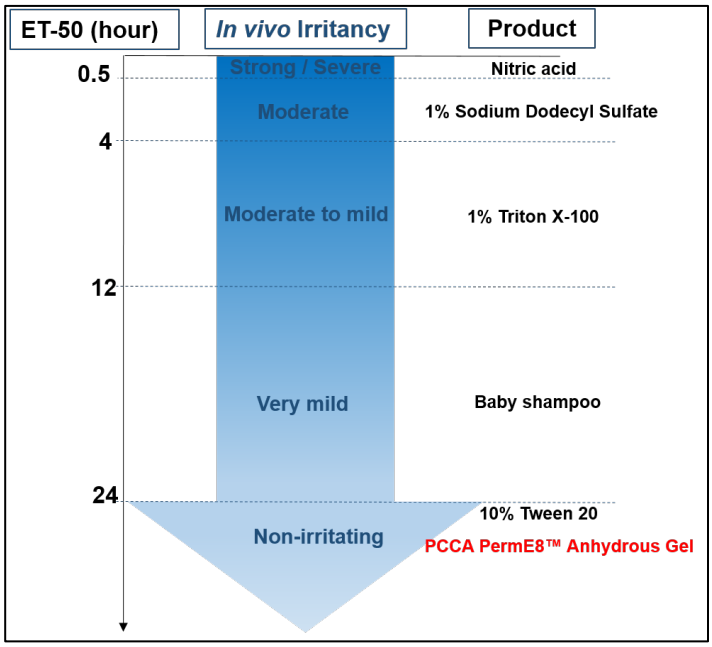

Figure 3. Correlation of *in vitro* and *in vivo* irritancy. (Modified based on MatTek protocol).

References:

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5. MatTek Corporation (2017) Protocol: *MTT Effective Time 50 (ET-50).* (MK-24-007-0001) Ashland, MA: USA.