PCCA EctoSeal P2G™ **TECHNICAL REPORT**

Evaluation of the Safety and Toxicological Profile on Reconstructed Human Epidermis

Introduction:

The epidermis is the outermost skin layer and it is increasingly used as a route of drug administration. Topical compounded medications must be non-toxic and non-irritant to the skin and, therefore, it is important to guarantee the safety of the bases used in compounding. The aim of this study was to evaluate the safety and toxicological profile of EctoSeal P2G™, in comparison to Poloxamer, using a 3-dimensional (3D) *in vitro* model of reconstructed human epidermis: EpiDerm™ by MatTek Corporation (Ashland, MA), a highly differentiated 3D model which consists of human-derived epidermal keratinocytes, cultured and differentiated to resemble the human epidermis.

Methodology:

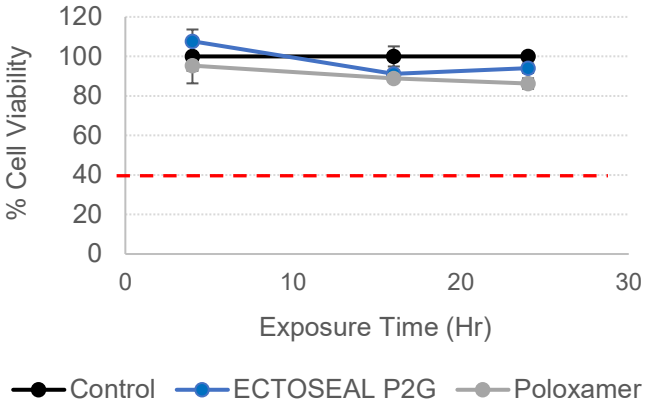
Upon receipt of the MTT-100 kit R, the EPI-200 cells (Lot 39169) were maintained in the supplied culture media and stored in accordance to the manufacturer's protocol until the initiation of the study. Following preparation of the cells, the EpiDerm™ tissues were treated in triplicate with 100 µL of the test product EctoSeal P2G 20% and another set of tissues were treated with Poloxamer 20% for 4, 16 and 24 hours. A triplicate set of EpiDerm™ tissues was also left untreated to serve as negative control. Following the exposure period, the dosing solutions were removed and the cells were analyzed for cell viability by the MTT Effective Time 50 (ET_{50}) assay, which consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) by the cells. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple formazan derivative. MTT is therefore an indicator of cell viability as the tissues are evaluated for their ability to reduce soluble-MTT (yellow) to formazan-MTT (purple).

Results and Discussion:

Viability of the epidermis tissue cells following exposure to the test products is represented by the absorbance of the respective extracts and expressed in percentage relative to the negative control, as follows: % Cell Viability=100 x [OD(test product) / OD(negative control)]. The greater the absorbency of the extracts, the greater the amount of MTT reduced by succinate dehydrogenase and, as a result, the higher the percent cell viability within the tissue.

Upon 24 hours of study, the viability of the cells exposed to EctoSeal P2G was superior to 90%. Similarly, the viability of the cells exposed to Poloxamer, a surfactant widely used in wound care, was superior to 85%, as demonstrated in the Table and Figure below.

Exposure Time (Hr)	**% Cell Viability**		
	Control (mean ± SD)	EctoSeal P2G (mean ± SD)	Poloxamer (mean ± SD)
4	100.00±13.63	107.68±1.03	95.34±2.58
16	100.00±5.09	91.18±1.99	88.89±1.40
24	100.00±1.18	94.02±2.42	86.29±2.63



The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50%, which is represented by a red dashed line in the Figure above. The general guideline for correlation of *in vitro* and *in vivo* results states that products with an ET_{50} of 24 hours are expected to be non-irritant. According to the results obtained, the ET_{50} of both EctoSeal P2G and Poloxamer is superior to 24 hours and, therefore, both products have a good safety and toxicological profile.

In conclusion, the proprietary compounding base EctoSeal P2G does not cause toxicity to the epidermis tissue. As a result, compounded medicines including this new proprietary compounding base may be applied to the skin without causing any toxicity to the epidermis tissue.