Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 2: Safety and Toxicological Profile by the MTT Assay*

SUMMARY: The evaluation of the safety and toxicological profile by the MTT assay is part of the safety assessment for PCCA Ellage™ Anhydrous Vaginal. This study builds on the HET-CAM assay (Part 1) by demonstrating that the toxic exposure time (ET_{50}) of PCCA Ellage is superior to 24 hours. It is concluded that PCCA Ellage has a good safety and toxicological profile on the vaginal mucosa, comparable to two reference OTC vaginal lubricants, and it is thus expected to be clinically safe *in vivo*.

Introduction:

The vaginal mucosa is a promising site for delivery of medication in the treatment of several conditions (e.g., vaginitis, infections) and also in hormone replacement therapy. Vaginal drug delivery, however, faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released. Mucoadhesive vaginal dosage forms are preferred over conventional gels and creams in order to prolong the contact time between the medication and the mucosal tissue, thus avoiding leakage and messiness. Considering the increased residence time, it is important to ensure that these mucoadhesive dosage forms are non-toxic and non-irritating to the vaginal mucosa [1].

The aim of this study was to evaluate the safety and toxicological profile of PCCA Ellage™ Anhydrous Vaginal, a mucoadhesive base designed specifically to remain in the vaginal mucosa for a longer period of time, in comparison to a positive control, the spermicide Gynol II, and two reference over-the-counter (OTC) vaginal lubricants (OTCs I&II). A 3-dimensional (3D) model of the human vaginal mucosa was used for the *in vitro* testing (Figure 1).

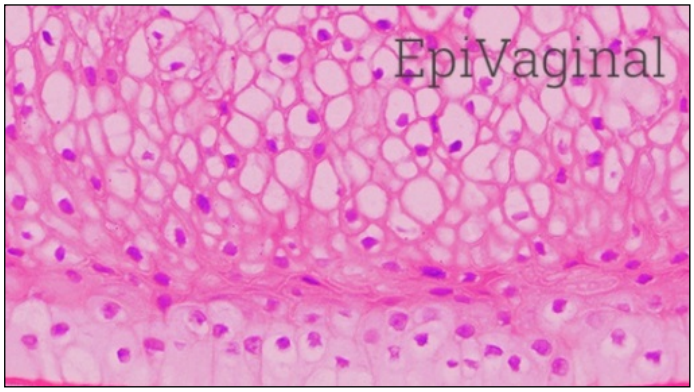


Figure 1. *Illustration of the EpiVaginal™ tissue model (adapted from MatTek Life Sciences, 2020)* [2].

Methodology:

The EpiVaginal™ (VEC-100) by MatTek Life Sciences (Ashland, MA) is a highly differentiated tissue that closely parallels the *in vivo* vaginal epithelial tissue. It is therefore an ideal *in vitro* research tool for safety and toxicological testing of feminine products [2].

The VEC-100 cells 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 EpiVaginal tissues were treated in duplicate with 100 µL of the test products (PCCA Ellage, OTCs I&II, and the control) for 1, 4, 16 and 24 hours. A set of EpiVaginal tissues remained untreated (in duplicate) to serve as a 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.

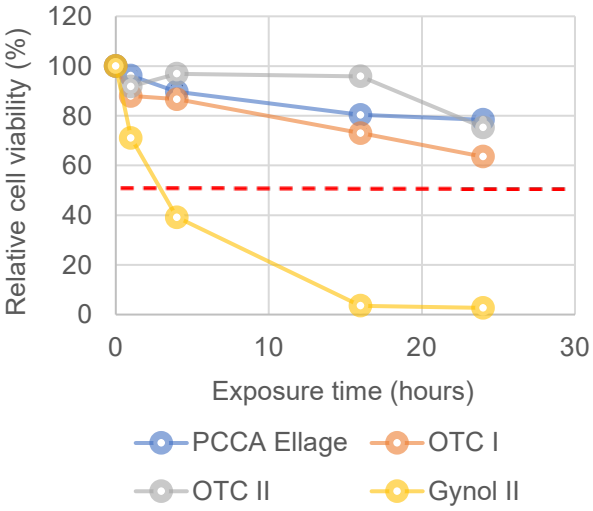
The MTT ET_{50} assay consists of measuring the reduction of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) by the cells. Succinate dehydrogenase enzymes within the mitochondria of viable cells have the ability to reduce soluble yellow tetrazonium salt of MTT to an insoluble purple 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) [3].

The MTT solution was prepared in the provided medium and added to the basal side of each tissue, followed by an incubation period of the tissues for 3 hours at 37°C. The purple formazan product was then extracted using the provided extractant, which was previously applied to both the apical and basal side of the tissues. Sample absorbance was read at 570 nm and reference absorbance at 650 nm with CLARIOstar – BMG Labtech Plate reader.

Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 2: Safety and Toxicological Profile by the MTT Assay*

Table 1, Figure 2. *Safety and toxicological profiles of PCCA Ellage, OTCs I&II and Gynol II for up to 24 hours.*

Time (hrs)	Relative cell viability (% mean ± SD)			
	PCCA Ellage	OTC I	OTC II	Gynol II
0	100.00±3.17	100.00±3.17	100.01±3.17	100.00±3.17
1	96.13±2.01	87.95±1.03	91.81±1.60	84.75±3.21
4	89.71±0.21	86.63±9.81	96.89±1.04	39.16±3.05
16	80.35±2.33	72.99±1.55	95.87±9.82	3.54±0.10
24	78.36±0.19	63.52±1.63	75.3±17.94	2.69±0.17



Results and Discussion:

The viability of the vaginal 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. 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 relative cell viability within the tissue.

At the start of the study (t=0 hours), the relative viability of the cells was 100% for all the tissues. Following 16 hours, the viability of the cells exposed to the positive control was less than 5%, which means that the vaginal tissue was no longer functional and thus confirms the toxicity of Gynol II. On the contrary, the viability of the cells exposed to PCCA Ellage was 78% following 24 hours, as shown in Table 1, Figure 2.

The toxic exposure time (ET_{50}) is the time when cell viability is reduced to 50%. The ET_{50} is represented by a red dashed line in Figure 2. According to the results obtained, the ET_{50} of the positive control is approximately 3 hours, as opposed to the ET_{50} of PCCA Ellage and the OTCs I&II which are superior to 24 hours (Table 1, Figure 2). This study demonstrates that PCCA Ellage has a good safety and toxicological profile on the vaginal mucosa, comparable to the reference OTC vaginal lubricants.

Conclusions:

The safety of vaginal compounded medicines is very important since damage to the vaginal mucosa weakens the natural defenses and increases the risk of infections such as HIV and herpes simplex [4].

The safety assessment for PCCA Ellage Anhydrous Vaginal was evaluated *in vitro* by the HET-CAM (Part 1) and the MTT (Part 2) assays. Both assessments have shown that PCCA Ellage is likely to remain at the site of action for a prolonged period of time without causing damage (irritancy/toxicity) to the vaginal tissue. As such, PCCA Ellage is expected to be clinically safe *in vivo*.

References:

1. Pereira, R. and Bruschi, M. (2012) 'Vaginal mucoadhesive drug delivery systems'. *Drug Development and Industrial Pharmacy*, 38 (6), p. 643-52.
2. MatTek Life Sciences (2020) EpiVaginal™. Available at: https://www.mattek.com/products/epivaginal/ (Accessed: 15 September 2020).
3. Wang, H., Cheng, H., Wang *et al.* (2010) 'An improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay for evaluating the viability of Escherichia coli cells'. *Journal of Microbiological Methods,* 82, p. 330-3.
4. Ayehunie, S., Cannon, C., LaRosa, K. *et al.* (2011) 'Development of an in vitro alternative assay method for vaginal irritation'. *Toxicology*, 279 (1-3), p. 130-8.