IN VITRO SAFETY PROFILE OF PERSONAL CARE PRODUCTS - USE OF AN *IN VITRO* TESTING PLATFORM BASED ON A RECONSTRUCTED VAGINAL TISSUE MODEL

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ABSTRACT

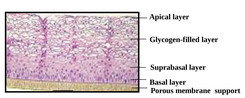
One of the common goals of industry is to confirm the safety of their products. Ethical concerns have led to the use of alternative testing methods in lieu of traditional testing methods. Several studies have shown good correlation between alternative test methods, traditional testing methods and human exposure. In the current study, the safety profile of three products with potential for vaginal exposure was assessed using the reconstructed human vaginal EpiVaginal™ model (MatTek Corporation, USA); the assay negative control (sterile, deionized water) and positive control (1% Triton® -X-100) were tested alongside. To increase the confidence in the test outcome, histopathology evaluation was conducted to assess the extent of cellular damage. Two liquid products were directly applied to the EpiVaginal™ tissues, while the wet wipe product was placed in direct contact with the tissue. Vaginal irritation expressed at ET_{50} values (3.32 and 12.71 hours) showed a higher irritation potential for the liquid formulations compared to the wipes (>24 hours). The lower irritation potential of the wipe product may be related to the availability of a rather limited amount of the liquid formulation in the wipes compared to the liquid formulations. Histology evaluations showed good correlation between the ET_{50} values and change in tissue structure. The results of this *in vitro* test methodology confirmed the safety profile of the products, should vaginal exposure occur during use. This two-endpoint testing platform (viability and histology) provided not only a correlative interpretation of the data, but also indication of the structural changes of the tissues exposed to the test article that are relevant to human exposure. Future plans include further exploring the capability of this *in vitro* testing platform for screening products before entering clinical trials.

INTRODUCTION

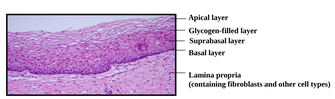
Repeated use of personal care products in the perineal region may induce irritation of the vaginal mucosa which could lead to other effects such as local infection. Therefore, the first step in the safety profile assessment is the testing of newly developed products for their irritation potential on the mucosal surface early in the research and development stage of product development. A second step consists of subsequent safety testing of products that are reformulated with the goal of reducing their irritation potential on vaginal tissue, particularly for products that are intended for regular use by the consumer. The *in vitro* test that is most frequently used to screen for vaginal mucosal irritation is based on reconstructed tissues that exhibit *in vivo*-like morphological and ultrastructural characteristics that provide a reproducible, consistent testing platform. Here we detail the results obtained in a screening study with products of interest to The Clorox Company. We used a combined approach using assessment of test products' cytotoxicity expressed as tissue viability and histology analysis to compare the safety profile of two different liquid products and a wet wipe. Our data supports the use of this methodology, based on the EpiVaginal™ (VEC-100) model from the MatTek Corporation, for pre-clinical screening of products intended for human use and addresses the reformulation needs of Product Development and Innovation groups that target the manufacturing of mild products.

MATERIALS & METHODS

Test System - EpiVaginal™ Tissue Model (MatTek Corporation)



EpiVaginal™ Tissue Model (MatTek Corporation)

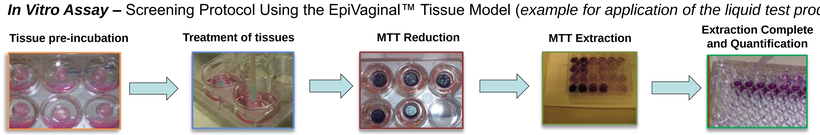


Native Human Vaginal Epithelium

Test Materials

- Two different types of Liquid products (Liquid #1 and Liquid #2)
- One type of wet wipe (Wipe #1) – the wipes were cut into ~0.5 cm diameter circles and applied to the tissues
- Test materials were tested at various exposure times and the viability (%) and ET_{50} values were compared

In Vitro Assay – Screening Protocol Using the EpiVaginal™ Tissue Model (*example for application of the liquid test products*)



ACKNOWLEDGMENTS

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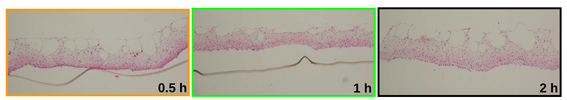
RESULTS

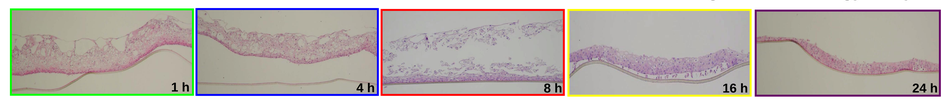
Positive control: 1.0% Triton® -X- 100



0.5 h: normal tissue morphology.

1 h and **2 h**: shrunken/condensed (pyknotic) nuclei associated with loss of viable cells.

Negative control: sterile, deionized water

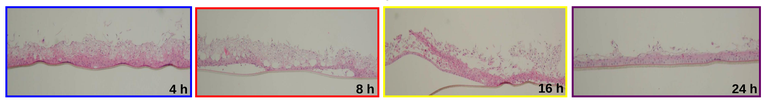


1 h and **4 h**: basal and suprabasal layers containing viable, nucleated cells and glycogen containing apical tissues layers were normal.

24 h: basal and suprabasal layers contained viable, nucleated cells, but the friable apical tissue layers are lost.

Figure 1 – Histology analysis

Wipe #1



4 h: the apical tissue layers were present with no signs of cytotoxicity. Tissues were viable and have normal tissue morphology.

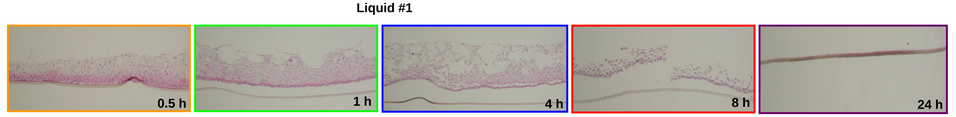
8 h: slight erosion of the apical layer was noted but the tissue had normal morphology.

16 h and **24 h**: tissues were dissociated and only in some spots few basal cells were visible.

Table 1 – Tissue viability results

Test material	ET_{50} value (hours)	Exposure time (h)							
		0.5	1	2	4	8	16	24	
Wipe #1	>24				93.7	78.8	128.3	94.0	Tissue Viability (%)
Liquid #1	3.32	94.1	101.3		42.1	16.2		7.7	
Liquid #2	12.71		84.6		87.3	62.7	43.7		
Positive control	0.93	98.9	44.5	34.8					

Liquid #1



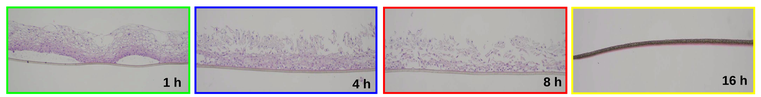
0.5 h and **1 h**: normal tissue morphology.

4 h: separation of the suprabasal layer from the basal layer, dissociation of the apical layer, and loss of suprabasal cell layer in some spots.

8 h: further deterioration of the tissue structure was noted; epithelium was dissociated, and the remaining few viable basal cell layer fragments were completely separated from the membrane support.

24 h: the epithelium was completely lost.

Liquid #2



1 h: slight separation from the underlying membrane support. Tissues were viable and had normal morphology.

4 h: dissociation of the apical layer.

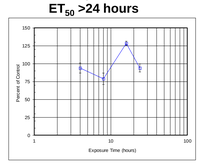
8 h: further deterioration of the tissue structure. With a few exceptions of viable cells in some spots, dissociation of the epithelium was noted.

16 h: epithelium completely lost.

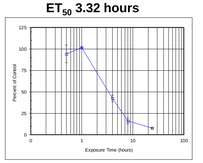
Positive control: 1.0% Triton® -X- 100
ET_{50} 0.93 hours

Figure 2 – Response curves

Wipe #1
ET_{50} >24 hours



Liquid #1
ET_{50} 3.32 hours



Liquid #2
ET_{50} 12.71 hours

CONCLUSIONS

1. **Our data showed a good correlation between the cytotoxicity assessment of the test articles (expressed as ET_{50} values) and the histology analysis performed for each exposure time (Figure 1, Figure 2).**
2. **Of the three products tested, the Wipe #1 was the least irritating. By comparison, Liquid #1 that was used to prepare the wet Wipe #1 had an ET_{50} value of 3.32 h and the histology analysis showed increased signs of toxicity starting with 4 h exposure time (Table 1). The lower irritation potential of the wipe product may be related to the availability of a rather limited amount of the liquid formulation in the wipes compared to the liquid formulation.**
3. **Of the two liquids tested, Liquid #2 was least irritating as demonstrated by the ET_{50} value of 12.71 h and by the histology analysis (Figure 1, Figure 2).**
4. **Our results show that the use of 3D tissue models represent a reliable testing platform for the screening of final formulations intended for human exposure to vaginal tissues. The tiered testing strategy used in our study involving cytotoxicity testing and histology analysis is a comprehensive approach used to compile the safety profile of products for feminine care.**

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