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™, while the wet tissue product was placed in direct contact with the tissue. Vaginal irritation expressed at ET₀ 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 ET₀ 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 perianal 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. In this study, the in vitro test that is most frequently used for screening 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 tissue. Our data supports the use of this methodology, based on the EpiVaginal™(VEC-100) model from the MatTek Corporation, for preclinical 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)

NatRef Human Vaginal Epithelium

NatRef Suprabasal Layer

Porous membrane Support

Native Human Vaginal Epithelium (MatTek Corporation)

Test Material:

- 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 ET₀ values were compared.

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

<table>
<thead>
<tr>
<th>Treatment of tissue</th>
<th>MTT Reduction</th>
<th>MTT Extraction</th>
<th>Extraction Control and Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test material</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Table 1 – Tissue viability results

<table>
<thead>
<tr>
<th>Test material</th>
<th>Wipe #1</th>
<th>Liquid #1</th>
<th>Liquid #2</th>
<th>Wipe #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure Time (h)</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Percent Control</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>ET₀ (h)</td>
<td>8</td>
<td>90</td>
<td>65</td>
<td>3.7</td>
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<tr>
<td>Viable cells (%)</td>
<td>96</td>
<td>95</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Positive control</td>
<td>99.6</td>
<td>99.5</td>
<td>100</td>
<td>98.6</td>
</tr>
<tr>
<td>Negative control</td>
<td>71</td>
<td>65</td>
<td>60</td>
<td>65</td>
</tr>
</tbody>
</table>

CONCLUSIONS

1. Our data showed a good correlation between the cytotoxicity assessment of the test articles (expressed as ET₀ 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 had an ET₀ value of 3.32 h and the histology analysis showed increased signs of toxicity starting with 4 h exposure (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 ET₀ 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.

CONCLUSIONS

REFERENCES


The MatTek Study Protocol – Vaginal Irritation Test for use with the VSC-100 Tissue Model 6/19/05.
