Evaluation of the Irritation Potential of PCCA Ellage™ Anhydrous Vaginal *Part 1: Hen's Egg Test-Chorioallantoic Membrane Assay*

SUMMARY: The evaluation of the irritation potential by the HET-CAM assay is part of the product safety assessment for PCCA Ellage™ Anhydrous Vaginal. A preliminary study was conducted at PCCA R&D and another study was outsourced to a specialized company. Both studies have shown that PCCA Ellage has no ocular irritation potential (IS<5). This finding strongly suggests that PCCA Ellage is also no irritant to the vaginal mucosal membrane and it is thus expected to be clinically safe *in vivo*.

At PCCA R&D, we respect animal welfare and we do not test our products on animals. Instead, we are proud to collaborate with institutions that provide alternatives to the use of animals for scientific purposes.

Introduction:

The evaluation of the irritation potential is part of the product safety assessment for PCCA Ellage Anhydrous Vaginal. There is evidence in the literature to suggest testing vaginal irritation with the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) assay, an *in vitro* alternative to the international standard Draize rabbit *in vivo* ocular irritation test. The irritation potentials for the eye and the vaginal mucosa are similar and, as such, any skin or eye irritant substance shall be directly labelled as a potential vaginal irritant [1,2].

The HET-CAM assay is a rapid, sensitive and inexpensive toxicity test that has been widely used to evaluate the potential ocular irritation of substances by measuring the ability to induce toxicity in the CAM of a chicken egg [1]. The HET-CAM test method recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is applicable to nonregulatory, validation or optimization of preclinical studies (NIH Publication No. 10-7553 – 2010).

The majority of vaginal products are intended to be self-administered and, as such, it is desirable that these products offer maximum comfort at the time of application and during the time of use [1]. PCCA Ellage is a mucoadhesive vaginal base that was developed to remain at the site of application. It is therefore very important to evaluate the irritation potential of this base in order to ensure the safety of the corresponding compounded medicines.

Aim & Methodology:

The aim of this study was to test the irritation potential of the vaginal base PCCA Ellage in comparison to positive and negative controls.

A preliminary study was conducted at PCCA R&D (Figures 1a and 1b), which was followed by the experiment V20-4095 at Consumer Product Testing Company, Inc. (CPT^{SM}) (Fairfield, NJ). The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) HET-CAM Recommended Test Method (NIH Publication No. 10-7553 – 2010) was the protocol followed at PCCA R&D with the negative and positive test controls, 0.9% NaCl and 0.1N NaOH, respectively. A modification of the HET-CAM Luepke and Kemper (1986) was the protocol followed by the outsourced facility [3] with popular eye cosmetics as negative test controls: Nivea Visage Liposome Eye Contour Gel and Pond's Revitalizing Eye Gel with Vitamin E. There are variable scoring schemes for the HET-CAM assay. The Irritation Scores (IS) adopted classifies the test products as no irritants for IS between 0 and 4.9; and as irritants for IS greater than 5 [4,5].

Figure 1. *Exposing the chorioallantoic membranes by the PCCA R&D team.*

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Results and Discussion:

The preliminary study at PCCA R&D has demonstrated that both PCCA Ellage and the 0.9% NaCl have no ocular irritation potentials (IS=0). Contrarily, the 0.1N NaOH is strongly irritative (IS=17) as shown by the lysis (vessels disintegration), hemorrhage (vessels bleeding) and coagulation (blood clotting) displayed in Figure 2. This experiment was extended for a total of 20 minutes and PCCA Ellage still presented no irritation potential.

The outsourced study by CPT[SM] yielded comparable results for PCCA Ellage (lot number 0527009), with an IS of 2.50 which is considered non-irritant by Gilleron *et al.* (IS=0-4.9) [4,5]. The negative controls were also classified as non-irritants with IS of 3.0 and 2.0 for the Nivea Visage Liposome Eye Contour Gel and the Pond's Revitalizing Eye Gel with Vitamin E, respectively. All irritation scores correspond to an average of 4 eggs tested per product. PCCA Ellage and the controls were all diluted to 50% in this experiment because previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye [6].

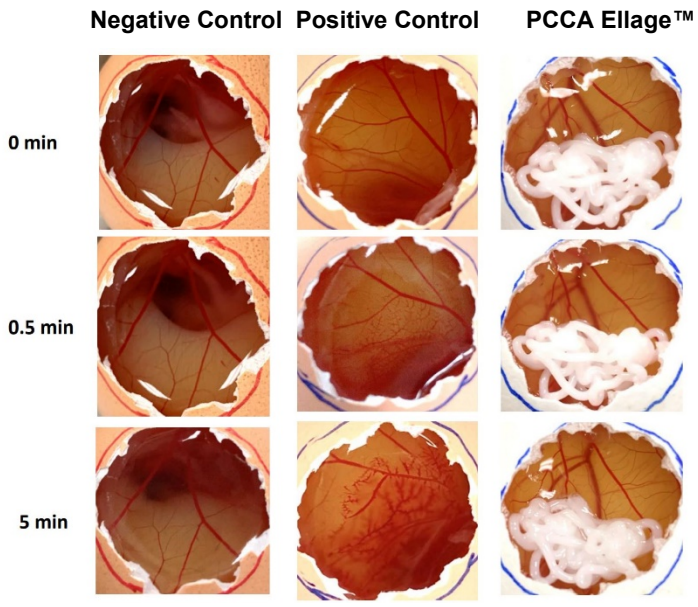

Figure 2. *Test eggs exposed to PCCA Ellage and controls (0.9% NaCl and 0.1N NaOH) for a contact time of 5 minutes.*

Conclusions:

The HET-CAM assay is perceived as an ideal *in vitro* test to evaluate the ocular irritation potential (topical toxicity) of substances. When transposed and applied to the vaginal irritation potential, this assay widens the preclinical safety assessment portfolio of vaginal products [1].

These preliminary and outsourced HET-CAM assays have shown that PCCA Ellage has no ocular irritation potential (IS<5). This finding strongly suggests that PCCA Ellage is also a non-irritant to the vaginal mucosal membrane and is thus expected to be clinically safe *in vivo*.

References:

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