



# Consumer Product Testing Co.

## FINAL REPORT

**CLIENT:**

Zenitech  
PO Box 44  
Old Greenwich, CT 06870

**ATTENTION:**

Carter LaVay

**TEST:**

The MatTek Corporation EpiOcular™ Tissue  
Model *In Vitro* Toxicity Testing System

**TEST ARTICLE:**

Zenigloss®, 04 Apr. 2001, Lot P01358

**EXPERIMENT  
REFERENCE NO.:**

V01-0077-1

Kathleen Alworth, B.A.  
Director of Quality Assurance

Steven Nitka  
Vice President  
Laboratory Director

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## QUALITY ASSURANCE UNIT STATEMENT

**Study No.:** V01-0077-1

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance with standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections may have been reported to management and the Study Director.

**Date of data inspection:** July 2, 2001

**Professional personnel involved:**

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Essam Eldeib, Ph.D.	-	Quality Assurance Associate

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures, study protocols and the Good Laboratory Practice principles.

**Objective:**

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiOcular *in vitro* toxicity testing system.

**Introduction:**

"MatTek's patented EpiOcular corneal Model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. The epidermal cells, which are cultured on specially prepared cell culture inserts using serum free medium, differentiate to form a multilayered structure which closely parallels the corneal epithelium . . . " This system " . . . provides a predictive, morphologically relevant *in vitro* means to assess ocular irritancy."<sup>1</sup>

EpiOcular, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

**Test Article:** Zenigloss<sup>®</sup>, 04 Apr. 2001, Lot P01358 (50%)

**Method:**

As per the sponsor's instructions, the test article was prepared as a 50% suspension in distilled water. This suspension exhibited a specific gravity greater than 0.95 g/ml. The 50% suspension was therefore diluted in distilled water (1 part test article suspension to 4 parts distilled water) and the concentration dosed was 10%. After the appropriate tissue preparation, 100 microliters of the test article and the negative control (distilled water) were added to the Millicells containing the EpiOcular samples. The six (6) well plates containing the dosed EpiOcular samples were then incubated at 37°C, five (5)% carbon dioxide and  $\geq$  90% humidity.

<sup>1</sup>MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721

**Method (continued):**

After the appropriate exposure period, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual material. Each was then rinsed a second and third time. Following the 3 rinses, each Millicell was submerged in 5 milliliters of assay media for 10 minutes, at room temperature. This final soak removed any residual, absorbed article. After the 10 minutes, excess liquid was shaken off and each EpiOcular tissue was placed into 300 microliters of MTT solution. The EpiOcular samples were then returned to the incubator.

After the three (3) hour MTT exposure each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts, which were then blotted on the bottom on paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate. Each insert was then immersed in two (2) milliliters of extraction, at room temperature, overnight. After the extraction procedure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of a negative control (distilled water) defined as 100%, the percent absorbencies of the test article were determined. The percentages listed below directly correlate with the cell metabolism in the EpiOcular samples.

**Results:**

<u>Article (% &amp; Exposure)</u>	<u>System</u>	<u>Percent Viability</u>	<u>Percent Inhibition</u>
Zenigloss <sup>®</sup> , 04 Apr. 2001, Lot P01358 (10% - 5 min.)	EpiOcular	104	-4
(10% - 1 hr.)	EpiOcular	104	-4
(10% - 4 hr.)	EpiOcular	106	-6

Using a semi-log scale, the percent viabilities for the test article were plotted on the linear y axis versus the dosing time on the log x axis. By interpolation, the time at which the percent viability would be 50% was determined (ET-50). As a general guideline (provided by MatTek) the following equation can be used to estimate the rabbit Draize eye score:

$$\text{Draize} = -4.74 + 101.7/(\text{ET}-50)^{0.5}$$

Based on the literature (Kay, J.H. and Calandra, J.C., "Interpretation of eye irritation tests," *J. Soc. Cosmetic Chem.*, 13, 281-289 (1962)), the ocular irritancy estimated potential has been categorized by MatTek into the following groups, based on the Draize score:

<u>Draize Score</u>	<u>Irritancy Classification</u>	<u>Example</u>	<u>EpiOcular ET-50 (min)</u>
0-15	Non-irritating, Minimal	PEG-75 Lanolin, Tween 20	>256 – 26.5
15.1 – 25	Mild	3% Sodium Dodecyl Sulfate	<26.5 – 11.7
25.1 – 50	Moderate	5% Triton X-100	<11.7 – 3.45
50.1 – 110	Severe, Extreme	5% Benzalkonium Chloride	<3.45

#### **Discussion:**

The Zenigloss<sup>®</sup>, 04 Apr. 2001, Lot P01358 test article elicited *in vitro* results, which place its ET-50 greater than 256 minutes. Therefore, at 50%, the test article's estimated Draize ocular irritation score is approximately 0 with a "non-irritating" irritancy classification.

#### **Conclusion:**

Under the conditions of this test, the results indicate that the Zenigloss<sup>®</sup>, 04 Apr. 2001, Lot P01358 has a "non-irritating" irritancy classification.