Consumer Product Testing Co.

EST. 1973

FINAL REPORT

CLIENT:	Aphex BioCleanse Systems, Inc.
ATTENTION:	David J. Weaver
TEST:	The MatTek Corporation EpiOcular™ Tissue Model *In Vitro* Toxicity Testing System
TEST ARTICLE:	Dermaphex Hand Sanitizer Lot 1039101
EXPERIMENT REFERENCE NO.:	Vll-0544

2/22/11

Steven Nitka
Vice President
Laboratory Director

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: Vl 1-0544

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed 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. The findings of this inspection may have been reported to management and the Study Director.

Quality Assurance:

Christine Hendricks 2/22/11
Signature/Date

70 New Dutch Lane• Fairfield, New Jersey 07004-2514 • (973) 808-7111 •Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

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."[1]

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: Dermaphex Hand Sanitizer Lot 1039101

Method:

The test article, at 100%, exhibited a specific gravity greater than 0.95 g/ml and it is water soluble. As per MatTek's protocol, the test article was diluted to 20% in distilled water. 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 90% humidity.

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 retl.lflled 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 using 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 the negative control (distilled water) defined as 100%, the percent absorbencies of the articles were determined. The percentages listed below directly correlate with the cell metabolism in the EpiOcular samples.

Results:

Article (% & **Exposure)**	**System**	**Percent Viability**	**Percent Inhibition**
Dermaphex Hand Sanitizer Lot 1039101			
(20% - 4 hrs.)	EpiOcular	41	59
(20% - 1 hr.)	EpiOcular	86	14
(20% - 20 mins.)	EpiOcular	99	1

When possible, using a semi-log scale, the percent viabilities for the articles 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:

Draize = -4.74 + 101.7/(ET-SOy0.5

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:

Draize Score	Irritancy Classification	Example	EpiOcular ET-50 (min)
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:

Under the conditions ohhis test, the Dermaphex Hand Sanitizer Lot 1039101 test article, at 20%, elicited *in vitro* results which indicate that its ET-50 is 181.2 minutes. Therefore, the test article, at 100%, has an estimated Draize ocular irritation score of "2.8" with a "minimally irritating" irritancy classification.

Conclusion:

Under the conditions of this test, the results indicate that the Dermaphex Hand Sanitizer Lot 1039101 test article, at 100%, has a "minimally irritating" irritancy classification.

Record Retention:

All records and documents pertaining to the conduct of this study shall be retained in the CPTC archives for a minimum of ten (10) years. At any time prior to the completion of the tenth archival year, a Sponsor may submit a written request to the CPTC QA Department to obtain custody of study records once the CPTC archive period has been completed. This transfer shall be performed at the Sponsor's expense. In the absence of a written request, study-related records shall be destroyed at the end of the CPTC archive period in a manner that renders them useless.

Professional personnel involved:

Steven Nitka, B.S.	Vice President Laboratory Director (Study Director)
Lillian Vazquez, B.S.	Laboratory Supervisor
Christine Hendricks	Quality Assurance Group Leader