



EST. 1975

Consumer Product Testing Co.

FINAL REPORT

CLIENT:

Optigenex, Inc.
750 Lexington Avenue
6th Floor,
New York, New York 10022

ATTENTION:

Jerry Wachs

TEST:

The Hen's Egg Test - Utilizing the Chorioallantoic
Membrane (HET-CAM)

TEST ARTICLE:

Eye Cream, Formula # 1636A, Date: 06/29/05

**EXPERIMENT
REFERENCE NO.:**

V05-0136

Christine Hendricks 8/1/05
Christine Hendricks
Quality Assurance Associate

Steven Nitka 8/1/05
Steven Nitka
Vice President
Laboratory Director

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



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QUALITY ASSURANCE UNIT STATEMENT

Study No.: V05-0136

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: August 1, 2005

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Christine Hendricks	-	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 and applicable study protocols.

Objective:

To evaluate the test article for irritancy potential utilizing the HET-CAM test. The test is a modification of that described by Kemper and Luepke.¹

Introduction:

The chick embryo has been used extensively in toxicology. "The chorioallantoic membrane (CAM) of the chick embryo is a complete tissue with organoid elements from all germ cell layers. The chorionic epithelium is ectodermal and the allantoic epithelium is endodermal. The mesoderm located between these epithelia is a complete connective tissue including arteries, capillaries, veins and lymphatic vessels. The CAM responds to injury with a complete inflammatory reaction, comparable to that induced in the rabbit eye test. It is technically easy to study, and is without nerves to sense pain."²

Test Article: Eye Cream, Formula # 1636A, Date: 06/29/05

Reference Articles: Almay One Coat Mascara
Maybelline Waterproof Ultra Eyeliner

¹Kemper, F.H. & Luepke, N.P., (1986). The HET-CAM Test: An Alternative to the Draize Test. *FD Chem. Toxic.* 24, p. 495 - 496.

²Leighton, J., Tchao, R., Verdone, J. & Nassauer, J. Macroscopic Assay of Focal Injury in the Chorioallantoic Membrane. In: *Alternative Methods in Toxicology*, Vol. 3, *In Vitro Toxicology E2*, pp. 357 - 369, Alan M. Goldberg, (ed.), Mary Ann Liebert Publishers, Inc., New York, 1985.

Method:

Fresh, fertile, White Leghorn eggs were obtained from Moyer's Chicks, Inc., in Quakertown, Pennsylvania. They were stored at this facility for up to seven (7) days, at approximately 50° F, before being incubated. For incubation the eggs were placed, on their sides, in a Kuhl incubator. The incubator is such that the eggs are automatically rotated once every hour. The temperature was controlled at 99° F ($\pm 1^\circ$) with a relative humidity of 60 - 70% for the ten (10) days of incubation. On day eight (8) the eggs were turned so that the acutely angled end faced down.

On day ten (10) each egg was removed from the incubator and placed in a Plexiglas work enclosure. This enclosure had been preheated and humidified so that its environment approached that of the incubator. A cut was made in the larger end of each egg, where the air sack is located. A Dremel® Moto-Flex Tool (model 232-5) equipped with a Dremel® Cut-Off Wheel (No. 409) was used to make each cut. Forceps were then used to remove the shell down to the shell-membrane junction. The inner egg membrane was then hydrated with a warm, physiological saline solution. The saline was removed after a two (2) to five (5) minute exposure. Utilizing pointed forceps, the inner egg membrane was then carefully removed to reveal the CAM.

The test or reference article, at a dosage of three-tenths of one milliliter (0.3 ml) of a liquid or three-tenths of one gram (0.3 g) of a solid, was then administered to each of four (4) CAM's. Twenty seconds later, the test or control article was rinsed from each CAM with five (5) milliliters of physiological saline. All CAM's were observed immediately prior to test article administration and at 30 seconds, two (2) and five (5) minutes after exposure to the test article. The reactions of the CAM, the blood vessels, including the capillaries, and the albumin were examined and scored for irritant effects as detailed below:

Effect	Time (min.)	Score		
		0.5	2	5
Hyperemia		5	3	1
Minimal Hemorrhage ("Feathering")		7	5	3
Hemorrhage (Obvious Leakage)		9	7	5
Coagulation and/or Thrombosis		11	9	7

The numerical, time dependent scores were totaled for each CAM. Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 32. The mean score was determined for all CAM's similarly tested.

Results:

Test Article (%)	CAM #	Scores @			
		0.5 min.	2 min.	5 min.	Total
Eye Cream, Formula # 1636A, Date: 06/29/05 (50%)	1	0	0	0	0
	2	0	3	0	3
	3	0	0	1	1
	4	0	3	0	3
				Average:	1.75

Reference Article (%)	CAM #	Scores @			
		0.5 min.	2 min.	5 min.	Total
Almay One Coat Mascara (50%)	1	0	0	1	1
	2	0	0	1	1
	3	0	0	0	0
	4	0	0	0	0
				Average:	0.50

Reference Article (%)	CAM #	Scores @			
		0.5 min.	2 min.	5 min.	Total
Maybelline Waterproof Ultra Eyeliner (50%)	1	0	0	1	1
	2	0	0	1	1
	3	0	0	1	1
	4	0	0	0	0
				Average:	0.75

Each article was then classified as indicated in the following:

Mean Score	Irritation Potential
0.0 - 4.9	Practically none
5.0 - 9.9	Slight
10.0 - 14.9	Moderate
15.0 - 32.0	Severe

Discussion:

Previous studies have shown that the CAM of the hen's egg is more sensitive to liquid irritants than is the rabbit eye. Therefore, dilutions of the liquid test and reference articles were used.

Historical *In Vivo* Results:

The reference products have historically been categorized as being practically non-irritating, eliciting scores approaching 0, at 24 hours, when dosed at 100% and tested using the Draize ocular irritation methodologies (Draize Scale: 0 – 110).

Conclusion:

Under the conditions of this test, the results indicate that the sponsor-submitted product, Eye Cream, Formula # 1636A, Date: 06/29/05, at 100%, would have practically no ocular irritation potential *in vivo*.