

FINAL REPORT

Study Title

**TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY  
USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT**

Test Substances

V  
J  
P  
T  
K  
R  
W

Reference Substance

H

Authors

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Study Completion Date

August 6, 2006

Performing Laboratory

Institute for In Vitro Sciences, Inc.  
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Study Number

05AE37, AE40-AE43, AE50, AE51, AG41.015033

Laboratory Project Number

4281

## TABLE OF CONTENTS

TABLE OF CONTENTS .....	2
STATEMENT OF COMPLIANCE .....	3
QUALITY ASSURANCE STATEMENT .....	4
SIGNATURE PAGE.....	5
TEST/REFERENCE SUBSTANCE RECEIPT .....	6
TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT	
INTRODUCTION.....	8
MATERIALS AND METHODS .....	9
RESULTS AND DISCUSSION .....	12
APPENDIX A	
SP015033 (PROTOCOL) .....	1-9
PROTOCOL ATTACHMENT 1 .....	1
PROTOCOL AMENDMENT I .....	1-2
PROTOCOL AMENDMENT II.....	1
PROTOCOL AMENDMENT III.....	1-2
APPENDIX B (RAW DATA) .....	B1-B13

## STATEMENT OF COMPLIANCE

The Topical Application Ocular Irritation Screening Assay Using The EpiOcular™ Human Cell Construct of the test substances, V, J, P (sample 1 and sample 2), T, K, R, and W (sample 1 and sample 2), and the reference substance, H (sample 1 and sample 2), was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test and reference substances have not been determined by the testing facility.

The stability of the test and reference substances under the test conditions has not been determined by the testing facility and is not included in the final report.

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Greg Mun, B.A.  
Study Director

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Date

## QUALITY ASSURANCE STATEMENT

Study Title: Topical Application Ocular Irritation Screening Assay Using the EpiOcular™ Human Cell Construct

Study Number: 05AE37, AE40-AE43, AE50, AE51, AG41.015033

Study Director: Greg Mun, B.A.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160) and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

<b>Phase Inspected</b>	<b>Audit Date(s)</b>	<b>Reported to Study Director</b>	<b>Reported to Management</b>
Protocol and Initial Paperwork	07-Dec-05	07-Dec-05	08-Dec-05
Rinsing of the Tissues/ Addition to MTT	07-Dec-05	07-Dec-05	08-Dec-05
Final Report and Data	28-30 June & 05-Jul-06	05-Jul-06	02-Aug-06

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

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Amanda K. Ulrey, RQAP-GLP  
Quality Assurance

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Date

**SIGNATURE PAGE**

**TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY  
USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT**

Initiation Date: December 7, 2005

Completion Date: August 6, 2006

Sponsor:

Sponsor's Representative:

Testing Facility: Institute for In Vitro Sciences, Inc.  
21 Firstfield Road, Suite 220  
Gaithersburg, MD 20878

Archive Location: Institute for In Vitro Sciences, Inc.  
Gaithersburg, MD 20878

Study Director:

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Greg Mun, B.A.

Date

### TEST/REFERENCE SUBSTANCE RECEIPT

IIVS Test/Reference Substance Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
05AE37	V	clear light green non-viscous liquid	8/1/05	room temperature
05AE40 (sample 1)	H	cloudy very light yellow non-viscous liquid	8/1/05	room temperature
05AE40 (sample 2) <sup>+</sup>	H	clear colorless non-viscous liquid	3/8/06	room temperature
05AE41	J	clear blue non-viscous liquid	8/1/05	room temperature
05AE42 (sample 1)	P	cloudy light orange non-viscous liquid	8/1/05	room temperature
05AE42 (sample 2) <sup>^</sup>	P	clear light orange non-viscous liquid	3/8/06	room temperature
05AE43	R	clear colorless non-viscous liquid	8/1/05	room temperature
05AE50	T	clear colorless non-viscous liquid	8/22/05	room temperature
05AE51 (sample 1)	W	clear colorless non-viscous liquid	8/22/05	room temperature
05AE51 (sample 2) <sup>^</sup>	W	clear colorless non-viscous liquid	3/8/06	room temperature
05AG41	K	fine white powder	9/30/05	room temperature

\* - Protected from exposure to light

<sup>+</sup> - Second sample used for comparison with the first sample in an additional definitive assay.

<sup>^</sup> - Second samples were prepared at IIVS from the new test substances (256X concentrates) provided by the Sponsor and were tested in an additional definitive assay.

**TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY  
USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT**

## INTRODUCTION

The EpiOcular™ Human Cell Construct was used to assess the potential ocular irritation of the test and reference substances. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to the test and reference substances for various exposure times<sup>1</sup>. The duration of exposure resulting in a 50% decrease in MTT conversion in test substance-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (ET<sub>50</sub>).

The purpose of this study was to evaluate the potential toxicity of the test and reference substances as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to each test or reference substance for various exposure times. The laboratory phase for the first valid definitive trial was conducted from December 7, 2005 to December 8, 2005 at the Institute for In Vitro Sciences, Inc.

As instructed by the Study director following a consultation with the Sponsor, the reference substance, H (sample 1 and sample 2), and the test substances, P (sample 2) and W (sample 2), were tested in a second valid definitive trial from April 5, 2006 to April 6, 2006 at the Institute for In Vitro Sciences, Inc. The results of both valid definitive assays are included in this report. All of the test and reference substances were tested in at least one valid screening assay to determine the duration of exposure to each test substance, which resulted in the ET<sub>50</sub> endpoint.

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<sup>1</sup> Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

## MATERIALS AND METHODS

### Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ Human Cell Construct Kit (MatTek Corporation), the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing, EpiOcular™ Assay Medium was warmed to approximately 37°C. Nine-tenths mL of Assay Medium were aliquoted into the appropriate wells of six-well plates. The six-well plates were labeled to indicate test substance and exposure time. The constructs were inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the six-well plates. The constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO<sub>2</sub> in air (standard conditions) for at least one hour. Following at least one hour, the medium was aspirated and 0.9 mL of fresh Assay Medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated.

### Test/Reference Substance Preparation

As instructed by the Sponsor, the test substances, V, J, P, T, K, and R, and the reference substance, H, were tested as provided by the Sponsor (neat).

For the test substance, K, an entire packet was diluted in 1.25 gallons of deionized water on the day of use. This formed the full strength (100%) test substance for testing. Since the inner package of K was not coded, the dilution was performed and documented by some one not performing the assay. For the remainder of this report, the diluted test substance is referred to as the test substance.

In the second definitive assay, the test substances, P (sample 2), and W (sample2), were prepared by diluting the concentrates (256X) in sterile, deionized water (Quality Biological). Each 1X test substance was prepared by diluting 0.5 mL of the test substance concentrate in 127.5 mL of water.

### Assessment of Direct Test/Reference Substance Reduction of MTT

Each test/reference substance was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) containing 2 mM L-glutamine (MTT Addition Medium) to assess its ability to directly reduce MTT. Approximately 100 µL of each test or reference substance were added to 1 mL of the MTT solution and the mixtures were incubated in the dark at standard conditions for approximately one hour. A negative control, 100 µL of sterile, deionized water (Quality Biological), was tested concurrently. If the MTT solution color turned blue/purple, the test substance was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test substance and the medium.

The test substances, V, J, P, R, T, W, and K, and the reference substance, H, were not observed to reduce MTT in the absence of viable cells.

### pH Determination

The pH of each liquid test or reference substance was measured using pH paper for both definitive assays. Initially, the test and reference substances were added to pH paper (EMD Chemicals Inc./EM Science) with a 0-14 pH range in 1.0 pH unit increments to approximate a narrow pH range. Next, each neat test or reference substance was added to pH paper with a narrower range of 0-6 pH, 5-10, and/or 7.5-14 pH range with 0.5 pH unit increments, to obtain a more accurate pH value. The pH values obtained from the narrower range pH paper are presented in Table 1 and Table 2. The pH of the test substance, J, could not be determined because the test substance discolored the pH paper.

### MTT Assay

The test and reference substances, H, P, and W, were tested in two valid definitive assays. Both the results of the first definitive assay (Trial 1, performed from December 7, 2005 to December 8, 2005) with an exception for the test article, P, and the second definitive assay (Trial 2, performed from April 5, 2006 to April 6, 2006) were considered valid and are presented in this report

In Trial 1, the EpiOcular™ cultures were treated in duplicate with the test substance, V and the reference substance, H, at four exposure times of 1, 5, 10, and 20 minutes. The EpiOcular™ cultures were treated in duplicate with the test substances, J and W, at four exposure times of 5, 10, 20, and 45 minutes. The EpiOcular™ cultures were treated in duplicate with the test substances, P, R, T, and K, at four exposure times of 20, 45, 90, and 240 minutes. One hundred microliters of the liquid test substance were applied to each EpiOcular™ human cell construct. Duplicate cultures of the negative control (exposure time control), 100 µL of sterile, deionized water (Quality Biological), were exposed for 15 and 240 minutes. Duplicate cultures of the positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), were exposed for 15 and 45 minutes. The exposed cultures were then incubated for the appropriate amount of time at standard conditions.

In Trial 2, the EpiOcular™ cultures were treated in duplicate with the reference substance, H (both sample 1 and sample 2), at five exposure times of 1, 5, 10, 12, and 20 minutes. The EpiOcular™ cultures were treated in duplicate with the test substance, W (sample 2), at four exposure times of 5, 20, 45, and 90 minutes. The EpiOcular™ cultures were treated in duplicate with the test substance, P (sample 2), at four exposure times of 20, 45, 90, and 240 minutes. Duplicate cultures of the negative control were exposed for 15, 240, 960, and 1440 minutes. Duplicate cultures of the positive control were exposed for 15 and 45 minutes. The exposed cultures were then incubated for the appropriate amount of time at standard conditions.

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline (Ca<sup>++</sup>Mg<sup>++</sup>Free-DPBS) and the wash medium was decanted. After rinsing, the tissues were transferred to 5 mL of Assay Medium for a 10 to 20-minute soak at room temperature to remove any test substance absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Medium was prepared no more than 2 hours before use. Three-tenths mL of MTT

solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with Ca<sup>++</sup>Mg<sup>++</sup>Free-DPBS. The trays were incubated for approximately three hours at standard conditions.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the Millicell® inserts was decanted into the well from which the Millicell® insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred microliters of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD<sub>550</sub>) of each well was measured with a Molecular Devices Vmax plate reader.

### Presentation of Data

The raw absorbance values were captured. The mean OD<sub>550</sub> value of the blank control wells was calculated. The corrected mean OD<sub>550</sub> of the exposure time controls was determined by subtracting the mean OD<sub>550</sub> of the blank control from their mean OD<sub>550</sub> values. The corrected OD<sub>550</sub> of the individual test substance exposure times and the positive control exposure times was determined by subtracting the mean OD<sub>550</sub> of the blank control from their OD<sub>550</sub> values. All calculations were performed using an Excel spreadsheet. The following percent of control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected OD}_{550} \text{ of Test Substance or Positive Control Exposure Time}}{\text{appropriate corrected mean OD}_{550} \text{ Negative Control}} \times 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test substance or positive control exposure time on the abscissa. The ET<sub>50</sub> value was interpolated from each plot. To determine the ET<sub>50</sub>, two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation  $y=m(x) + b$ . Finally, to determine the ET<sub>50</sub>, the equation was solved for  $y=50$ . If all of the exposure time points show greater than 50% survival, the ET<sub>50</sub> value is presented as greater than the maximum exposure time.

### Criteria for a Valid Test

The assay results were accepted since the ET<sub>50</sub> value of the positive control fell within two standard deviations of the historical mean (updated every three months) and the corrected mean OD<sub>550</sub> value for the minimum negative control exposure time was within 20% of the corrected mean OD<sub>550</sub> value for the maximum negative control exposure time.

## RESULTS AND DISCUSSION

### MTT Assay

In the first definitive assay, the EpiOcular™ cultures were treated in duplicate with the test substance, V, and the reference substance, H, at four exposure times of 1, 5, 10, and 20 minutes. The test substances, J and W, were exposed for 5, 10, 20, and 45 minutes. The test substances, P, R, T, and K, were exposed for 20, 45, 90, and 240 minutes. The negative control was exposed in duplicate for 15 and 240 minutes. Table 1 summarizes the ET<sub>50</sub> results of the Topical Application Ocular Irritation Screening Assay using the EpiOcular™ Human Cell Construct for the test/reference substances and the positive control, 0.3% Triton®-X-100. The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.5 – 39.1 minutes), and the corrected mean OD<sub>550</sub> value for the minimum negative control exposure time (1.488) was within 20% of the corrected mean OD<sub>550</sub> value for the maximum negative control exposure time (up to 240 minutes) (1.463), the assay results were accepted.

Following a consultation with the Sponsor, a second definitive assay was performed. The additional definitive assay was performed to evaluate the potential effects of the following discrepancies. First, the physical description of the reference substance, H, was cloudy very light yellow non-viscous liquid, which differed from the original physical description (clear). The cloudiness of the reference substance may have been caused by contamination. Therefore, the reference substance (sample 1) and a new sample provided by the Sponsor (sample 2) were tested in parallel for comparison at five exposure times of 1, 5, 10, 12, and 20 minutes. Second, the physical description of the test substance, P, was cloudy light orange non-viscous liquid, which differed from the original physical description (clear). The Sponsor concluded that the cloudiness was caused by the limited stability of the test substance. A new test substance (256X concentrate) was received and a new sample (sample 2) was prepared at IIVS. The new prepared sample was tested at four exposure times of 20, 45, 90 and 240 minutes. For the test substance, P, only the results from Trial 2 were considered valid and are presented in this report. Third, the pH of the test substance, W, was measured at 6.0, which differed from the Sponsor's pH record of 7.76 and 8.06. A new test substance (256X concentrate) was received and a new sample (sample 2) was prepared at IIVS. The new prepared sample was tested at four exposure times of 5, 20, 45, and 90 minutes. A new pH evaluation indicated that the pH of the test substance, W (sample 1), used in the original testing was in fact 7.5. For the test substance, W, the results of both sample 1 and sample 2 were considered valid and are presented in this report.

In the second definitive assay, the EpiOcular™ cultures were treated in duplicate with the reference substance, H (sample 1 and sample 2), and the test substances at the exposure times mentioned above. The negative control was exposed in duplicate for 15, 240, 480, and 1440 minutes. Table 2 summarizes the ET<sub>50</sub> results of the second definitive assay for the test/reference substances and the positive control, 0.3% Triton®-X-100. The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.6 – 39.0 minutes) (positive control updated every three months), and the corrected mean OD<sub>550</sub> value for the minimum negative control exposure time (1.498) was within 20% of the corrected mean OD<sub>550</sub> value for the maximum negative control exposure time (up to 240 minutes) (1.449), the assay results were accepted.

**Table 1**

<b>Assay Date</b>	<b>IIVS Test/Reference Substance Number</b>	<b>Sponsor's Designation</b>	<b>Conc.</b>	<b>ET<sub>50</sub> (minutes)</b>	<b>pH</b>
12/7/05	05AE37	V	Neat	12.0	11.0
	05AE40*	H*	Neat	9.4	12.0
	05AE41	J	Neat	19.3	DpH
	05AE42	P	Neat	NR	9.5
	05AE43	R	Neat	> 240	6.0
	05AE50	T	Neat	31.6	2.0
	05AE51	W	Neat	39.6	6.0 (7.5 <sup>#</sup> )
	05AG41^	K^	Neat^	> 240	4.0
	Positive Control	0.3% Triton <sup>®</sup> -X-100	NA	22.6	NA

**Table 2**

<b>Assay Date</b>	<b>IIVS Test/Reference Substance Number</b>	<b>Sponsor's Designation</b>	<b>Conc.</b>	<b>ET<sub>50</sub> (minutes)</b>	<b>pH</b>
4/5/06	05AE40 (sample 1)*	H*	Neat	9.8	12.0
	05AE40 (sample 2)*+	H)* <sup>+</sup>	Neat	9.1	12.0
	05AE42	P	Neat	74.0	9.5 (10.0 <sup>#</sup> )
	05AE51	W	Neat	43.3	5.0 (6.0 <sup>#</sup> )
	Positive Control	0.3% Triton <sup>®</sup> -X-100	NA	26.0	NA

\* - Reference Substance

^ - An entire packet of the test substance, K, was diluted in 1.25 gallons of deionized water on the day of the experiment

+ - A second reference substance tested concurrently with the original reference substance (sample 1) for comparison

# - The pH value obtained on June 16, 2006 using the pH paper. The pH value was subsequently verified with an accumet<sup>®</sup> BASIC pH Meter. The test articles, P and W, were past their expiration date (April 18, 2006).

NR – The result was considered invalid because the test substance was presumed unstable at the time of testing. See Table 2 for the reported ET<sub>50</sub> result.

DpH – Discolored pH Paper, the pH could not be determined because the test substance discolored the pH paper

NA - Not Applicable

## **APPENDIX A**

## **APPENDIX B**

EPIOCLAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: V  
 TEST ARTICLE: 05AE37

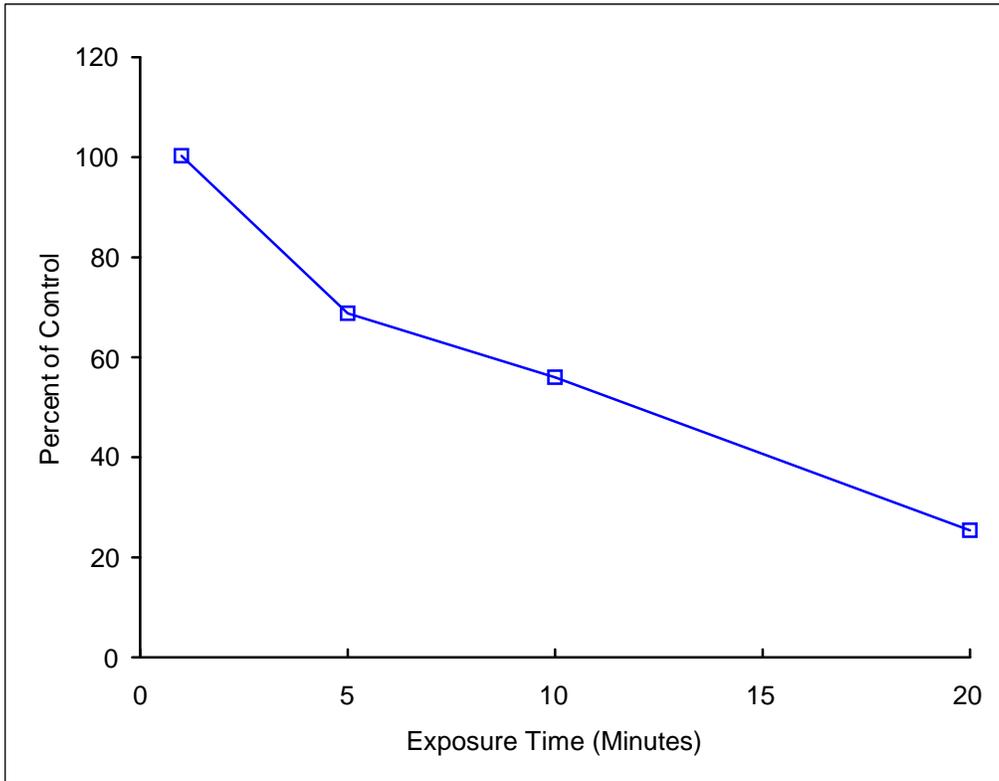
Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = 12.0 Minutes

TRIAL 1  
 CONCENTRATION: 100%.

			y = Percent Viable	
			x = Exposure Time	
			slope=rise/run=(y1-y2)/(x1-x2)	
			y intercept=y-(slope*x)	
			X	Y
TIME	PERCENT			
EXPOSURE	VIABLE			
(Minutes)				
1	100.3	1	10.0	1
5	68.8	2	20.0	2
10	56.0	3	11.960784	3
20	25.4			
			slope =	-3.06
			y intercept =	86.6

V  
 CONCENTRATION: 100%.  
 TRIAL 1



EPIOCLAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: H\*  
 TEST ARTICLE: 05AE40

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = 9.4 Minutes

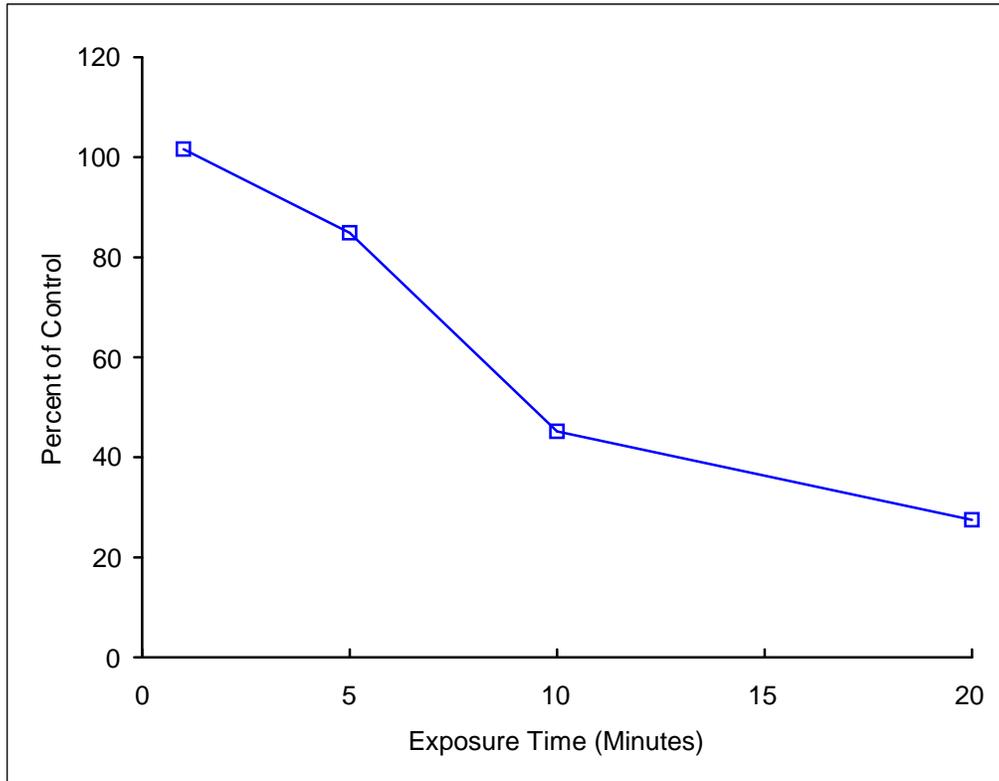
TRIAL 1  
 CONCENTRATION: 100%.

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
1	101.6	1	84.9
5	84.9	2	45.2
10	45.2	3	50
20	27.5		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 $\text{slope} = -7.94$   
 $y \text{ intercept} = 124.6$

\*- Reference substance

H\*  
 CONCENTRATION: 100%.  
 TRIAL 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: J  
 TEST ARTICLE: 05AE41

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = 19.3 Minutes

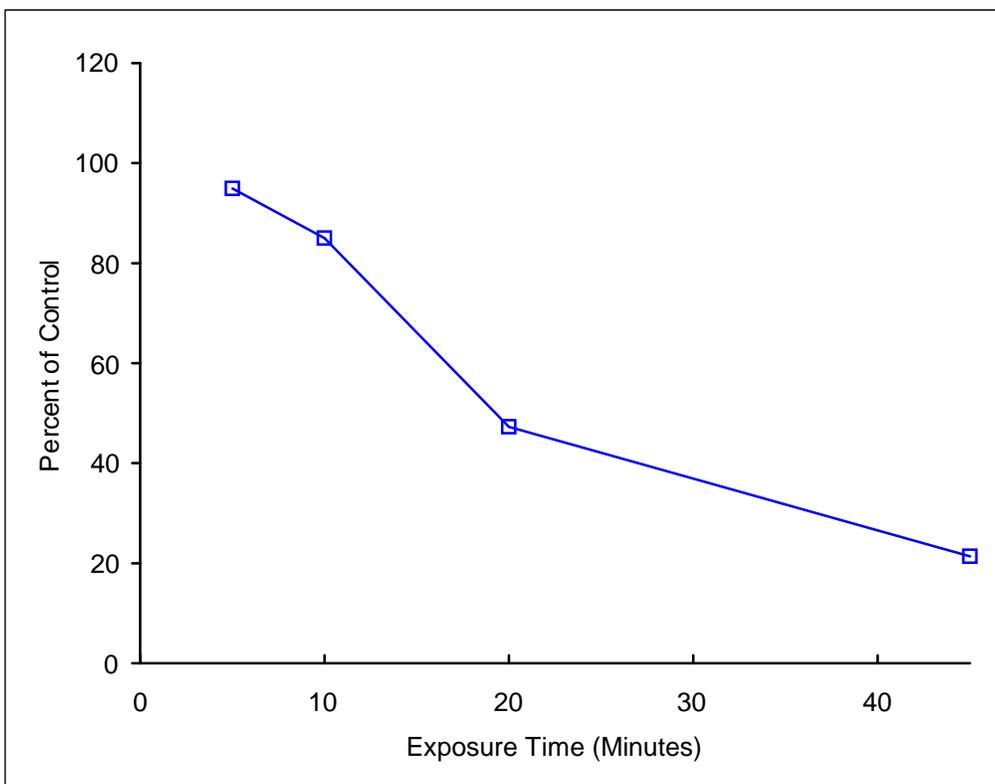
TRIAL 1  
 CONCENTRATION: 100%.

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
5	94.9	10.0	85
10	85.0	20.0	47.3
20	47.3	19.28382	50
45	21.4		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 $\text{slope} = -3.77$   
 $y \text{ intercept} = 122.7$

J

CONCENTRATION: 100%.  
 TRIAL 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: R  
 TEST ARTICLE: 05AE43

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

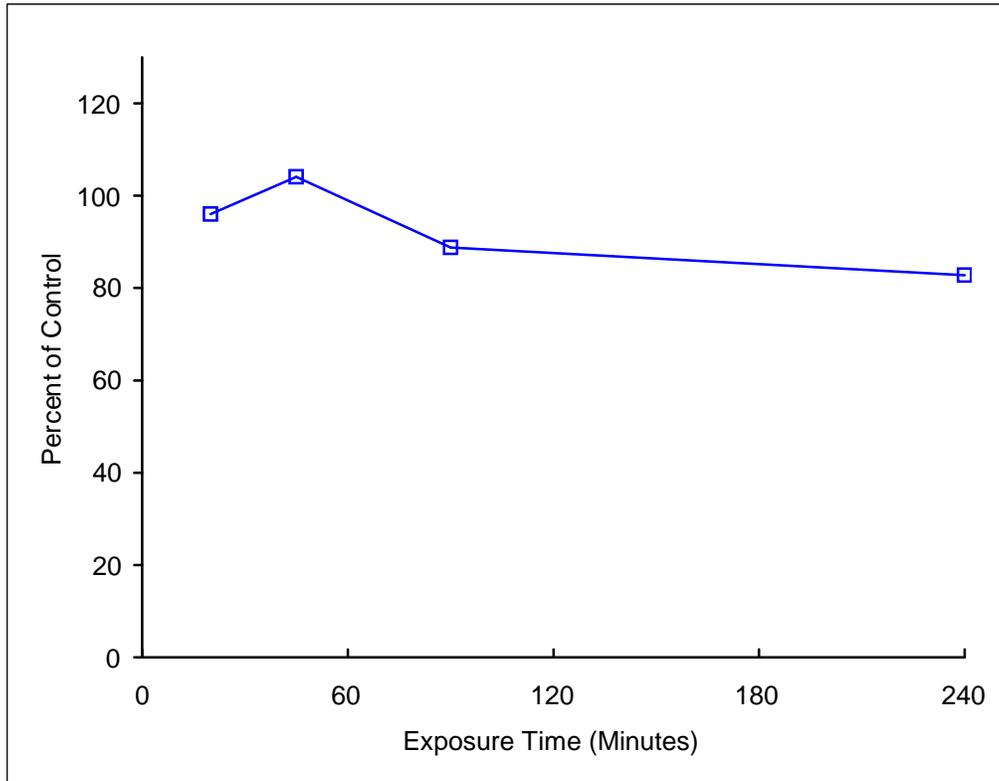
ET<sub>50</sub> = > 240 Minutes

TRIAL 1  
 CONCENTRATION: 100%.

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
20	96.0	1	82.8
45	104.1	2	82.8
90	88.8	3	50
240	82.8		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 slope = #DIV/0!  
 y intercept = #DIV/0!

R  
 CONCENTRATION: 100%.  
 TRIAL 1



EPIOCLAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: T  
 TEST ARTICLE: 05AE50

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

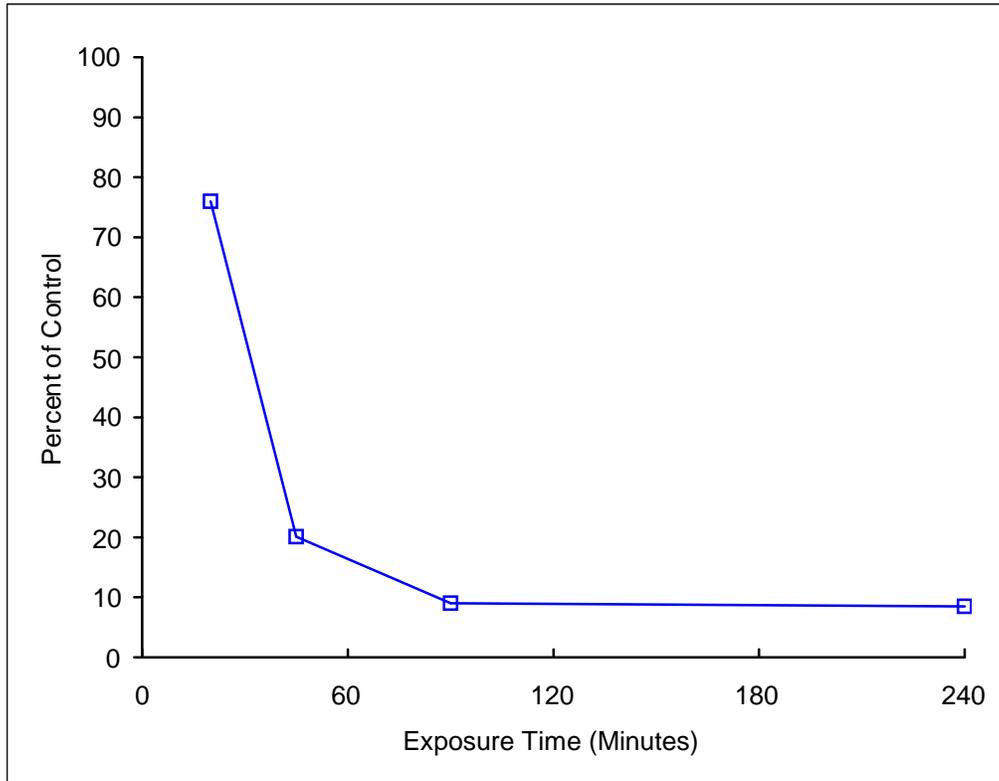
ET<sub>50</sub> = 31.6 Minutes

TRIAL 1  
 CONCENTRATION: 100%.

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
20	76.0	1	76
45	20.1	2	20.1
90	9.0	3	50
240	8.5		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 $\text{slope} = -2.236$   
 $y \text{ intercept} = 120.72$

T  
 CONCENTRATION: 100%.  
 TRIAL 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: W  
 TEST ARTICLE: 05AE51

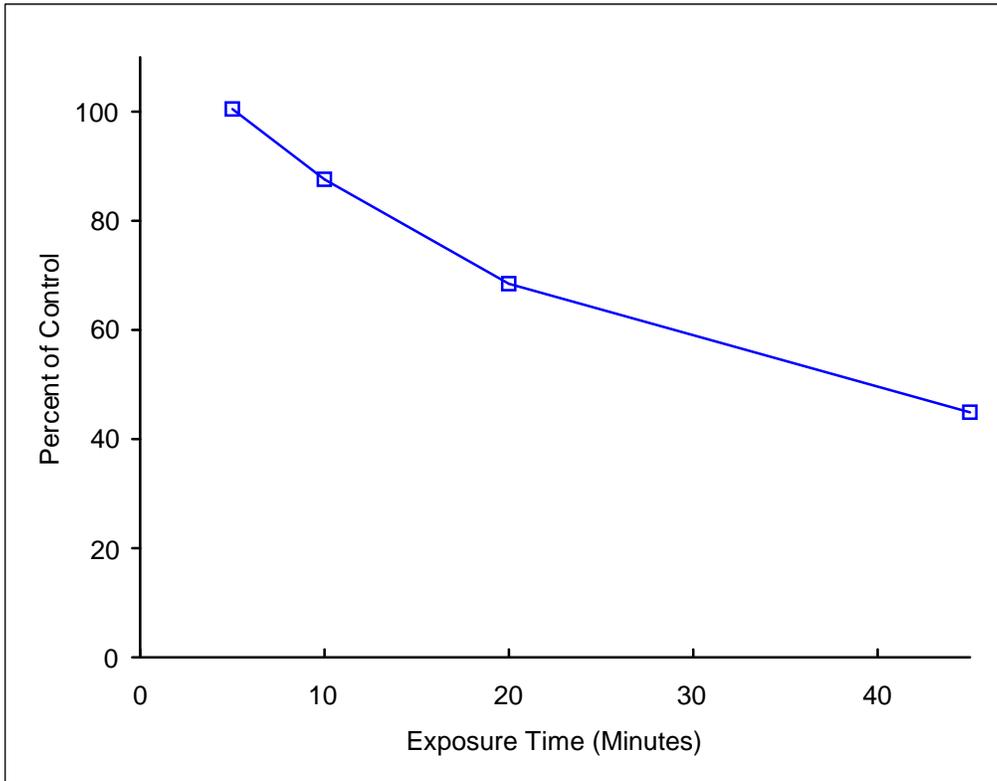
Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = 39.6 Minutes

TRIAL 1  
 CONCENTRATION: 100%.

			y = Percent Viable	
			x = Exposure Time	
			slope=rise/run=(y1-y2)/(x1-x2)	
			y intercept=y-(slope*x)	
			X	Y
TIME	PERCENT			
EXPOSURE	VIABLE			
(Minutes)				
5	100.5	1	20.0	1
10	87.6	2	45.0	2
20	68.5	3	39.597458	3
45	44.9			
			slope =	-0.944
			y intercept =	87.38

W  
 CONCENTRATION: 100%.  
 TRIAL 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05  
 TEST MATERIAL: K  
 TEST ARTICLE: 05AG41

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = > 240 Minutes

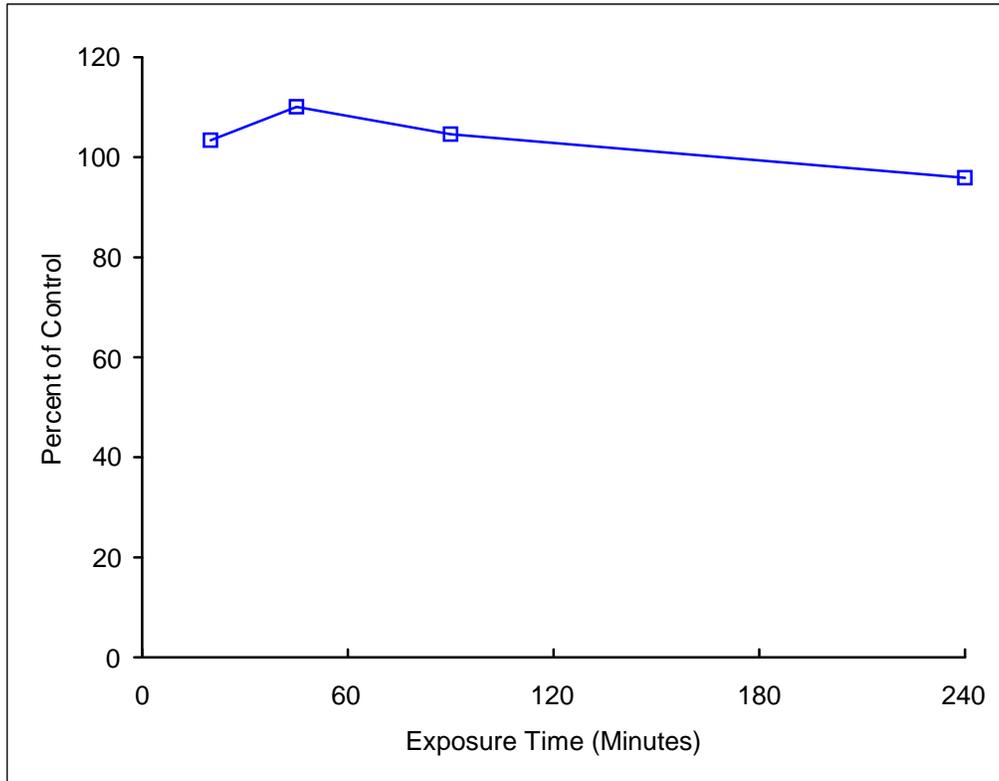
TRIAL 1  
 CONCENTRATION: 100%\*

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
20	103.4	1	95.9
45	110.1	2	95.9
90	104.6	3	50
240	95.9		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 $\text{slope} = \#DIV/0!$   
 $y \text{ intercept} = \#DIV/0!$

\*- As instructed by sponsor, 1 packet of the test article was diluted in 1.25 gallons of deionized water

K  
 CONCENTRATION: 100%\*  
 TRIAL 1



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 7-Dec-05

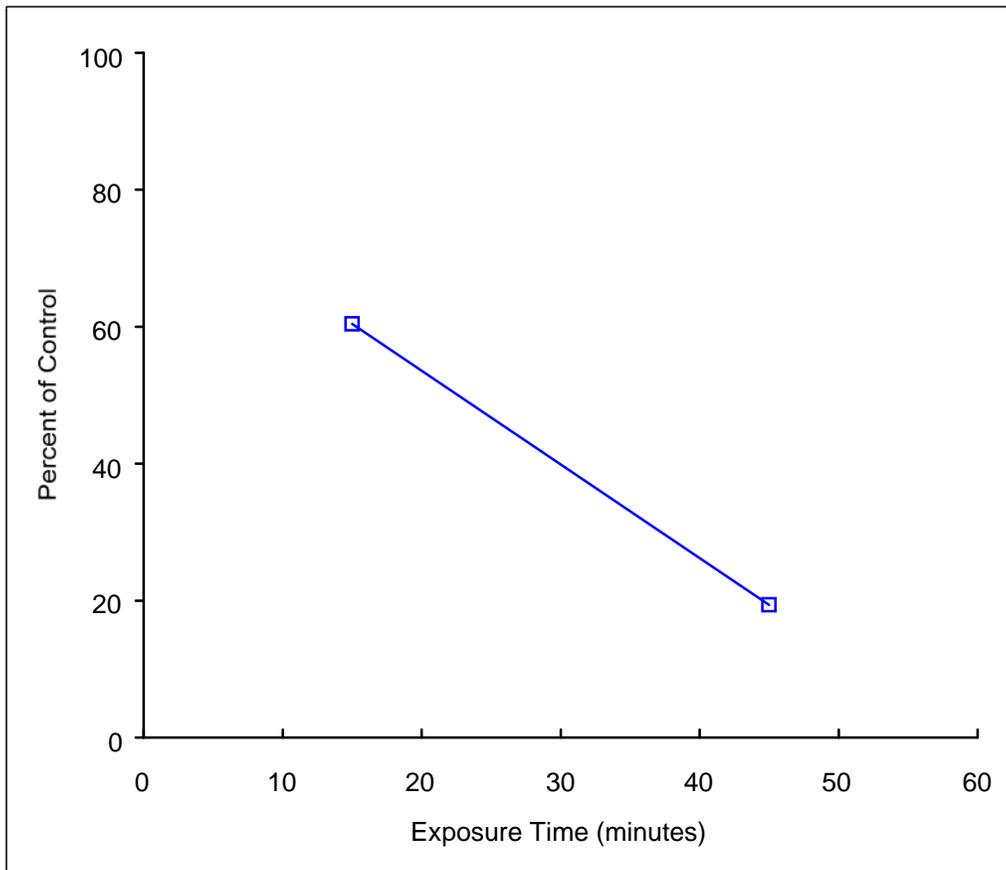
TEST MATERIAL: 0.3% TRITON®-X-100

ET<sub>50</sub> = 22.6 Minutes

			y = Percent Viable			
			x = Exposure Time			
			slope=rise/run=(y1-y2)/(x1-x2)			
			y intercept=y-(slope*x)			
			X		Y	
TIME	PERCENT		1	15.0	1	60.4
EXPOSURE	VIABLE		2	45.0	2	19.4
(Minutes)			3	22.609756	3	50
			slope =		-1.366667	
			y intercept =		80.9	

0.3% TRITON®-X-100

7-Dec-05



EPIOCLAR BIOASSAY

EXPERIMENT DATE: 5-Apr-06  
 TEST MATERIAL: H (sample 1)  
 REFERENCE SUBSTANCE: 05AE40

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

ET<sub>50</sub> = 9.8 Minutes

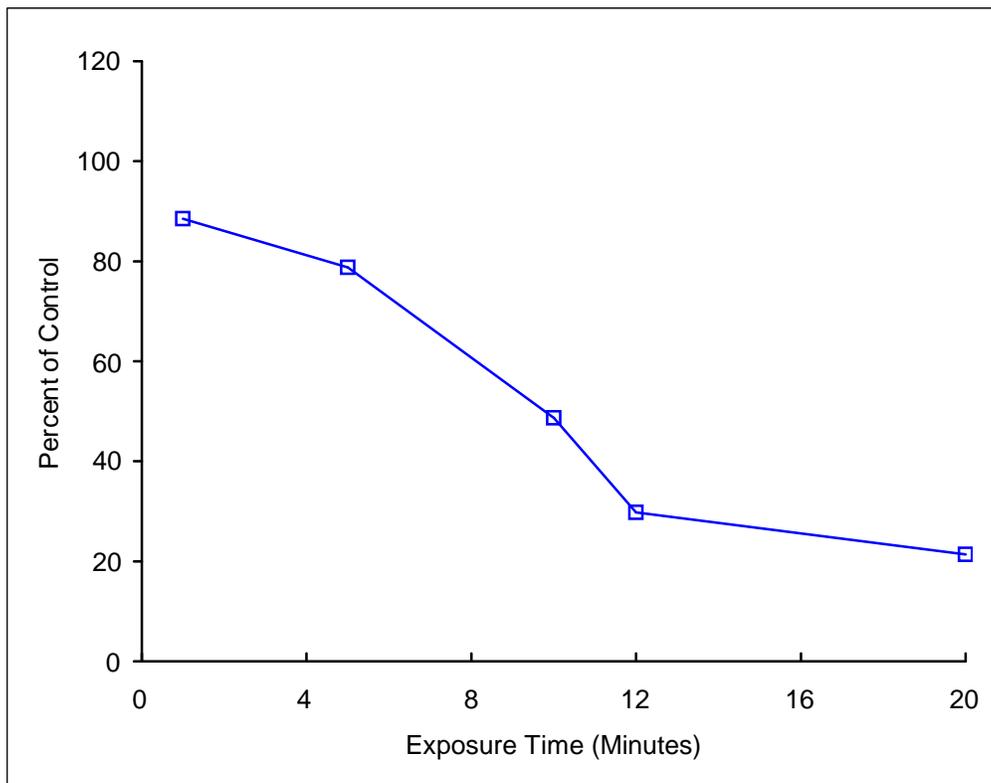
TRIAL 2  
 CONCENTRATION: 100%

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
1	88.5	1	78.8
5	78.8	2	48.7
10	48.7	3	50
12	29.8		
20	21.4		

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$   
 $\text{slope} = -6.02$   
 $y \text{ intercept} = 108.9$

H (sample 1)

CONCENTRATION: 100%  
 TRIAL 2



EPIOCLAR BIOASSAY

EXPERIMENT DATE: 5-Apr-06  
 TEST MATERIAL: H (sample 2)  
 REFERENCE SUBSTANCE: 05AE40

Study No.  
 05AE37, AE40-AE43, AE50, AE51, AG41.015033

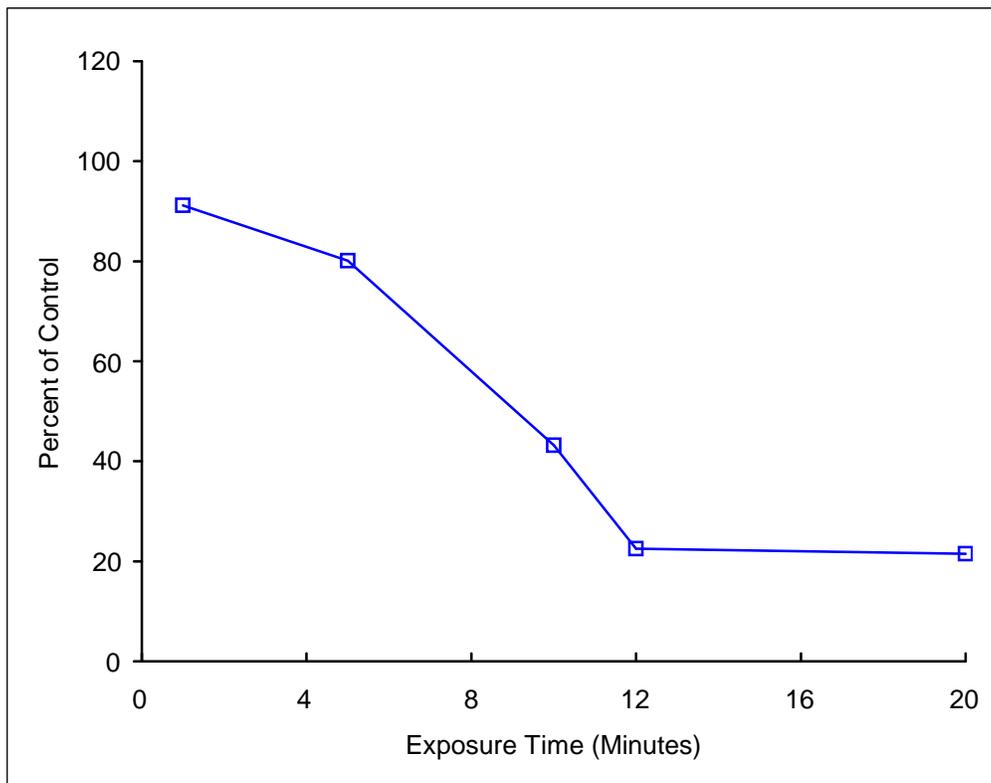
ET<sub>50</sub> = 9.1 Minutes

TRIAL 2  
 CONCENTRATION: 100%

TIME EXPOSURE (Minutes)	PERCENT VIABLE	X	Y
1	91.2	1	80.1
5	80.1	2	43.2
10	43.2	3	50
12	22.5		-7.38
20	21.5		117

$y = \text{Percent Viable}$   
 $x = \text{Exposure Time}$   
 $\text{slope} = \text{rise/run} = (y_1 - y_2) / (x_1 - x_2)$   
 $y \text{ intercept} = y - (\text{slope} * x)$

H (sample 2)  
 CONCENTRATION: 100%  
 TRIAL 2



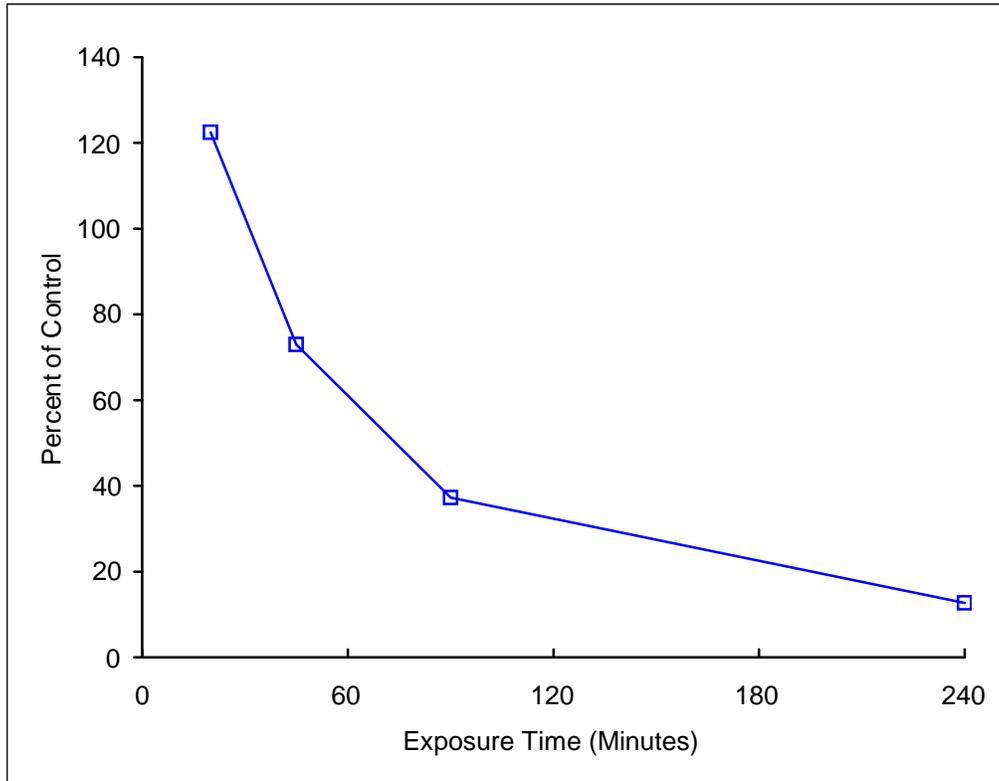
EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 5-Apr-06 Study No.  
 TEST MATERIAL: P 05AE37, AE40-AE43, AE50, AE51, AG41.015033  
 TEST SUBSTANCE: 05AE42  
 (prepared at IIVS from concentrate) ET<sub>50</sub> = 74.0 Minutes

TRIAL 2  
 CONCENTRATION: 100%.

			y = Percent Viable	
			x = Exposure Time	
			slope=rise/run=(y1-y2)/(x1-x2)	
			y intercept=y-(slope*x)	
			X	Y
TIME	PERCENT		1	45.0
EXPOSURE	VIABLE		2	90.0
(Minutes)			3	73.991597
20	122.5			
45	73.0			
90	37.3			
240	12.7			
			slope =	-0.793333
			y intercept =	108.7

P  
 CONCENTRATION: 100%.  
 TRIAL 2



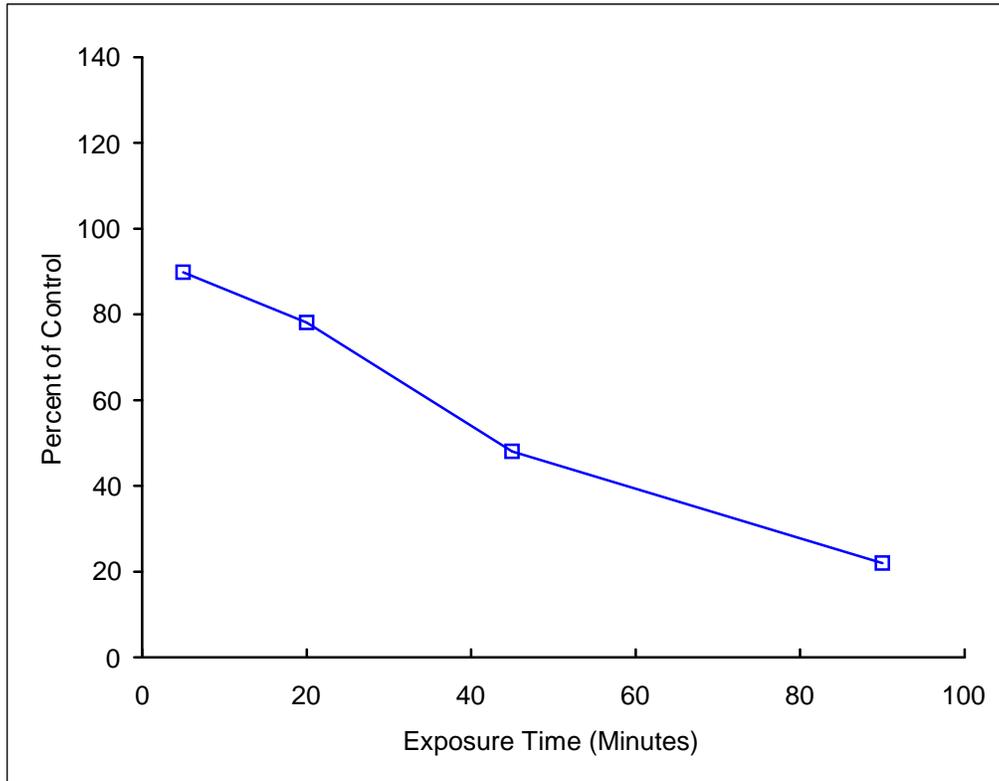
EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 5-Apr-06 Study No.  
 TEST MATERIAL: W 05AE37, AE40-AE43, AE50, AE51, AG41.015033  
 TEST SUBSTANCE: 05AE51  
 (prepared at IIVS from concentrate) ET<sub>50</sub> = 43.3 Minutes

TRIAL 2  
 CONCENTRATION: 100%.

			y = Percent Viable	
			x = Exposure Time	
			slope=rise/run=(y1-y2)/(x1-x2)	
			y intercept=y-(slope*x)	
			X	Y
TIME	PERCENT		1	1
EXPOSURE	VIABLE		2	2
(Minutes)			3	3
5	89.8	1	20.0	78.1
20	78.1	2	45.0	48
45	48.0	3	43.33887	50
90	22.0			
			slope =	-1.204
			y intercept =	102.18

W  
 CONCENTRATION: 100%.  
 TRIAL 2



EPIOCULAR™ BIOASSAY

EXPERIMENT DATE: 5-Apr-06

TEST MATERIAL: 0.3% TRITON®-X-100

ET<sub>50</sub>= 26.0 Minutes

TIME EXPOSURE (Minutes)	PERCENT VIABLE
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15	66.3
45	21.8

y = Percent Viable  
x = Exposure Time  
slope=rise/run=(y1-y2)/(x1-x2)  
y intercept=y-(slope\*x)

	X	Y
1	15.0	66.3
2	45.0	21.8
3	25.988764	50

slope = -1.483333  
y intercept = 88.55

0.3% TRITON®-X-100

5-Apr-06

