Evaluation of a Sample Provided by

Bio-Organic Catalyst, Inc.

Utilizing the Ocular Irritection® Test Method

September 25, 2015

INVITRO INTERNATIONAL

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September 25, 2015

Jay Johnston
Bio-Organic Catalyst, Inc.
711 W. 17th Street, Suite E-6
Costa Mesa, CA 92627

Dear Mr. Johnston:

Enclosed is a copy of the final report detailing the results of our study of the material that was sent to us for analysis by the Irritection® Assay System.

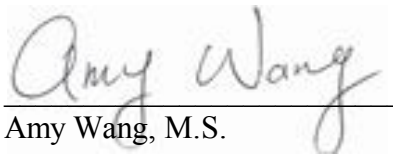
We are delighted that you have selected InVitro International to perform this analysis for you. We look forward to being able to provide additional services for you in the future.

Sincerely,

W. Richard Ulmer
President & CEO

UTILIZATION OF THE IRRITECTION® ASSAY SYSTEM TO EVALUATE A SAMPLE PROVIDED BY BIO-ORGANIC CATALYST, INC.

Study Completion Date:	September 25, 2015
Client:	Bio-Organic Catalyst, Inc. 711 W. 17th Street, Suite E-6 Costa Mesa, CA 92627
Contact:	Jay Johnston
Phone Number:	(949) 515-1301
Testing Laboratory:	InVitro International 330 E. Orangethorpe Ave., Ste. D Placentia, CA 92870 Phone: (949) 851-8356 Fax: (949) 851-4985

Director of R&D, QA:  9/25/2015

Amy Wang, M.S. Date

Approved by:
President & CEO of
InVitro International, Inc.  9/25/2015

W. Richard Ulmer Date

EXECUTIVE SUMMARY

A single sample was evaluated with the Irritection Assay System in order to predict its potential for ocular and dermal irritation. The results of the study indicated that the sample of EcoSystem Plus-EcoCatalyst-700-50 was a minimal ocular irritant and thus a GHS/EU CLP Classification of No Category. However, the pH of the sample, 3.2 at 10%, is lower than the optimal pH for the Irritection Assay System. Consequently, there is potential for irritancy underestimation on this sample.

AN EVALUATION OF A SAMPLE PROVIDED BY BIO-ORGANIC CATALYST, INC.

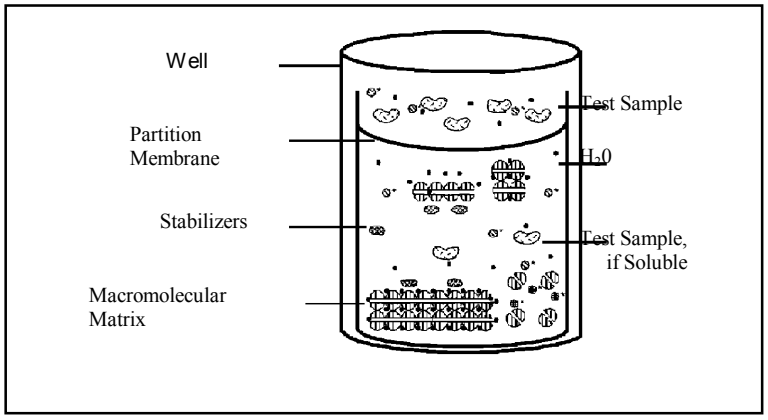
STUDY OBJECTIVE

A single sample provided by Bio-Organic Catalyst, Inc. was evaluated with the Irritection® Assay System in order to predict its potential to cause ocular and dermal irritation. To achieve this objective, the sample was subjected to a standardized testing process as described under Materials and Methods.

BACKGROUND

The proprietary Ocular Irritection® assay is a standardized and quantitative *in vitro* test which utilizes changes of relevant macromolecules to predict the acute ocular irritancy of chemicals and chemical formulations. This assay, depicted schematically in Figure 1 below, is based on the principle that chemical compounds will promote measurable changes in target biomolecules and macromolecular structures. Previous studies have clearly demonstrated that the processes of protein denaturation and disaggregation that are induced in this *in vitro* assay mimic the effects that are produced when these types of irritants are applied to the eye. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

Figure 1. The Ocular Irritection Model



The Ocular Irritection® assay system provides significant benefits when compared to the *in vivo* Draize test method. Additionally, the Ocular Irritection® *in vitro* assay has completed the validation study to meet the standards as defined by the OECD and UN Globally Harmonized System (UN GHS) for classification and has demonstrated high levels of both sensitivity and reproducibility. Of additional relevance, the Ocular Irritection assay method can be very helpful when comparing multiple samples at varying concentrations or volumes. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection from formulation development to final product selection.

MATERIALS AND METHODS

The Ocular Irritection® assay is a quantitative *in vitro* test method that mimics an acute ocular irritation test. For best results, test samples should be optimally in the pre-defined applicability domain, having a pH range of 4-9. First, the test sample is characterized by measuring the pH and foam fraction in order to select the proper sample handling method. To perform this standardized assay, the test sample is either applied to a synthetic biobarrier composed of a semi-permeable membrane or applied directly to the reagent solution. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing glycoproteins. Reaction of the test sample with these proteins and macromolecular complexes promotes conformational changes that may be readily detected as an increase in the turbidity of the protein solution. The turbidity may be detected spectrophotometrically at a wavelength of 405 nm.

The irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE) score. This score is defined by comparing the increase in optical density (OD_{405}) produced by the test material to a standard curve that is constructed by measuring the increase in OD_{405} produced by a set of Calibration substances. These Calibrators have been selected for use in this test because their irritancy potential has been previously documented in a series of *in vivo* investigations. The predicted *in vivo* classification, based on this scoring system, is shown in Table. Test samples producing an IDE score of less than or equal to 12.5 are to be considered UN GHS/EU CLP non-irritants. Test samples that produce IDE score greater than 12.5 are to be classified as UN GHS/EU CLP irritants. Furthermore, test samples producing an IDE score greater than 30.0 are likely to be considered UN GHS/EU CLP Category 1.

Table 1. Prediction Model of the Ocular Irritection® Assay

Irritection Score	Degree of ocular irritancy	UN GHS/EU CLP Classification
0-12.5	Minimal	Non-irritant (No Category)
>12.5-30.0	Mild	Irritant (Category 1/ Category 2)
>30.0-51.0	Moderate	
>51.0	Severe	

A detailed description of the Ocular Irritection® test procedure may be found in InVitro International's Irritection® Assay System Instruction Manual. All data are calculated and analyzed via a computer program which determines assay result acceptance based upon qualification parameters defined in the program. In general, the program has been designed to accept sample data as qualified if the following criteria are met: the OD_{405} values of Calibrators and internal Quality Control samples fall within previously specified ranges; sample blanks are less than 500 optical density (OD) units; the net sample OD_{405} is greater than -15; and an Inhibition Check is negative. These software printouts are included in Appendix I.

RESULTS

The results of this analysis provided a predicted *in vivo* classification for the test sample. The complete software printouts are included in Appendix I.

Table 2 presents a summary of results for the Bio-Organic Catalyst, Inc. sample studied.

Table 2. Summary of Ocular Irritection Results

IVI #	Sample Description	Conc.	IDE Score	Ocular Irritancy Classification
EE9439	EcoSystem Plus-EcoCatalyst-700-50	0.31%	2.9	Minimal Irritant
		0.63%	3.6	Minimal Irritant
		1.25%	4.7	Minimal Irritant
		2.5%	7.6	Minimal Irritant
		5%	10.2 [a]	Minimal Irritant

[a] Maximum Qualified Score

DISCUSSION

A single sample, provided by Bio-Organic Catalyst, Inc. was evaluated with the Irritection® Assay System in order to predict its potential to cause ocular irritation.

The Ocular Irritection test process determined that this test sample should be classified as a surfactant material. Therefore, the standard Ocular Irritection surfactant handling procedure was performed. The following concentrations of neat sample were applied directly to the reagent solution for analysis: 0.31, 0.63, 1.25, 2.5 and 5%. The results of the study indicated that the sample of EcoSystem Plus-EcoCatalyst-700-50 was classified as a minimal ocular irritant with an IDE score of 10.2, and these findings lead to a UN GHS/EU CLP classification of No Category (Non-irritant). However, the pH of the sample, 3.2 at 10%, is lower than the optimal pH for the Irritection Assay System. Consequently, there is potential for irritancy underestimation on this sample.

APPENDIX I

ASSAY REPORT - ORIGINAL

Sample Description	:	EcoSystem+EcoCatalyst700	Date	:	09/25/15
Sample Number	:	EE9439	Time	:	10:38:25
Product Type	:	@50% dilution	Technician Name	:	Amy
Assay Method	:	Ocular	Kit Lot Number	:	IO 090214
Protocol	:	Irritection Ocular (New)	Reagent temperature	:	25.0
Incubation Time	:	24.0 hours	Reagent pH Before Activation	:	7.89
Plate Layout	:	1 Sample/5 Concentrations	Reagent pH After Activation	:	6.46
Instrument Type	:	Dynex MRX	Sample pH at 10%	:	3.20
Wavelength	:	405nm	Assay Number	:	170
Comment	:		Assay Qualification	:	Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
0.31 %	98	-1	99	2.9	Minimal	Qualified
0.63 %	123	1	122	3.6	Minimal	Qualified
1.25 %	160	1	159	4.7	Minimal	Qualified
2.5 %	263	3	260	7.6	Minimal	Qualified
5 %	351	5	346	10.2	Minimal	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	132	0.0	73 - 277	Range qualified
Cal 1	426	12.5	126 - 454	Range qualified
Cal 2	1056	30.0	450 - 1270	Range qualified
Cal 3	2009	51.0	1425 - 2295	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	316	9.3	7.2 - 20.8	Range qualified
QC 2	1062	30.1	23.6 - 35.6	Range qualified

Sample Inhibition Check Results:

Concentration / Inhibition Check OD

0.31 % / 1839 0.63 % / 1699 1.25 % / 1680 2.5 % / 1487 5 % / 1578

* Mean value from assay data history
** Mean value from protocol defaults or adjusted value due to calibrator zero substitution
[ ] Value before substitution