Evaluation of a Sample Provided by

ChemFree Corporation

Utilizing the Irritection® Assay System

November 24, 2015

INVITRO INTERNATIONAL

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November 24, 2015

Chad C Parson
ChemFree Corporation
8 Meca Way
Norcross, GA 30093

Dear Mr. Parson:

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 CHEMFREE CORPORATION

Study Completion Date: November 24, 2015

Client: ChemFree Corporation
8 Meca Way
Norcross, GA 30093

Contact: Chad C Parson

Phone Number: (770) 356-5368

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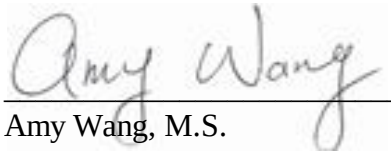
Director of R&D, QA:  11/24/2015

Amy Wang, M.S. Date

Approved by:
President & CEO of
InVitro International, Inc.  11/24/2015

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 SW-LF was a minimal ocular irritant and thus a GHS/EU CLP Classification of No Category. The dermal results demonstrated that the sample was a dermal non-irritant.

AN EVALUATION OF A SAMPLE PROVIDED BY CHEMFREE CORPORATION

STUDY OBJECTIVE

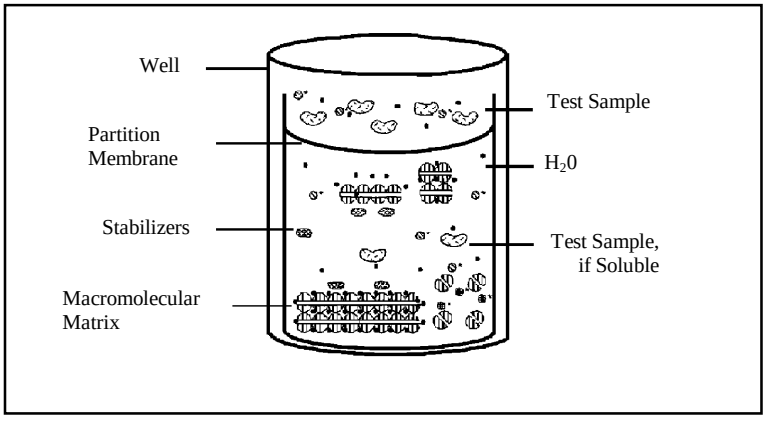
A single sample provided by ChemFree Corporation was evaluated with the Irritection® Assay System in order to predict its potential to cause ocular and dermal irritation. To achieve this objective, the sample was subjected to a standardized testing process as described under Materials and Methods.

BACKGROUND

The proprietary Ocular and Dermal Irritection assays are standardized and quantitative *in vitro* acute ocular and dermal irritation tests which utilize changes of relevant macromolecules to predict acute ocular and dermal irritancy of chemicals and chemical formulations.

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.

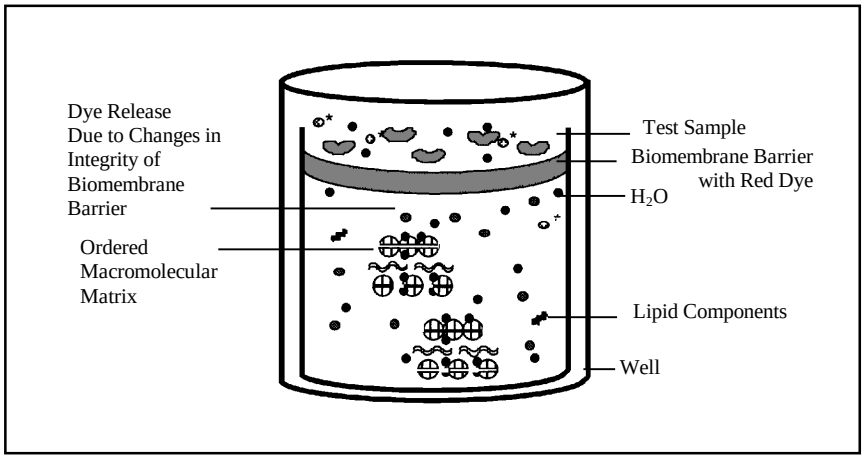
Figure 1. The Ocular Irritection Model



The Dermal Irritection assay, depicted schematically in Figure 2, 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 skin. Consequently, this *in vitro* test may be employed to predict the *in vivo* toxic effects of chemicals and formulations.

Figure 2. The Dermal 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 and Dermal Irritection assay methods can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, these tests serve as extremely useful screening tools that facilitate all stages of raw material selection, formulation development and final product selection.

MATERIALS/METHODS

The Ocular and Dermal Irritection assays are quantitative *in vitro* test methods that mimic acute ocular and dermal irritation tests. 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. With the Dermal Irritection test, dye that has been dissociated from the biobarrier during transit of the applied sample may be detected spectrophotometrically at a wavelength of 450 nm.

The ocular irritancy potential of a test sample is expressed as an Irritection Draize Equivalent (IDE), whereas the dermal irritancy potential of a test sample is expressed as a Human Irritancy

Equivalent (HIE) score. These scores are defined by comparing the increase in optical density ($OD_{405/450}$) produced by the test material to a standard curve that is constructed by measuring the increase in OD produced by a set of Calibration substances. These Calibrators have been selected for use in these tests because their irritancy potential has been previously documented in a series of *in vivo* investigations. The predicted *in vivo* classification, based on these scoring systems, is shown in Tables 1 and 2. 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.

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	

Table 2. Relationship of Human Irritancy Equivalent (HIE) Score to Irritancy Classification for the Dermal Irritection Test Method.

Human Irritancy Equivalent (HIE)	Predicted Dermal Irritancy Classification
0.00 - 0.90	Non-Irritant
0.90 - 1.20	Non-Irritant/Irritant
1.20 - 5.00	Irritant

A detailed description of the Ocular and Dermal Irritection test procedures 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 values of Calibrators and internal Quality Control samples fall within previously specified ranges; sample blanks are less than 500 OD units; the net sample OD is greater than -15; and an Inhibition Check is negative.

RESULTS

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

Tables 3 and 4 present a summary of results for the ChemFree Corporation sample studied.

Table 3. Summary of Ocular Irritection Results

IVI Number	Sample Description	Conc.	IDE Score	Predicted Ocular Irritancy Classification
EE9479	SW-LF	0.31%	2.9	Minimal Irritant
		0.63%	5.8 [a]	Minimal Irritant
		1.25%	3.2	Minimal Irritant
		2.5%	3.1	Minimal Irritant
		5%	3.1	Minimal Irritant

[a] Maximum Qualified Score

Table 4. Summary of the Dermal Irritection Results

IVI Number	Sample Description	Conc.	HIE Score	Predicted Dermal Irritancy Classification
S5521	SW-LF	1%	0.59	Non-Irritant
		5%	0.53	Non-Irritant
		10%	0.70	Non-Irritant
		25%	0.61	Non-Irritant
		50%	0.76 [a]	Non-Irritant

[a] Maximum Qualified Score

DISCUSSION

A single sample, provided ChemFree Corporation, was evaluated with the Irritection Assay System in order to predict its potential to cause ocular and dermal 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 SW-LF was classified as a minimal ocular irritant with an IDE score of 5.8, and these findings lead to a UN GHS/EU CLP classification of No Category.

A standard concentration-dependent dose-response study was performed with the Dermal Irritection test method. The following concentrations of neat sample were applied for analysis: 1, 5, 10, 25 and 50%. The results demonstrated that the sample was predicted to be a dermal non-irritant with a HIE score of 0.76.

In summary, the Ocular and Dermal Irritection test methods successfully classified the ocular and dermal irritation potential of this sample.

APPENDIX I

ASSAY REPORT - ORIGINAL

Sample Description	:	SW-LF	Date	:	11/20/15
Sample Number	:	EE9479	Time	:	13:58:01
Product Type	:		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	:	7.95
Plate Layout	:	4 Samples/5 Concentrations	Reagent pH After Activation	:	6.48
Instrument Type	:	Dynex MRX	Sample pH at 10%	:	7.48
Wavelength	:	405nm	Assay Number	:	184
Comment	:		Assay Qualification	:	Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
0.31 %	72	-1	73	2.9	Minimal	Qualified
0.63 %	147	-1	148	5.8	Minimal	Qualified
1.25 %	83	1	82	3.2	Minimal	Examine concentration curve
2.5 %	82	3	79	3.1	Minimal	Examine concentration curve
5 %	79	0	79	3.1	Minimal	Examine concentration curve

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	146	0.0	73 - 277	Range qualified
Cal 1	318	12.5	126 - 454	Range qualified
Cal 2	912	30.0	450 - 1270	Range qualified
Cal 3	1953	51.0	1425 - 2295	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	406	15.1	7.2 - 20.8	Range qualified
QC 2	997	31.7	23.6 - 35.6	Range qualified

Sample Inhibition Check Results:

Concentration / Inhibition Check OD

0.31 % / 1682 0.63 % / 1632 1.25 % / 1448 2.5 % / 1577 5 % / 1601

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

ASSAY REPORT - ORIGINAL

Sample Description	:	SW-LF	Date	:	11/24/15
Sample Number	:	S5521	Time	:	11:50:59
Product Type	:		Technician Name	:	Amy
Assay Method	:	Dermal	Kit Lot Number	:	ID 100114
Protocol	:	Surfactant-450 (Nov. 2010)	Reagent temperature	:	25.0
Incubation Time	:	24.0 hours	Reagent pH Before Activation	:	10.10
Plate Layout	:	2 Sample/5 concentrations	Reagent pH After Activation	:	8.17
Instrument Type	:	Dynex MRX	Sample pH at 10%	:	7.48
Wavelength	:	450nm	Assay Number	:	130
Comment	:		Assay Qualification	:	Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
1 %	121	1	120	0.59	Non-Irritant	Qualified
5 %	109	2	107	0.53	Non-Irritant	Qualified
10 %	145	3	142	0.70	Non-Irritant	Qualified
25 %	124	1	123	0.61	Non-Irritant	Qualified
50 %	156	1	155	0.76	Non-Irritant	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	121	0.00	0 - 150	Range qualified
Cal 1	203	1.00	60 - 260	Range qualified
Cal 2	508	2.00	330 - 886	Range qualified
Cal 3	1157	4.00	810 - 1430	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	172	0.85	0.11 - 0.95	Range qualified
QC 2	802	2.91	0.94 - 3.60	Range qualified

Sample Inhibition Check Results:

Concentration / Inhibition Check OD
50 % / 1774

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