

**Evaluation of a Sample
Provided by
ChemFree Corporation
Utilizing the
Irritection[®] Assay System**

May 28, 2010

INVITRO

INTERNATIONAL

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May 28, 2010

Onofre Ortiz
ChemFree Corporation
8 Meca Way
Norcross, GA 30093

Dear Mr. Ortiz:

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: May 28, 2010

Client: ChemFree Corporation
8 Meca Way
Norcross, GA 30093

Contact: Onofre Ortiz

Phone Number: (770) 356-5368

Testing Laboratory: InVitro International
17751 Sky Park East, Suite G
Irvine, CA 92614
Phone: (949) 851-8356
Fax: (949) 851-4985

Director of R&D, QA:

Amy Wang 5/28/10
Amy Wang, M.S. Date

Approved by:
President & CEO of
InVitro International, Inc.

W. Richard Ulmer 5/28/10
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 ocular results indicated that the sample of SW-1 Degreasing Solution was a minimal ocular irritant. 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.

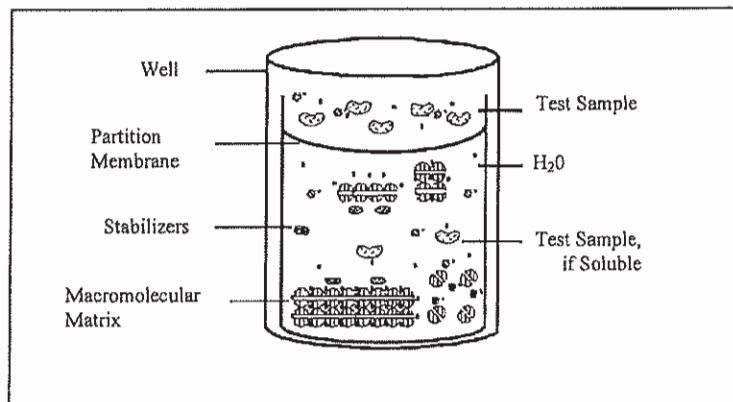
To achieve this objective, standard concentration-dependent dose-response studies were performed with the Ocular and Dermal Irritection test methods.

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.

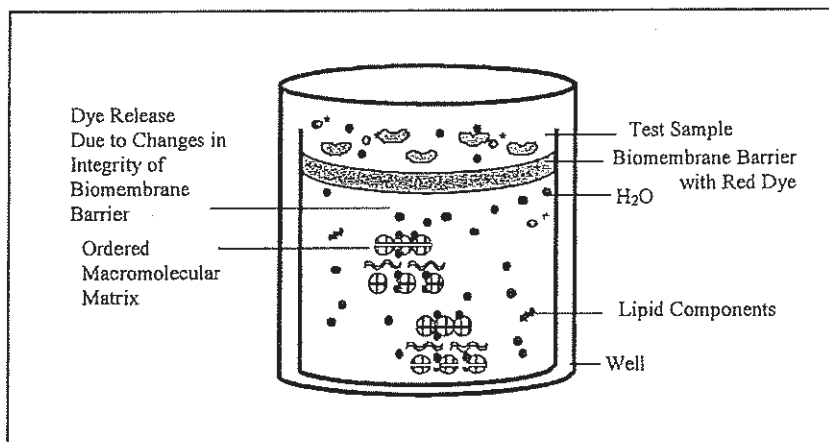
The Ocular Irritection assay, depicted schematically in Figure 1 below, provides significant advances over the *in vivo* Draize test method. The Draize eye irritation assay has been criticized because of the large variability of results obtained from different laboratories that have analyzed the same specimen.

Figure 1. The Ocular Irritection Model



The Dermal Irritation 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 Irritation Model



The quantitative Ocular and Dermal Irritation *in vitro* assays have been found to be highly reproducible. Of even greater relevance, the Ocular and Dermal Irritation 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 Irritation assays are quantitative *in vitro* test methods that mimic acute ocular and dermal irritation tests. To perform the Ocular Irritation standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. To perform the Dermal Irritation standardized assay, the test sample is applied to a similar synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly ordered globulins and 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. With the Ocular Irritation test, turbidity may be detected spectrophotometrically at a wavelength of 405 nm. With the Dermal Irritation 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 Irritation 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.

Table 1. Relationship of Irritection Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritection Test Method.

Irritection Draize Equivalent (IDE) Score	Predicted Ocular Irritancy Classification
0.0 - 12.5	Minimal Irritant
12.5 - 30.0	Mild Irritant
30.0 - 51.0	Moderate Irritant
51.0 - 80.0	Severe Irritant

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	Dose	IDE Score	Predicted Ocular Irritancy Classification
E8234	SW-1 Degreasing Solution	1 %	9.3	Minimal Irritant
		5 %	8.8	Minimal Irritant
		10 %	9.2	Minimal Irritant
		25 %	8.9	Minimal Irritant
		50 %	9.5 ^a	Minimal Irritant

^a Maximum Qualified Score**Table 4.** Summary of the Dermal Irritection Results

IVI Number	Sample Description	Dose	HIE Score	Predicted Dermal Irritancy Classification
S4956	SW-1 Degreasing Solution	1 %	0.72	Non-Irritant
		5 %	0.66	Non-Irritant
		10 %	0.72	Non-Irritant
		25 %	0.75 ^a	Non-Irritant
		50 %	0.64	Non-Irritant

^a Maximum Qualified Score

DISCUSSION

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.

A standard concentration-dependent dose-response study was performed with the Ocular Irritection test method. The following concentrations of neat sample were applied for analysis: 1, 5, 10, 25 and 50 %. The results of the study indicated that the sample of SW-1 Degreasing Solution was classified as a minimal irritant with an IDE score of 9.5.

A similar concentration-dependent dose-response study was performed with the Dermal Irritection test method. The results demonstrated that the sample was predicted to be a non-irritant with a HIE score of 0.75.

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-1 Degreasing Solution	Date	: 05/27/10
Sample Number : E8234	Time	: 15:14:01
Product Type :	Technician Name	: Amy
Assay Method : Ocular	Kit Lot Number	: IO 061909
Protocol : Surfactant-405	Reagent temperature	: 25.0
Incubation Time : 5.0 hours	Reagent pH Before Activation	: 8.06
Plate Layout : 1 Sample/5 Concentrations	Reagent pH After Activation	: 5.86
Instrument Type : Dynex MRX	Sample pH at 10%	: 8.05
Wavelength : 405nm	Assay Number	: 1
Comment :	Assay Qualification	: Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
1 %	188	-1	189	9.3	Minimal	Qualified
5 %	180	0	180	8.8	Minimal	Qualified
10 %	187	-1	188	9.2	Minimal	Qualified
25 %	179	-2	181	8.9	Minimal	Qualified
50 %	191	-2	193	9.5	Minimal	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	233	0.0	105 - 315	Range qualified
Cal 1	255	12.5	100 - 402	Range qualified
Cal 2	791	30.0	450 - 1160	Range qualified
Cal 3	1642	51.0	1629 - 2715	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	169	8.3	3.3 - 18.5	Range qualified
QC 2	712	27.4	23.2 - 39.3	Range qualified

Sample Inhibition Check Results:

Concentration / Inhibition Check OD

1 % / 1642 5 % / 1630 10 % / 1619 25 % / 1603 50 % / 1591

* 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-1 Degreasing Solution	Date	: 05/25/10
Sample Number : S4956	Time	: 14:57:51
Product Type :	Technician Name	: Amy
Assay Method : Dermal	Kit Lot Number	: ID 042710
Protocol : Surfactant-450	Reagent temperature	: 25.0
Incubation Time : 24.0 hours	Reagent pH Before Activation	: 10.00
Plate Layout : 1 Sample/5 Concentrations	Reagent pH After Activation	: 7.88
Instrument Type : Dynex MRX	Sample pH at 10%	: 8.05
Wavelength : 450nm	Assay Number	: 1
Comment :	Assay Qualification	: Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
1 %	184	-2	186	0.72	Non-Irritant	Qualified
5 %	170	-1	171	0.66	Non-Irritant	Qualified
10 %	185	-1	186	0.72	Non-Irritant	Qualified
25 %	195	1	194	0.75	Non-Irritant	Qualified
50 %	169	2	167	0.64	Non-Irritant	Qualified

Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
Cal 0	78	0.00	0 - 100	Range qualified
Cal 1	259	1.00	60 - 260	Range qualified
Cal 2	530	2.00	330 - 886	Range qualified
Cal 3	920	4.00	810 - 1430	Range qualified

Quality Control Values:

Designation	OD	Irritancy Score	Range Limit (Score)	Qualification
QC 1	135	0.52	0.11 - 0.95	Range qualified
QC 2	809	3.43	0.94 - 3.60	Range qualified

Sample Inhibition Check Results:

Concentration / Inhibition Check OD

1 % / 1753 5 % / 1939 10 % / 1696 25 % / 1128 50 % / 940

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Director of R&D, QA:

Amy Wang, M.S. Date

Approved by:
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STUDY OBJECTIVE

A single sample provided by ChemFree Corporation was evaluated with the Irritecton[®] Assay System in order to predict its potential to cause ocular and dermal irritation.

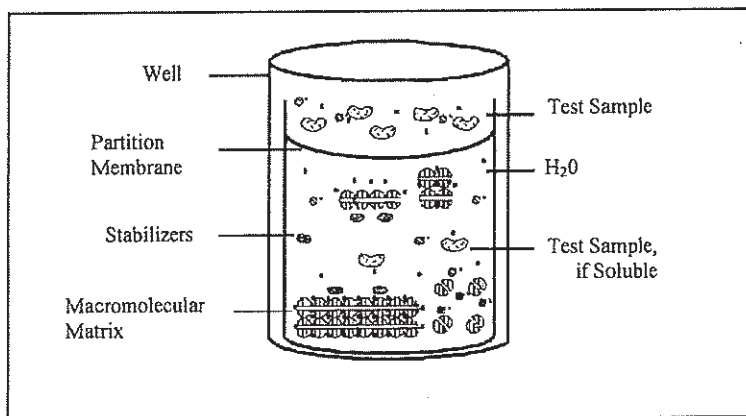
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The proprietary Ocular and Dermal Irritecton assays are standardized and quantitative *in vitro* acute ocular and dermal irritation tests which utilize changes of relevant macromolecules to predict acute ocular and dermal irritancy of chemicals and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

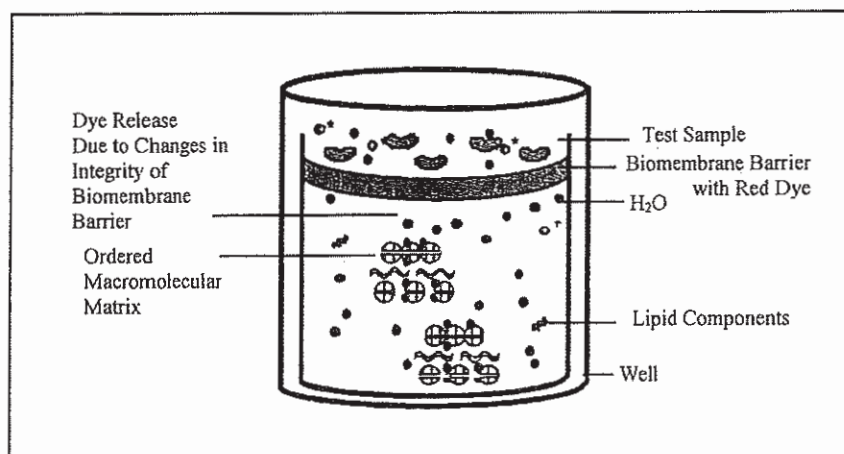
The Ocular Irritecton assay, depicted schematically in Figure 1 below, provides significant advances over the *in vivo* Draize test method. The Draize eye irritation assay has been criticized because of the large variability of results obtained from different laboratories that have analyzed the same specimen.

Figure 1. The Ocular Irritecton 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 quantitative Ocular and Dermal Irritection *in vitro* assays have been found to be highly reproducible. Of even greater 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. To perform the Ocular Irritection standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. To perform the Dermal Irritection standardized assay, the test sample is applied to a similar synthetic biobarrier that is coated with a dye-containing keratin-collagen matrix. Following application, the sample is absorbed by and permeates through this synthetic biobarrier to gradually come into contact with a proprietary solution containing highly ordered globulins and 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. With the Ocular Irritection test, 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.

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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.

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30.0 - 51.0	Moderate Irritant
51.0 - 80.0	Severe Irritant

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.

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		10 %	9.2	Minimal Irritant
		25 %	8.9	Minimal Irritant
		50 %	9.5 ^a	Minimal Irritant

^a Maximum Qualified Score**Table 4.** Summary of the Dermal Irritection Results

IVI Number	Sample Description	Dose	HIE Score	Predicted Dermal Irritancy Classification
S4956	SW-1 Degreasing Solution	1 %	0.72	Non-Irritant
		5 %	0.66	Non-Irritant
		10 %	0.72	Non-Irritant
		25 %	0.75 ^a	Non-Irritant
		50 %	0.64	Non-Irritant

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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.

A standard concentration-dependent dose-response study was performed with the Ocular Irritection test method. The following concentrations of neat sample were applied for analysis: 1, 5, 10, 25 and 50 %. The results of the study indicated that the sample of SW-1 Degreasing Solution was classified as a minimal irritant with an IDE score of 9.5.

A similar concentration-dependent dose-response study was performed with the Dermal Irritection test method. The results demonstrated that the sample was predicted to be a non-irritant with a HIE score of 0.75.

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

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Sample Number : E8234	Time	: 15:14:01
Product Type :	Technician Name	: Amy
Assay Method : Ocular	Kit Lot Number	: IO 061909
Protocol : Surfactant-405	Reagent temperature	: 25.0
Incubation Time : 5.0 hours	Reagent pH Before Activation:	8.06
Plate Layout : 1 Sample/5 Concentrations	Reagent pH After Activation	: 5.86
Instrument Type : Dynex MRX	Sample pH at 10%	: 8.05
Wavelength : 405nm	Assay Number	: 1
Comment :	Assay Qualification	: Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
1 %	188	-1	189	9.3	Minimal	Qualified
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Calibrator Values:

Designation	OD	Irritancy Score	Range Limit (OD)	Qualification
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ASSAY REPORT - ORIGINAL

Sample Description :	SW-1 Degreasing Solution	Date :	05/25/10
Sample Number :	S4956	Time :	14:57:51
Product Type :		Technician Name :	Amy
Assay Method :	Dermal	Kit Lot Number :	ID 042710
Protocol :	Surfactant-450	Reagent temperature :	25.0
Incubation Time :	24.0 hours	Reagent pH Before Activation :	10.00
Plate Layout :	1 Sample/5 Concentrations	Reagent pH After Activation :	7.88
Instrument Type :	Dynex MRX	Sample pH at 10% :	8.05
Wavelength :	450nm	Assay Number :	1
Comment :		Assay Qualification :	Qualified

Sample Results:

Dose	Sample OD	Blank OD	Net OD	Irritancy Score	Irritancy Classification	Qualification
1 %	184	-2	186	0.72	Non-Irritant	Qualified
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25 %	195	1	194	0.75	Non-Irritant	Qualified
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Calibrator Values:

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