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Date: **27 February 2003**

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REMARKS: ☐ Urgent ☒ For your review ☐ Reply ASAP ☐ Please comment

Hello Thomas:

Here is the Dermatological and Ocular study for ChemFree SW-3

TM

Evaluation of a Sample Provided by

ChemFree Corporation

Utilizing the Irritection® Assay System

October 25, 2001

INVITRO INTERNATIONAL

UTILIZATION OF THE IRRITECTION® ASSAY SYSTEM TO EVALUATE A SAMPLE PROVIDED BY CHEMFREE CORPORATION

Study Completion Date: October 25, 2001

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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-3 Truck Grade Degreasing Solution was a mild 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.

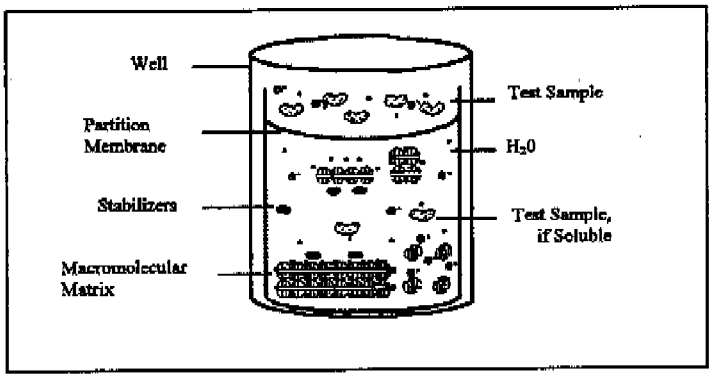
To achieve this objective, standard concentration-dependent dose-response studies were performed with the Ocular and Dermal Irritection test methods.

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.

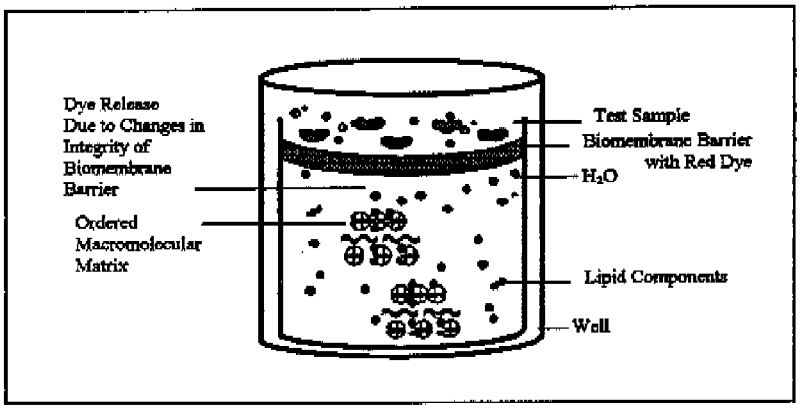
The Ocular Irritection assay, depicted schematically in Figure 1 below, provides significant advances over the *in vivo* Draize test method. The Draize eye irritation assay has been criticized because of the large variability of results obtained from different laboratories that have analyzed the same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

Figure 1. The Ocular Irritection Model



The Dermal 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.

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.

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
E6647	SW-3 Truck Grade Degreasing Solution	1%	14.5	Mild Irritant
		5%	14.7	Mild Irritant
		10%	15.4	Mild Irritant
		25%	15.9	Mild Irritant
		50%	17.0*	Mild Irritant

* Maximum Qualified Score

Table 4. Summary of the Dermal Irritection Results

IVI Number	Sample Description	Dose	HIE Score	Predicted Dermal Irritancy Classification
S3777	SW-3 Truck Grade Degreasing Solution	1%	0.61*	Non-Irritant
		5%	0.61	Non-Irritant
		10%	0.56	Non-Irritant
		25%	0.55	Non-Irritant
		50%	0.47	Non-Irritant

* 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-3 Truck Grade Degreasing Solution was classified as a mild ocular irritant with an IDE score of 17.0.

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 0.61.

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