

Ocular Irritation Results

Client: UNSW (FSI)

Date of Test: 22/10/2020

Contact: Dewen Zhou

Our Ref: IR02p72-1

Sample Desc: FSI - Defend

Client o/n:

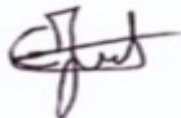
Results:	Dose	IDE Score	Predicted Irritancy Classification
	25 mg	5.50	Minimal Irritant
	50 mg	7.90	Minimal Irritant
	75 mg	7.10	Minimal Irritant
	100 mg	7.60	Minimal Irritant
	125 mg	10.00	Minimal Irritant
Maximum Qualified Score: *		10.00	

Interpretation: UN GHS/EU CLP Non Irritant

Key:

Irritation Draize Equivalent (IDE)	Predicted Ocular Irritancy Classification	UN GHS/EU CLP Classification
0.0 - 12.5	Minimal Irritant	Non-irritant [No Category]
12.6 - 30.0	Mild Irritant	Irritant [Category 1/Category 2]
30.1 - 51.0	Moderate Irritant	
51.1 - 80.0	Severe Irritant	

Signed:



IR02p72- Evgenia Platarou

Ocular Irritation Results

Ocular Irritation Study Report

STUDY OBJECTIVE

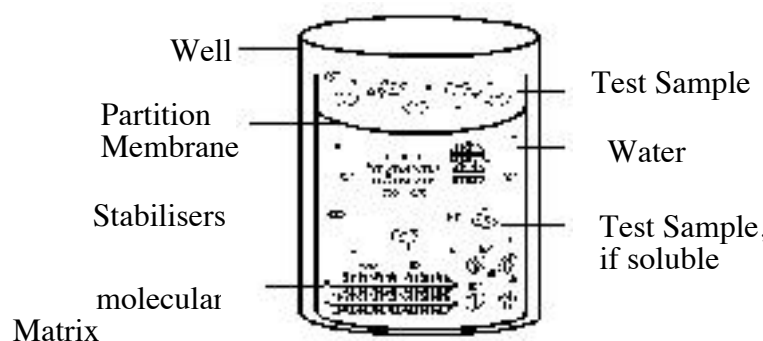
samples provided by UNSW (FSI) were evaluated with the Irritation Assay System in order to predict potential to cause irritation. The test was conducted on 22/10/2020.

To achieve this objective, a standard concentration-dependent dose-response study was performed using the Ocular Irritation test method.

BACKGROUND

The proprietary Irritation 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 Irritation Model



Additionally, the Irritation assay system provides significant benefits when compared to the in vivo Draize test method. The quantitative Irritation in vitro assay has been found to be highly reproducible. Of even greater relevance, the Irritation assay method can be readily employed to evaluate multiple samples at varying volumes or concentrations. Thus, the test serves as an extremely useful screening tool that facilitates all stages of raw material selection, formulation development and final product selection. The optical density was detected spectrophotometrically at a wavelength of 405 nm

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MATERIALS AND METHODS

The Irritation assay is a quantitative *in vitro* test method that mimics an acute dermal irritation test. To perform this standardized assay, the test sample is applied to a synthetic biobarrier composed of a semi-permeable membrane. 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 irritancy potential of a test sample is expressed as an Irritation Draize Equivalent (IDE) score. This score is defined by comparing the changes in optical density (OD405) produced by the test material to a standard curve that is constructed by measuring the increase in OD405 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 1. 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. Relationship of Irritation Draize Equivalent (IDE) Score to Irritancy Classification for the Ocular Irritation Test Method

Irritation Draize Equivalent (IDE)	Predicted Ocular Irritancy Classification	UN GHS/EU CLP Classification
0.0 - 12.5	Minimal Irritant	Non-irritant [No Category]
12.6 - 30.0	Mild Irritant	Irritant [Category 1/Category 2]
30.1 - 51.0	Moderate Irritant	
51.1 - 80.0	Severe Irritant	

A detailed description of the Irritation test procedure may be found in InVitro International's Irritation® Assay System Instruction Manual or at www.invitrointl.com. 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 optical density (OD) units; the net sample OD is greater than -15; and an Inhibition Check is negative.