STUDY REPORT: Example_Report_Surfactant

Study ID: 20XX_XX_example	**Test Item:** EXAMPLE ITEM

DATE OF FINAL REPORT: XX/XX/20XX

Evaluation of ocular irritation potential using OCULAR IRRITECTION (OI®) test method (OECD 496)

TEST FACILITY:

C.A.O. _Laboratorio di Biologia Cellulare
applicata e Tossicologia In Vitro per la
determinazione della sicurezza e dell'efficacia
di prodotti destinati all'uso umano
Via G. Pastore, 3 20056 Trezzo Sull'Adda (MI)

SPONSOR:

Customer
Via XXXXXXX
CAP,….. City……

Note: The results reported in the present report refer exclusively to the tested test item. This study cannot be reproduced in part without written permission of the laboratory.

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1. STUDY SPECIFIC INFORMATION

STUDY ID:	**2020_04_example**
TITLE:	*Evaluation of ocular irritation potential using OCULAR IRRITECTION (OI®) test method (OECD 496)*
SPONSOR:	**CUSTOMER**
TESTING FACILITY:	**C.A.O.**
TEST ITEM:	EXAMPLE ITEM
DATE OF RECEIPT OF TEST ITEM:	15/04/2020
START EXPERIMENTATION DATE:	15/04/2020
END EXPERIMENTATION DATE:	16/04/2020
REPORTS DATE:	21/04/2020

1.1 Guideline-Regulations

The test methods described in this report were performed according to methods described in the following documents:

- OECD guideline 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage (2019)
- DB-ALM Protocol n°157_Ocular Irritection®Assay System

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1.2 Signatures

***PERFORMED BY*:** Dr. Ilaria Losini

Date:

***STUDY DIRECTOR*:** Dr. Ilaria Losini

Date:

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2. SUMMARY

Test item EXAMPLE ITEM provided by **CUSTOMER** was evaluated with OCULAR IRRITECTION (OI®) test method (OECD496) to predict its potential to cause ocular irritation. The results of this study may be summarized as follows:

SAMPLE DESCRIPTION	Maximal Qualified Score (MQS)	Predicted UN GHS classification
EXAMPLE ITEM	49.8	Category 1

According to the OECD Guideline 496, the test item EXAMPLE ITEM can be considered an **UN GHS Category 1 substance.**

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3. PURPOSE OF THE STUDY

The purpose of the study was to evaluate the potential to cause ocular irritation of the test item EXAMPLE ITEM provided by **CUSTOMER.**

4. TEST ITEM

4.1 Identification

SPONSOR IDENTIFICATION:	EXAMPLE ITEM
C.A.O INTERNAL CODE:	Example_Report_1
DESCRIPTION:	EXAMPLE ITEM
BATCH NUMBER:	N.D
STORAGE CONDITION:	Room Temperature
EXPIRY DATE:	N.D

Safety data sheet: N.D **Certificate of Analyses**: N.D

The integrity of supplied data to the identity, purity and stability of the test item is responsibility of the Sponsor.

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4.2 Preparation of test item

The test item should be classified as a surfactant material. Therefore, a Ocular Irritection surfactant handling procedure was performed. In order to be submitted to the test system, the test item has undergone a dilution in order to obtain the following concentrations: 0.3125, 0.625, 1.25, 2.5, 5%. The dilution of the test item was performed using demineralized water according to the table below:

Concentration	Amount of test item	Amount of water	Total amount
5 %	0,500 ml	9,5 ml	10 ml
2.5 %	2,5 ml sol 5%	2,5 ml	5 ml
1.25 %	2,5 ml sol 2.5%	2,5 ml	5 ml
0.625 %	2,5 ml sol 1.25%	2,5 ml	5 ml
0.3125 %	2,5 ml sol 0.625%	2,5 ml	5 ml

5. SCIENTIFIC BACKGROUND

The in vitro macromolecular test method Ocular Irritection® consists of two components: a macromolecular matrix and a membrane disc for the controlled delivery of the test chemical to the macromolecular matrix. It is an acellular biochemical test system and does not address the cytotoxicity aspect of ocular toxicity. The macromolecular matrix serves as the target for the test chemical and is composed of a mixture of proteins, glycoproteins, carbohydrates, lipids and low molecular weight components forming a gel matrix. The protein oligomers which are part of the matrix self-associate to form larger fibrils that are held together by non-covalent forces. The macromolecular matrix, when rehydrated with a buffered salt solution, forms a highly ordered and transparent structure. Test chemicals causing ocular damage are known to produce denaturation of collagen and saponification of lipids (e.g., by alkalis), coagulation and precipitation of proteins (e.g., by acids) and/or dissolvance of lipids (e.g., by solvents). Test chemicals producing protein denaturation, unfolding and changes in conformation will lead to the disruption and disaggregation of the highly organized macromolecular reagent matrix, and produce turbidity of the macromolecular reagent. Such phenomena are quantified, by measuring the changes in light scattering (at a wavelength of 405 nm using a spectrometer), which is compared to the standard curve established in parallel by measuring the increase in OD produced by a set of calibration substances. The standard curve is used for deriving an Irritection Draize Equivalent (IDE) Score for each tested dose/concentration of the test chemical. The highest IDE Score of the five tested doses/concentrations of a test chemical, namely Maximal Qualified Score (MQS), is then used to determine an UN GHS ocular hazard category based on pre-defined cut-off values.

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5.1 Test System

For the determination of ocular irritation potential of a test item was used the commercial kit, specially developed for the purpose Ocular Irritection® test system supplied by Vitro International and distributed by INT.EGRA. for Europe, with the following lot number and expiry date:
Lot No. IO 050119

Expiry 05/2021

Kit contains:
Ocular Reagent powder (1 bottle)
Ocular Hydrating solution (1 bottle)
Ocular Blanking buffer (1 bottle)
Ocular Activator_A (1 vial)
Four Calibratiors Solution: Cal0, Cal1, Cal2, and Cal3
Two Quality Controls Solutuion: QC1 and QC2
Ocular Inhibition check(I) (1 vial)
24 Membrane Discs with 24-well assay plate lot # IO100815
Wooden stirring Sticks
Whatman filter paper, 12.5 cm diameter
Range Specification Data Sheet

6. EXPERIMENTAL PROCEDURE

6.1 Control Chemicals

Concurrent controls should be tested in parallel to the test chemical. In the case of Ocular Irritection®, these include 4 calibrating chemicals and two quality control (QC) chemicals provided within the commercial kit . The calibrating chemicals include four chemicals with UN GHS classification ranging from No Category to Category 1and cover a defined range of OD responses which are used to derive the standard curve for Irritection Draize Equivalent (IDE) Score determination. The two QC chemicals have defined ranges of IDE scores associated with their irritation potential which falls close to the prediction model cut-offs.

6.2 Dosage, route of administration and analytical replies

For the test item were tested 125 µl of the following 5 substance concentrations: 0.3125, 0.625, 1.25, 2.5, 5%. Each concentration of the test substance was dispensed directly to the reagent solution and to the blanking buffer and covered with a

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Membrane Discs. 125 µl of quality control QC1 and QC2, and the four calibrator CAL0, Cal1, Cal2 and CAL3 are applied neat onto the membrane disc placed over the matrix reagent.

6.3 Steps

According OECD Guideline OECD guideline 496: In vitro Macromolecular Test Method for Identifying Chemicals Inducing Serious Eye Damage and Chemicals not Requiring Classification for Eye Irritation or Serious Eye Damage (2019) the following steps were performed:

Day 1

REAGENT PREPARATION

As a basis of the Ocular Irritection® in vitro macromolecular test method, a macromolecular matrix is prepared by dissolving the reagent powder provided within the kit into a hydrating solution, and filtering the dissolved reagent. The resulting pH was measured: 8.026. Furthermore, the reagent solution (as well as the blanking buffer conducted in parallel for each tested dose/concentration) was activated using an activator buffered solution, to reduce the pH of the reagent solution and initiate formation of the ordered macromolecular matrix. The resulting pH of the activated reagent solution was measured: 6.528. Aliquots of the activated protein matrix reagent solution are transferred to a 24-well plate.

TEST ITEM EXPOSURE PROCEDURE

The Reagent solution and the Ocular Blanking activated buffer were dispensed in the wells of 24-well plate supplied with the kit, in an established layout based on the number of wells required to analyze the four calibrators, the 2 quality controls and 4 volumes of the test item. The 5 concentration of the test substance was dispensed directly to the reagent solution and to the blanking buffer and covered with a Membrane Discs.125 µl of quality control QC1 and QC2, and the four calibrator CAL0, Cal1, Cal2 and CAL3 are applied neat onto the membrane disc placed over the matrix reagent

INCUBATION

The macromolecular matrix of the Ocular Irritection® test method is exposed to the test chemicals and concurrent controls for 24.0 ± 0.5 hours in an incubator maintained at 25 ± 1°C.

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Following incubation test chemicals and controls are transferred to a 96 well plate for OD reading at 405nm. The process of transfer is described in detail and illustrated in the protocol within the kit . The raw OD readings from each well are obtained and the IDE scores for the QCs and test chemicals are calculated by the software. MQS for a test chemical is determined from a single test run qualified as appropriate based on the analysis of the OD scores for the calibrators and QC chemicals as well as aspects of the dose response generated with the five tested doses/concentrations of test chemical.

6.4 Data recording

The data of optical density measured by the spectrophotometer, are recorded automatically by Irritection Software.

6.5 Interpretation of Results and Prediction Model

The Irritection Software program serve as the user interface to plate reader. The program automatically receives the optical density reading from plate reader and then convert the data to the Irritection Draize Equivalent (IDE) score. The optical density (OD405) obtained with a qualified test chemical is compared to the standard curve obtained with the set of calibrators, to derive an Irritection Draize Equivalent (IDE) Score, for each tested dose/concentration. The highest obtained IDE score, named the Maximal Qualified Score (MQS), is then used to predict the ocular hazard potential of the test chemical according to the UN GHS classification system.
In the case of the Ocular Irritection® in vitro macromolecular test method the Prediction Model described in table 1 is used.

Maximal Qualified Score (MQS)	Predicted UN GHS classification
0.0 – 12.5	No Category
>12.5 – 30	No Prediction can be made
>30	Category 1

Tab.1 *Ocular Irritection prediction® model*

If the MQS result is > 12.5 – 30.0 No final Prediction Can be made from this result in isolation This is because a considerable number of in vivo UN GHS Category 1 chemicals showed MQS within this interval and were therefore under-predicted with the macromolecular test assay. In addition, considerable number of in vivo UN GHS No Category showed MQS within this interval i.e. were over-predicted For final classification of chemicals with MQS in the interval > 12.5 – 30.0, further information and/or testing with other test methods will be required according to the IATA

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guidance document. Consideration would need to be given to all possible mechanisms of ocular toxicity that may be relevant to he test chemical, based on existing data and knowledge as outlined in GD263 when deriving a classification.

6.6 Acceptance criteria of the study

All data are calculated and analyzed via a computer program which determines assay result acceptance based upon qualification parameters defined in the program

(Irritection Software). In general, the Irritection Software has been designed to accept sample data as qualified if the following criteria are met: The values obtained for all four calibrators and for at least one of two Quality Controls are within the pre-established accepted ranges; or the values obtained for any three of four calibrators, and for both Quality Controls are within the pre-established accepted ranges. Sample blanks are less than 500 OD units; the net sample OD is greater than -15; and an Inhibition Check is negative.

6.7 Acceptance criteria of the result

The Irritection Software employs an internal algorithm to assess the test result and compare to those that would be expected for an ideal dose-response curve. The statement “Qualified” in the Sample result section means that the data for the tested sample obey the expected behavior of typical dose-repose curve. The irritancy of the test sample is judged to be defined by highest qualified score calculated by the Irritection software: Maximum Qualified Score (MQS)
MQS is selected from data because is the irritancy score that correlates most closely with the in vivo irritancy properties of test item.

7. STUDY RESULTS

7.1 Control Items

The data (OD) obtained for 4 Calibrator and 2 QC were within the accepted limits as specified by the Range Specification Data Sheet. On basis of these finding, the data generated for the test item EXAMPLE ITEM were accepted.

7.2 Test Items

The results obtained for test item are shown in the following table.

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Sample	Protocol	Dose	IDE Score
EXAMPLE ITEM	OECD496 (Surfactant)	0.3125%	17.7
		0.625%	30.7
		1.25%	37.8
		2.5%	45.2
		5%	49.8[a]

[a] Maximum Qualified Score

8. CONCLUSIONS

Test item EXAMPLE ITEM provided by **CUSTOMER** was evaluated with OCULAR IRRITECTION (OI®) test method (OECD496) . The following concentrations of sample were applied for analysis: 0.3125, 0.625, 1.25, 2.5, 5%. The results demonstrated that the sample EXAMPLE ITEM can be considered a **Category 1 substance** with a Maximum Qualified Score of **49.8**.

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