# EpiOcular™ Eye Irritation Test (EpiOcular-EIT) for Identification of Materials Not Requiring Classification and Labeling for Eye Irritation or Serious Eye Damage

Yulia Kaluzhny, Helena Kandárová, Laurence d'Argembeau-Thornton, Thoa Truong, Paul Kearney, PatrickHayden, and Mitchell Klausner

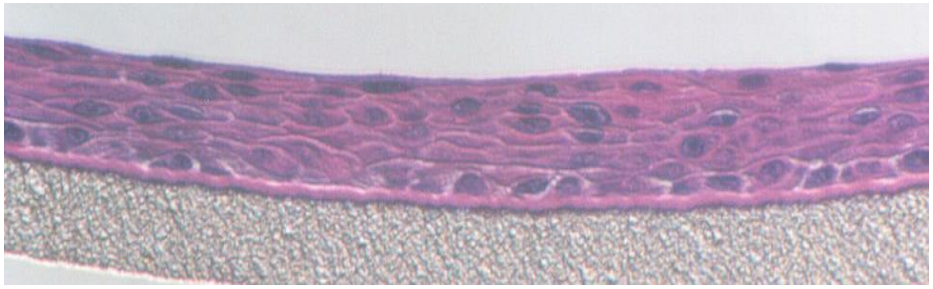

MatTek CORPORATION

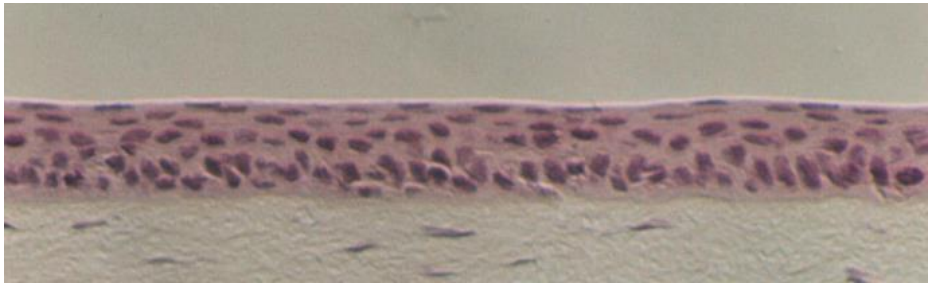
# Why We Need Safety Tests for Products


*Fig. 1.* To depict the need for governmental regulations over cosmetics, the U.S. Food and Drug Administration prepared this poster in 1933 from photographs of a 38-year-old Ohio woman. The patient's first photo was taken an hour before eyelash dyeing, and the other was obtained during the following month showing bilateral staphylococcal corneal ulcers that resulted in vision of light perception. (Reprinted from Lamb RdeF[109] with permission of Farrar and Reinhart.)

*Fig. 3.* John H. Draize, PhD, the FDA pharmacologist who developed methods for assessing the ocular and dermal toxicity of drugs and cosmetics (Courtesy of the U.S. Food and Drug Administration History Office).

# EpiOcular: Reconstructed human Cornea-like Epithelium



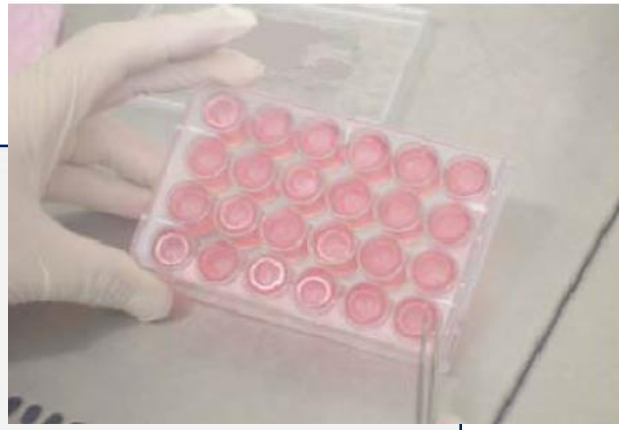


**A**) *in vitro* EpiOcular tissue (OCL-200)

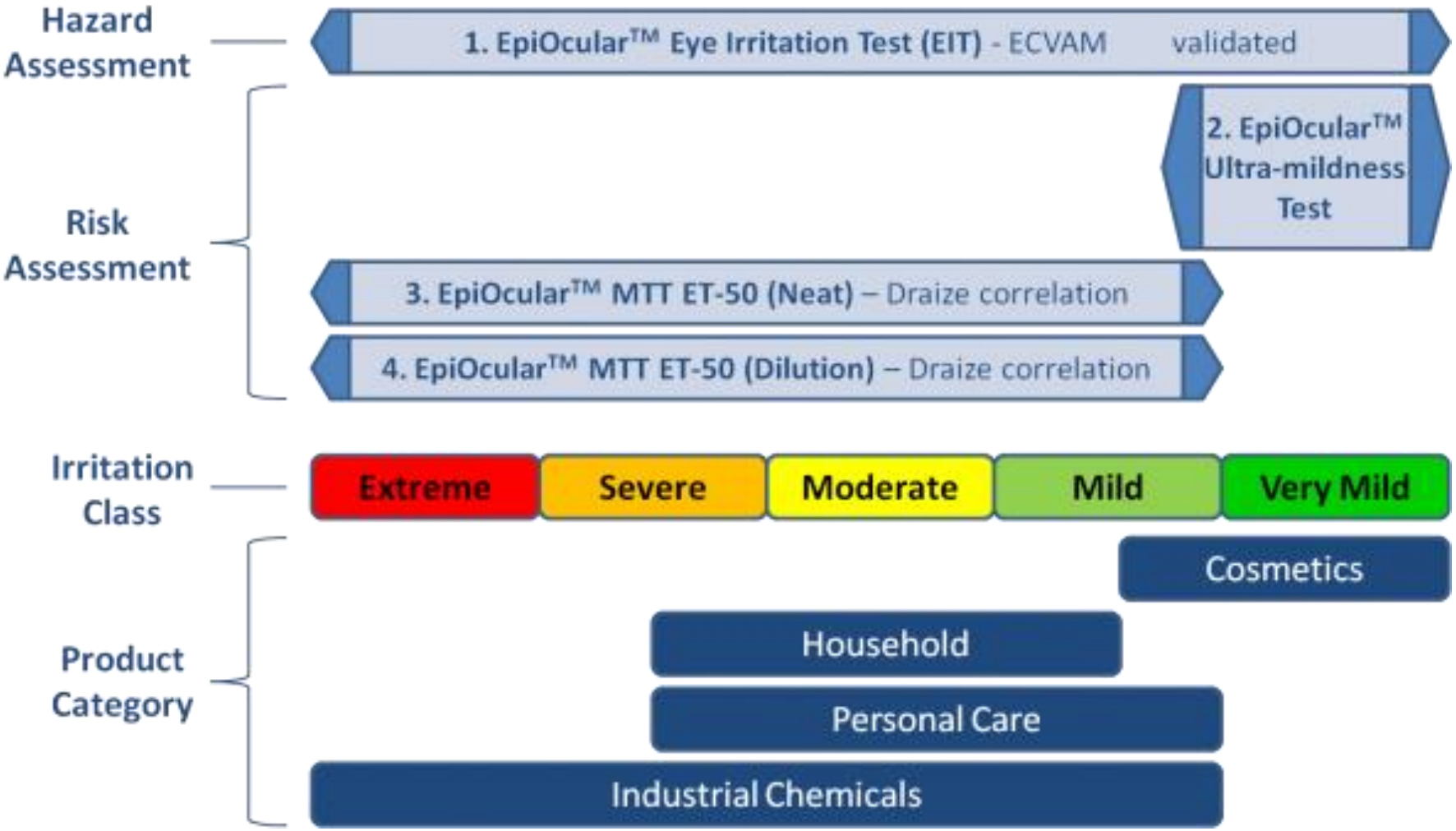
**B**) rabbit corneal epithelium.

- **Test system**: non-keratinized multi-layered epithelium reconstructed from primary human epidermal keratinocytes.
- **H&E** stained cross-section of the EpiOcular tissue (NHEK) reveals **highly organised basal and supra-basal cell layers** followed by cells which flatten out as the apical surface is approached, similar to *in vivo* corneal tissue.
- **Endpoints usualy measured**: cytotoxicity (MTT assay), permeation, release of inflammatory mediators
- **Applicability and limitations:** Applicable to all types of chemicals and also to intensely coloured chemicals (with use of HPLC/UPLC-spectrophotometry)

# EpiOcular: Reconstructed human Cornea-like Epithelium



# EpiOcular Test Methods



# EpiOcular™ Eye Irritation Test (EIT): OECD TG 492

# EpiOcular EIT Test Development

- EpiOcular EIT Protocol was developed by MatTek Corporation in 2007, as a reaction on ECVAM's request for a simple and straightforward assay that could be used in the framework of REACH and 7th Amendment to the Cosmetic Legislation (on demand protocol).

- EIT was developed for classification and labeling substances for regulatory purposes of chemicals including raw cosmetic ingredients**.**

- The protocol and its prediction model was designed to discriminate between ocular irritant / corrosive materials (GHS Categories 1 and 2 combined) and those that require no labeling (GHS No Category).

- In total **112 chemicals** were tested during the development of the protocol by MatTek.

COLIPA
THE EUROPEAN COSMETICS ASSOCIATION
= Cosmetics Europe
the personal care association

Now Cosmetics Europe

EUROPEAN CENTRE FOR THE VALIDATION OF ALTERNATIVE METHODS
ECVAM

# COLIPA Pre-Validation of EpiOcular™ Protocol: Technology Transfer - 2007

COLIPA The European Cosmetics Association

MARY KAY

AVON the company for women

Institute for In Vitro Sciences, Inc.

WELLA

BDF Beiersdorf

LVMH Moët Hennessy · Louis Vuitton

Pierre Fabre

- Protocol transfer was tested together with IIVS and pre-validation was done in 7 mainly cosmetic oriented laboratories.

- Independent **in house validation study** with 60 industrial chemicals was performed by **BASF**. Later on, BASF also conducted testing of pesticides formulations using the EIT method and received excellent results.

**Detailed information in:**

**In-house Validation of the EpiOcular™ Eye Irritation Test and its Combination with the Bovine Corneal Opacity and Permeability Test for the Assessment of Ocular Irritation**.

Kolle, S.N., Kandárová, H., Wareing,B. van Ravenzwaay,B., Landsiedel, R.(2011). ATLA **39**, 365–387, 2011 (60 chemicals).

# EpiOcular EIT Test was Adopted by OECD as TG 492 in 2015

OECD/OCDE

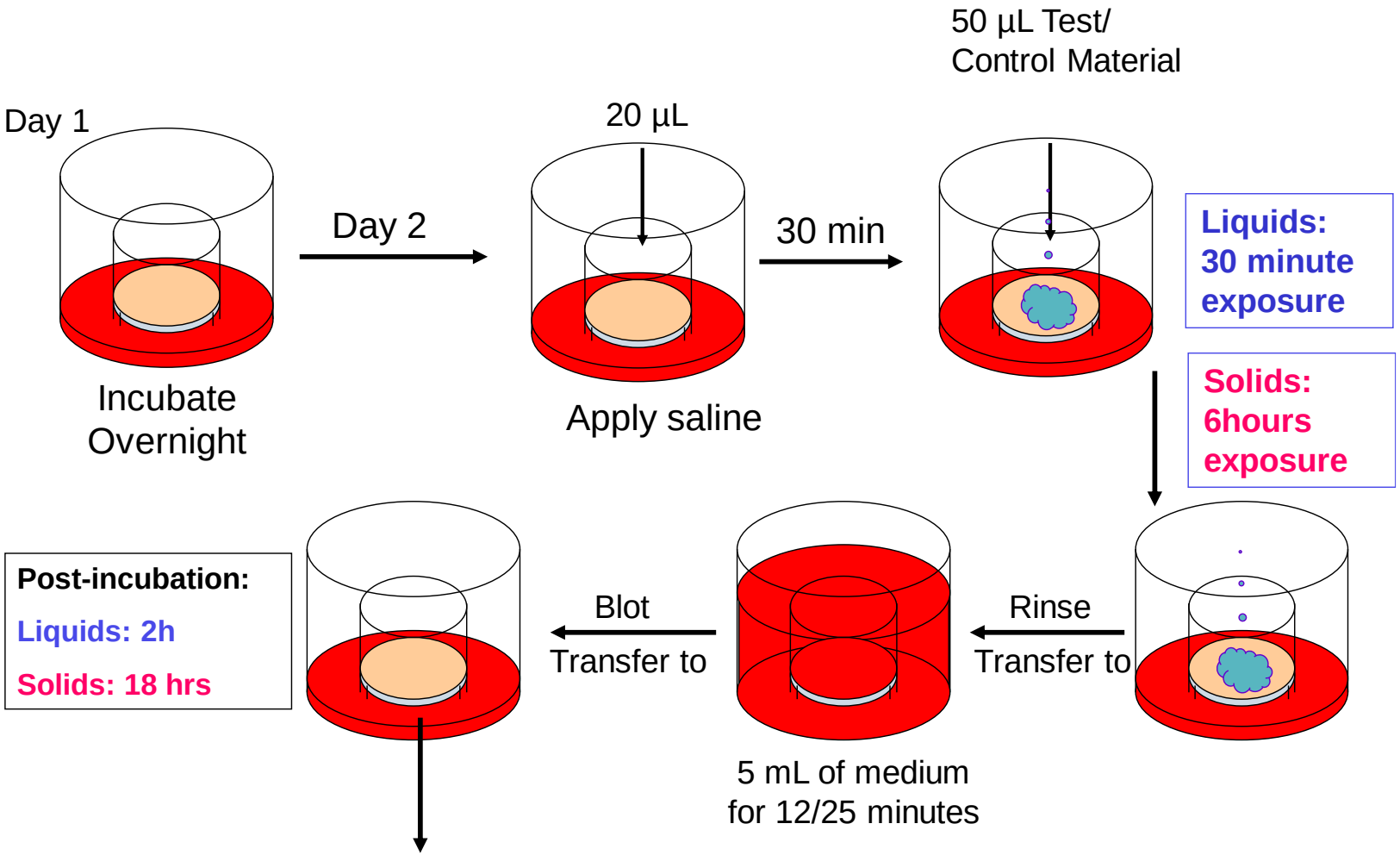
492

Adopted:
28 July 2015

## OECD GUIDELINE FOR THE TESTING OF CHEMICALS

**Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage**

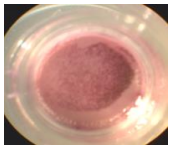
# EpiOcular™ EIT Protocol



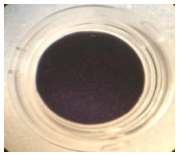
# EpiOcular™ EIT Protocol

MTT assay (3hours)

Dead tissue



Viable tissue



## Prediction model:

Eye Irritating chemicals are cytotoxic in specific test conditions of the in vitro eye irritation test

**If viability > 60%, Non Irritant**

**If viability < 60 %, Irritant ("no conclusion can be made" within the interpretation of the OECD TG 492)**

OECD TG 492 can be utilized as part of a tiered testing strategy to identify chemicals that do not require classification and labeling.

All compounds predicted as irritants are undergoing further testing (e.g. using BCOP, ICE…) to identify the irritation potency/severity or to exclude false positive prediction.

# EpiOcular™ EIT Protocol

Eye Irritation Test (EIT) for Hazard Identification of Eye Irritating Chemicals using Reconstructed Human Cornea-like Epithelial (RhCE) Tissue Model

Yulia Kaluzhny[1], Helena Kandárová[2], Laurence d'Argembeau-Thornton[1], Paul Kearney[1], Mitchell Klausner[1]

[1]MatTek Corporation, [2]MatTek In Vitro Life Science Laboratories

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0:05 Title
1:43 Reconstructed Human Cornea-like Epithelial (RhCE) Tissue Treatment Preparation (Day 0)
3:08 Pre-treatment and Test Article Exposure (Day 1)
4:42 Rinsing
6:13 MTT Viability Assay (Liquid Test Day 1; Solid Test Day 2)
8:19 Results: Representative EIT Results for 10 Test Articles
9:36 Conclusion

Summary

We have developed an eye irritation test which utilizes a three dimensional reconstructed human cornea-like epithelial (RhCE) tissue model. The test is able to discriminate between ocular irritant and corrosive materials (GHS Categories 1 and 2 combined) and those that do not require labeling (GHS No Category).

**Date Published:** 8/23/2015, Issue 102; **doi:** 10.3791/52979

**Keywords:** Bioengineering, Issue 102, alternative methods, cornea, EpiOcular, EURL ECVAM, eye irritation, in vitro, MTT viability assay, ocular irritation, REACH, reconstructed ocular tissue model, RhCE

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# EpiOcular assays for testing chemicals and formulations – ET-50 protocols

http://www.jove.com/video/52979/eye-irritation-test-eit-for-hazard-identification-eye-irritating

# EpiOcular time-to-toxicity (ET-50) protocols

- Protocols exist for more than 25 years
- Suitable for the assessment of the irritation potency, tolerance and mildness of a cosmetic formulations – AVON and Mary Kay use the EpiOcular assay for the mildness testing of their products for more than 20 years!
- Specific protocols were developed for surfactants and surfactant based formulations. Validation was conducted by IIVS and Colgate in 1998. Data from the validation were submitted to ECVAM in 2000 and resubmitted in 2004. Applicability domain increase was requested. New studies initiated within CON4EI project (2015-2017).
- Accepted by EPA for the assessment of Antimicrobial cleaning products. Level of irritation is assessed as tissue viability in MTT assay, determination of ET-50 value.
- Different test design and PM than EpiOcular EIT – more time-points required, but more information can be obtained compared to the EIT.

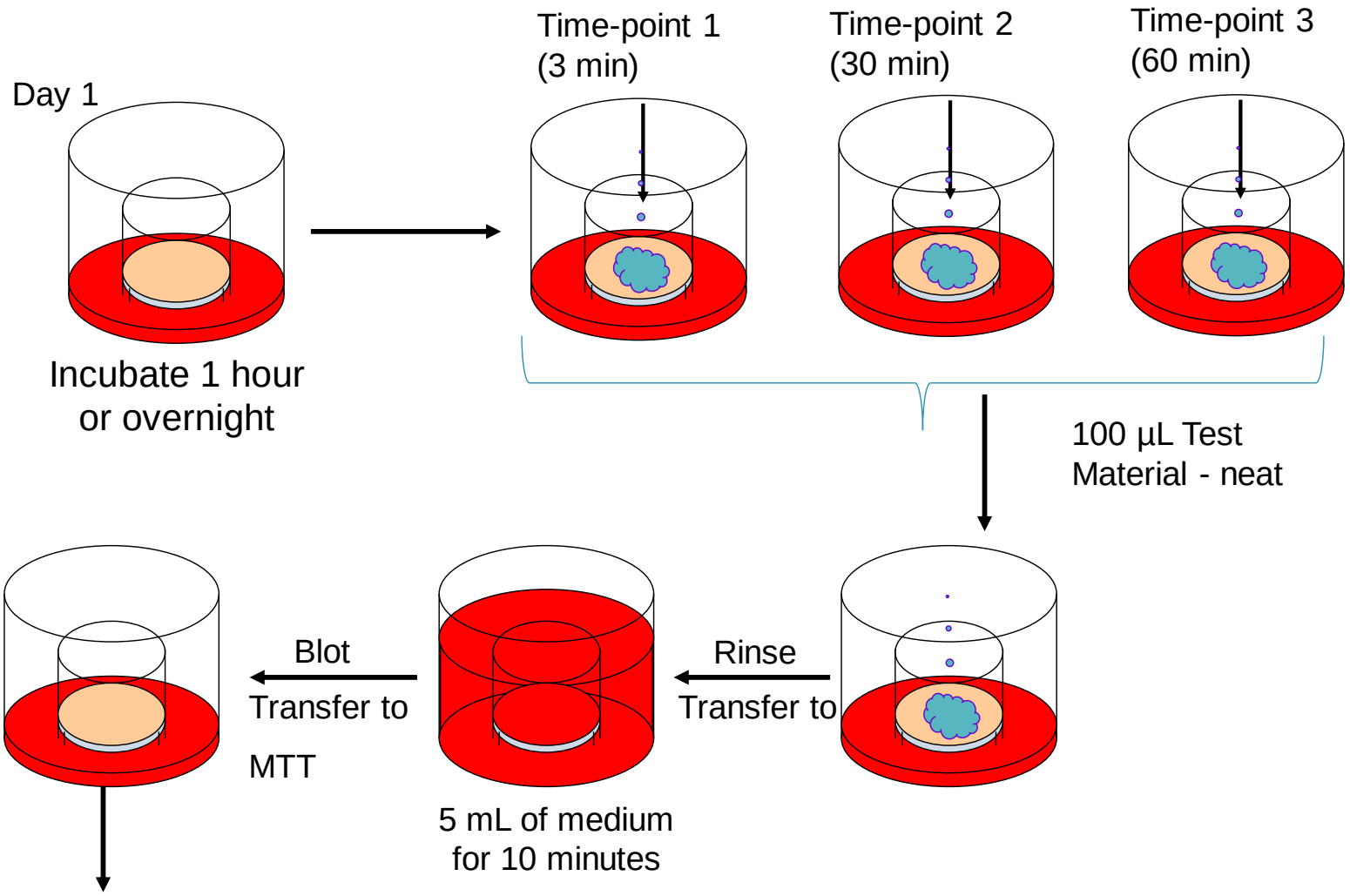
# EpiOcular time-to-toxicity (ET-50) protocols

- Neat Method: used for non-water soluble materials or for exposing test articles undiluted.

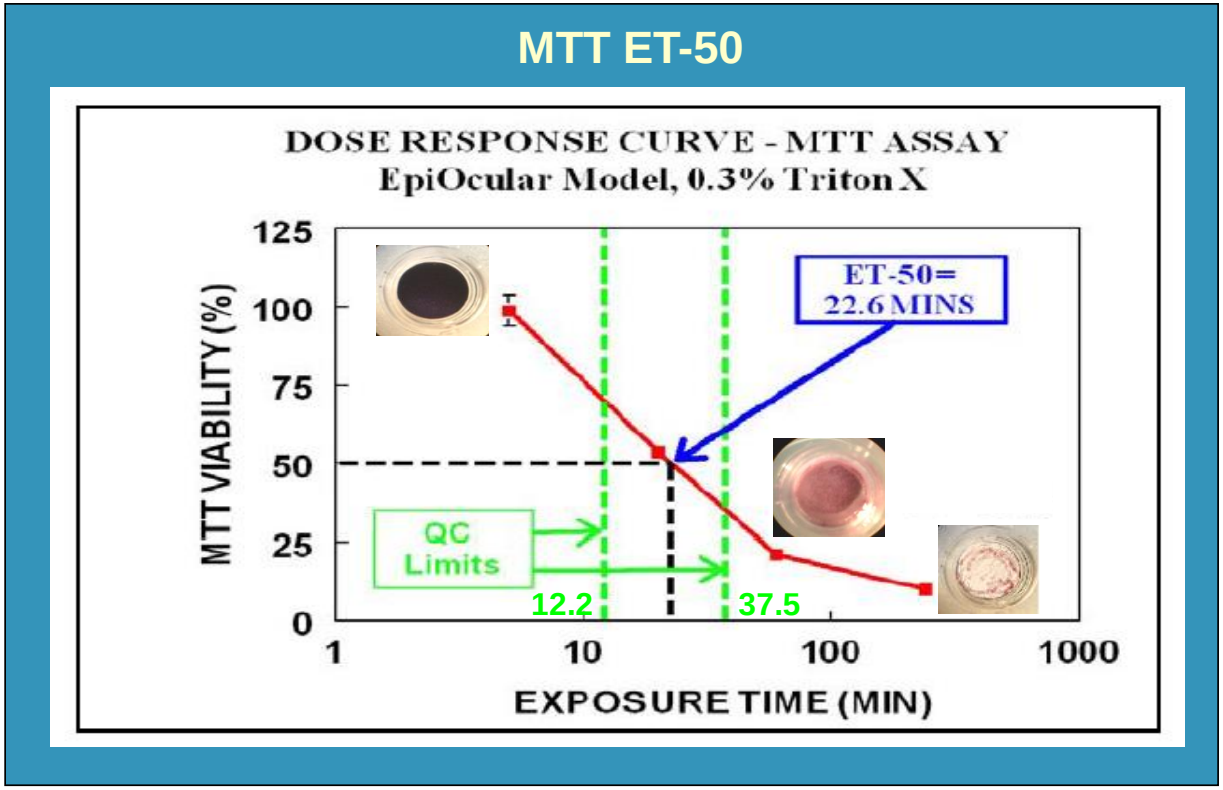
- Dilution Method: applicable to water-soluble materials with a specific gravity of ≥ 0.95 and requires an initial dilution of the test article to 20 % in water. Recommended for surfactants and rinse-off cosmetics.

- Sub-Draize Mildness Testing: involves applying neat test articles to the EpiOcular tissue model and is used to differentiate between materials for which standard Rabbit Eye Draize testing is insensitive (eye care cosmetics).

# Example of the ET-50 Protocol: Neat Method



# Example of the ET-50 Protocol: Neat Method



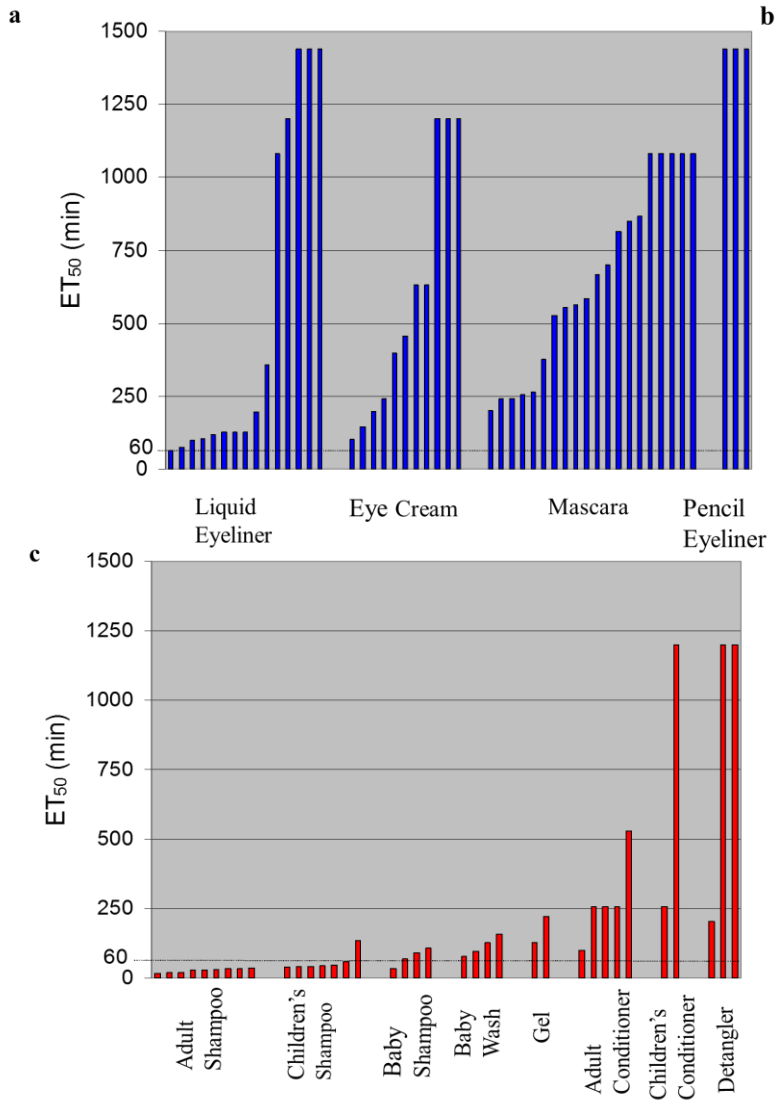

| Draize Score | Irritancy Classification | Example | EpiOcular ET-50 (min) |
|---|---|---|---|
| 0-15 | Non-irritating, Minimal | PEG-75 Lanolin, Tween 20 | >60 |
| 15.1-25 | Mild | Pareth 25-12 | 30-60 |
| 25.1-50 | Moderate | 1% Triton X-100 | 3-29.99 |
| 50.1-110 | Severe, Extreme | 5% Benzalkonium Chloride | <3 |

# Example of the ET-50 Protocol: Neat Method

Assessment of Ocular Irritation Ranges of Market-Leading Cosmetic and Personal-Care Products Using an *In Vitro* Tissue Equivalent – SOT 2002 – The Toxicologist 66, 243, 2002. N E McCain, R R Binetti, S D Gettings, and B C Jones, **Avon Products**, Inc., Suffern, NY, USA

| Formulation Category | Number of Samples | Mean ET50 (min) | Median ET50 (min) | Range of ET50 Scores (min) |
|---|---|---|---|---|
| **Surfactant Formulas** | | | | |
| Adult Shampoo | 9 | 27.0 | 28.8 | 17.1-35.1 |
| Children's Shampoo | 7 | 58.2 | 45.4 | 37.9-135.4 |
| Baby Shampoo | 4 | 74.9 | 80.3 | 31.9-107.1 |
| Baby Wash | 4 | 114.5 | 112.6 | 76.9-155.9 |
| **Eye Area Cosmetics** | | | | |
| Liquid Eyeliner | 15 | 532.2 | 127.0 | 64-1440 |
| Eye Cream | 11 | 581.8 | 454.0 | 102-1200 |
| Mascara | 20 | 655.1 | 626.5 | 199-1080 |
| Pencil Eyeliner | 3 | 1440.0 | 1440.0 | 1440 |
| **Creams/Lotions** | | | | |
| Adult Lotion/Cream | 17 | 511.4 | 440.8 | 234-1440 |
| Sunscreen | 15 | 673.6 | 486.0 | 252-1440 |
| Baby Lotion | 2 | 1200.0 | not applicable | 1200 |
| **Non-Surfactant Haircare** | | | | |
| Children's Styling Gel | 2 | 172.7 | not applicable | 126-219.3 |
| Adult Conditioner | 5 | 279.6 | 256.0 | 100-530 |
| Children's Conditioner | 2 | 728.0 | not applicable | 256-1200 |
| Children's Hair Detangler | 3 | 868.4 | 1200.0 | 205.1-1200 |

# Example of the ET-50 Protocol: Neat Method




All of the formulations tested in this study, with the exception of six adult shampoos, had individual $ET_{50}$ scores greater than 31 minutes and are classified as mild to minimal/non-irritating. The six adult shampoos with $ET_{50}$ values less than 31 minutes are classified as moderate irritants.

# Summary

- **EpiOcular EIT test protocol has been adopted on July 28, 2015** as the new **OECD TG 492**. This test enables reliable identification of substances that do not require labelling.
    - If a chemical classifies as NI in the EIT, no further testing is required.
    - In case of positive results from the EIT it is necessary to conduct further *in vitro* testing to exclude corrosive nature of the test article or false positive prediction. Such a test could be BCOP, ICE or STE if the applicability domain is not limited.
- AVON, Mary Kay and some other **major cosmetic companies have established a reference database** of *in vitro* ocular irritation using the **EpiOcular ET-50 method** for a cross-section of marketed cosmetic and personal care products.
- The *in vitro* EpiOcular tissue model used in the ET-50 test design is able to distinguish differences between moderate "**mild" and "ultra-mild" formulations**.

# Training in performing the OECD TG 491 and the OECD TG 492 in Brazil

**Project Leader**: Dr. Talita Miguel Marin

RENAMA Rede Nacional de Métodos Alternativos

**The Brazilian National Alternative Methods Network (RENAMA)**

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## Inscrições até 01 de outubro de 2018

**Métodos de avaliação do potencial de irritação e corrosão ocular que utilizam modelos *in vitro* de córnea (OECD TG 491 e 492),**

**Coordenadora:** Dra. Talita Miguel Marin

**Data:** 26/11/18 à 30/11/18.

**Local:** Laboratório Nacional de Biociências – LNBio/ CNPEM
Rua Giuseppe Máximo Scolfaro 10.000, Cidade Universitária, *Campinas, São Paulo*.

**Inscrições (Brasil):**
https://goo.gl/forms/JOAhtv4BapFBn8rm1

**Conteúdo:** capacitar os participantes para a realização do ensaio TG OECD 491 para identificar substâncias que causam danos severos oculares e substâncias que não produzem dano. O curso também abordará o ensaio baseado em córnea humana reconstituída (TG OECD 492) e as novas metodologias envolvidas para emular a resposta humana (human-on-a-chip).

**Público-alvo:** Estudantes de pós-graduação, profissionais e pesquisadores que atuem na área de Métodos Alternativos e/ou experimentação animal nos países do MERCOSUL tanto do setor público quanto privado.

**BRAZILIAN CENTER FOR RESEARCH IN ENERGY AND MATERIALS – CNPEM**
**Campinas, São Paulo**

**Project Leader**: Dr. Talita Miguel Marin

MatTek CORPORATION

# Thank you for your attention!

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