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PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.

SERIOUS EYE DAMAGE AND EYE IRRITATION

15 February 2018

Aims of webinar series

- Update 2014-2015 webinar series
- Live and recorded webinars
- Reflects significant progress in use and acceptance of non-animal methods
- Describe methods and testing strategies that can be used to meet REACH data requirements

Webinars in this series

Perspectives on the Development, Evaluation, and Application of *in Silico* Approaches for Predicting Toxicity Recorded	Dr. Grace Patlewicz, US EPA Prof. Mark Cronin, Liverpool John Moores University
3R Approach to Acute Oral Toxicity Recorded	Dr. Kimmo Louekari, ECHA
Skin Irritation and Corrosion 25 January 2018, 4–5 pm GMT	Dr. Gertrude-Emilia Costin, Institute for In Vitro Sciences Dr. Costanza Rovida, TEAM Mastery and CAAT-Europe
Skin Sensitisation 1 February 2018, 4–5 pm GMT	Dr. Susanne Kolle, BASF SE Dr. Silvia Casati, EURL ECVAM
Serious Eye Damage and Eye irritation 15 February 2018, 4–5 pm GMT	Dr. Kim Norman, Burt's Bees Dr. Els Adriaens, Adriaens Consulting

Speakers

Dr Kim Norman obtained her PhD in cell and developmental biology from Vanderbilt University and is a diplomate of the American Board of Toxicology and a European Registered Toxicologist. She is currently a senior scientist at Burt's Bees, focusing on the regulatory compliance of cosmetics and personal-care products, and previously worked as a toxicologist at the Institute for In Vitro Sciences on non-animal toxicological studies. She has participated in numerous international meetings and training activities to promote the use and regulatory acceptance of non-animal methods of safety assessment.

Dr Els Adriaens studied biology, completed a PhD in the Faculty of Pharmaceutical Sciences at Ghent University, and subsequently obtained a master's degree in statistical data analysis. She has been a statistical data analysis consultant since 2008, currently with Adriaens Consulting BVBA, and specialises in setting up, analysing, and reporting on validation studies (in the domain of *in vitro* alternatives to eye and skin irritation and sensitisation) and clinical post-marketing studies (mainly for medical devices). She has also taught various basic statistics courses.

Chair – Andrew Turley – Science Editor – Chemical Watch
Gilly Stoddart – Director – PETA International Science Consortium

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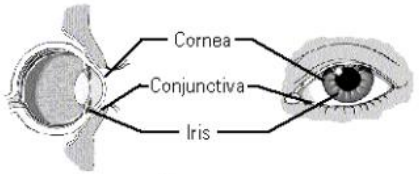
Outline

- The traditional *in vivo* Draize rabbit eye test: understanding what we're trying to replace
- Framework for full replacement
- Use of *in vitro* methods under REACH
- Available alternative methods
- Potential combinations of *in vitro* methods in testing strategies

Draize rabbit eye test (OECD TG 405)

- Corneal opacity (**CO**: score 0 to 4)
- Iris lesions (**IR**: score 0 to 2)
- Conjunctiva redness (**CR**: score 0 to 3)
- Conjunctiva chemosis (**CC**: score 0 to 4)



Human eye

Observe tissues for up to 21 days

1 | 2 | 3 | 7 | 14 | 21 → persistence Cat 1

Calculate for each rabbit mean CO, mean IR, mean CR, and mean CC values over days 1 - 3

Draize rabbit eye test (OECD TG 405)

UN GHS / EU CLP Classification

No Category (not classified)	**Category 2B/2A** Eye irritation (rev 7/21 days)	**Category 1** Serious eye damage
• **CO** < 1, and • **IR** < 1, and • **CR** < 2, and • **CC** < 2 in 2/3, 3/4, 3/5 or 4/6	• 1 ≤ **CO** < 3, or • 1 ≤ **IR** ≤ 1.5, or • **CR** ≥ 2, or • **CC** ≥ 2 in 2/3, 3/4, 3/5 or 4/6	**Severity** (mean scores days 1-3) • **CO** ≥ 3, or • **IR** > 1.5 In 2/3, 3/4, 3/5 or 4/6 **Persistence at day 21 in at least 1 rabbit** CO, IR, CR and/or CC > 0 **CO = 4** in any rabbit at any time

Replace the regulatory *in vivo* Draize eye test

- To date, no single *in vitro* method exists that covers the three UN GHS categories and can fully replace the *in vivo* Draize rabbit eye test.
- Comprehensive in depth analyses of historical *in vivo* rabbit eye data revealed that several key causes explain why only partial replacement has been accomplished until now.
  - Evaluation of the Draize within-test variability → propose acceptable target values for false negative and false positive rates for alternative methods
  - Which endpoints are most important in driving UN GHS/EU CLP classification for serious eye damage/eye irritation → selection of appropriate reference chemicals

Arch Toxicol (2014) 88:701–723
DOI 10.1007/s00204-013-1156-8

IN VITRO SYSTEMS

Retrospective analysis of the Draize test for serious eye damage/ eye irritation: importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of in vitro test methods

Els Adriaens · João Barroso · Chantra Eskes · Sebastian Hoffmann · Pauline McNamee · Nathalie Alépée · Sandrine Bessou-Touya · Ann De Smedt · Bart De Wever · Uwe Pfannenbecker · Magalie Tailhardat · Valérie Zuang

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Available at: https://link.springer.com/content/pdf/10.1007%2Fs00204-013-1156-8.pdf

Arch Toxicol (2017) 91:521–547
DOI 10.1007/s00204-016-1679-x

REVIEW ARTICLE

Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD)

João Barroso[1,2] · Uwe Pfannenbecker[3] · Els Adriaens[4] · Nathalie Alépée[5] · Magalie Cluzel[6] · Ann De Smedt[7] · Jalila Hibatallah[8] · Martina Klaric[1] · Karsten R. Mewes[9] · Marion Millet[10] · Marie Templier[10] · Pauline McNamee[11]

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PMCID: PMC5461467
NIHMSID: NIHMS858848

Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008–2014 REACH Data

Thomas Luechtefeld,[1] Alexandra Maertens,[1] Daniel P. Russo,[2] Costanza Rovida,[4] Hao Zhu,[2,3] and Thomas Hartung[1,4]

Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5461467/pdf/nihms858848.pdf

Prevalence of outcomes of the Draize rabbit eye test

- Reference Chemicals Databases (RCD): chemicals put together mainly to support validation studies
  - Eye Irritation Reference Chemicals Data Bank (ECETOC)
  - Database form ZEBET (Spielmann et al., 1996)
  - Database from Laboratoire National de la Santé (LNS) (Gautheron et al., 1992)
- European New Chemicals Database (NCD): chemicals registered by multiple industry sectors since 1981
- REACH registrations 2008 – 2014 (Leuchtefeld et al., 2017)

Data source (number of studies)	**UN GHS/EU CLP** (proportion of studies)		
	No Cat.	Cat. 2	Cat. 1
RCD (274) [a]	60.2	17.2	22.6
NCD (1860) [a]	82.6	10.4	6.9
REACH registrations 2008-2014 (1841) [b]	72.9	16.0	10.1

[a] valid studies; [b] studies for which mode eye irritation category could be extracted

Variability of the Draize rabbit eye test

- Within-test variability RCD/NCD databases
  - Over-classification error for No Cat. and Cat. 2 is negligible (<1%)
  - Cat. 2 chemicals: at least 12% could be equally identified as No Cat.
  - Cat. 1 chemicals: at least 11% could be equally identified as Cat. 2
- Between-test variability REACH registrations 2008 – 2014 (Leuchtefeld et al., 2017; based on all substances with at least two Draize tests and extractable eye irritation category, n=491)
  - Over-classification error for No Cat. and Cat. 2 is negligible: e.g. prior No Cat., 94% probability of future No Cat.
  - Cat. 2 chemicals: most probable repeat test outcome is No Cat.
  - Cat. 1 chemicals: prior Cat. 1, 74% probability of future Cat. 1 and 10.4% probability of No Cat.

Variability of the Draize rabbit eye test

- Both studies (within-test and between-test variability) suggest a high over-predictive power of the Draize eye test

- These findings should be considered when defining acceptance levels of FN's and FP's in the development and validation of alternative test methods/testing strategies

DRD - importance of Drivers of Classification

- Draize eye test Reference Database (DRD) – 681 independent Draize eye studies
  - Eye Irritation Reference Chemicals Data Bank (ECETOC)
  - Database form ZEBET (Spielmann et al., 1996)
  - Database from Laboratoire National de la Santé (LNS) (Gautheron et al., 1992)
  - Database developed by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to support the retrospective evaluations of the BCOP, ICE, IRE, and HET-CAM that were performed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (ICCVAM 2007, 2010);
  - Database developed by EURL ECVAM to support the prospective validation study of RhCE-based test methods performed by EURL ECVAM and Cosmetics Europe
  - Five studies that were not included in the other databases but that were used in the Cosmetics Europe study on the use of HPLC/UPLC-spectrophotometry in Reconstructed human Tissue (RhT)-based test methods (Alépée et al. 2015).

Conclusion analyses of Drivers of Classification

Distribution of *in vivo* studies in the DRD according to their UN GHS/EU CLP classification and the main effect driving the classification (cells with grey background indicate the most important drivers)

Category 1 28.1% (n=165)						**Category 2** [a] 13.5% (n=79)			**No Category** 58.4% (n=343)			
Severity [a] *in ≥ 60% of the animals* 27.3% (n=45)		Persistence on Day 21 *in at least one animal* 46.7% (n=77)			Severe CO *in at least one animal* 20.6% (n=34)	Severity [a] *in ≥ 60% of the animals*			*in at least one observation time in at least one animal*		*in all observation times in all animals*	
CO mean ≥ 3	**IR mean > 1.5**	**CO**	**Conj**	**IR**	**CO=4**	**CO mean ≥ 1**	**Conj mean ≥ 2**	**IR mean ≥ 1**	**CO > 0 ****	**CO > 0**	**CO = 0 ****	**CO = 0**
73.3% (n=33)	26.7% (n=12)	80.5% (n=62)	19.5% (n=15)	0% (n=0)	100% (n=34)	60.8% (n=48)	38% (n=30)	1.3% (n=1)	8.7% (n=30)	13.1% (n=45)	1.7% (n=6)	76.4% (n=262)

[a] Mean scores calculated from gradings at 24, 48, and 72 hours after instillation of the test chemical; ** at least one animal with a mean score of days 1-3 above the classification cut-off for at least one endpoint

Conclusion analysis of *in vivo* drivers of classification

- Iritis rarely drives classification on its own (< 4% of the chemicals)

  No Need to address iritis *in vitro*

- Cat. 2 chemicals
  - 54-75% classified based on corneal opacity (11-20% CO without CR/CC)
  - 75-81% classified based on conjunctiva redness (23-41% CR without CO)
  - conjunctiva chemosis rarely drives classification on its own (~2%)

  In vitro methods must be able to identify conjunctiva redness

- Cat. 1 chemicals
  - 50-70% classified based on persistence without severity (mostly CO: >80%)
  - 28-36% classified based on severity of effects (days 1 to 3) (mostly CO: >85%)

  In vitro methods to address persistence are required

Use of *in vitro* methods under REACH (Annex VII and VIII)

ANNEX VII

STANDARD INFORMATION REQUIREMENTS FOR SUBSTANCES MANUFACTURED OR IMPORTED IN QUANTITIES OF ONE TONNE OR MORE (1)

8.2.	Serious eye damage/eye irritation	8.2. The study/ies do(es) not need to be conducted if: — the substance is classified as skin corrosion, leading to classification as serious eye damage (Category 1), or — the substance is classified as skin irritation and the available information indicates that it should be classified as eye irritation (Category 2), or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5) and the available information indicates that it should be classified as serious eye damage (Category 1), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature.
8.2.1.	Serious eye damage/eye irritation, *in vitro*	8.2.1. If results from a first *in vitro* study do not allow a conclusive decision on the classification of a substance or on the absence of eye irritation potential, (an) other *in vitro* study/ies) for this endpoint shall be considered.'

ANNEX VIII

STANDARD INFORMATION REQUIREMENTS FOR SUBSTANCES MANUFACTURED OR IMPORTED IN QUANTITIES OF 10 TONNES OR MORE (1)

8.2.	Serious eye damage/eye irritation	8.2. An *in vivo* study for eye corrosion/irritation shall be considered only if the *in vitro* study(ies) under point 8.2.1 in Annex VII are not applicable, or the results obtained from these study(ies) are not adequate for classification and risk assessment. The study does not need to be conducted if: — the substance is classified as skin corrosion, or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is spontaneously flammable in air or in contact with water or moisture at room temperature.'

Use of *in vitro* methods under REACH (Annex XI)

ANNEX XI

GENERAL RULES FOR ADAPTATION OF THE STANDARD TESTING REGIME SET OUT IN ANNEXES VII TO X

1.4. ***In vitro* methods**

Results obtained from suitable *in vitro* methods may indicate the presence of a certain dangerous property or may be important in relation to a mechanistic understanding, which may be important for the assessment. In this context, 'suitable' means sufficiently well developed according to internationally agreed test development criteria (e.g. the European Centre for the Validation of Alternative Methods (ECVAM)) criteria for the entry of a test into the prevalidation process). Depending on the potential risk, immediate confirmation requiring testing beyond the information foreseen in Annexes VII or VIII or proposed confirmation requiring testing beyond the information foreseen in Annexes IX or X for the respective tonnage level may be necessary.

If the results obtained from the use of such *in vitro* methods do not indicate a certain dangerous property, the relevant test shall nevertheless be carried out at the appropriate tonnage level to confirm the negative result, unless testing is not required in accordance with Annexes VII to X or the other rules in this Annex.

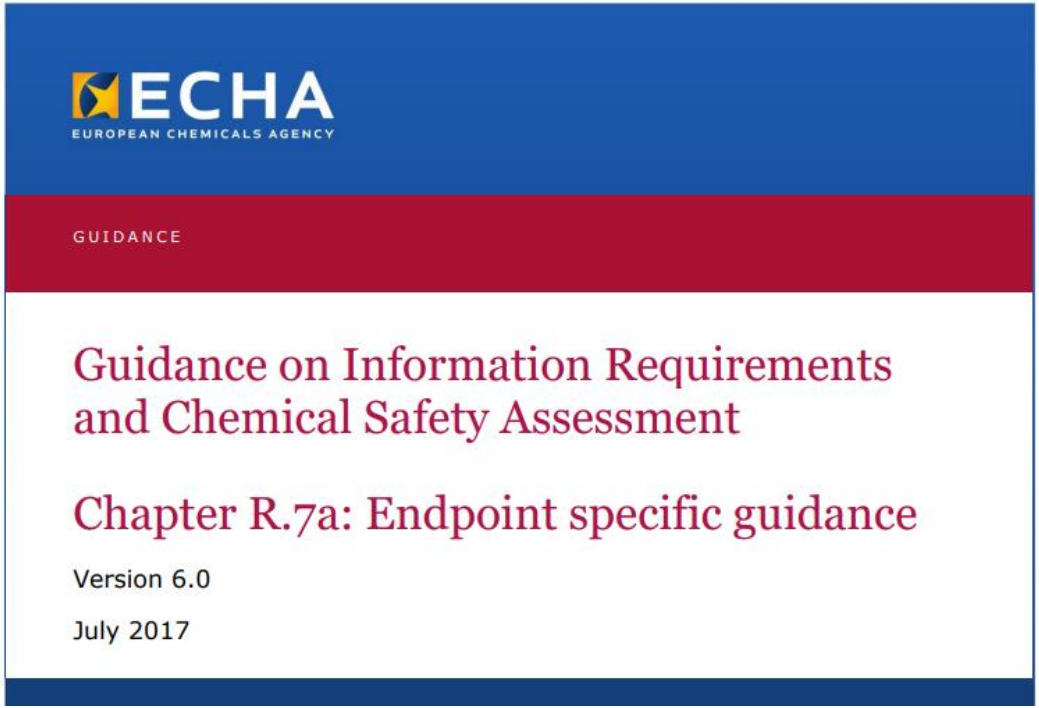
Such confirmation may be waived, if the following conditions are met:

(1) results are derived from an *in vitro* method whose scientific validity has been established by a validation study, according to internationally agreed validation principles;

(2) results are adequate for the purpose of classification and labelling and/or risk assessment; and

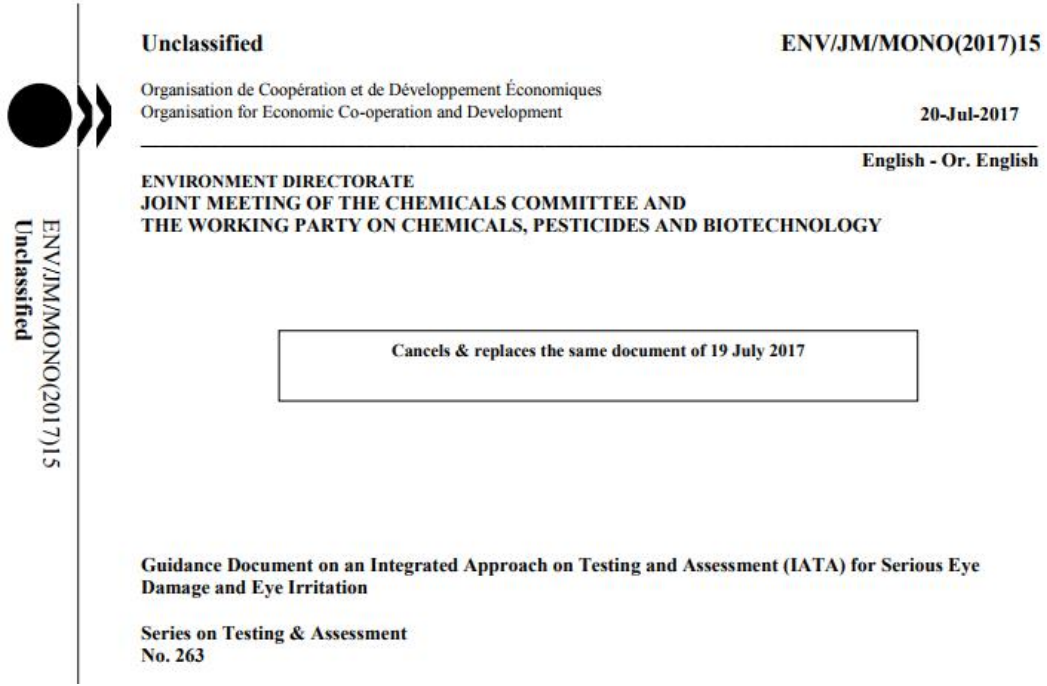
(3) adequate and reliable documentation of the applied method is provided.

ECHA Guidance



- Section R7.2 on irritation/corrosion
- Provides guidance on how to fulfil REACH information requirements using different types of information, including alternative methods
- Includes a general integrated approach to testing and assessment
- Updated in July 2017

OECD Integrated approach on testing and assessment



Unclassified ENV/JM/MONO(2017)15

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

20-Jul-2017

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 19 July 2017

Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation

Series on Testing & Assessment
No. 263

ENV/JM/MONO(2017)15
Unclassified

Available at:
http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2017)15&doclanguage=en

Alternatives to replace the Draize eye test

Organotypic Assays

- Bovine Corneal Opacity and Permeability Assay (BCOP)
- Isolated Chicken Eye (ICE)
- Isolated Rabbit Eye (IRE)
- Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM)

Cytotoxicity and Cell-Function Based Assays

- Fluorescein Leakage (FL)
- Cytosensor Microphysiometer (CM)
- Short Time Exposure (STE)

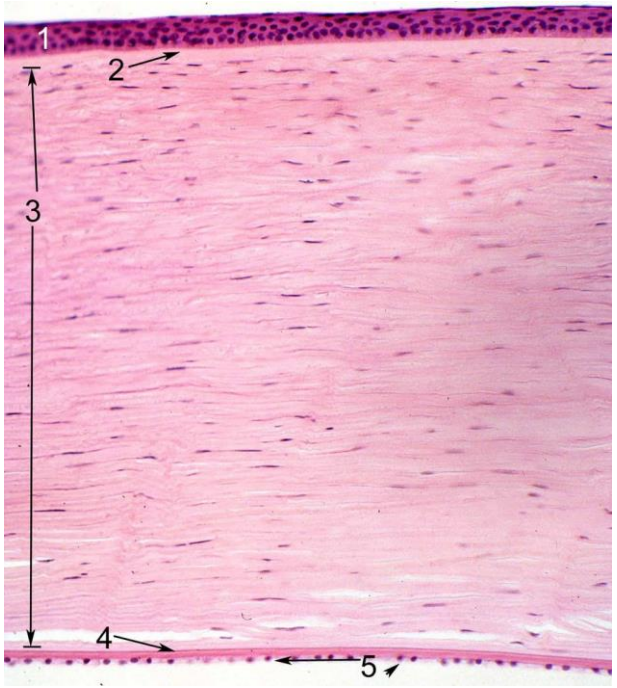
Reconstructed Human Tissue Models

- EpiOcular™ Eye Irritation Test (EIT)
- SkinEthic™ HCE Eye Irritation Test (HCE EIT)

In Chemico Assays

- Ocular Irritection®

Common modes of chemical action in ocular toxicity



Histologic section of human cornea. 1- epithelium, 2- Bowman's layer, 3 – Stroma; 4- Descemet's membrane, 5- endothelium

Image from eyepathology.blogspot.com

Cell Membrane Lysis

- Surface active agents solubilize membrane lipids
- Organic solvents extract lipids

Protein Coagulation/Denaturation

- Acids and certain solvents

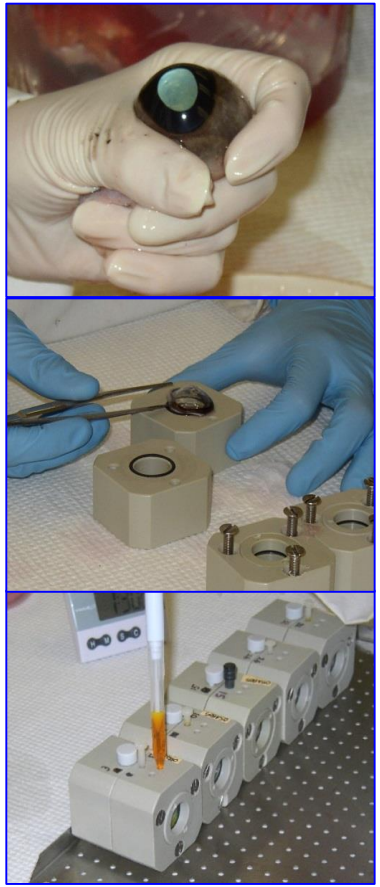
Saponification

- Alkali (often progressive)

Chemical Reactivity

- Reactive materials such as bleaches and peroxides

Bovine Corneal Opacity and Permeability (BCOP)



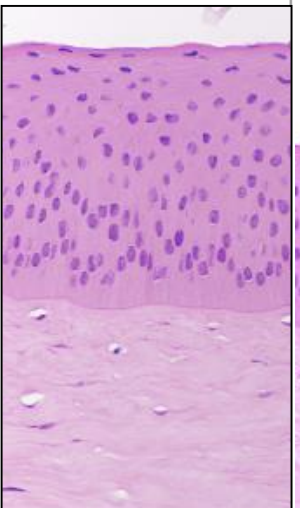
- **Test system:** corneas isolated from bovine eyes obtained from abattoir animals
- **Endpoints measured:** corneal opacity and permeability
- **Protocol:** liquids (neat) and surfactants (10%) exposed for 10 min plus 2 hours post-exposure incubation; solids (20%) exposed for 4 hours without post-exposure incubation
- **Status:** validated and accepted for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2 (OECD TG 437), US EPA cat. I / II / III
- **Applicability and limitations:** according to TG 437
  - ❖ No Cat.: high FPs in general
  - ❖ Cat. 1: high FPs for alcohols and ketones
  - ❖ Cat. 1: high FNs for solids, but **46% (6/13) FNs** for chemicals classified based on **persistence** without severity

Tutorial on the BCOP: https://www.youtube.com/watch?v=TiZbp5KDHI8

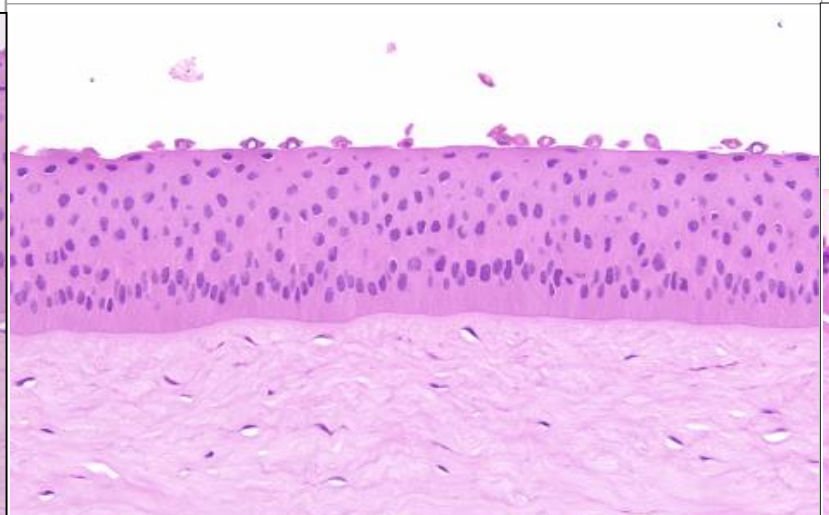
Histopathology on tissues

- Histopathology may be used to obtain more information on the degree of damage and depth of penetration

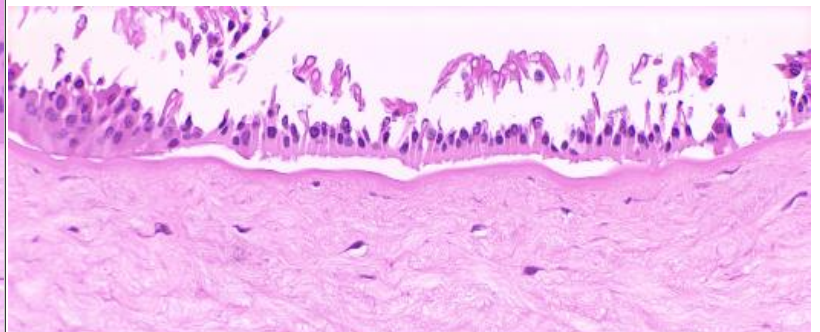
Control Cornea



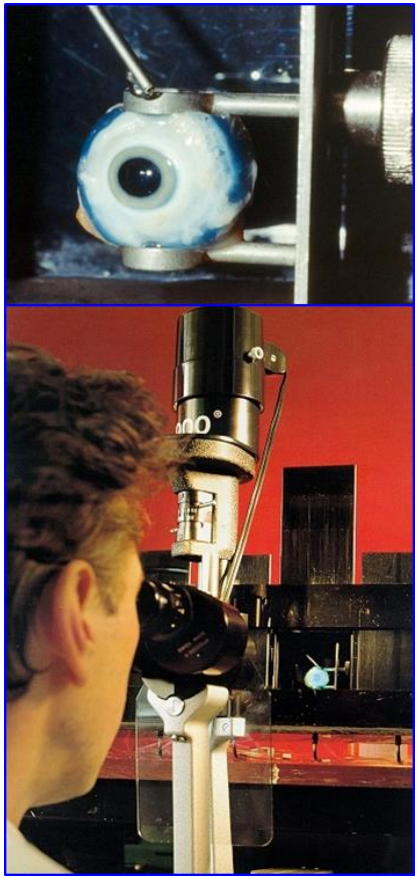
a) 1.5% SLS 10-minute exposure
Opacity = 1.7 OD_{490}= 0.302



b) 5% SLS 30-minute exposure
Opacity = 7.7 OD_{490}= 2.54



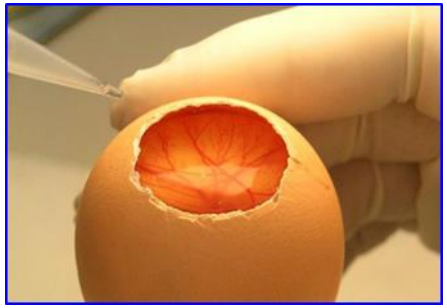
Isolated Chicken Eye (ICE)

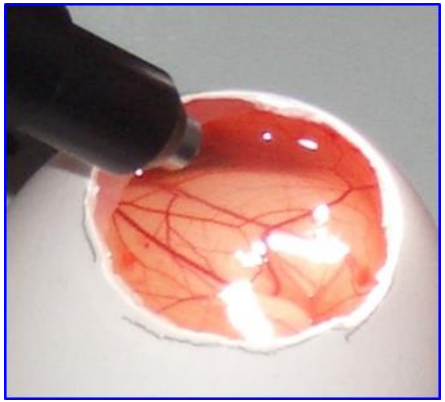


- **Test system:** chicken eyes isolated from abattoir animals
- **Endpoints measured:** corneal opacity, fluorescein retention, corneal swelling and morphological damage
- **Protocol:** test chemicals exposed neat for 10 sec and assessed during a 4 hour period
- **Status:** validated and regulatory accepted for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2 (OECD TG 438)
- **Applicability and limitations:** according to TG 438,
  - ❖ Cat. 1: high FPs for alcohols
  - ❖ Cat. 1: high FNs for solids, but **75% (9/12) FNs** for chemicals classified based on **persistence** without severity
  - ❖ Cat. 1: high FNs for surfactants; histopathology shown to improve predictions for non-extreme pH detergent and cleaning products (Cazelle et al. 2014)

    → 75% sensitivity, 73% specificity, 73% accuracy

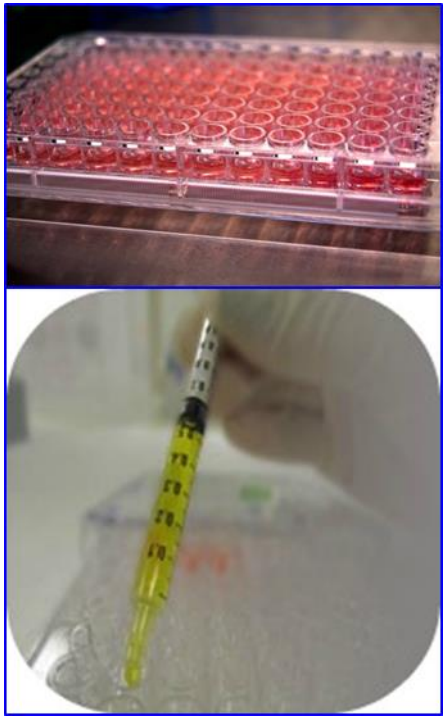
Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM)





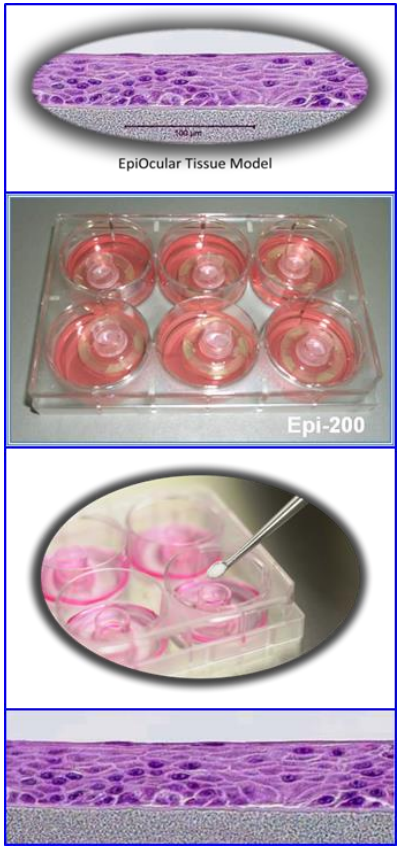
- **Test system**: chorioallantoic membrane of chicken eggs at the 10th day of embryonation
- **Endpoints measured**: coagulation (to id Cat. 1); coagulation, haemorrhage and "lysis" (to id No Cat.)
- **Protocols**:
  - Cat. 1: time to develop effects during 5 min exposure, e.g. mean time of coagulation (mtc), Spielmann et al. 1991
  - No Cat.: effects observed at different fixed time points (0.5, 2 and 5 min), Luepke 1985
- **Status**: validated but not recommended by ICCVAM; International workshop held in 2012 and currently undergoing additional validation
- **Applicability and limitations**:
  - Only method directly addressing conjunctival effects
  - Chemicals that affect the membrane or the read-out such as sticky materials, coloured chemicals, solids that cause physical abrasion
  - Alcohols (fixatives) may be wrongly predicted

Short Time Exposure (STE)



- **Test system:** confluent monolayer of SIRC cells
- **Endpoints measured:** cytotoxicity (MTT assay)
- **Protocol:** test chemicals exposed at 5% and 0.05% for 5 min
- **Status:** validated and recommended for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2; OECD TG 491
- **Applicability and limitations:**
  - ❖ No Cat.: high FNs for highly volatile chemicals
  - ❖ Cat. 1: high FNs in general
  - ❖ Not applicable to test chemicals that are not soluble or do not form stable suspension in solvent for ≥ 5 min

EpiOcular™ Eye Irritation Test (EIT)



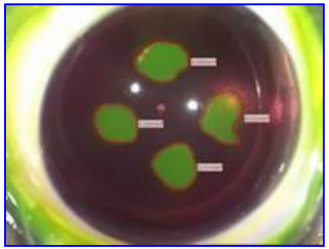
- **Test system:** non-keratinized multi-layered epithelium reconstructed from primary human epidermal keratinocytes
- **Endpoints measured:** cytotoxicity (MTT assay)
- **Protocol:** liquids (50 µL) exposed for 30 min followed by 2 h post-exposure incubation; solids (50 mg) exposed for 6 h followed by 18 h post-exposure incubation
- **Status:** validated and recommended for identifying UN GHS No Cat., but not Cat. 2 or Cat. 1; OECD TG 492
- **Applicability and limitations:**
  - ❖ Applicable to all types of chemicals
  - ❖ Intensely coloured chemicals addressed with HPLC/UPLC-spectrophotometry

SkinEthic™ Eye Irritation Test (HCE EIT)

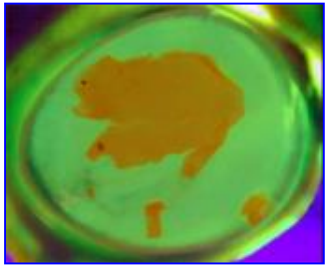
Images: www.episkin.com

- **Test system:** model composed of transformed human corneal keratinocytes; reconstructed tissue forms a stratified epithelium similar to the human cornea
- **Endpoints measured:** cytotoxicity (MTT assay)
- **Protocol:** liquid/viscous substances (30 µL) applied for 30 min; solids (30 mg) applied, then 4 h incubation
- **Status:** validated and recommended for identifying UN GHS No Cat., but not Cat. 2 or Cat. 1; OECD TG 492
- **Applicability and limitations:**
  - ❖ Applicable to a broad range of chemicals
  - ❖ MTT reducing/or coloured test substances viability corrected accordingly

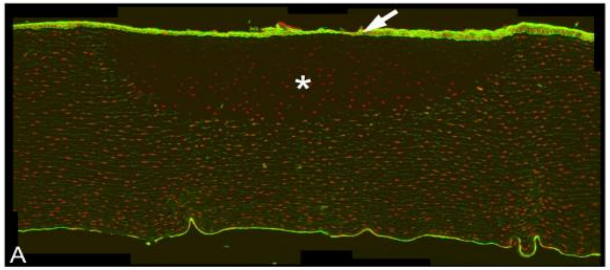
Methods under development for persistence



- **Ex-Vivo Eye Irritation Test (EVEIT)**
  - Developed by ACTO e.V. & IHT, Univ. Aachen, Germany
  - Uses excised rabbit corneas
  - Monitors full-thickness corneal recovery (epithelium and stroma) over 3 days using non-invasive OCT following 60 min exposure to solids and 30 sec to liquids



- **Porcine Cornea Ocular Reversibility Assay (PorCORA)**
  - Developed by MB Research Laboratories, USA
  - Uses excised porcine corneas
  - Monitors corneal epithelial recovery over 21 days by fluorescein stain retention following 5 min exposure



- **Initial Depth of Corneal Injury Assessment**
  - Developed by James Maurer and James Jester
  - Propose initial depth of injury is predictive of the degree and duration of injury
  - Corneal evaluation by histopathology and live/dead staining

Methods overview

BCOP
ICE
CM (surfactants)
STE
EIT
Ocular Irritection®

Testing strategy

BCOP
ICE
FL
IRE
HET-CAM
CM (aqueous soluble)
STE
EIT
Ocular Irritection®

Practical considerations

- Is the sample to be tested for regulatory classification and labelling?
  - If so, what is the most appropriate assay system(s) and what is the regulatory guidance
- Consider the following:
  - physicochemical properties of the sample: liquid/solid, viscosity, charge, pH
  - solubility: some assays are only compatible with water soluble samples
  - ingredient/formulation: assess expected eye damage
- Explore availability of selected method(s), ensure proper assay performance
- Prepare the appropriate protocol which adheres to OECD guidance for selected method
- Ensure proper training on the method (e.g. with method developer) before conducting routine testing
- Conduct the assay(s) under Good Laboratories Practices (GLPs) compliance
  - negative controls, positive controls, assay acceptance criteria
  - concurrently tested benchmarks or reference samples may be useful

Selection of chemicals according to the DRD principles

- Cefic LRI-AIMT6-VITO CON4EI project (lead: VITO)
  - Multicentre project: 80 eye reference chemicals, selected from the DRD, were tested with 8 alternative methods for Serious Eye Damage/Eye irritation with main purpose - development of Testing Strategies for UN GHS No Cat. vs. Cat. 1 and Cat. 2
  - Opportunity: broaden the knowledge of reliability, applicability domains, identify strengths & limitations
  - Chemical selection: majority rule (effect observed in ≥ 60% of the animals) was applied for all important Cat. 1 drivers of classification (CO persistence D21 and CO=4)
  - Focus for this example: OECD adopted test methods on serious eye damage/eye irritation
    - OECD TG 437 (BCOP OP-KIT) and BCOP LLBO (different device to measure opacity)
    - OECD TG 492 (EpiOcular™ EIT and SkinEthic™ HCE EIT)

Distribution of the chemicals

UN GHS – Driver	Liquid	Solid	Grand Total
Cat 1	17	21	38
CO mean ≥ 3	7	7	14
CO pers D21	4	8	12
CO=4	6	6	12
Cat 2	13	14	27
CO mean ≥ 1	8	5	13
Conj mean ≥ 2	5	9	14
No Cat (CO = 0)	8	7	15
Grand Total	**38**	**42**	**80**

Top-Down approach

First step: First test method is used to identify Cat 1

UN GHS	Cat 1 prediction	Cat 2 prediction	No cat prediction
Cat 1	True Cat 1 ≥ 70%	FN < 30%	
Cat 2 / No Cat	FP < 30% / < 10%	True Not Cat 1	

Second test (different mechanism) used to identify Cat. 1 (**Step 2**):

- Increase Cat 1 sensitivity
- Keep FP low

In case no decision can be made, go the second or third test to identify No Cat. (**Step 2 or 3**)

UN GHS	Classified	NC prediction
Cat 1 / Cat 2	True classified	FN < 10%
No Cat	FP < 40%	True No Cat

→ Not 'No Cat' → Cat 2

Suggested max values FN & FP based on:

- in depth analysis of historical Draize data (Adriaens et al., 2014; Barroso et al., 2017),
- criteria set by VMG (validation RhCE-based methods EURL ECVAM and Cosmetics Europe),
- REACH registrations 2008–2014 with repeat Draize studies (Luechtefeld et al., 2017)

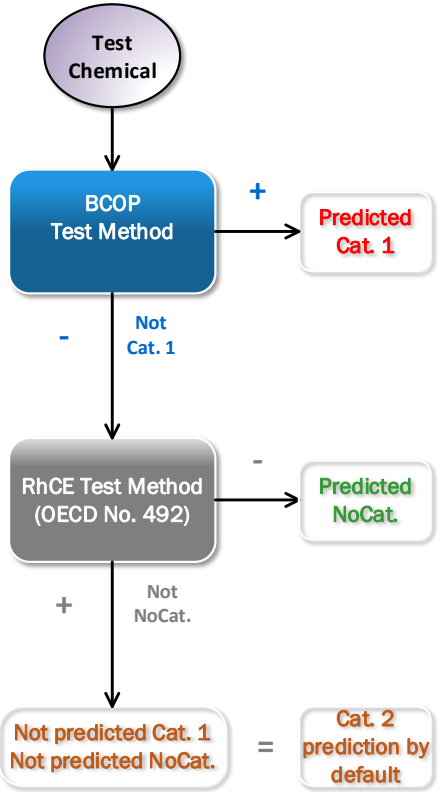
Identification Cat. 1

Test method	False Positive (Over-predicted Cat 2 & No Cat)			True Cat. 1	False Negative Cat 1 (Under-predicted Cat 1)		
	No Cat. (N=15)	Cat. 2 (N=27)	**Overall (N=42)**	**(N=38)**	**Not Cat. 1 (N=38)**	No Pred./ Cat. 2 (N=38)	No Cat. (N=38)
Required values for two-tiered approach [A]	< 10%	< 30%		**≥ 70%**			
BCOP OP-KIT IVIS > 55	0%	**24.1%**	**15.5%**	**61.8%**	**38.2%**	32.9%	5.3%
BCOP LLBO IVIS > 125	3.3%	**42.6%**	**28.6%**	**77.6%**	**22.3%**	19.7%	2.6%
BCOP LLBO Opacity > 145	0%	**27.8%**	**17.9%**	**71.1%**	**28.9%**	NA	NA

Identification No Cat.

Test method	True No Cat	False Positive (Over-predicted No Cat)			False Negative (Under-predicted Cat 1 & Cat 2)		
	N=15	**Overall (N=15)**	No Pred Cat 2 (N=15)	Cat 1 (N=15)	Overall (N=65)	Cat 2 (N=27)	Cat 1 (N=38)
Required values for two-tiered approach [A]	**≥ 60%**				< 10%		
SkinEthic™ HCE EIT	**100%**	0%	NA	NA	3.1%	7.4%	0%
EpiOcular EIT	**86.7%**	13.3%	NA	NA	3.1%	7.4%	0%
BCOP OP-KIT IVIS ≤ 3	**70%**	30%	30%	0%	10%	16.7%	5.3%
BCOP LLBO IVIS ≤ 20	**63.3%**	36.7%	33.3%	3.3%	5.4%	9.3%	2.6%

Example Two-step TOP-DOWN approach



Endpoints BCOP

Opacity + 15 x Permeability (OD) = IVIS

- OP-KIT: Cat. 1 prediction IVIS > 55
- LLBO: Cat. 1 prediction Opacity > 145

Endpoint RhCE

Cell Viability (%) cut-off values :

Test methods	No Category
EpiOcular™ EIT (for both protocols)	Mean viability > 60%
SkinEthic™ HCE EIT (for the liquids' protocol)	Mean viability > 60%
SkinEthic™ HCE EIT (for the solids' protocol)	Mean viability > 50%

Example Two-step TOP-DOWN approach

BCOP OP-KIT (IVIS > 55: identify Cat. 1)

UN GHS	Testing strategy		
	Cat. 1	Cat. 2	No Cat.
Cat. 1	**47 (62%)**	29 (38%)	0 (0%)
Cat. 2	13 (24%)	**37 (69%)**	4 (7%)
No Cat.	0 (0%)	0-4 (0-13%)	**26-30 (87-100%)**

RhCE (Viability: identify No Cat)

Accuracy = 69-71%

BCOP LLBO (Opacity > 145: identify Cat. 1)

UN GHS	Testing strategy		
	Cat. 1	Cat. 2	No Cat.
Cat. 1	**54 (71%)**	22 (29%)	0 (0%)
Cat. 2	15 (28%)	**35 (65%)**	4 (7%)
No Cat.	0 (0%)	0-4 (0-13%)	**26-30 (87-100%)**

RhCE (Viability: identify No Cat)

Accuracy = 72-74%

Conclusions CON4EI

- Testing strategy performs better than a stand-alone method
  - under-predictions often related to low water solubility
  - over-predictions more often Cat. 2 CO Severity
- BCOP LLBO higher sensitivity than BCOP OP-KIT for identifying Cat. 1 vs. Not Cat. 1 (Example of two-step Top (BCOP LLBO optimized cut-off) – Down (RhCE) approach: correct identification of Cat 1 = 71% (vs. 62% BCOP TG 437)
- RhCE (EpiOcular™ EIT and SkinEthic™ HCE EIT) recommended as a first step in a testing strategy to identify chemicals that do not require classification (validation studies: specificity 63% and 70%) and FNR below 10% (CON4EI: 87-100% specificity, all No Cat. chemicals from subgroup CO=0)
- Performance of Bottom-Up approach was similar for the different strategies

Conclusions

- *In vitro* methods are the standard information requirement for REACH (the *in vivo* test is a Annex VIII Column 2 adaptation)
- Annex XI describes general rules for adapting the standard testing regime set out in Annexes VII to X
- Consult the ECHA endpoint specific guidance and the OECD IATA
- Consider the applicability domain of the *in vitro* tests and the properties of your substance before initiating new tests to select the most appropriate tests
- It is estimated that for at least 70% of the substances one single *in vitro* test method will be sufficient to derive a final conclusion on serious eye damage/eye irritation, if method is carefully chosen
- More information on eye irritation/corrosion is available at: https://www.piscltd.org.uk/eye-irritation-2/

Webinars in this series

Perspectives on the Development, Evaluation, and Application of *in Silico* Approaches for Predicting Toxicity Recorded	Dr. Grace Patlewicz, US EPA Prof. Mark Cronin, Liverpool John Moores University
3R Approach to Acute Oral Toxicity Recorded	Dr. Kimmo Louekari, ECHA
Skin Irritation and Corrosion 25 January 2018, 4–5 pm GMT	Dr. Gertrude-Emilia Costin, Institute for In Vitro Sciences Dr. Costanza Rovida, TEAM Mastery and CAAT-Europe
Skin Sensitisation 1 February 2018, 4–5 pm GMT	Dr. Susanne Kolle, BASF SE Dr. Silvia Casati, EURL ECVAM
Serious Eye Damage and Eye irritation 15 February 2018, 4–5 pm GMT	Dr. Kim Norman, Burt's Bees Dr. Els Adriaens, Adriaens Consulting