ChemicalWatch
GLOBAL RISK & REGULATION NEWS

Serious Eye Damage and Eye Irritation Webinar

4 December 2014, 4:00pm GMT

Today's webinar

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This webinar will:

- Discuss the drivers of in vivo classification for serious eye damage and eye irritation in order to define what is required to achieve full replacement of the regulatory animal test;
- Look at the available in vitro methods and how they can be used alone or in combination in testing strategies such as the top-down or bottom-up approaches.

Speakers

- **Dr Kimberly Norman**, Institute for In Vitro Sciences

- **Dr Joao Barroso**, EURL ECVAM

- Chair: **Philip Lightowlers**, Chemical Watch

- Chair: **Dr Gilly Stoddart**, PETA International Science Consortium, Ltd

Questions

- Please submit questions during the webinar using your chat box

- Any unanswered questions can be raised on our Forum following the webinar: http://forum.chemicalwatch.com/

Upcoming Webinars

Webinar 4: Skin Sensitisation

January 28, 2014

11am ET, 4pm GMT

Silvia Casati, EURL ECVAM

Susanne Kolle, BASF

Webinar 5: February 2015: Mammalian acute toxicity (3T3 neutral red assay)

Webinar 6: March 2015: Ecotoxicity (fish embryo test)

Please contact the PETA International Science Consortium, Ltd., for assistance in avoiding animal testing

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Serious Eye Damage and Eye Irritation Webinar

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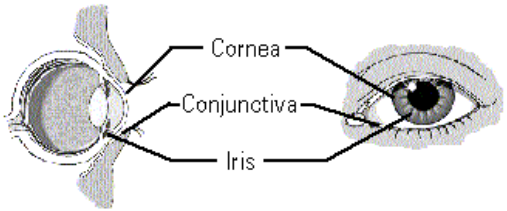
Outline

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

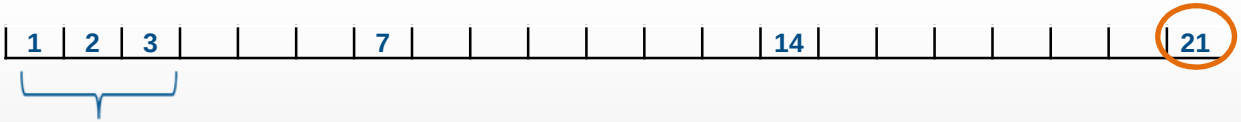
Draize rabbit eye test (OECD TG 405)

- 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

Tissue observation for up to 21 days



→ persistence Cat 1

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

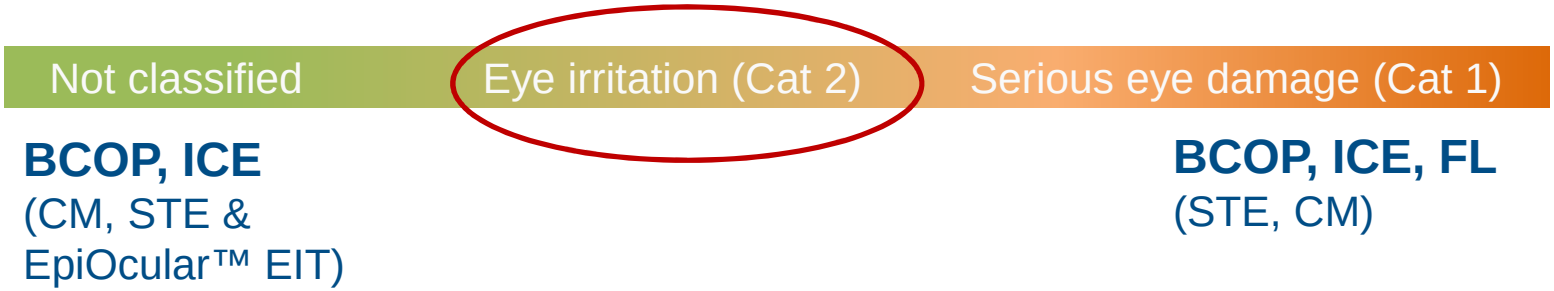
Draize rabbit eye test (OECD TG 405)

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

At present only partial replacement with *in vitro* methods has been achieved.



To better understand the reason for this, in depth analyses of historical *in vivo* rabbit data were performed.

- Which **endpoints** are most important in **driving** UN GHS/EU CLP classification for serious eye damage/eye irritation
- Evaluation of the Draize **within-test variability** → propose acceptable target values for false negative and false positive rates for alternative methods

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

Received: 2 July 2013 / Accepted: 29 October 2013 / Published online: 28 December 2013

Available at: http://link.springer.com/article/10.1007%2Fs00204-013-1156-8

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Overview of historical *in vivo* data sources

- **Reference Chemicals Databases (RCD)**
  - Eye Irritation Reference Chemicals Data Bank (ECETOC)
  - Database from ZEBET (Spielmann *et al.*, 1996)
  - Database from Laboratoire National de la Santé (LNS) (Gautheron *et al.*, 1992)

  Composition: limited number of chemicals that were put together in databases mainly to support validation studies

- **European New Chemicals Database (NCD)**

  Composition: contains all chemicals registered by multiple industry sectors since 1981

Data source	Number of valid studies	UN GHS/EU CLP (proportion of valid studies)		
		NC	Cat 2	Cat 1
RCD	274	**60.2**	17.2	22.6
NCD	1860	**82.6**	10.4	6.9

Conclusions from analysis of *in vivo* drivers of irritation

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

Ø **No need to address iritis *in vitro***

§ Cat 2 chemicals:

75%-54% classified based on corneal opacity (11-20% CO without CR/CC)

81%-75% classified based on conjunctiva redness (23-41% CR without CO)

conjunctiva chemosis rarely drives classification on its own (~2%)

Ø ***In vitro* methods must be able to identify conjunctiva redness**

§ Cat 1 chemicals:

50-70% classified based on persistence without severity (mostly CO: >80%)

36-28% classified based on severity of effects (days 1 to 3) (mostly CO: >85%)

Ø ***In vitro* methods to address persistence are required**

Within-test variability (No Cat. chemicals)

Source	Total	CO = 0	0 < CO < 1
RCD	**N=165**	**85.5**	**14.5**
NCD	**N=1537**	**95.1**	**4.9**

NCD: 1537 studies available with 3 animals: 1537 x 3 = 4611 animals

Sample 10.000 times 3 animals from the pool of 4611 animals → determine class

Resampling probabilities of
10.000 theoretical chemicals

Source	N	Predicted UN GHS class		
		No Cat	Cat 2	Cat 1
RCD	606	**99.9**	0.1	0.0
NCD	4611	**99.9**	0.1	0.0

Conclusions from analysis of *in vivo* within-test variability

§ Effect of Draize within-test variability on classification:

- 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

Probabilities may certainly increase if between-laboratory variability would be considered

Resampling suggests a high over-predictive power of the Draize eye test

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

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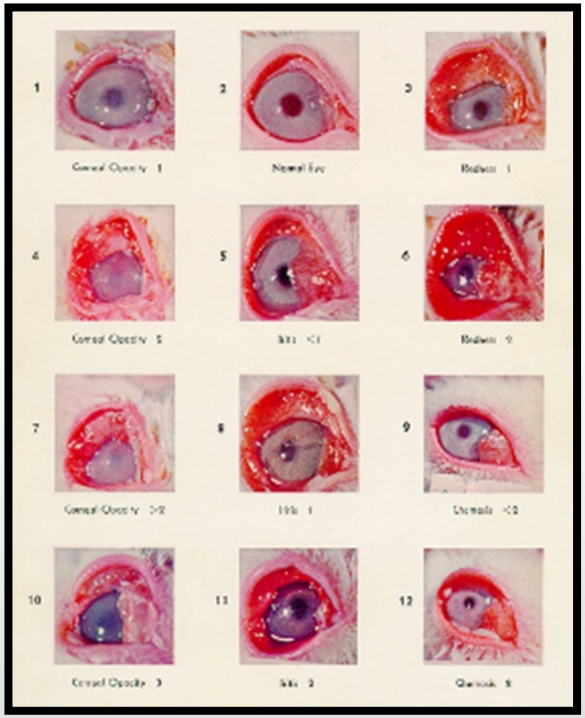
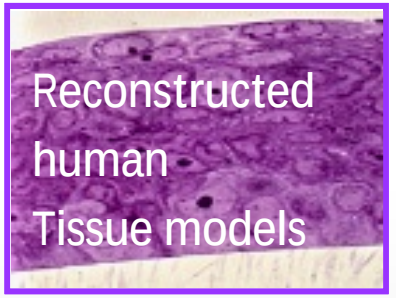
Replace the regulatory *in vivo* Draize eye test

No single *in vitro* method will be able to replace the animal test

Draize rabbit test



In vitro test methods



In vitro protein & mucosal protein assays

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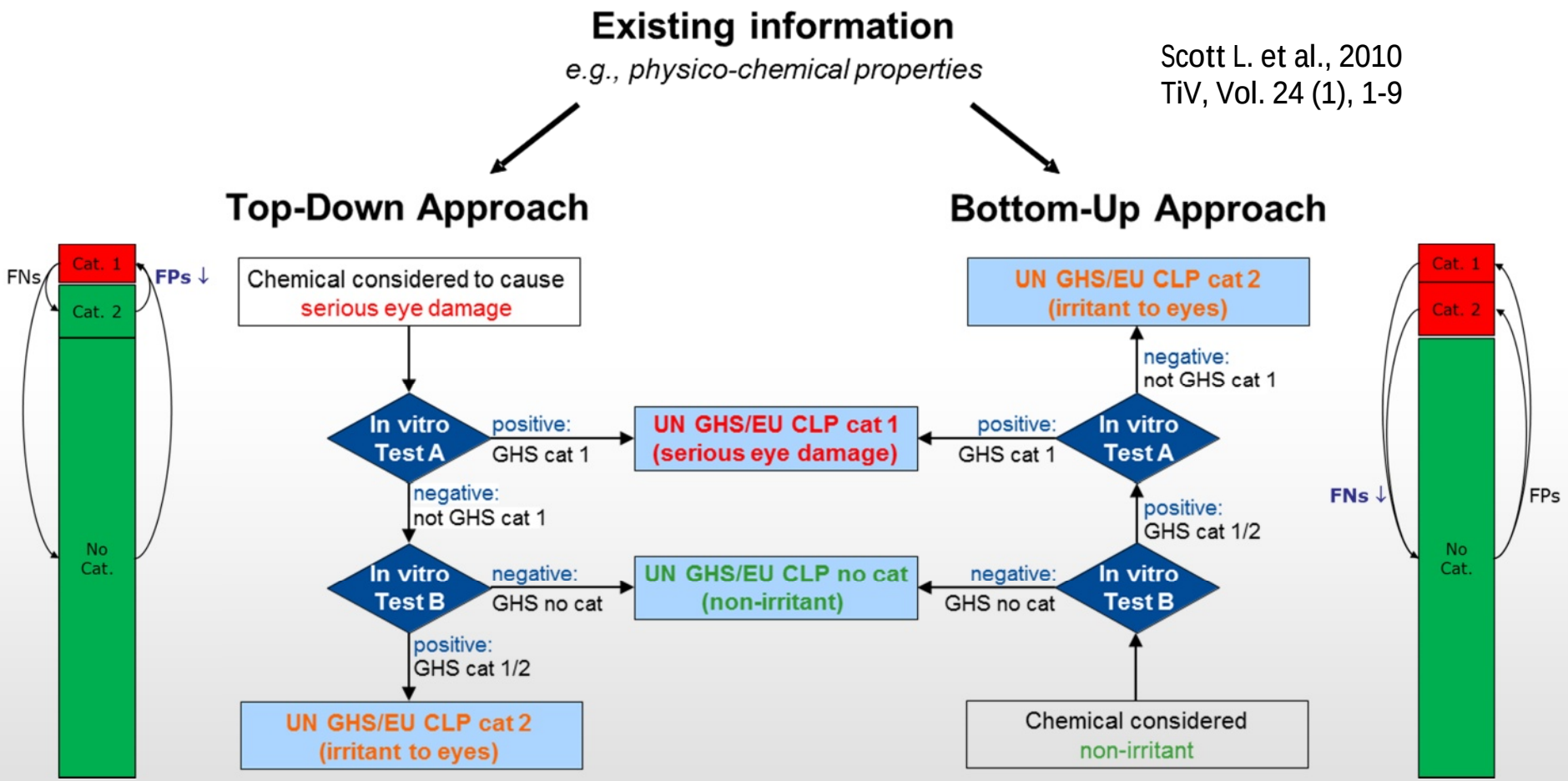
Conceptual framework for a testing strategy

(ECVAM Expert Meeting, Feb 2005)



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)

In Chemico Assays

- Ocular Irritection®

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QSAR modelling

Software tools

- Toxtree
- OECD QSAR Toolbox
- Derek Nexus
- TOPKAT
- Molcode QSARModel
- Multi-CASE

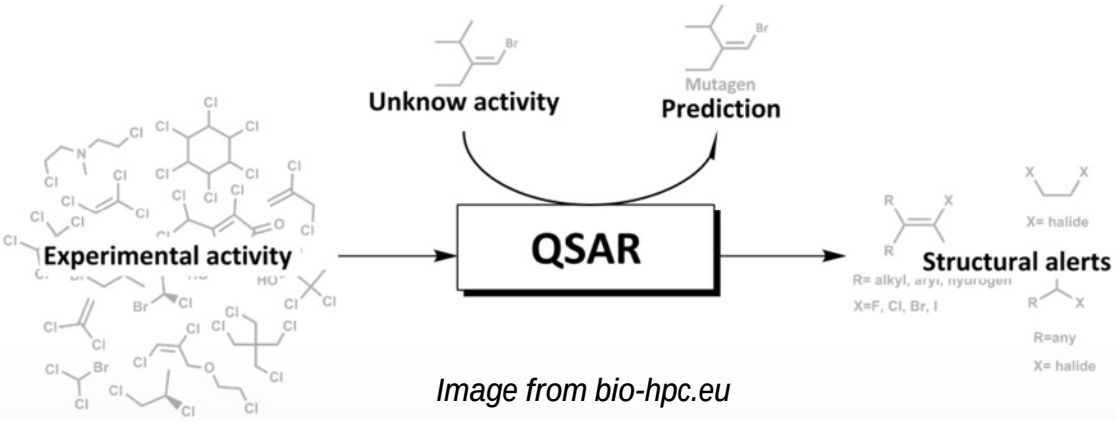
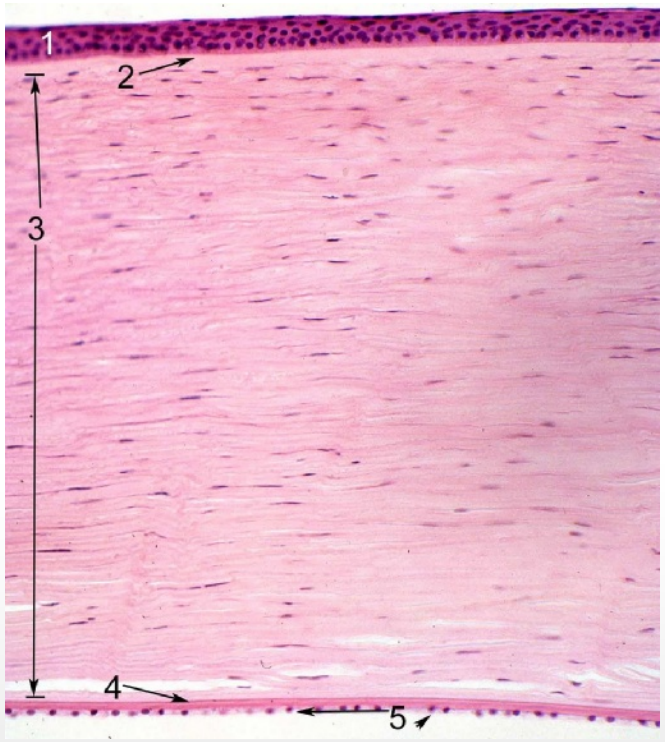


Image from bio-hpc.eu

Applicability

- These tools may be used in a WoE approach or tiered testing strategy.
- Predictions should be evaluated using information on the model characteristics
- For classification and labelling, the BfR rulebase provides information that is closest to the regulatory goal

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

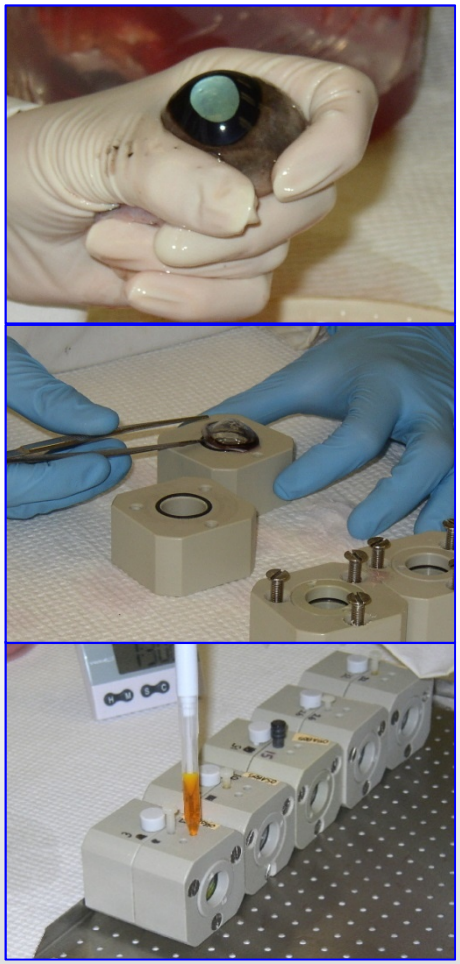
Saponification

- 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 regulatory accepted for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2 (OECD TG 437), US EPA cat. I / II
- **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:
http://ec.europa.eu/enterprise/epaa/international-activities-3rs/index_en.htm

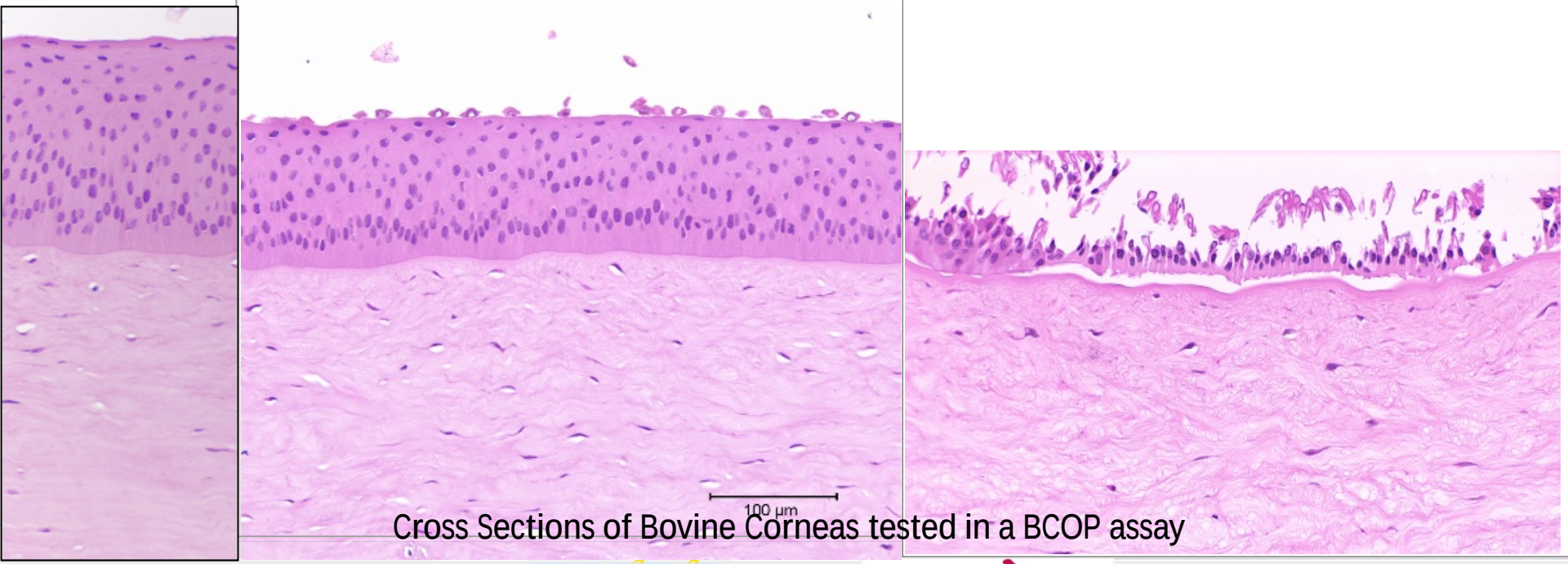
Histopathology on tissues

- 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



Cross Sections of Bovine Corneas tested in a BCOP assay

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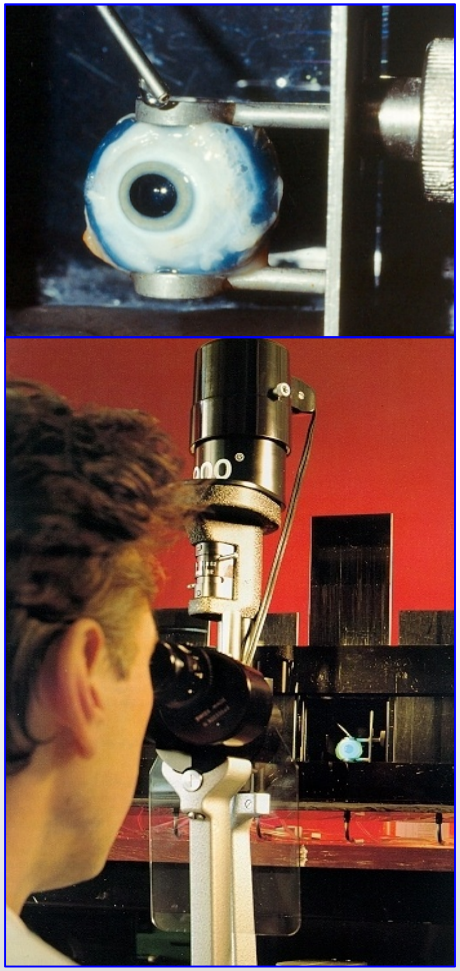
Isolated Chicken Eye (ICE)



- **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)
    - à 75% sensitivity, 73% specificity, 73% accuracy

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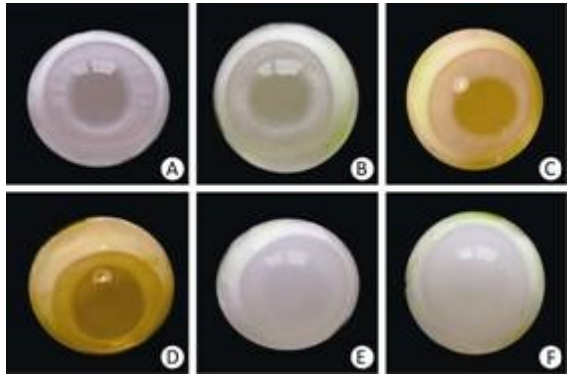
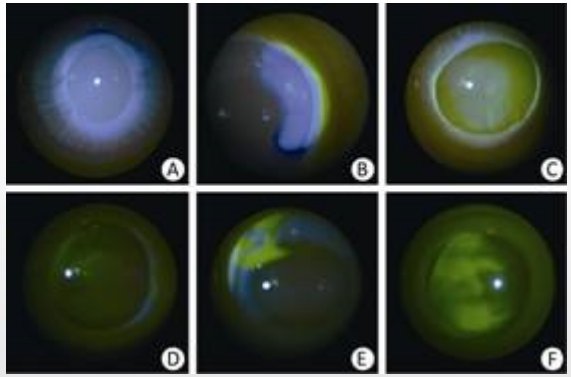
Isolated Rabbit Eye (IRE)



Corneal Opacity Scoring



Fluorescein Penetration into Cornea

Images from Xiang et al. (2010)

- **Test system:** rabbit eyes, may be isolated from abattoir animals, animals used for skin testing
- **Endpoints measured:** corneal opacity, corneal swelling, fluorescein penetration, assessment of epithelial integrity
- **Protocol:** Liquids- 0.1 mL applied onto center of cornea for 10 sec; solids- 0.1 applied over the cornea for 10 sec; rinsed and monitored at various times over a 4 hour observation period
- **Status:** Evaluated in several international validation studies; may be used for identifying UN GHS Cat. 1
- **Applicability and limitations:**
  - Broad range of solid and liquid test substances may be evaluated.
  - Cannot assess reversibility of corneal lesions.

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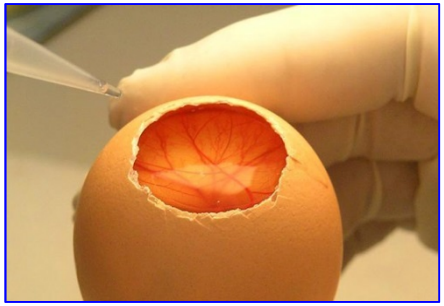
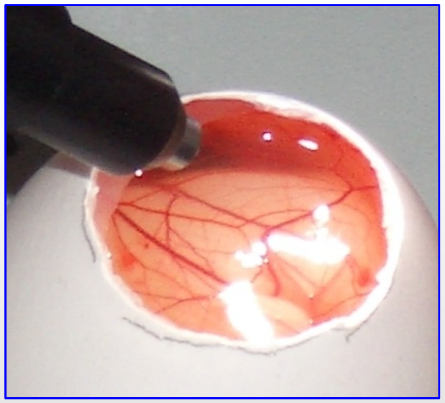
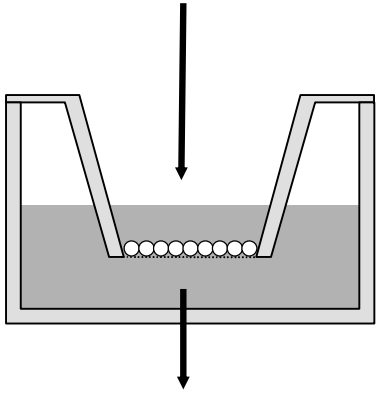
Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM)

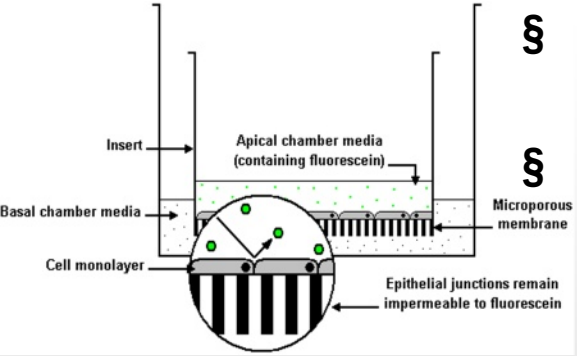




- **Test system**: chorioallantoic membrane of chicken eggs at the 10th day of embryonation
- **Endpoints measured**: coagulation (to id Cat. 1); coagulation, haemorrhage and "lysis" (to id No Cat.)
- **Protocols**:
  - Cat. 1: time to develop effects during 5 min exposure, e.g. mean time of coagulation (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

Fluorescein Leakage (FL)





- **Test system:** confluent monolayer of MDCK CB997 tubular epithelial cells
- **Endpoints measured:** trans-epithelial permeability to fluorescein
- **Protocol:** 1 min exposure to various concentrations followed by 30 min incubation with fluorescein
- **Status:** validated and regulatory accepted for identifying UN GHS Cat. 1, but not Cat. 2 nor No Cat. (OECD TG 460)
- **Applicability and limitations:** according to TG 460,
  - Only applicable to test chemicals that are soluble or that form a stable suspension at ≥ 250 mg/mL (or at FL_{20} ≤ [X] < 100 mg/mL)
  - Not applicable to strong acids and bases, cell fixatives and highly volatile chem.
  - Coloured and viscous chemicals may be wrongly predicted
  - High FNs in general

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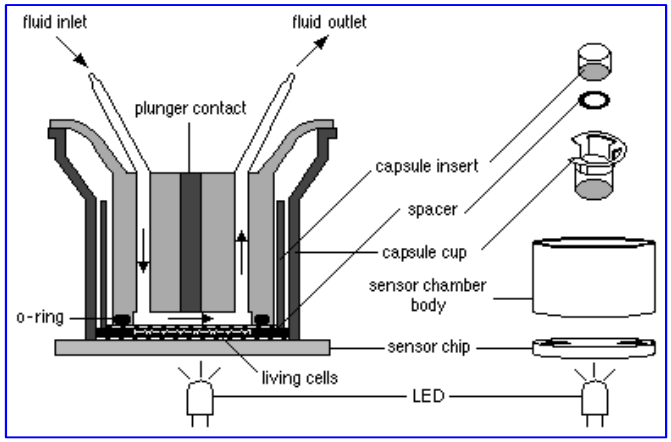
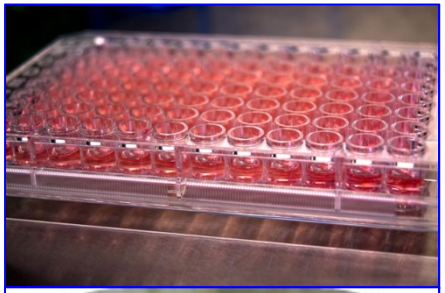
Cytosensor Microphysiometer (CM)

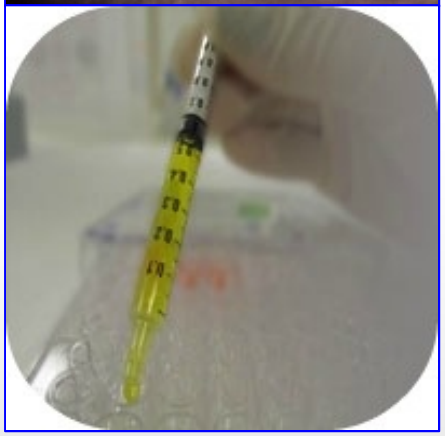


- **Test system:** sub-confluent monolayer of mouse L929 fibroblasts
- **Endpoints measured:** cellular metabolic rate (acidification)
- **Protocol:** cells exposed progressively to increasing concentrations of the test chemical (13.5 min exposures, followed by rinseout and 25 sec metabolism measurement)
- **Status:** validated and recommended for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2; Draft OECD TG under discussion (US EPA for categories III and IV)

- **Applicability and limitations**:
  - Cat. 1: applicable to test chemicals that are soluble or that form a stable suspension at > 2 mg/mL (or at $MRD_{50} \leq [X] < 2$ mg/mL) for ≥ 20 min
  - No Cat.: applicable to surfactants that are soluble or that form a stable suspension at > 10 mg/mL for ≥ 20 min
  - No Cat.: high FPs in general

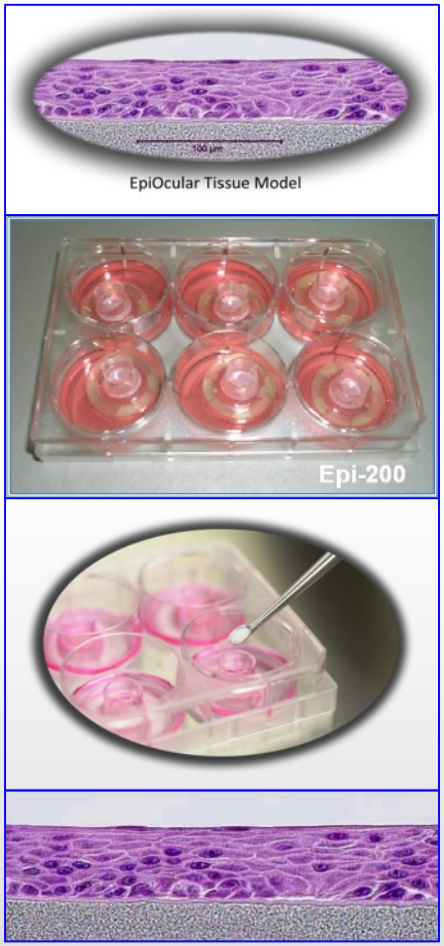
Short Time Exposure (STE)





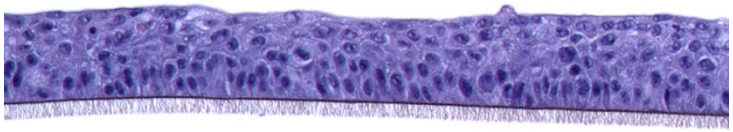
- **Test system:** confluent monolayer of SIRC cells
- **Endpoints measured:** cytotoxicity (MTT assay)
- **Protocol:** test chemicals exposed at 5% and 0.05% for 5 min
- **Status:** validated and recommended for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2; Draft OECD TG under discussion
- **Applicability and limitations:**
  - No Cat.: high FNs for highly volatile chemicals with vapour pressure > 6 kPa and non-surfactant solids
    à excluded from applicability domain
  - 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 (neat) exposed for 30 min followed by 2 h post-exposure incubation; solids (neat) exposed for 6 h followed by 18 h post-exposure incubation
  - *solids protocol optimised during validation study: exposure increased from 90 min (77% sensitivity) to 6 h (94% sensitivity)*
- **Status:** validated and recommended for identifying UN GHS No Cat., but not Cat. 2 nor Cat. 1; Draft OECD TG under discussion
- **Applicability and limitations:**
  - Applicable to all types of chemicals
  - Intensely coloured chemicals addressed with HPLC/UPLC-spectrophotometry

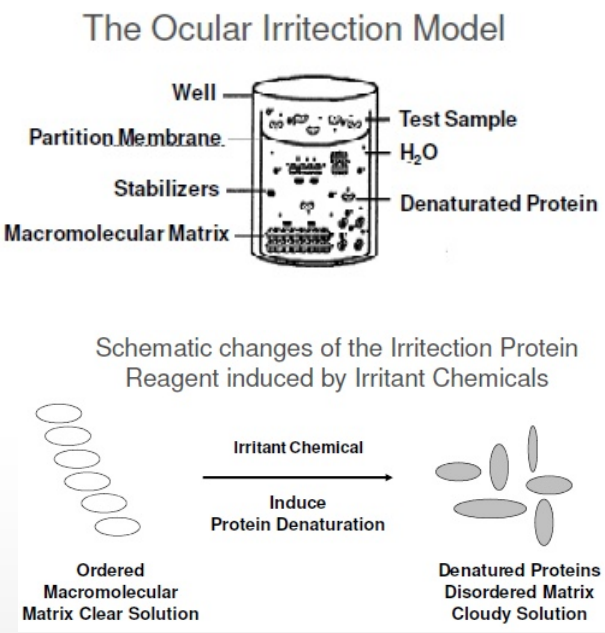
SkinEthic™ Human Corneal Epithelium (HCE)



- **Test system:** multi-layered epithelium prepared from immortalised human corneal epithelial cells

- **Endpoints measured:** cytotoxicity (MTT assay)
- **Protocols:** test chemicals exposed neat for:
  - Short-time Exposure (SE): 10 min without post-exposure incubation
  - Long-time Exposure (LE): 60 min followed by 16 h post-exposure incubation
- **Status:** not considered valid due to poor sensitivity, but high reproducibility (> 92%).
  - SE: 43% sensitivity, 57% FNs; 89% specificity, 11% FPs; 66% accuracy
  - LE: 72% sensitivity, 28% FNs; 66% specificity, 34% FPs; 69% accuracy
  - Undergoing optimisation and external validation by the method developer

Ocular Irritection®



- **Test system:** macromolecular matrix composed of lipids, (glyco-)proteins, carbohydrates and low MW components that mimics the highly ordered structure of the cornea
- **Endpoints measured:** turbidity at 405 nm ("opacity")
- **Protocol:** 24 h exposure to 5 different amounts of chemical; different protocols for surfactants & non-surfactants
- **Status:** has undergone external validation; currently under evaluation by EURL ECVAM for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2

- **Applicability and limitations (still under evaluation):**
  - Fast, simple, inexpensive and readily available (2-year shelf-life)
  - Not applicable to chemicals with pH < 4 or pH > 9, oils, water-insoluble organic chemicals, non-ionic surfactants and intensely coloured chemicals
  - No Cat.: mispredictions obtained with chemicals containing acrylate, carboxamide or cycloalkene organic functional groups

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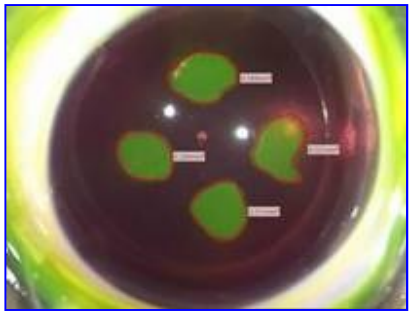
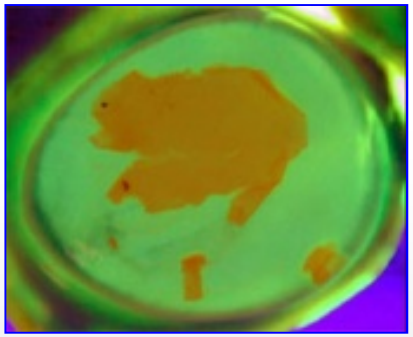
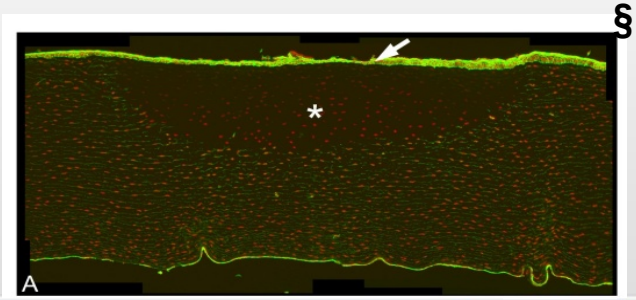
Methods under development for persistence





- **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

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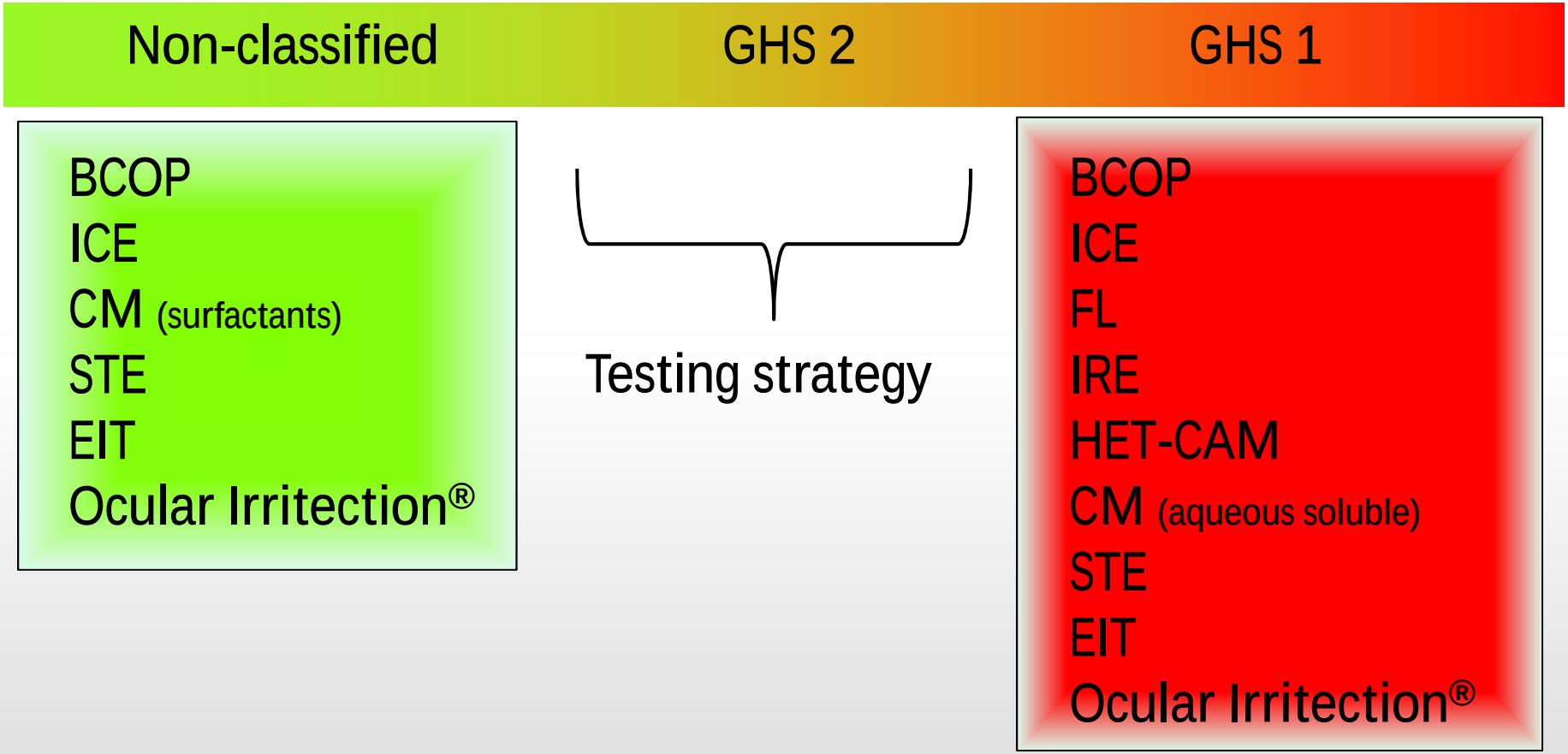
Methods overview



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

Use of *in vitro* methods under REACH

ANNEX VII

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

8.2.	Eye irritation The assessment of this endpoint shall comprise the following consecutive steps: (1) an assessment of the available human and animal data, (2) an assessment of the acid or alkaline reserve, (3) *in vitro* study for eye irritation.	8.2.	Step 3 does not need to be conducted if: — the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes, or — the substance is flammable in air at room temperature;

ANNEX VIII

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

8.2.	Eye irritation		
8.2.1.	*In vivo* eye irritation	8.2.1.	The study does not need to be conducted if: — the substance is classified as irritating to eyes with risk of serious damage to eyes, or — the substance is classified as corrosive to the skin and provided that the registrant classified the substance as eye irritant, or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is flammable in air at room temperature.

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Use of *in vitro* methods under REACH

ANNEX VII

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

8.2.	Eye irritation The assessment of this endpoint shall comprise the following consecutive steps: (1) an assessment of the available human and animal data, (2) an assessment of the acid or alkaline reserve, (3) *in vitro* study for eye irritation.	8.2.	Step 3 does not need to be conducted if: — the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes, or — the substance is flammable in air at normal temperature;

ANNEX VIII

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

8.2.	Eye irritation		
8.2.1.	*In vivo* eye irritation	8.2.1.	The study does not need to be conducted if: — the substance is classified as irritating to eyes with risk of serious damage to eyes, or — the substance is classified as corrosive to the skin and provided that the registrant classified the substance as eye irritant, or — the substance is a strong acid (pH ≤ 2,0) or base (pH ≥ 11,5), or — the substance is flammable in air at room temperature.

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Use of *in vitro* methods under REACH

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.

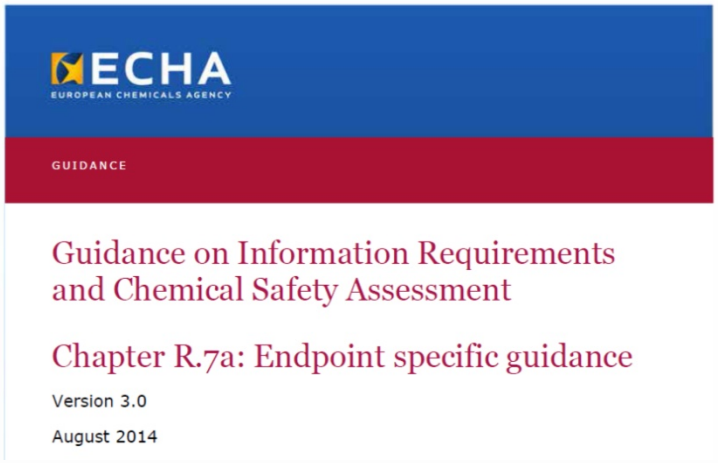
Such confirmation may be waived, if the following conditions are met:

(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.

REACH Guidance on IR&CSA



Guidance on Information Requirements and Chemical Safety Assessment, Endpoint specific guidance (Chapter R.7a), **Section R.7.2 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
- Update ongoing: http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

Project proposal for the development of a Guidance Document on an IATA for serious eye damage/eye irritation submitted to OECD by US and EC

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Other useful documents on the use of alternatives


ECHA
EUROPEAN CHEMICALS AGENCY

Eye irritation/corrosion

TITLE OF THE TEST GUIDELINES (YEAR OF APPROVAL):

Bovine Corneal Opacity test Method (BCOP), **EU B.47, OECD 437** (adopted in 2009, revised in 2013 by OECD)

Isolated Chicken Eye Test (ICE), **EU B.48, OECD 438** (adopted in 2009 and revised in 2013 by OECD)

Fluorescein leakage method, **OECD 460** (adopted in 2012)

Cytosensor Microphysiometer (Draft OECD TG under discussion).

Acute Eye Irritation/Corrosion, **EU B.5, OECD 405** (adopted in 1981 and revised in 1987, 2002, and 2012 by OECD)

Note: the latest version of the test guideline should always be used independent of whether it is published by EU or OECD.

Accepted *in vitro* methods for serious eye damage/eye irritation and how to use them under REACH:

http://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_eye_irritation_en.pdf


European Commission

JRC SCIENCE AND POLICY REPORTS

Alternative methods for regulatory toxicology – a state-of-the-art review

State-of-the-art review on alternative methods produced by the JRC for ECHA:

http://newsletter.echa.europa.eu/home/-/newsletter/entry/5_14_alternative-methods-to-avoid-testing-on-animals-an-important-new-review

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Predictive capacity for the identification of No Cat.

Test Method	Accuracy	Sensitivity	False Negatives	Specificity	False Positives
BCOP	69%	100%	0%	31%	69%
ICE	82% (83%)	99% (100%)	1% (0%)	67% (67%)	33% (33%)
EpiOcular™ EIT	80%	96%	4%	63%	37%
STE	85% (90%)	88% (98%)	12% (2%)	80% (81%)	20% (19%)
CM	68%	100%	0%	47%	68%
Ocular Irritection®	76% (81%)	91% (96%)	9% (4%)	60% (64%)	40% (36%)
HET-CAM	69%	100%	0%	36%	64%

Require further testing

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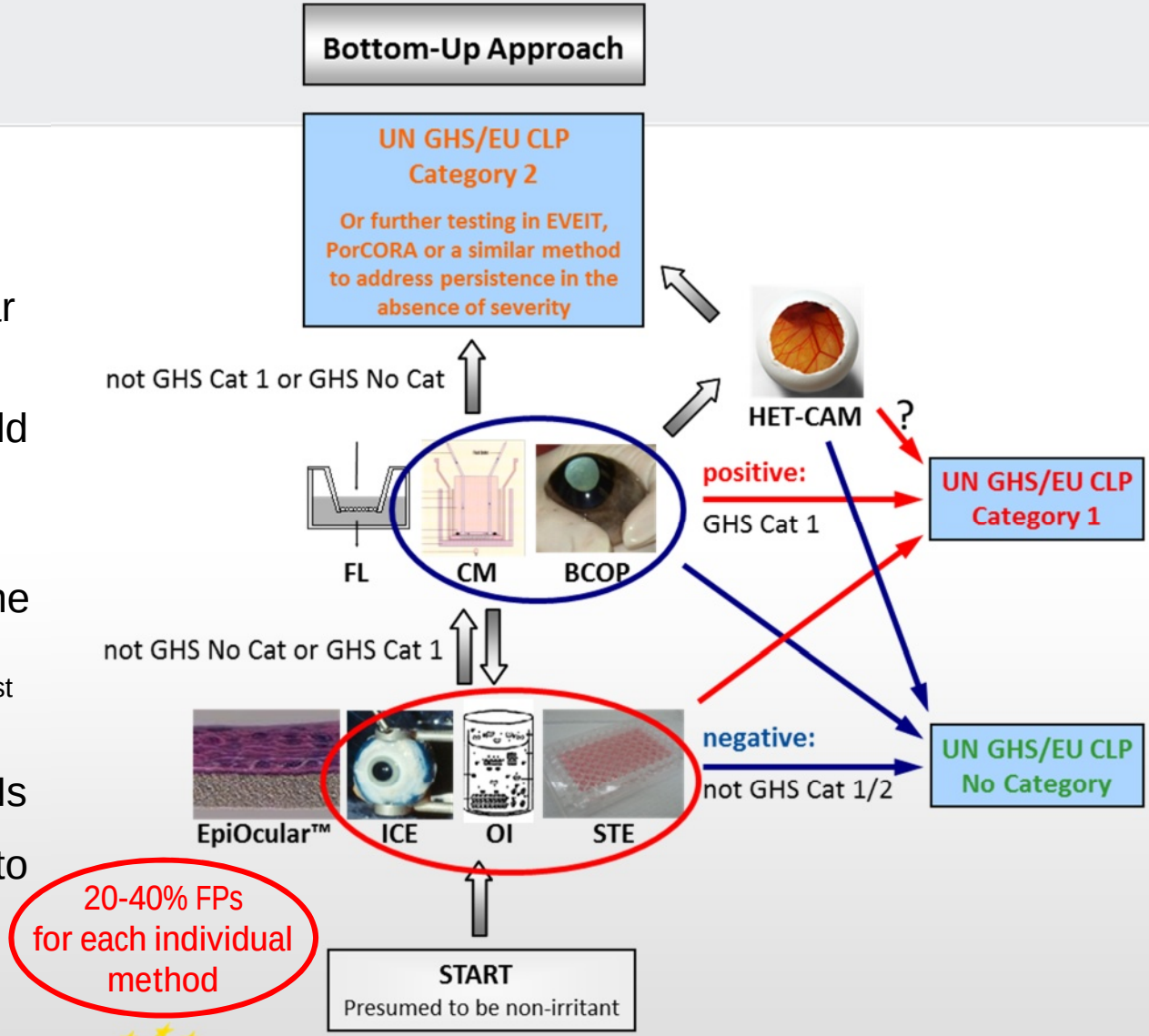
- Start bottom-up approach with one of the methods showing lower FPs, i.e. EpiOcular™ EIT, ICE, Ocular Irritection® or STE
- 60-80% of the No Cat. should be identified with a single method and with < 5% FNs
- In some cases more than one method will be needed (in case of a +ve result in the 1st tier) to decrease FPs, but with almost no impact on FNs
- Most methods can be used to identify both Cat. 1 and No Cat., thus increasing efficiency of the strategy



Predictive capacity for the identification of Cat. 1

Test Method	Accuracy	Sensitivity	False Negatives	Specificity	False Positives
BCOP	79% (85%)	86% (92%)	14% (8%)	75% (80%)	25% (20%)
ICE	86% (94%)	52% (71%)	48% (29%)	94% (96%)	6% (4%)
FL	77%	44%	56%	93%	7%
STE	85%	53%	47%	99%	1%
CM	90%	79%	21%	98%	2%
Ocular Irritection®	75% (76%)	53% (56%)	47% (44%)	81% (82%)	19% (18%)

Require further testing, but mostly persistence without severity!

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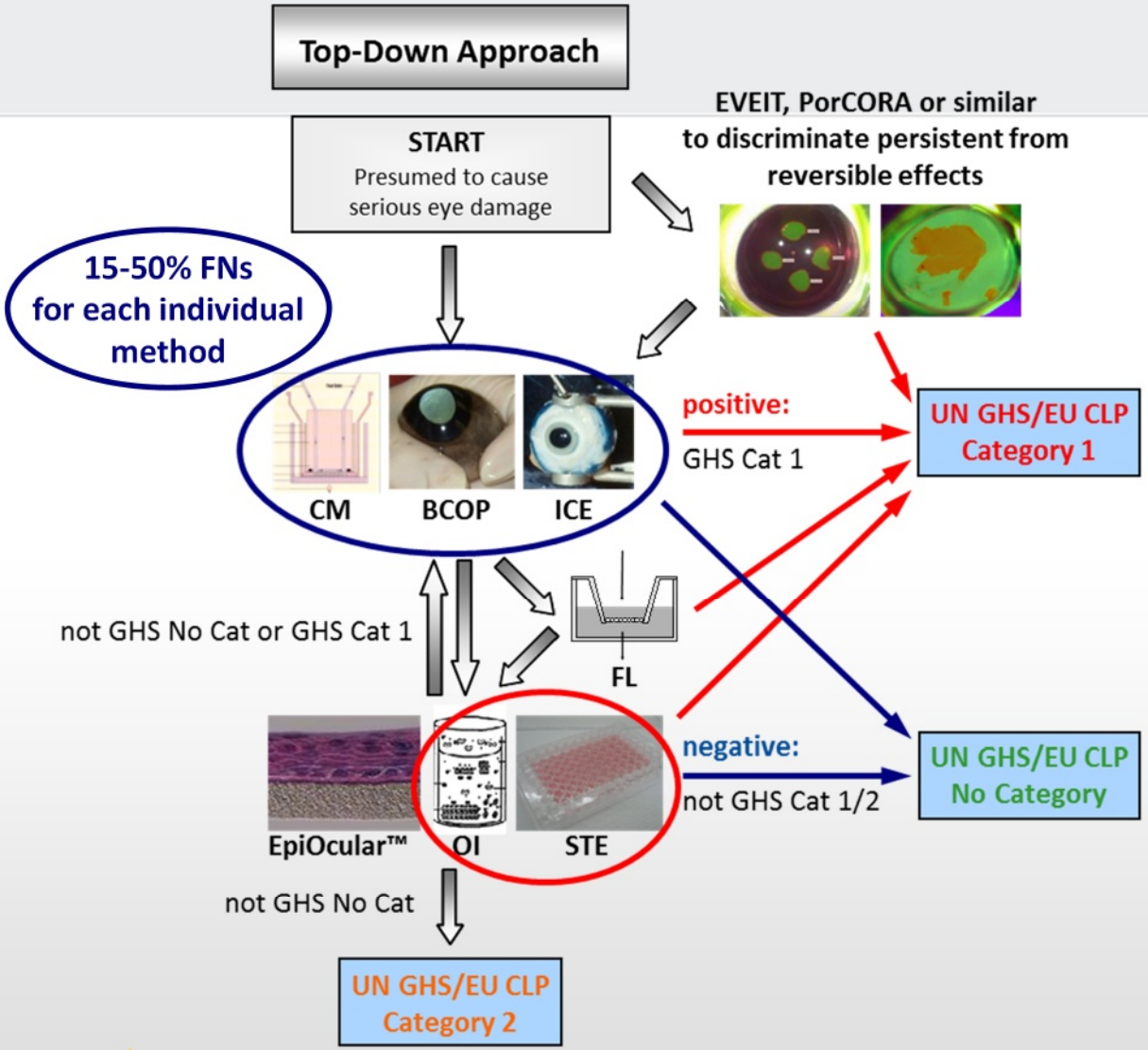
- Start top-down approach with one of the methods showing lower FNs, i.e. CM or BCOP; ICE for surfactants
- 15-50% FNs (under-predicted Cat. 1) in each of these methods, mostly chemicals classified *in vivo* based on persistence without severity
- Persistence of effects may be detected with methods such as EVEIT or PorCORA
- Increased coverage in the detection of No Cat. and Cat. 1 could lead to higher confidence in a default Cat. 2 classification in the last tier



Conclusions

- *In vitro* methods are the standard information requirement under REACH for substances produced between 1 and 10 tons/year
- For substances produced between 10-100 tons/year the *in vivo* test is currently the standard requirement but this can be adapted with the use of *in vitro* methods using the provisions of Annex XI, point 1.4
- 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
- For 20-30% of the substances a combination of 2 or more methods may be need
- Full replacement may be achieved in the majority of cases if due consideration is given to persistence vs. reversibility

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Thank you for attending

What did you think about the webinar? Please take part in our email survey (in your inbox now)

A downloadable recording of this presentation (with slides) will be available shortly.

If you have any questions, please contact Lorna (lorna@chemicalwatch.com)

NEXT

Skin sensitization, Wednesday 28 January, 4pm (UK time)

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