Alternative approaches for the assessment of serious eye damage/eye irritation

Webinar Series on the Use of New Approach Methodologies (NAMs) in Risk Assessment

João Barroso (Joint Research Centre, EURL ECVAM)

3 November 2021

Joint
Research
Centre

Historic events on serious eye damage/eye irritation

No single in vitro method capable of replacing the Draize rabbit eye test!

90's

Major international validation studies (EC/HO, COLIPA, CTFA, IRAG, BFA/BMBF, MHW/JCIA)

2002

EU Regulatory Acceptance Four organotypic assays accepted for C&L of severe irritants (R41), **although not validated** (BCOP, ICE, HET-CAM, IRE)

2005-2017

ECVAM workshop to identify test strategies for eye irritation (2005)

Several validation studies (ICCVAM, ECVAM, JaCVAM, KoCVAM and external)

OECD *in vitro/ex vivo* Test Guidelines

2017

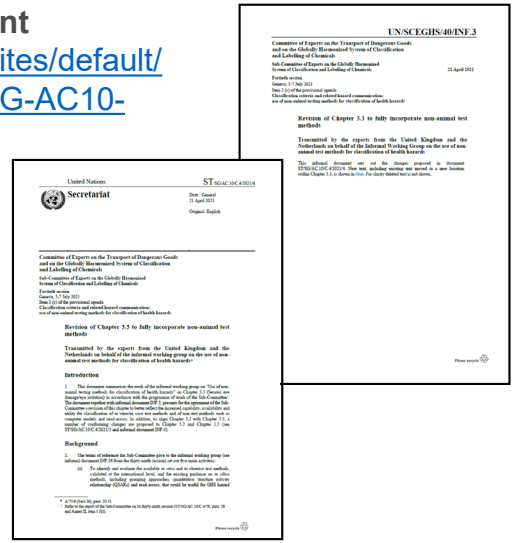
IATA for Serious Eye Damage and Eye Irritation **(OECD GD 263)**

2021 (2023)

Introduction of ***in vitro/ex vivo* classification criteria** in GHS

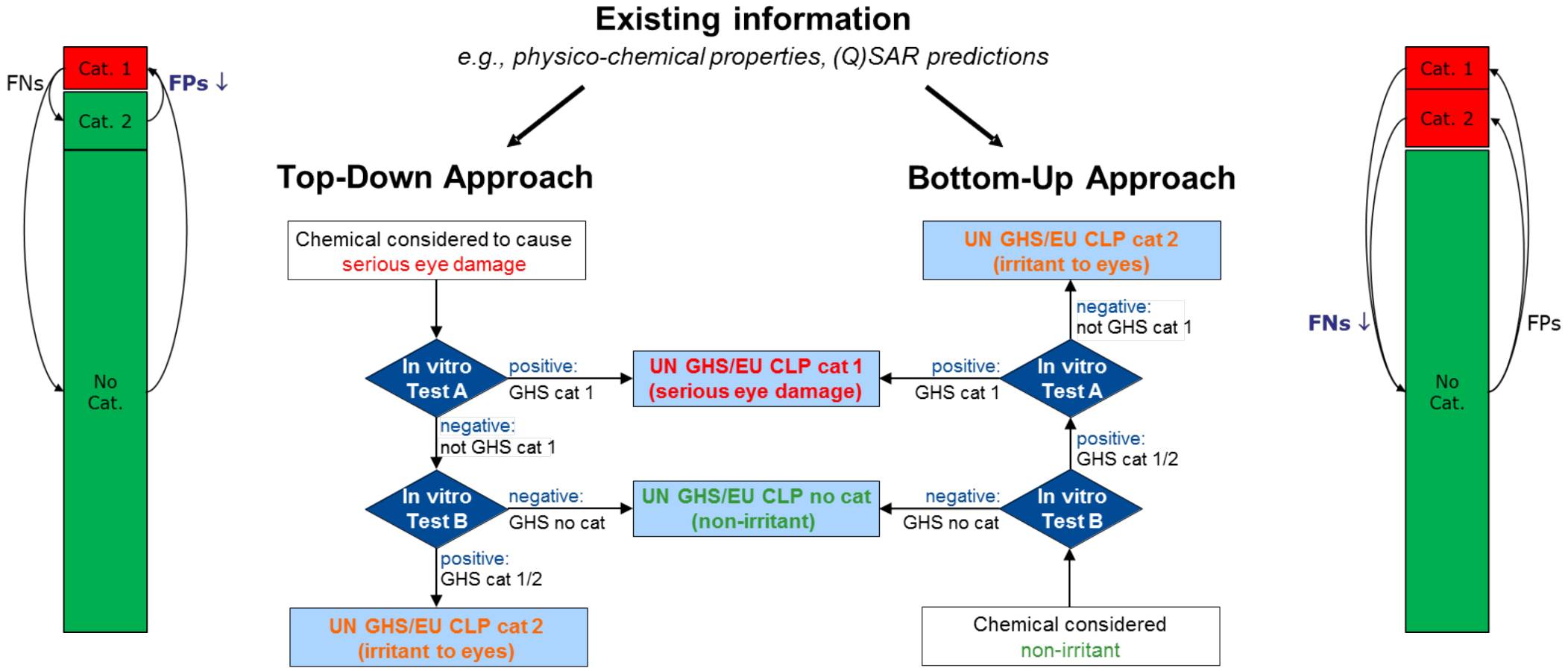
Informal Document
https://unece.org/sites/default/files/2021-04/UN-SCEGHS-40-INF3_0.pdf

Working Document
https://unece.org/sites/default/files/2021-04/ST-SG-AC10-C4-2021-4e.pdf



European Commission

Conceptual framework for a testing strategy



Scott *et al*. 2010, TiV 24: 1-9

European Commission

Non-animal test methods for ocular toxicity

Organotypic assays (full thickness corneal models)

- **TG 437:** Bovine Corneal Opacity and Permeability (BCOP) assay using OP-KIT (+ Histopathology)
- **TG 437:** BCOP using a Laser Light-Based Opacitometer (LLBO) (+ Histopathology)
- **TG 438:** Isolated Chicken Eye (ICE) assay + Histopathology
- Isolated Rabbit Eye (IRE) assay
- Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) assay
- Ex-Vivo Eye Irritation Test (EVEIT)
- Porcine Cornea Ocular Reversibility Assay (PorCORA)

Reconstructed human Cornea-like Epithelium (RhCE) assays (epithelium models)

- **TG 492:** EpiOcular™ Eye Irritation Test (EIT)
- **TG 492:** SkinEthic™ Human Corneal Epithelium (HCE) EIT
- **TG 492:** LabCyte Cornea-Model24 EIT
- **TG 492:** MCTT HCE™ EIT
- **TG 494:** Vitrigel®-EIT Method
- EpiOcular™ ET-50 and SkinEthic HCE TTT
- EyeIRR-IS assay

In chemico assays

- **TG 496:** Ocular Irritection® assay
- OptiSafe™ assay

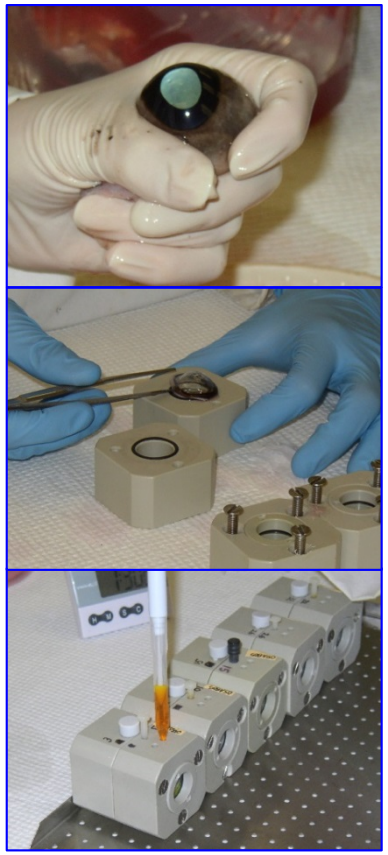
Cytotoxicity and cell-function assays (squamous epithelium models)

- **TG 491:** Short Time Exposure (STE) assay
- **TG 460:** Fluorescein Leakage (FL) assay
- Cytosensor Microphysiometer (CM) assay
- Neutral Red Release (NRR) assay

Organotypic assays

Full-thickness corneal models

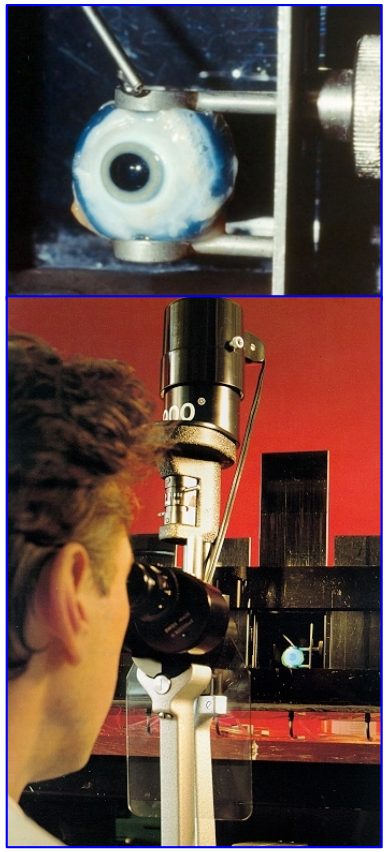
Bovine Corneal Opacity and Permeability (BCOP) assay OP-KIT and LLBO (TG 437)



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

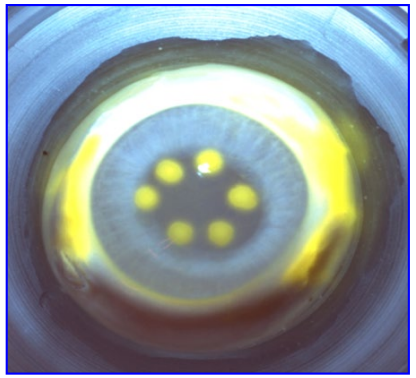
- **Test system:** corneas isolated from bovine eyes obtained from abattoir animals
- **Exposure:** 10 min for liquids (neat) and surfactants (10% w/v); 4 hours for non-surfactant solids (20% w/v)
- **Endpoints measured:** corneal opacity and loss of barrier function (permeability)
- **Status:** validated and regulatory accepted for identifying UN GHS Cat 1 (since 2009) and No Cat (since 2013), but not Cat 2 (OECD TG 437); LLBO introduced in 2020; Histopathology under discussion at OECD
- **Applicability and limitations:** according to TG 437,
  - Applicable to all types of chemicals
  - 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, while only 25% for solids (4/16) and all persistent

Isolated Chicken Eye (ICE) assay (TG 438)

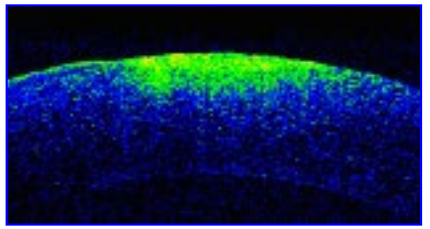


- **Test system:** chicken eyes isolated from abattoir animals
- **Exposure:** all chemicals exposed neat for 10 min
- **Endpoints measured:** corneal opacity, fluorescein retention, corneal swelling, morphological damage and histology
- **Status:** validated and regulatory accepted for identifying UN GHS Cat 1 (since 2009) and No Cat (since 2013), but not Cat 2 (OECD TG 438). Histology endpoint and revision of criteria for No Cat in 2018
- **Applicability and limitations:** according to TG 438,
  - Applicable to all types of chemicals
  - 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, while 55% for solids (6/11) and all persistent
  - Cat 1: high FNs for surfactants; histopathology can improve predictions for non-extreme pH (2 < pH < 11.5) detergents and surfactants inducing persistent non-severe effects *in vivo*

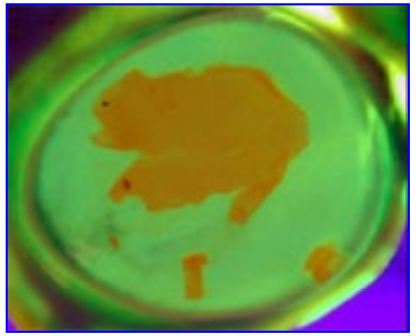
Methods for identifying persistent effects



Ex-Vivo Eye Irritation Test (EVEIT)



- Developed by ACTO & IHT, Univ. Aachen, Germany
- Uses excised rabbit corneas
- Monitors full-thickness corneal recovery (epithelium and stroma) over 3 days using non-invasive Optical Coherence Tomography (OCT) following 60 min exposure to solids and 30 sec to liquids
- Grading on days 1 and 3 used to discriminate between Cat 1, Cat 2 and NC



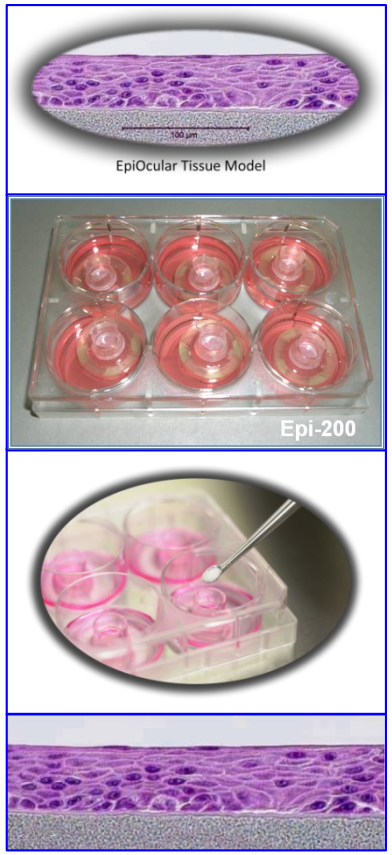
Porcine Cornea Ocular Reversibility Assay (PorCORA)

- 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

Reconstructed human Cornea-like Epithelium (RhCE) assays

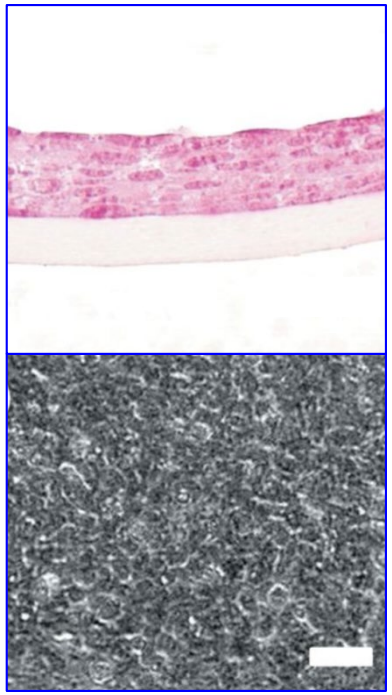
Epithelium models

EpiOcular™ EIT; SkinEthic™ HCE EIT; LabCyte EIT; MCTT HCE™ EIT (TG 492)



- **Test system:** reconstructed non-keratinized multi-layered human corneal epithelium
- **Exposure:** single fixed exposure time of 1-30 min for liquids (neat) and 3-24 hours for solids (neat)
- **Endpoints measured:** cytotoxicity (MTT, WST-8 or WST-1 assay)
- **Status:** validated and regulatory accepted since 2015 (EpiOcular), 2017 (SkinEthic), 2018 (LabCyte) and 2019 (MCTT) for identifying UN GHS No Cat, but not Cat 2 and Cat 1 (OECD TG 492)
- **Applicability and limitations:** according to TG 492,
  - Test items applied neat to model real-life exposures
  - Applicable to all types of chemicals, including agrochemical formulations
  - Applicable to intensely coloured chemicals (with use of HPLC/UPLC-spectrophotometry)

Vitrigel®-EIT Method (TG 494)

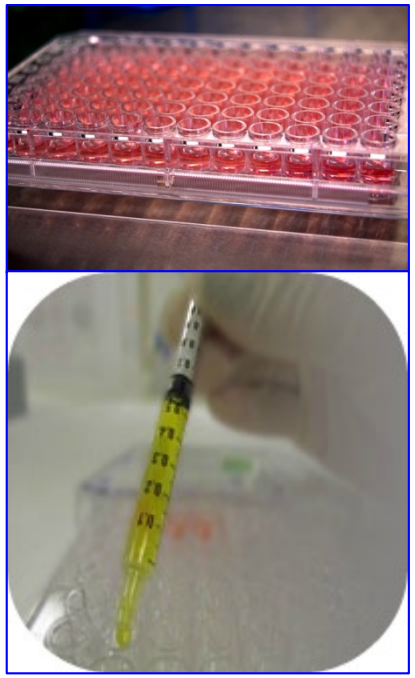


- **Test system:** reconstructed non-keratinized multi-layered human corneal epithelium
- **Exposure:** 3 min at 2.5% (w/v)
- **Endpoints measured:** damage to epithelial barrier function measured by time-dependent changes in Transepithelial Electrical Resistance (TEER)
- **Status:** validated and regulatory accepted since 2019 for identifying UN GHS No Cat, but not Cat 2 and Cat 1 (OECD TG 494); Applicability domain revised in 2021
- **Applicability and limitations:** according to TG 494,
  - Not applicable to chemicals with pH ≤ 5 in 2.5% (w/v) solution
  - Not applicable to chemicals showing rapid phase separation in solution (i.e. if difference of absorbance at 660 nm of 2.5% (w/v) test chemical preparation after 3 min is > 0.1)

Cytotoxicity and cell-function assays

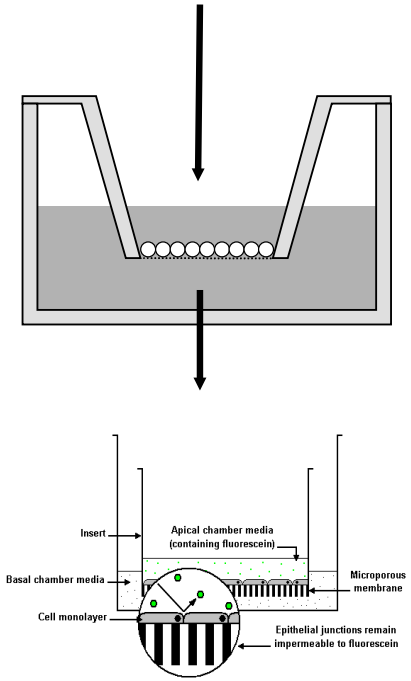
Squamous epithelium models

Short Time Exposure (STE) assay (TG 491)



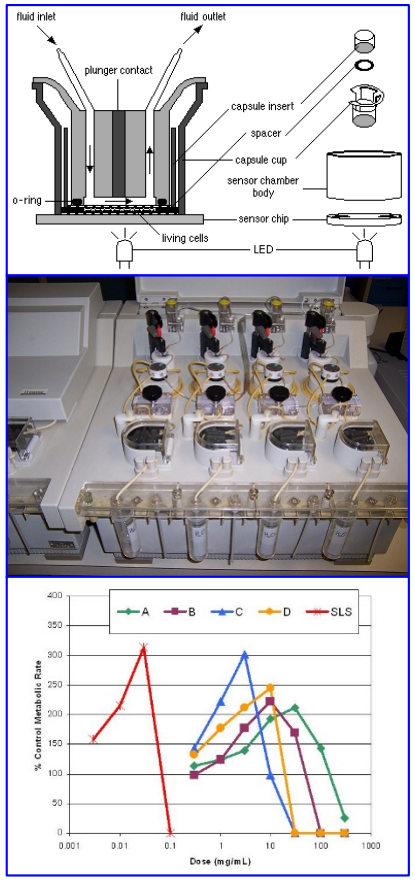
- **Test system:** confluent monolayer of SIRC cells
- **Exposure:** 5 min at 5% and 0.05%
- **Endpoints measured:** cytotoxicity (MTT assay)
- **Status:** validated and regulatory accepted since 2015 for identifying UN GHS Cat 1 and No Cat, but not Cat 2 (OECD TG 491)
- **Applicability and limitations:** according to TG 491,
  - Applicable only to soluble chemicals and those that form a stable suspension during testing
  - No Cat: not applicable to non-surfactant solids
  - No Cat: highly volatile chemicals (vapour pressure > 6 kPa) can be correctly tested using mineral oil instead of saline as solvent (2020 revision)
  - Cat 1: high FNs in general

Fluorescein Leakage (FL) assay (TG 460)



- **Test system:** confluent monolayer of MDCK CB997 tubular epithelial cells
- **Exposure:** a series of 5 concentrations exposed each for 1 min
- **Endpoints measured:** trans-epithelial permeability to fluorescein
- **Status:** validated and regulatory accepted since 2012 for identifying UN GHS Cat 1, but not Cat 2 and No Cat (OECD TG 460)
- **Applicability and limitations:** according to TG 460,
  - Applicable only to soluble chemicals and those that form a stable suspension during testing
  - Not applicable to strong acids and bases, cell fixatives and highly volatile chemicals
  - Coloured and viscous chemicals may be mispredicted
  - High FNs in general

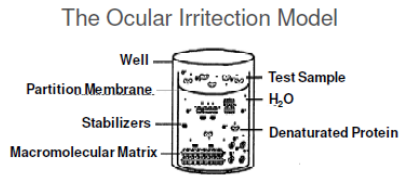
Cytosensor Microphysiometer (CM) assay

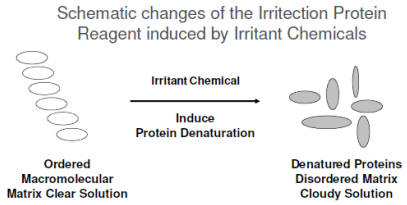


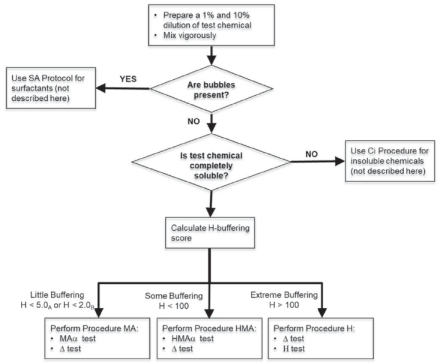
- **Test system:** sub-confluent monolayer of mouse L929 fibroblasts
- **Exposure:** sequential exposure to seven increasing concentrations of the test chemical, each for 13.5 min followed by washing and measurement (20 min cycle duration)
- **Endpoints measured:** cellular metabolic rate (acidification)
- **Status:** validated and recommended for identifying UN GHS Cat. 1 and No Cat., but not Cat. 2; Accepted by US EPA for Cat. III and IV
- **Applicability and limitations:**
  - Cat. 1: applicable only to soluble chemicals and those that form a stable suspension during testing
  - No Cat.: applicable only to soluble surfactants and those that form a stable suspension during testing
  - No Cat.: high FPs in general

In chemico assays

Ocular Irritection® (TG 496) & OptiSafe™ assays







- **Test system:** transparent macromolecular matrix composed of a mixture of proteins, glycoproteins, carbohydrates, lipids and low MW components that mimics the highly ordered structure of the cornea
- **Exposure:** 5 different doses exposed each for 24 hours (OI) or 18 hours (OS)
- **Endpoints measured:** turbidity at 405 nm ("opacity")
- **Status:** OI: validated and regulatory accepted since 2019 for identifying UN GHS Cat 1 and No Cat, but not Cat 2 (OECD TG 496); OS: validated by ICCVAM for identifying UN GHS No Cat and EPA IV in 2020
- **Applicability and limitations:** according to TG 496,
  - Fast, simple, inexpensive and readily available (2-year shelf-life)
  - Do not address the cytotoxicity and reversibility aspects of ocular toxicity
  - OI not applicable to chemicals with pH < 4 or pH > 9 in 10% water solution; OS not applicable to surfactants; Both not applicable to chemicals interfering with the test system (e.g. intensely coloured chemicals)
  - Cat 1: high FNs for chemicals with persistent non-severe effects

Predictive capacity for identifying GHS Cat 1

Test Method		Accuracy	Sensitivity	False Negatives	Specificity	False Positives
BCOP (TG 437)	OP-KIT LLBO	79% (85%) 78% (85%)	86% (92%) 76% (87%)	14% (8%) 24% (13%)	75% (80%) 79% (83%)	25% (20%) 21% (17%)
ICE (TG 438)		83%/87%*	53%/71%*	47%/29%*	93% (96%)	7%/8%*
STE (TG 491)		83%	49%	51%	99%	1%
FL (TG 460)		77%	44%	56%	93%	7%
Irritection (TG 496)		75% (76%)	54% (56%)	46% (44%)	81% (81%)	19% (19%)

* Values obtained when considering histopathology for non-extreme pH (2 < pH < 11.5) detergents and surfactants (n = 30 out 172)

Require further testing (mostly persistent non-severe effects *in vivo*)

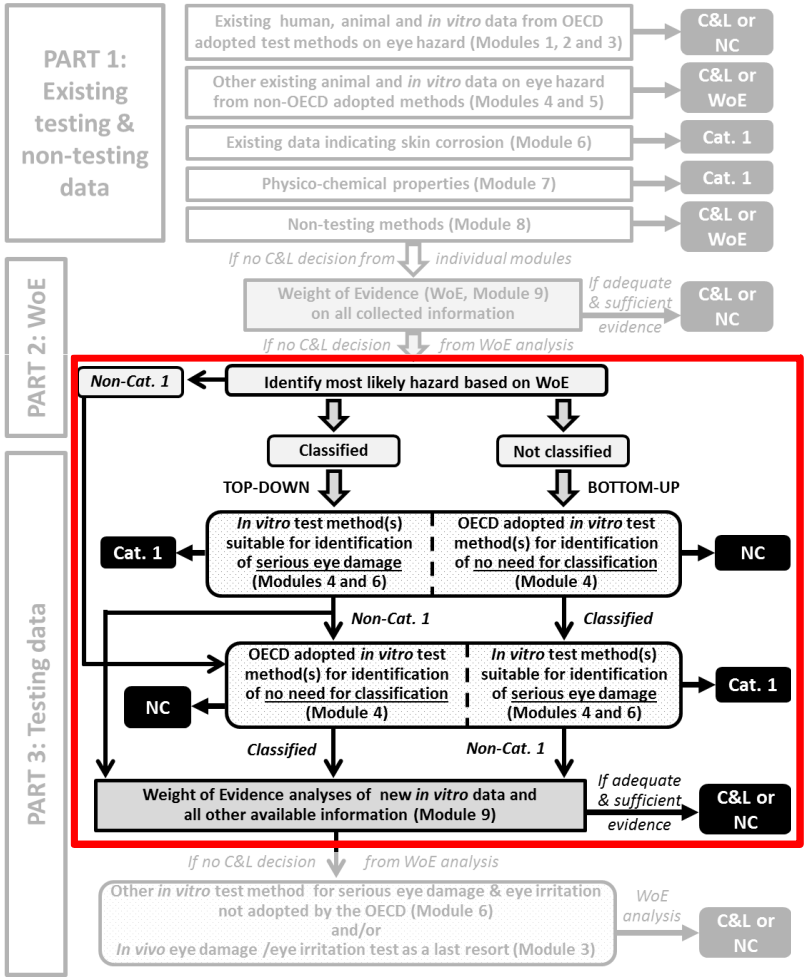
Predictive capacity for identifying GHS No Cat

Test Method		Accuracy	Sensitivity	False Negatives	Specificity	False Positives
BCOP (TG 437)	OP-KIT LLBO	69% 83%	100% 94%	0% 6%	31% 55%	69% 45%
ICE (TG 438)		88%	97%	3%	76%	24%
RhCE EIT (TG 492)	EpiOcular SkinEthic MCTT	80%/82%* 84% 86%	96%/91%* 95% 99%	4%/9%* 5% 1%	63%/72%* 72% 69%	37%/28%* 28% 31%
Vitrigel-EIT (TG 494)		81%	96%	4%	67%	33%
STE (TG 491)		85% (91%)	88% (98%)	12% (2%)	81% (84%)	19% (16%)
Irritection (TG 496) OptiSafe		75% (76%) 79%	91% (93%) 100%	9% (7%) 0%	59% (59%) 58%	41% (41%) 42%

* Values obtained for 97 liquid agrochemical formulations

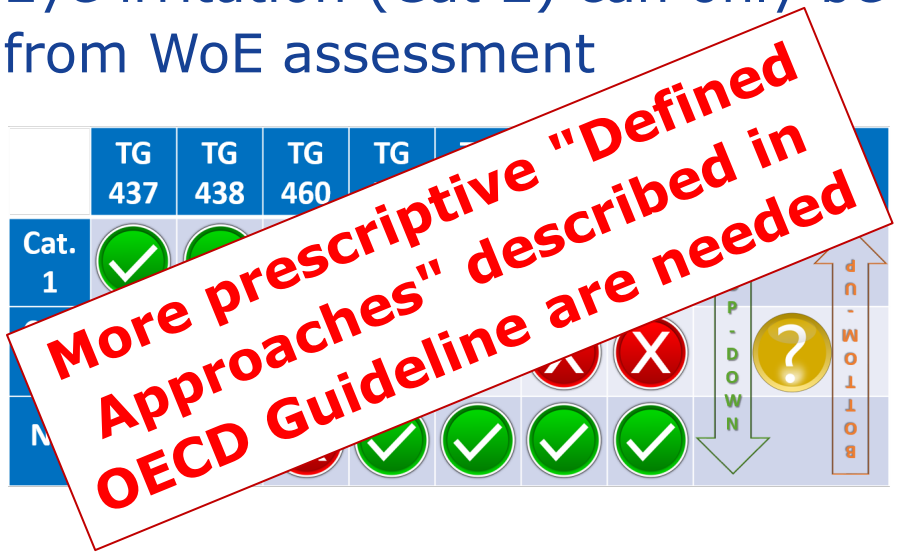
Require further testing

Integrated Approach on Testing and Assessment (IATA)



OECD Guidance Document No. 263

- TGs 437, 438, 460, 491, 496
  - Eye damage (Cat 1) or **No prediction can be made**
- TGs 437, 438, 491, 492, 494, 496
  - Not classified (No Cat) or **No prediction can be made**
- Eye irritation (Cat 2) can only be concluded from WoE assessment

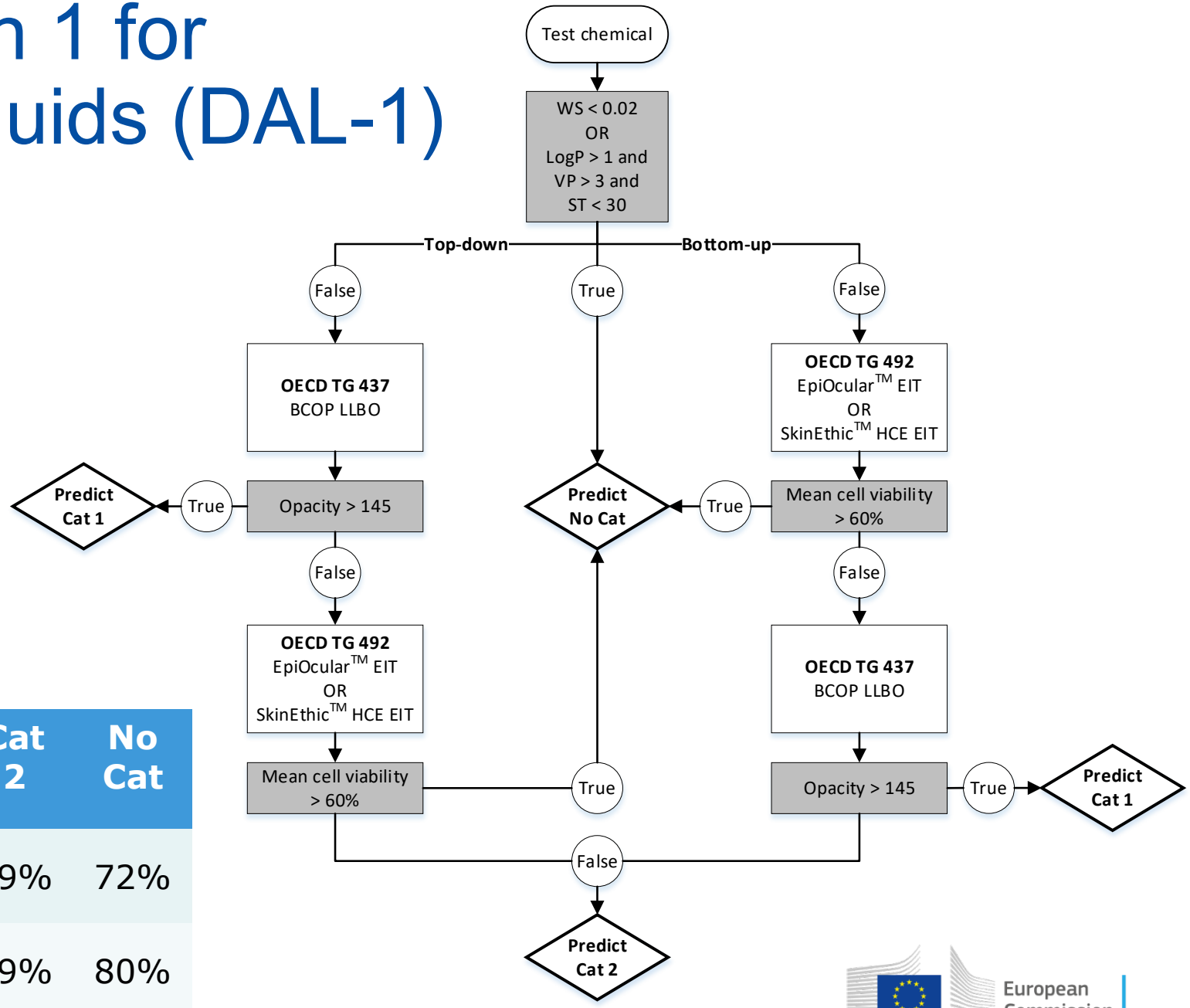


European Commission

Defined Approach 1 for non-surfactant liquids (DAL-1)

- Two Defined Approaches based on top-down/bottom-up approach currently under discussion at OECD
- DAL-1 uses phys. chem. properties, BCOP LLBO, and EpiOcular™ EIT or SkinEthic™ HCE EIT

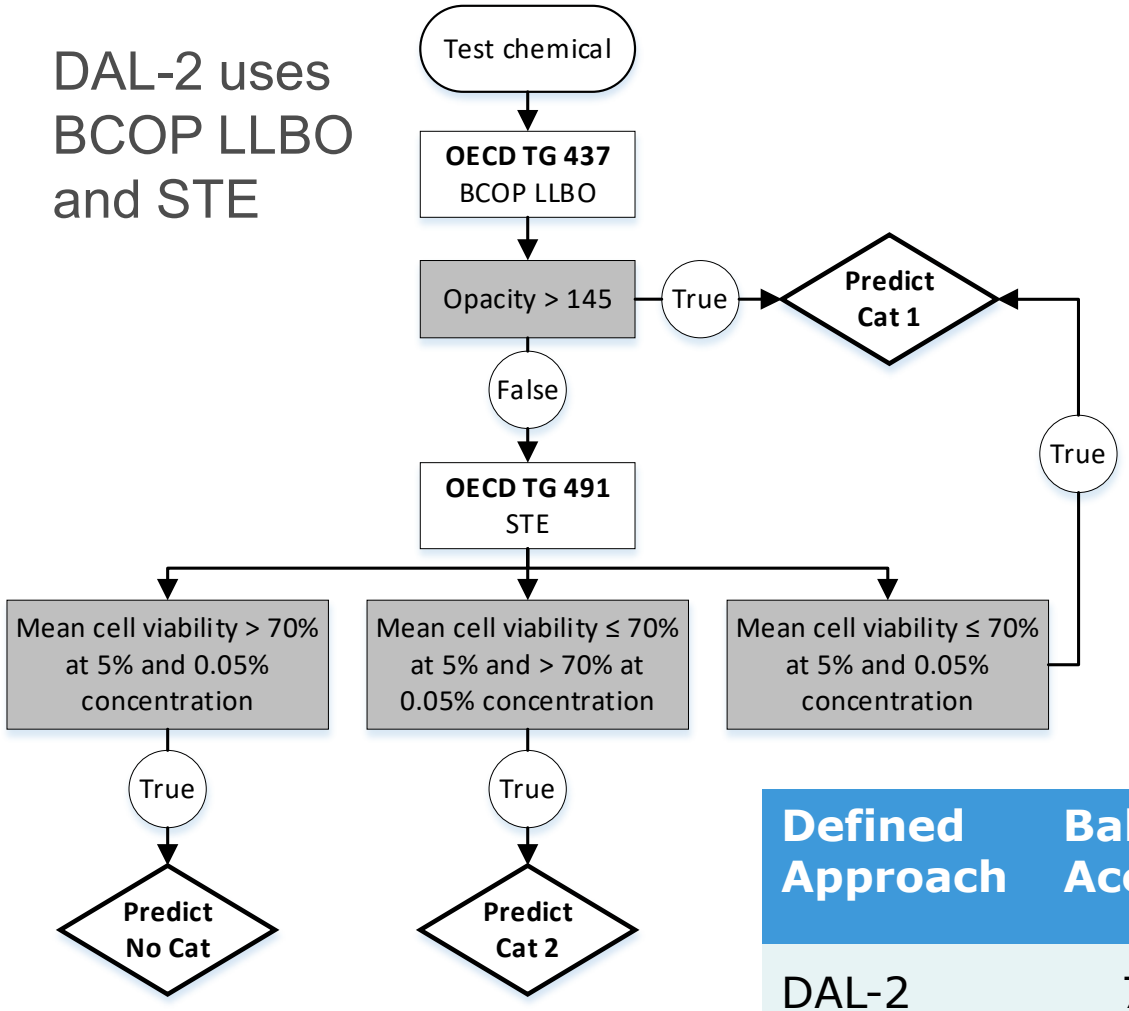
Defined Approach	Balanced Accuracy	Cat 1	Cat 2	No Cat
DAL-1 with EpiOcular	69%	77%	59%	72%
DAL-1 with SkinEthic	75%	77%	69%	80%

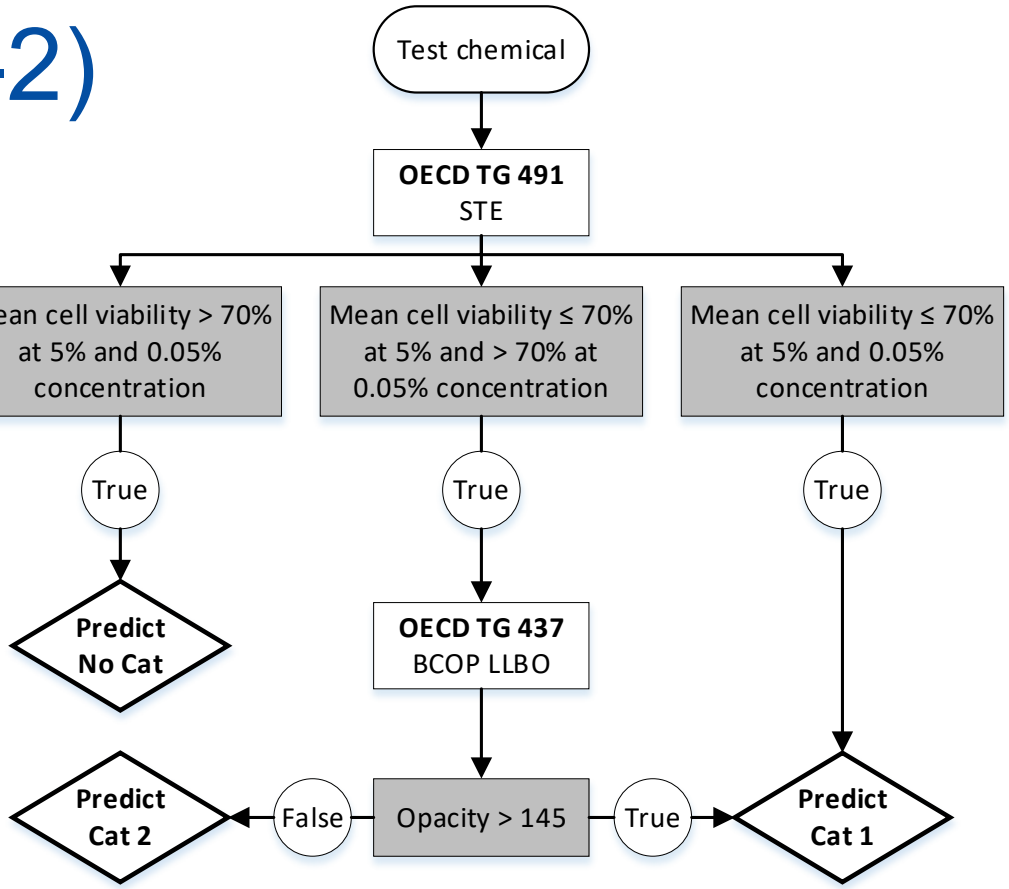


European Commission

Defined Approach 2 for non-surfactant liquids (DAL-2)

- DAL-2 uses BCOP LLBO and STE

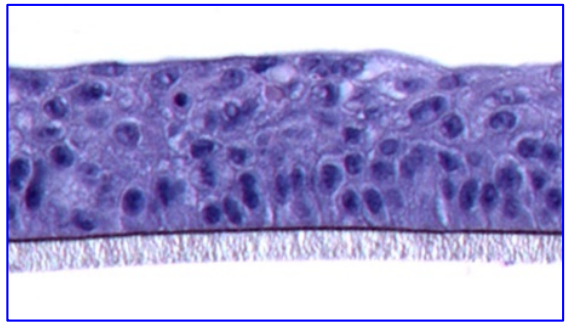


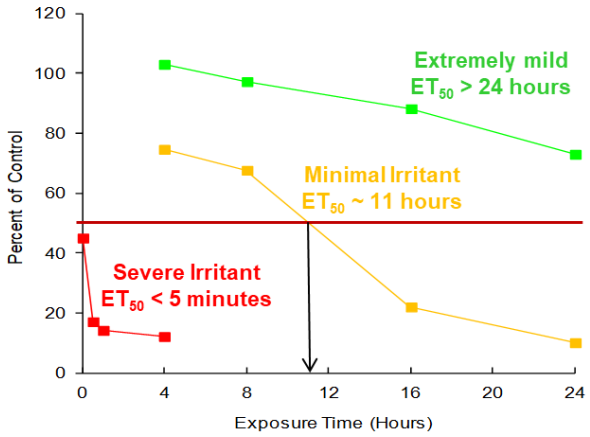


Defined Approach	Balanced Accuracy	Cat 1	Cat 2	No Cat
DAL-2	74%	81%	56%	85%

European Commission

Time-to-toxicity in RhCE models



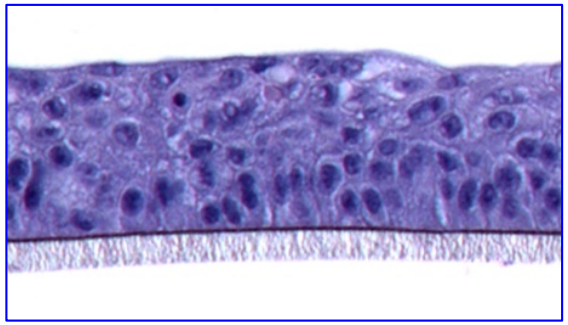


ET_{50} (estimated time to reduce viability to 50% of control), plot relative viability over exposure time

- Multiple exposure time protocol provides continuum of responses across eye irritation spectrum
- EpiOcular™ ET-50 accepted by US EPA to discriminate between EPA III and IV
- SkinEthic™ HCE Time To Toxicity (TTT) validated by L'Oréal, independently peer reviewed and currently under discussion at OECD to identify UN GHS Cat 1, Cat 2 and No Cat (draft TG 492B)
  - Exposure liquids: 5 min neat, 16 min 20% w/v, 120 min 20% w/v
  - Exposure solids: 30 min neat, 120 min neat
  - Tissue viability measured with MTT assay

Protocol	Accuracy	Cat 1	Cat 2	No Cat
TTL	81%	85%	80%	79%
TTS	67%	75%	55%	72%
TTT	74%	79%	69%	75%

EyeIRR-IS



Toxicology in Vitro 71 (2021) 105072

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

The EyeIRR-IS assay: Development and evaluation of an *in vitro* assay to measure the eye irritation sub-categorization of liquid chemicals

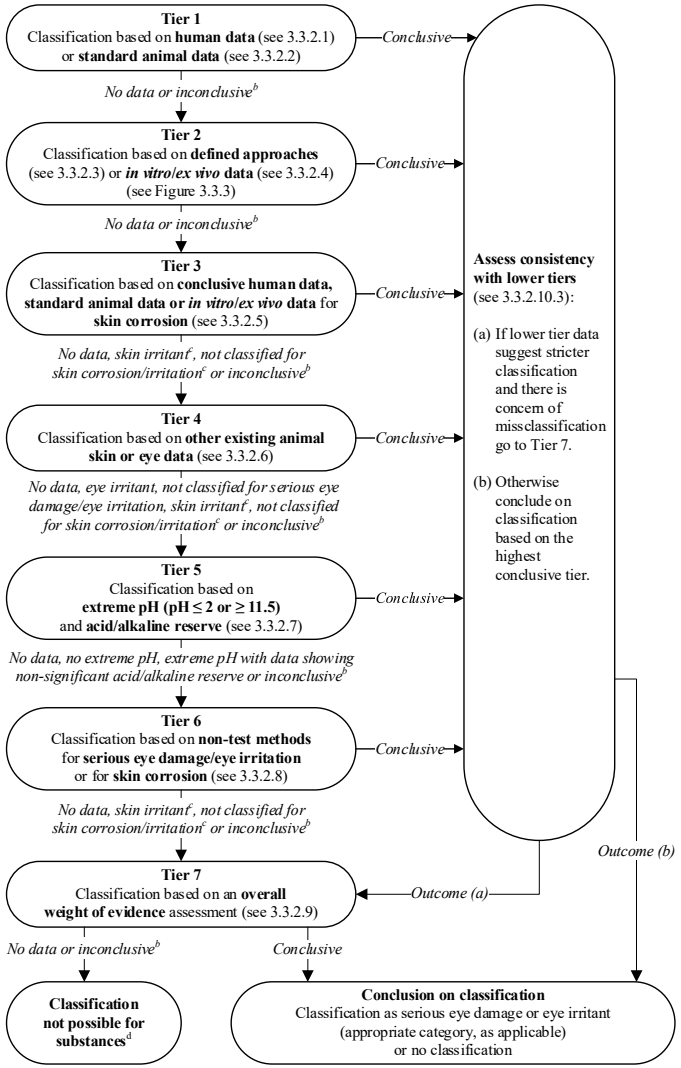
Françoise Cottrez [a], Virginie Leblanc [b], Elodie Boitel [a], Hervé Groux [a], Nathalie Alépée [b,*]

[a] ImmunoSearch, Grasse, France
[b] L'Oréal Research and Innovation, Aulnay sous bois, France

- Developed by ImmunoSearch, France
- Genomic approach using the SkinEthic™ HCE model and analysis of expression of 10 gene biomarkers by RT-PCR
- Proposed for identification of UN GHS No Cat, Cat 2 and Cat 1
- Appears to correctly predict persistent non-severe effects *in vivo*

Test Method	Accuracy	Cat 1	Cat 2	No Cat
EyeIRR-IS	83%	94%	67%	89%

Revision of chapter 3.3 of UN GHS on serious eye damage/eye irritation



- Non-animal classification criteria introduced
- Continues to follow a tiered approach
- *In vitro*/*ex vivo* data given a higher weight: placed in Tier 2 above skin corrosion and other animal data
- *In vitro*/*ex vivo* data can be used to identify no classification on their own
- Defined Approaches featured in Tier 2 (given higher weight than individual *in vitro*/*ex vivo* methods)
- Criterion for extreme pH with non-significant acid/alkaline reserve clarified for both substances and mixtures
- Principle of test method neutrality applied: validated methods/approaches other than OECD Guidelines can also be used for classification

Conclusions

- Several partial replacement methods available
- For ~70% of the substances one single *in vitro* method may be sufficient to conclude No Cat or Cat 1
- For the remaining substances a combination of 2 or more methods may be needed
- Identification of Cat 2 still remains a challenge but DAs and some individual methods (e.g. EVEIT, RhCE TTT, EyeIRR-IS) may provide long-waited full replacement solution
- Acknowledging the uncertainty associated with reference animal method is key to success

Thank you

European Commission