

Use of Predictive Animal-free Toxicology Testing Approaches to Develop Safer Products

Amy J. Clippinger, PhD

Towards Safer Design Strategies:
Using toxicology tools and concepts within chemistry courses
and programs

22nd Annual Green Chemistry & Engineering Conference

June 20, 2018

Photo credit: MatTek Corp.

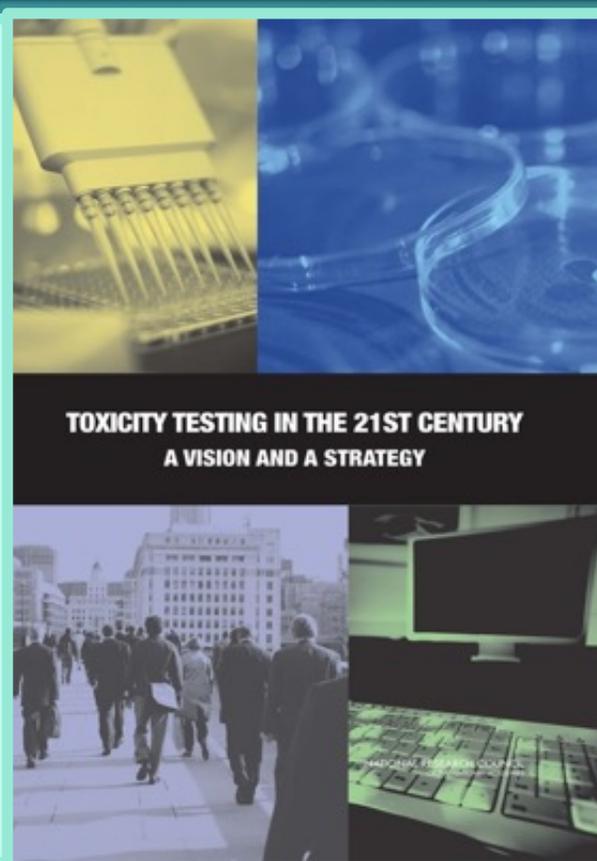
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Outline

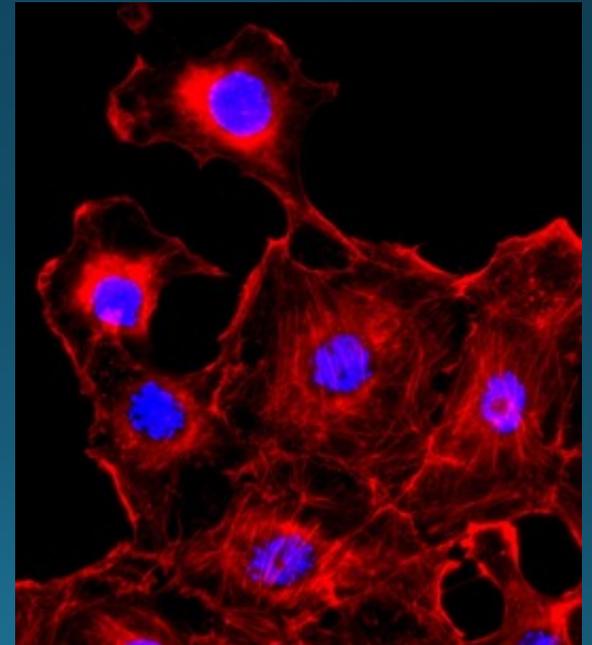
- Drivers of *in vitro* and *in silico* testing
- Eye irritation testing
 - Reproducibility of animal tests
 - Existing *in vitro* methods
 - Antimicrobial cleaning products
 - Agrochemical formulations
- Training resources

“Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin...The envisioned change is expected to generate more robust data on the potential risks to humans posed by exposure to environmental agents and to expand capabilities to test chemicals more efficiently.”
-National Research Council, Toxicity Testing in the 21st Century: A Vision and A Strategy (2007)



What drives the use of non-animal methods?

- ✓ Protection of human health and the environment
- ✓ Ethics
- ✓ Time
- ✓ Money



Reproducibility of Animal Tests



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

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 Pfannenbecker³ · Els Adriaens⁴ · Nathalie Alépée⁵ ·
Magalie Cluzel⁶ · Ann De Smedt⁷ · Jalila Hibatallah⁸ · Martina Klaric¹ ·
Karsten R. Mewes⁹ · Marion Millet¹⁰ · Marie Templier¹⁰ · Pauline McNamee¹¹

Published in final edited form as:
ALTEX, 2016 ; 33(2): 123–134. doi:10.14573/altex.1510053.

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

Thomas Luechtefeld¹, Alexandra Maertens¹, Daniel P. Russo², Costanza Rovida⁴, Hao Zhu^{2,3}, and Thomas Hartung^{1,4}

Reproducibility of the Draize Eye Test

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Thomas Luechtefeld¹, Alexandra Maertens¹, Daniel P. Russo², Costanza Rovida⁴, Hao Zhu^{2,3}, and Thomas Hartung^{1,4}

- Data submitted to the European Chemicals Agency
- 491 substances with at least 2 Draize eye tests
- Conditional probabilities of Draize evaluations based on a previous test result

Prior GHS category	1	2A	2B	NC
1 (serious eye damage)	73%	16.1%	0.4%	10.4%
2A (irritant)	4.2%	32.9%	3.5%	59.4%
2B (mild irritant)	0.2%	4%	15.5%	80.2%
NC (non-irritant)	1.1%	3.5%	1.5%	93.9%

from Luechtefeld et al., *ALTEX* 33(2), 2016.

Reproducibility of the Draize Eye Test

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NC (non-irritant)	1.1%	3.5%	1.5%	93.9%

- Some Category 1 could be NC in a subsequent test
- Category 2A and 2B more likely to be NC than Category 2 in a subsequent test

from Luechtefeld et al., ALTEX 33(2), 2016.

Existing *In Vitro* Methods

In Vitro OECD Test Methods for Serious Eye Damage and Eye Irritation

- Monolayer cell cultures
 - Short time exposure assay (OECD TG 491)
 - Fluorescein leakage (OECD TG 460)
- Reconstructed human three-dimensional tissues
 - EpiOcular™ (OECD TG 492)
- *Ex vivo* organotypic models
 - Bovine corneal opacity and permeability (OECD TG 437)
 - Isolated chicken eye (OECD TG 438)

Monolayer Cell Culture Systems

- Primary or immortalized epithelial cells [Statens Seruminstitut Rabbit Cornea (SIRC) corneal epithelial cells or Madin-Darby canine kidney (MDCK) epithelial cells]
- Measure cell viability (MTT assay) or permeability to a fluorescein dye
- Assumes that chemicals causing eye damage/irritation will induce cytotoxicity or disrupt epithelial barrier function in the corneal epithelium and/or conjunctiva

OECD/OCDE

491

Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Short Time Exposure *In Vitro* Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

OECD/OCDE

460

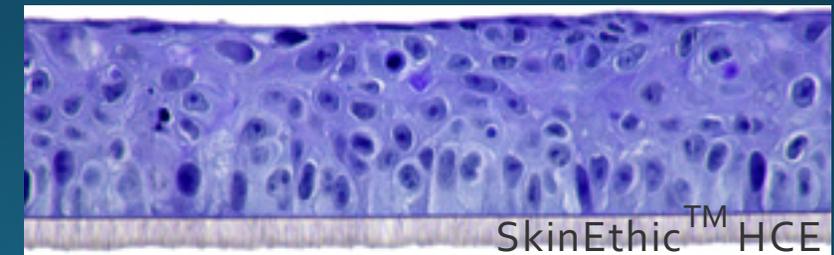
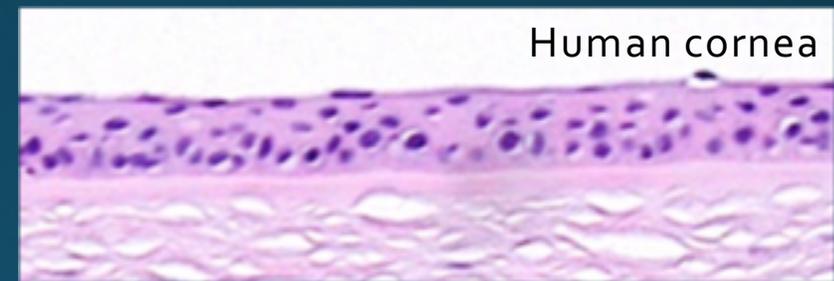
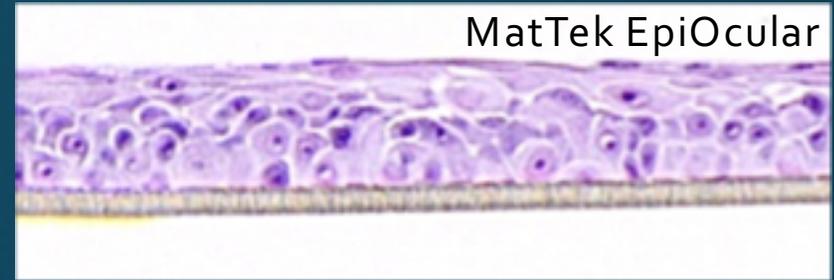
Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants

Reconstructed Human Corneal Epithelium Models

- Commercially-available 3D human tissue constructs:
 - EpiOcular (MatTek Corp)
 - SkinEthic (EpiSkin)
- Assess cell viability (MTT assay)
- Assumes that chemicals causing eye damage/irritation will induce cytotoxicity in the corneal epithelium and/or conjunctiva



OECD/OCDE

492

Adopted:
9 October 2017

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

Ex Vivo Bovine Corneal or Chicken Whole Eye Models

- Evaluate effects *ex vivo* using corneas (bovine) or whole eyes (chicken) taken from animals used in food production
- Assess corneal injury:
 - Opacity (measure the amount of light transmitted through the cornea)
 - Permeability (measure the amount of fluorescein dye that penetrates through the cornea)
 - Swelling (ICE)

OECD/OCDE 437
Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage



Images courtesy of the Institute for In Vitro Sciences

OECD/OCDE 438
Adopted:
9 October 2017

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage



Images courtesy of Menk Prinsen

Example:
Antimicrobial Cleaning Products

Background

- The majority of cleaning products do not have to go through a registration process before they are marketed. Companies decide how to assure safety – generally without using animals.
- However, if the product has an “antimicrobial” claim, it is regulated by the EPA’s Office of Pesticide Programs (OPP) and animal testing, including eye irritation testing, is required.
- Both EPA and industry wanted a strategy to replace the rabbit eye irritation test.

Parties involved



2004-2009

Paired data submitted by industry. Following analysis, a testing strategy was proposed

2013

US EPA policy issued

2009

US EPA OPP pilot program initiated

2015

Policy update

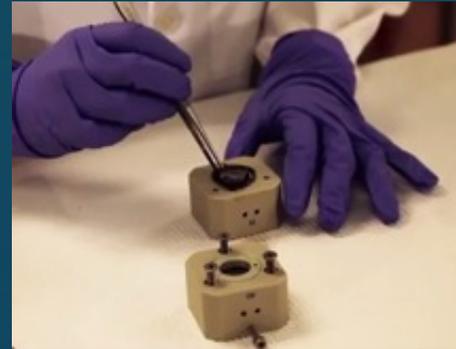
- The EPA policy allows the use of the alternate framework for testing AMCPs and, on a case-by-case basis, for other classes of pesticides and pesticide products

EPA OPP toxicity category	Signal words	Hazard statements
I	DANGER	Corrosive (causes irreversible eye damage). Wear appropriate protective eyewear such as goggles, face shield, or safety glasses.
II	WARNING	Causes substantial but temporary eye injury. Wear appropriate protective eyewear such as goggles, face shield, or safety glasses.
III	CAUTION	Causes moderate eye irritation. May wear protective eyewear, if appropriate (not required).
IV	CAUTION (optional)	No statements are required. However, the registrant may choose to use category III labeling.

The assays

BOVINE CORNEAL OPACITY
AND PERMEABILITY ASSAY
OECD test guideline 437

categories I, II, or III

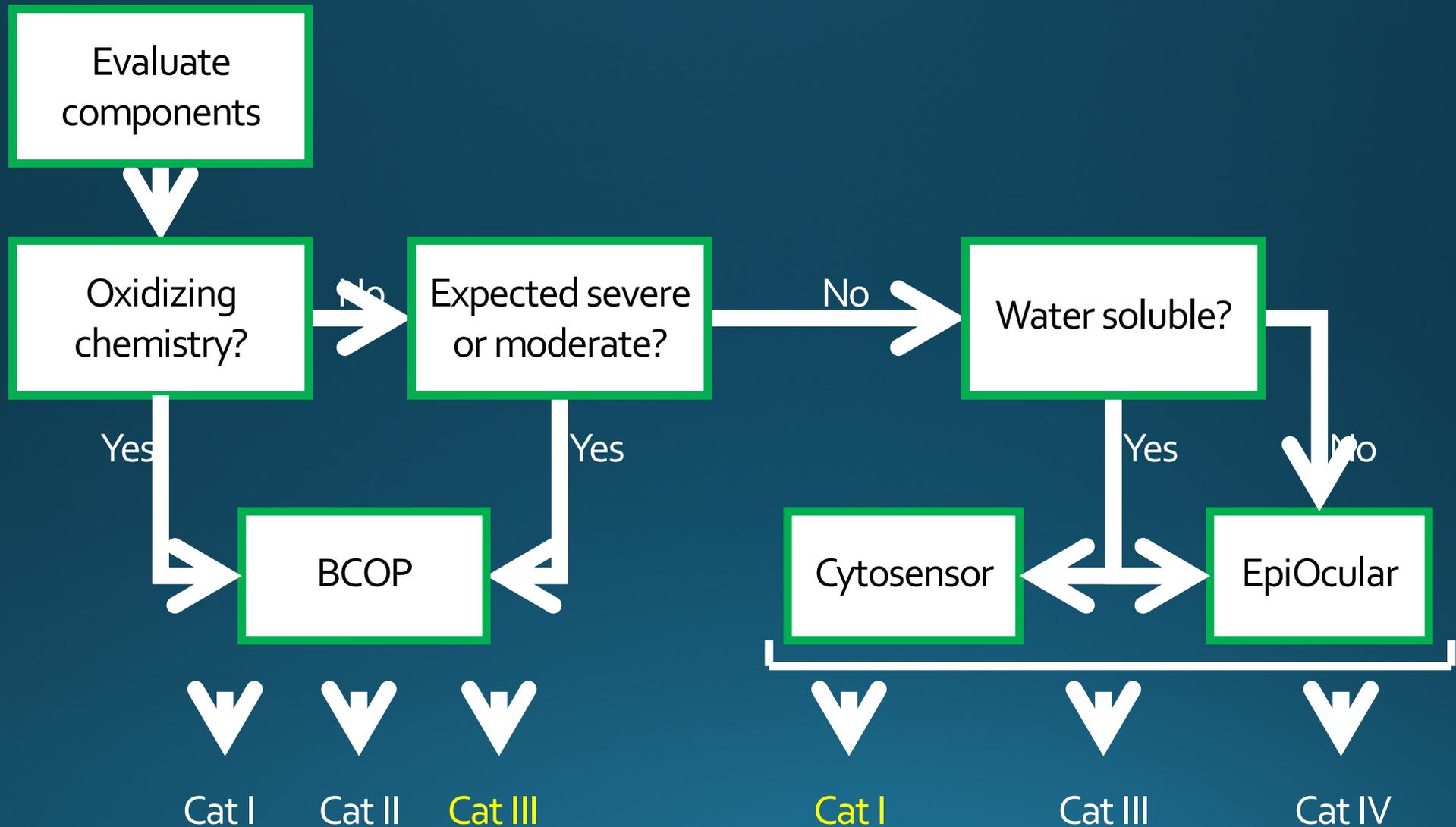


CYTOSENSOR™ MICROPHYSIOMETER
OECD draft test guideline
category I, III, or IV

EPIOCULAR™ ASSAY (MatTek Corp.)
OECD test guideline 492; time to toxicity assay
category I, III, or IV



Decision tree: selection and evaluation of assays for eye hazard labeling



Program utilization

**USE OF AN ALTERNATE TESTING FRAMEWORK FOR
CLASSIFICATION OF EYE IRRITATION POTENTIAL OF EPA
PESTICIDE PRODUCTS**

3-2-2015

Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington DC, 20460



Framework is underutilized: the vast majority of registrations have used the animal test

Example: Agrochemical Formulations



The EpiOcular™ Eye Irritation Test is the Method of Choice for the *In Vitro* Eye Irritation Testing of Agrochemical Formulations: Correlation Analysis of EpiOcular Eye Irritation Test and BCOP Test Data According to the UN GHS, US EPA and Brazil ANVISA Classification Schemes

Regulatory Toxicology and Pharmacology 81 (2016) 407–420

Susanne N. Kolle,¹ Maria Cecilia Rey Moren van Ravenzwaay¹ and Robert Landsiedel¹



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Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Tiered application of the neutral red release and EpiOcular™ assays for evaluating the eye irritation potential of agrochemical formulations

Regulatory

Cont

Raja S. Settivari ^{a,*}, Ricardo Acosta Amado ^b, Marco Corvaro ^c, Nicolo R. Visconti ^a, Lynn Kan ^a, Edward W. Carney ^a, Darrell R. Boverhof ^a, Sean C. Gehen ^b



ELSEVIER

Regulatory

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

Lacking applicability of *in vitro* eye irritation methods to identify seriously eye irritating agrochemical formulations: Results of bovine cornea opacity and permeability assay, isolated chicken eye test and the EpiOcular™ ET-50 method to classify according to UN GHS

Susanne N. Kolle ^{a,*}, Andrew Van Cott ^b, Bennard van Ravenzwaay ^a, Robert Landsiedel ^a

- Crop Life America companies
 - BASF, Dow AgroSciences, Bayer, Syngenta, DuPont
- NICEATM analyzed paired data for approximately 200 agrochemical formulations
- Existing rabbit *in vivo* + *in vitro* data from one or more assays:
 - Bovine corneal opacity and permeability
 - Isolated chicken eye
 - EpiOcular (MatTek Corp)
 - Neutral red release
 - Chorioallantoic membrane vascular assay

Results

- A tiered approach using EO and NRR seems promising, but not sufficient to identify all hazard categories
- Preliminary data suggest the current BCOP protocol may not be useful for testing agrochemical formulations
- Overall, there is a need to conduct prospective *in vitro* testing

Prospective Testing of Agrochemical Formulations

Co-organized by NICEATM and the PETA-ISC, with Validation Management Team members from

- EPA Office of Pesticide Products
- Canada's Pesticide Management Regulatory Authority
- ICCVAM
- EURL ECVAM
- Industry

Coded formulations and existing data donated by companies

- BASF
- Bayer
- Corteva (formerly Dow AgroSciences and DuPont)
- FMC
- Monsanto
- Syngenta

Prospective Testing of Agrochemical Formulations

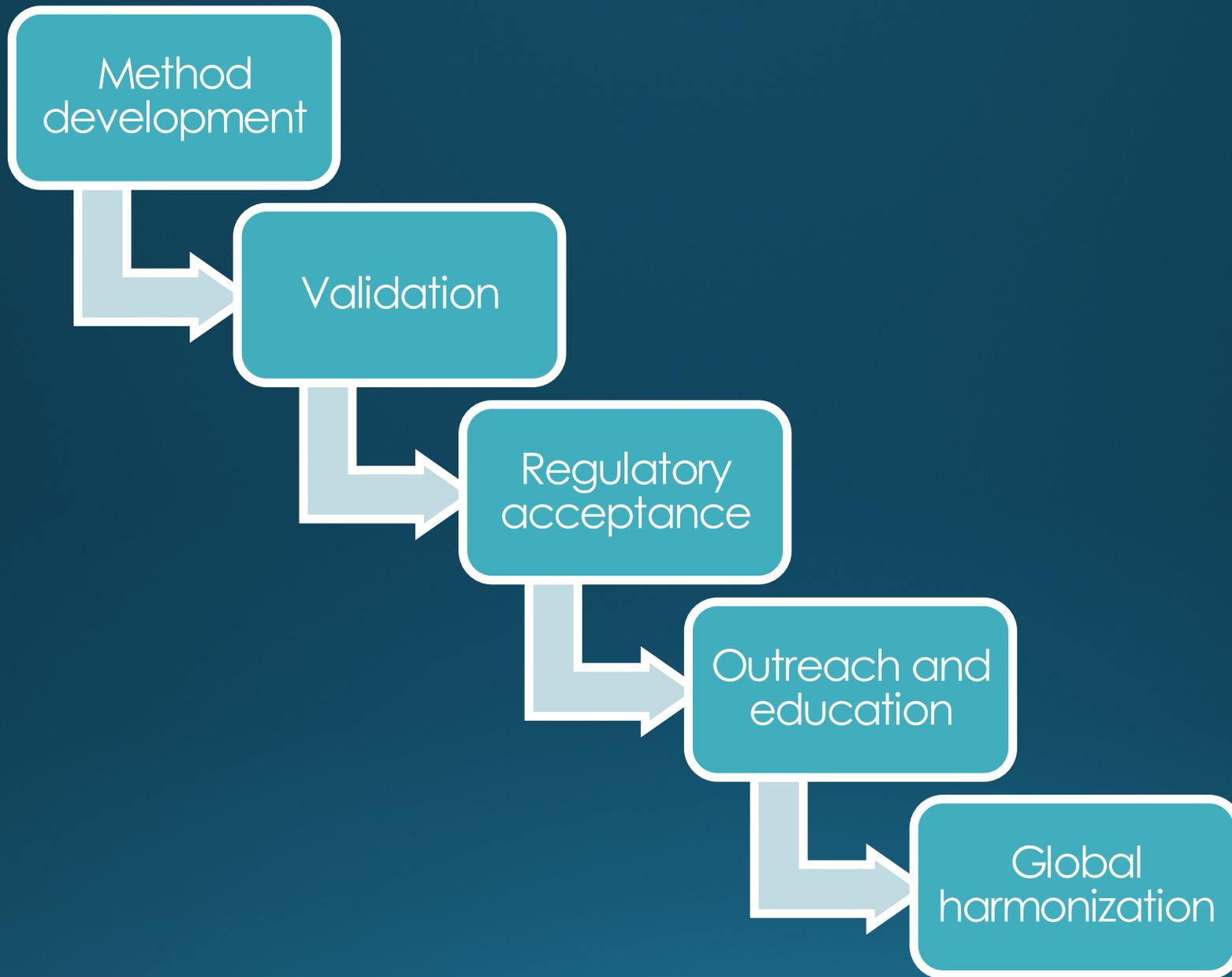
PHASE 1

6 agrochemical formulations tested to demonstrate proof-of-concept

- BCOP
- ICE
- Neutral red release
- EpiOcular (time to toxicity and TG 492 protocols)
- PorCORA (porcine cornea reversibility assay)

PHASE 2

Comprehensive assessment of applicability with a larger set (n=40) of formulations



Clippinger AJ, Hill E, Curren R, Bishop PL. Bridging the gap between regulatory acceptance and industry use of non-animal methods. 2016;33(4):453-458

Training resources

TOXICITY ENDPOINT	REPLACEMENT, REDUCTION, OR REFINEMENT METHOD		OECD
SKIN CORROSION	Integrated approach on testing and assessment (IATA)		OECD guidance document (GD) 203, published in 2014
	<i>In vitro</i> membrane barrier test Corrositex		OECD test guideline (TG) 435, revised in 2015
	Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 431, revised in 2016
		EpiDerm™ (MatTek, US)	OECD TG 431, revised in 2016
		SkinEthic™ (L'Oréal, France)	OECD TG 431, revised in 2016
		epiCS® (CellSystems, Germany)	OECD TG 431, revised in 2016
Rat skin transcutaneous electrical resistance (TER) test		OECD TG 430, revised in 2015	
SKIN IRRITATION	Integrated approach on testing and assessment (IATA)		OECD GD 203, published in 2014
	Reconstructed human epidermis (RhE) test	EpiSkin™ (L'Oréal, France)	OECD TG 439, revised in 2015
		EpiDerm™ (MatTek, US)	OECD TG 439, revised in 2015
		SkinEthic™ (L'Oréal, France)	OECD TG 439, revised in 2015
		LabCyte EPI-MODEL (J-TEC, Japan)	OECD TG 439, revised in 2015
SKIN ABSORPTION/ PENETRATION	<i>In vitro</i> diffusion method		OECD TG 428, published in 2004
SKIN SENSITISATION	Adverse outcome pathway (AOP) for skin sensitisation		OECD series on testing and assessment 168, published in 2012
	Guidance on reporting of defined approaches and individual information sources to be used within integrated approaches to testing and assessment (IATA)		OECD GD 256 (Annex 1, Annex 2), published in 2016
	ARE-Nrf2 luciferase test method (e.g. KeratinoSens™ assay)		OECD TG 442D, published in 2015
	Direct peptide reactivity assay (DPRA)		OECD TG 442C, published in 2015
	Human cell line activation test (h-CLAT)		OECD TG 442E, revised in 2017
	IL-8 Luc assay		OECD TG 442E, published in 2017
	U937 Skin Sensitization Test (U-SENS™)		OECD TG 442E, published in 2017
	Murine local lymph node assay (LLNA)*	Reduced LLNA (rLLNA)	OECD TG 429, revised in 2010
		LLNA	OECD TG 429, revised in 2010
		LLNA: BrdU-ELISA, non-radioactive	OECD TG 442B, published in 2010
LLNA: DA, non-radioactive		OECD TG 442A, published in 2010	

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Training Videos & Webinars

Eye Irritation and Corrosion

- Alternative Methods to Animal Testing: Ocular Irritation Assays (recorded for the course “Replacing regulatory experiments on animals: an introduction to *in silico* models and *in vitro* test methods”; 2017)
Click [HERE](#) for video.
- REACH webinar series: Serious Eye Damage and Eye Irritation (co-hosted by the PETA International Science Consortium, 2014)
Click [HERE](#) for webinar.
- OECD test guideline training video: OECD Test Guideline 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage (EpiOcular).
Click [HERE](#) for video.
- OECD test guideline training video: OECD Test Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage.
Click [HERE](#) for video.
- Institute for In Vitro Sciences: Bovine Corneal Opacity and Permeability Training Video (2015)
Click [HERE](#) for webinar. ([Español](#))
- Institute for In Vitro Sciences: Serious Eye Damage and Eye Irritation (2013)
Click [HERE](#) for webinar.
- Institute for In Vitro Sciences: Short Time Exposure (STE) Assay: Method and Use (2013)
Click [HERE](#) for webinar.
- Institute for In Vitro Sciences: Current Perspectives and New Directions Using the BCOP Assay—Applications in the Regulatory Arena (2011)
Click [HERE](#) for webinar.

Home > News & Resources > Documents & Resources > IIVS BCOP Training Video – English

IIVS BCOP Training Video – English

November 1, 2015

This 13- minute video demonstrates how to perform the BCOP assay according to the Test Guidelines set forth for the assay by the Organization for Economic Co-operation and Development (OECD TG 437). The video focuses on steps that are critical to the success of the assay such as handling of the isolated cornea and removal of the test material from the cornea at the conclusion of the exposure time.



Training opportunities



Awards

AWARD TO ATTEND *IN VITRO* TOXICOLOGY WORKSHOP

The PETA International Science Consortium Ltd. will provide an early career scientist with the opportunity to attend the annual Institute for In Vitro Sciences Practical Methods for In Vitro Toxicology Workshop in January 2018.

The January 16–19, 2018, workshop will take place in Gaithersburg, Maryland. Attendees will participate in lectures by experts in the field of *in vitro* toxicology and gain hands-on laboratory experience in applying *in vitro* methods.

Apply Now to Gain Hands-on *In Vitro* Experience!

Graduate students who have completed their first year of studies and post-doctoral fellows who are interested in a career in toxicology are eligible to apply for this award. The Consortium will cover registration costs and provide a travel stipend for the award winner.

For more information, visit PISCLtd.org.uk/iivs-prize.

**Deadline
October 15, 2017**

For proposal application details, please visit PISCLtd.org.uk/iivs-prize.

For more information, please contact:
Katherine Greff
KatherineG@piscltd.org.uk



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Award for Early-Career Scientist to Attend ESTIV2018

The PETA International Science Consortium Ltd. is offering an early-career scientist the opportunity to attend the 20th International Congress on *In Vitro* Toxicology (ESTIV2018).

Students and early-career scientists who are submitting oral or poster presentations at ESTIV2018 can apply for this award. The Consortium will cover registration costs for the winner and contribute towards travel expenses and hotel accommodations.

**Deadline
17 June 2018**

For more details and to apply, see PISCLtd.org.uk/ESTIV2018-award.

For more information, please contact:
AnnaZ@piscltd.org.uk

PETA INTERNATIONAL
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Call for short proposals

Win free *in vitro*
respiratory tissue models

How will you use Epithelix tissues to
avoid testing on animals?

The PETA International Science Consortium and Epithelix invite you to apply to win MucilAir™ or SmallAir™ three-dimensional reconstructed human respiratory tissues from Epithelix. International researchers from any sector are encouraged to apply.

To enter, visit <https://www.piscltd.org.uk/epithelix-award/>

For more information, please contact:

Dr. Monita Sharma
MonitaS@PISCLtd.org.uk

Deadline: 31 October 2018

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Acknowledgements

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- BASF
- Bayer Crop Science
- Corteva (formerly DowAgro Sciences)
- Dupont
- PETA International Science Consortium
- Syngenta
- Clorox
- Colgate Palmolive
- Dial (Henkel)
- EcoLab
- JohnsonDiversey
- Procter & Gamble
- SC Johnson & Son
- The Institute for In Vitro Sciences
- The Accord Group

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