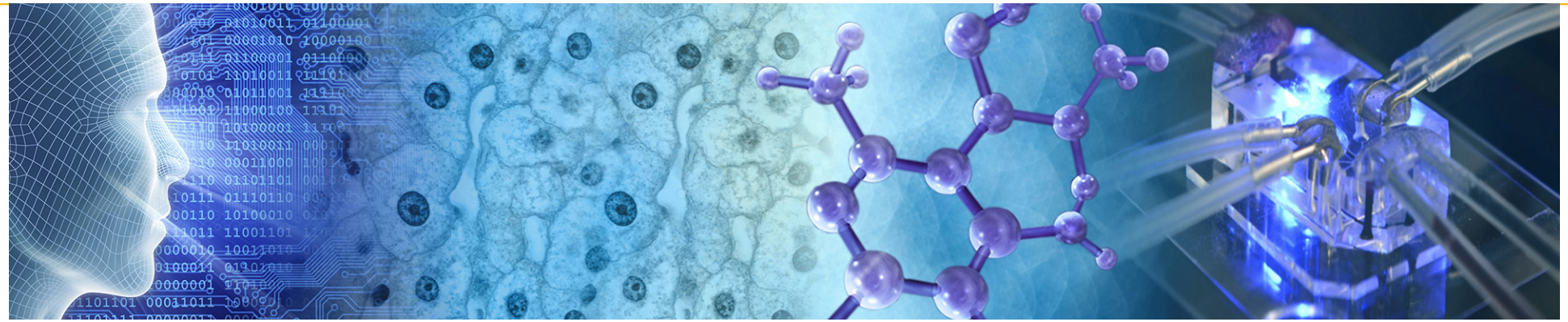
NTP
National Toxicology Program



Development of NAMs to predict acute toxicological responses

David Allen, PhD
ILS, Contractor supporting NICEATM

December 17, 2019

**ILS staff provide technical support for NICEATM, but do not represent NIEHS, NTP, or the official positions of any federal agency*

Animal Use in the 6-Pack

Endpoint	OECD Method	Estimated Animal Use
Acute oral toxicity	420 (Fixed Dose Procedure)	6-12
	423 (Acute Toxic Class)	5-12
	425 (Up-and-Down Procedure)	6-12
Acute dermal toxicity	402	8
Acute inhalation toxicity	403	Limit: 6
		Full: 30
	436 (ATC)	6-12
Skin irritation	404	1-3
Eye irritation	405	1-3
Skin sensitization	406 (Guinea pig Maximization Test)	15-30
	406 (Buehler Test)	30
	429 (Local Lymph Node Assay)	16-20
TOTAL		**36-86**

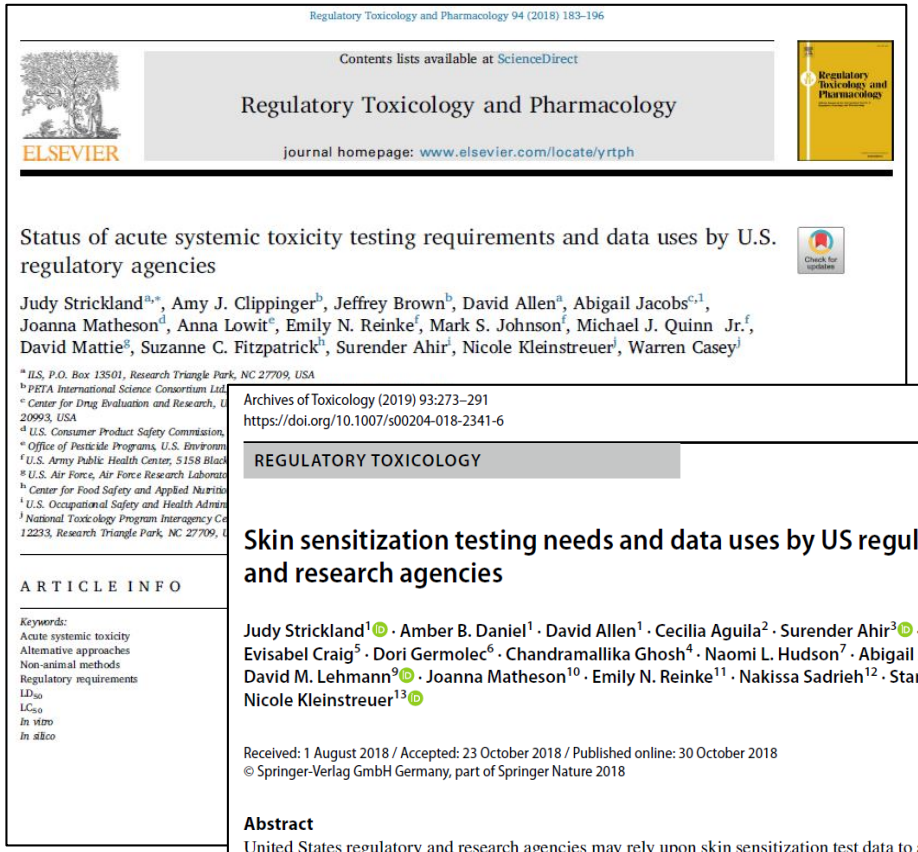
Annually Submitted Acute 6-Pack Studies – Formulations

Acute study*	2012*	2013*	2014*	2015*	2016*	2017*
Oral lethality	324	248	328	268	322	254
Dermal lethality	292	257	313	255	267	234
Inhalation lethality	264	217	248	254	270	246
Eye irritation	291	261	273	251	263	239
Skin irritation	270	254	268	258	259	238
Skin sensitization	247	237	262	267	255	240

- Much fewer studies are submitted each year for new active ingredients (n~10)
- Most animal use is for formulations and thus provides the largest opportunity for animal savings

*from EPA OPP Update - ICCVAM Public Forum 2018
https://ntp.niehs.nih.gov/iccvam/meetings/iccvam-forum-2018/08-epa-opp.pdf

Roadmap 101: Starting with the End User in Mind



Regulatory Toxicology and Pharmacology 94 (2018) 183–196

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

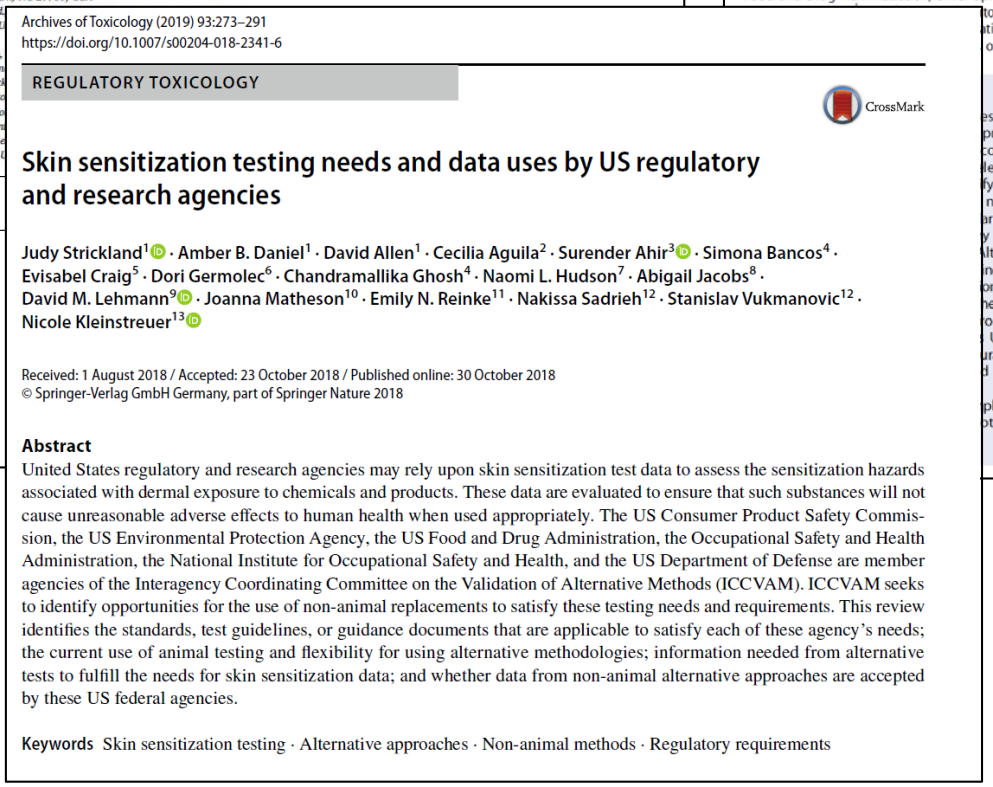
journal homepage: www.elsevier.com/locate/yrtph

Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies

Judy Strickland[a,*], Amy J. Clippinger[b], Jeffrey Brown[b], David Allen[a], Abigail Jacobs[c,1], Joanna Matheson[d], Anna Lowit[e], Emily N. Reinke[f], Mark S. Johnson[f], Michael J. Quinn Jr.[f], David Mattie[g], Suzanne C. Fitzpatrick[h], Surender Ahir[i], Nicole Kleinstreuer[j], Warren Casey[j]

ARTICLE INFO

Keywords:
Acute systemic toxicity
Alternative approaches
Non-animal methods
Regulatory requirements
LD_{50}
LC_{50}
In vivo
In silico



Archives of Toxicology (2019) 93:273–291
https://doi.org/10.1007/s00204-018-2341-6

REGULATORY TOXICOLOGY

CrossMark

Skin sensitization testing needs and data uses by US regulatory and research agencies

Judy Strickland[1] · Amber B. Daniel[1] · David Allen[1] · Cecilia Aguila[2] · Surender Ahir[3] · Simona Bancos[4] · Evisabel Craig[5] · Dori Germolec[6] · Chandramallika Ghosh[4] · Naomi L. Hudson[7] · Abigail Jacobs[8] · David M. Lehmann[9] · Joanna Matheson[10] · Emily N. Reinke[11] · Nakissa Sadrieh[12] · Stanislav Vukmanovic[12] · Nicole Kleinstreuer[13]

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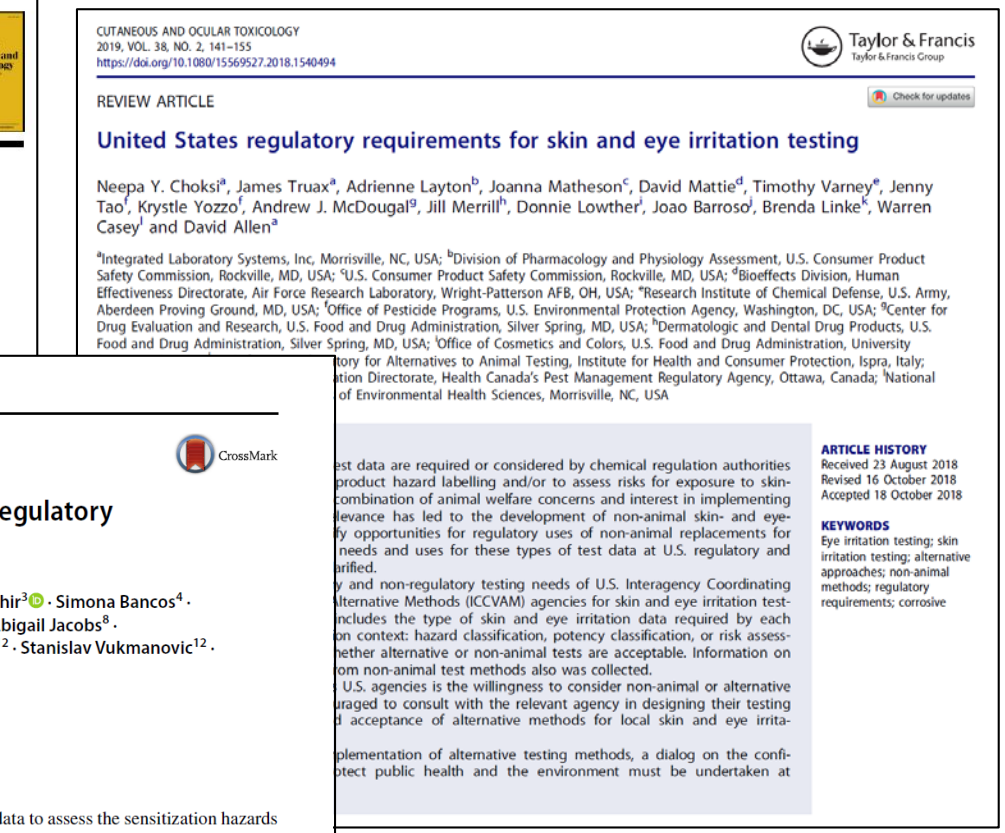
Abstract
United States regulatory and research agencies may rely upon skin sensitization test data to assess the sensitization hazards associated with dermal exposure to chemicals and products. These data are evaluated to ensure that such substances will not cause unreasonable adverse effects to human health when used appropriately. The US Consumer Product Safety Commission, the US Environmental Protection Agency, the US Food and Drug Administration, the Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, and the US Department of Defense are member agencies of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM seeks to identify opportunities for the use of non-animal replacements to satisfy these testing needs and requirements. This review identifies the standards, test guidelines, or guidance documents that are applicable to satisfy each of these agency's needs; the current use of animal testing and flexibility for using alternative methodologies; information needed from alternative tests to fulfill the needs for skin sensitization data; and whether data from non-animal alternative approaches are accepted by these US federal agencies.

Keywords Skin sensitization testing · Alternative approaches · Non-animal methods · Regulatory requirements



CUTANEOUS AND OCULAR TOXICOLOGY
2019, VOL. 38, NO. 2, 141–155
https://doi.org/10.1080/15569527.2018.1540494

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Check for updates

United States regulatory requirements for skin and eye irritation testing

Neepa Y. Choksi[a], James Truax[a], Adrienne Layton[b], Joanna Matheson[c], David Mattie[d], Timothy Varney[e], Jenny Tao[f], Krystle Yozzo[f], Andrew J. McDougal[g], Jill Merrill[h], Donnie Lowther[i], Joao Barroso[j], Brenda Linke[k], Warren Casey[l] and David Allen[a]

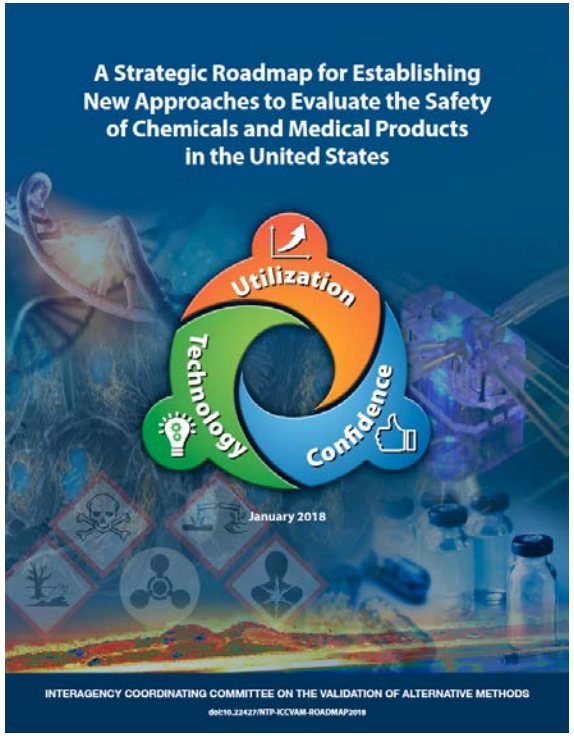
[a]Integrated Laboratory Systems, Inc, Morrisville, NC, USA; [b]Division of Pharmacology and Physiology Assessment, U.S. Consumer Product Safety Commission, Rockville, MD, USA; [c]U.S. Consumer Product Safety Commission, Rockville, MD, USA; [d]Bioeffects Division, Human Effectiveness Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH, USA; [e]Research Institute of Chemical Defense, U.S. Army, Aberdeen Proving Ground, MD, USA; [f]Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC, USA; [g]Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA; [h]Dermatologic and Dental Drug Products, U.S. Food and Drug Administration, Silver Spring, MD, USA; [i]Office of Cosmetics and Colors, U.S. Food and Drug Administration, University ...tory for Alternatives to Animal Testing, Institute for Health and Consumer Protection, Ispra, Italy; ...ation Directorate, Health Canada's Pest Management Regulatory Agency, Ottawa, Canada; [l]National ...of Environmental Health Sciences, Morrisville, NC, USA

...est data are required or considered by chemical regulation authorities ...product hazard labelling and/or to assess risks for exposure to skin- ...combination of animal welfare concerns and interest in implementing ...levance has led to the development of non-animal skin- and eye- ...ty opportunities for regulatory uses of non-animal replacements for ... needs and uses for these types of test data at U.S. regulatory and ...arified.
...y and non-regulatory testing needs of U.S. Interagency Coordinating ...lternative Methods (ICCVAM) agencies for skin and eye irritation test- ...includes the type of skin and eye irritation data required by each ...on context: hazard classification, potency classification, or risk assess- ...hether alternative or non-animal tests are acceptable. Information on ...om non-animal test methods also was collected.
... U.S. agencies is the willingness to consider non-animal or alternative ...uraged to consult with the relevant agency in designing their testing ...d acceptance of alternative methods for local skin and eye irrita-

...plementation of alternative testing methods, a dialog on the confi- ...otect public health and the environment must be undertaken at

ARTICLE HISTORY
Received 23 August 2018
Revised 16 October 2018
Accepted 18 October 2018

KEYWORDS
Eye irritation testing; skin irritation testing; alternative approaches; non-animal methods; regulatory requirements; corrosive



- Identifies requirements, needs, and decision contexts for each endpoint

Waiving Acute Dermal Toxicity Testing

Table 2. Results of comparison analysis for oral & dermal formulation acute studies				
Rat Dermal Hazard Category (mg/kg)	Rat Oral Hazard Category (mg/kg)			
	EPA I ≤50	EPA II >50 – ≤500	EPA III >500 – ≤5000	EPA IV >5000
EPA I ≤200	1	0	0	0
EPA II >200 – ≤2000	0	2	2	0
EPA III >2000 – ≤5000	0	23	133	28
EPA IV >5000	0	28	173	203
Total	**1**	**53**	**308**	**231**

EPA Guidance on Waiving Dermal Toxicity

US Environmental Protection Agency
Office of Pesticide Programs

Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis

November 9, 2016

3.0 Waiver Guidance.

The agency believes this retrospective analysis fully supports the conclusion that waivers may be granted for acute dermal toxicity studies for formulated pesticide products. Applicants should submit formal waiver requests as part of their registration application through existing processes[7]. Waiver requests should contain all relevant information to support the waiver (e.g., acute oral LD_{50} and dermal irritation study data) and cite this guidance.

https://www.epa.gov/sites/production/files/2016-11/documents/acute-dermal-toxicity-pesticide-formulations_0.pdf

Additivity Calculation for Agrochemical Formulations

The acute toxicity estimate (ATE) of ingredients should be considered as follows:

- Include ingredients present at 1% or greater with a known acute toxicity, which fall into any of the GHS acute toxicity categories.
- Ignore ingredients that are presumed not acutely toxic (e.g., water, sugar).
- Ignore ingredients if the oral limit test does not show acute toxicity at 2,000 mg/kg/body weight.

The ATE of the mixture is determined by calculation from the ATE values for all relevant ingredients according to the following formula below for Oral, Dermal or Inhalation Toxicity:

$$\frac{100}{ATE_{mix}} = \sum_{\eta} \frac{C_i}{ATE_i}$$

where:
C_i= concentration of ingredient i
n ingredients and i is running from 1 to n
ATE_i = Acute Toxicity Estimate of ingredient I

EPA Categories

I (≤ 50 mg/kg)
II (>50 ≤ 500 mg/kg)
III (>500 ≤ 5000 mg/kg)
IV (>5000 mg/kg)

	Van Cott et al. 2018 - Additivity				
In vivo	I	II	III	IV	Total
I	0	1	0	0	1
II	0	12	42	19	73
III	0	7	69	45	121
IV	0	0	5	10	15
Total	0	20	116	74	210

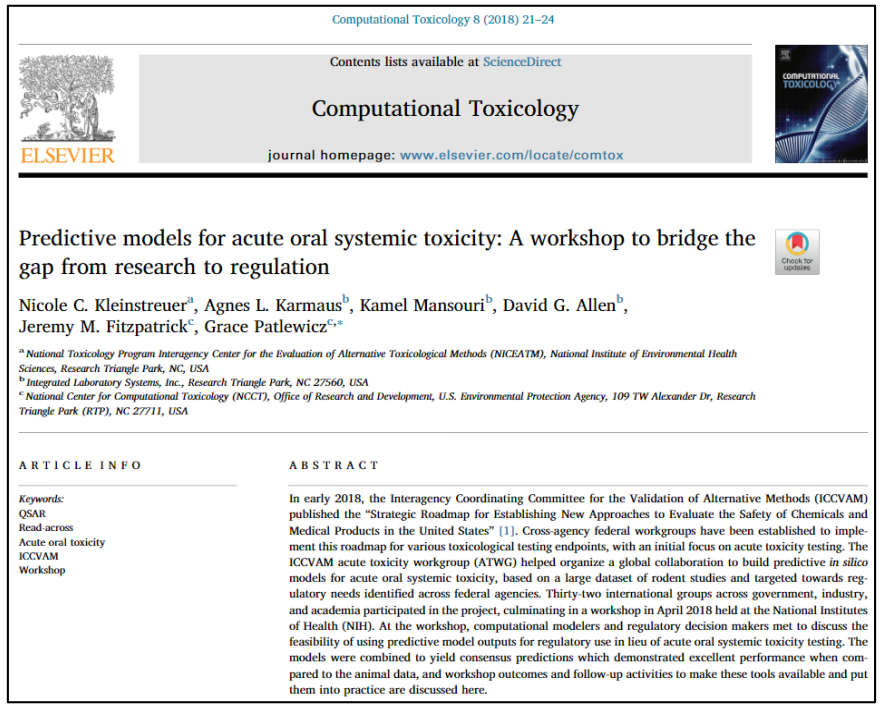
Correct classification: 43% (91/210)
Over classification: 6% (12/210)
Under classification: 51% (107/210)

	Corvaro et al. 2016 - Additivity				
In vivo	I	II	III	IV	Total
I	0	0	0	0	0
II	0	6	9	0	15
III	0	1	51	30	82
IV	0	1	9	92	102
Total	0	8	69	122	199

Correct classification: 75% (149/199)
Over classification: 6% (11/199)
Under classification: 19% (39/199)

- EPA pilot program: GHS Mixtures Equation Pilot
  - OPP has been accepting submissions of acute toxicity data paired with calculations to support evaluations of pesticide product formulations
  - Includes conventional pesticides and antimicrobial cleaning product
  - NICEATM data analyses ongoing and will compare to the trends seen above

ICCVAM Workshop on Acute Oral Toxicity Modeling



Computational Toxicology 8 (2018) 21–24

Contents lists available at ScienceDirect

Computational Toxicology

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

Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation

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[b] Integrated Laboratory Systems, Inc., Research Triangle Park, NC 27560, USA
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ARTICLE INFO

Keywords:
QSAR
Read-across
Acute oral toxicity
ICCVAM
Workshop

ABSTRACT

In early 2018, the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) published the "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" [1]. Cross-agency federal workgroups have been established to implement this roadmap for various toxicological testing endpoints, with an initial focus on acute toxicity testing. The ICCVAM acute toxicity workgroup (ATWG) helped organize a global collaboration to build predictive *in silico* models for acute oral systemic toxicity, based on a large dataset of rodent studies and targeted towards regulatory needs identified across federal agencies. Thirty-two international groups across government, industry, and academia participated in the project, culminating in a workshop in April 2018 held at the National Institutes of Health (NIH). At the workshop, computational modelers and regulatory decision makers met to discuss the feasibility of using predictive model outputs for regulatory use in lieu of acute oral systemic toxicity testing. The models were combined to yield consensus predictions which demonstrated excellent performance when compared to the animal data, and workshop outcomes and follow-up activities to make these tools available and put them into practice are discussed here.

Binary Models

Hazard

- Highly toxic (≤50 mg/kg)
- Toxic (>50-5000 mg/kg)

+ Nontoxic (>2000 mg/kg)

Continuous Model

Point estimates of LD50 values

Categorical Models

EPA Categories

Hazard

- I (≤ 50 mg/kg)
- II (>50 ≤ 500 mg/kg)
- III (>500 ≤ 5000 mg/kg)
- IV (>5000 mg/kg)

Packing Group

GHS Categories

- I (≤ 5 mg/kg)
- II (>5 ≤ 50 mg/kg)
- III (>50 ≤ 300 mg/kg)
- IV (>300 ≤ 2000 mg/kg)
- NC (> 2000 mg/kg)

Hazard

Hazard

- April 11-12, 2018 at NIH
- Scientists were invited to submit in silico models that use chemical structure information to predict LD50 values and hazard categories
  - Largest set of curated LD50 data ever assembled: ~21,000 LD50 values for ~15,000 chemicals
  - 139 Models, 35 Groups (Academic, Industry, Govt), 8 Countries
  - Attendance: 90 in-person, 170 Webcast
  - Consensus model developed

Acute Oral Lethality Consensus Modeling

CATMoS: **C**ollaborative **A**cute **T**oxicity **Mo**deling **S**uite

Initial models & predictions

- VT (32 models)
- NT (33 models)
- GHS (23 models)
- EPA (26 models)
- LD50 (25 models)

Combining models

Step 1

Weighted average /majority rule

Independent consensus models/predictions

- VT
- NT
- GHS
- EPA
- LD50

A consensus model per endpoint (~20-~30 models)

Weight of Evidence approach (WoE)

Step 2

Majority rule

Consistent consensus models/predictions

- VT
- NT
- GHS
- EPA
- LD50

Consensus representing all ~140 models

- Consensus models for individual chemicals leverage the strengths of each contributing in silico model and their overall predictions

CATMoS Performance Assessment

	Very Toxic		Non-Toxic		EPA		GHS	
	Train	Eval	Train	Eval	Train	Eval	Train	Eval
Sensitivity	0.87	0.70	0.88	0.67	0.81	0.62	0.80	0.58
Specificity	0.99	0.97	0.97	0.90	0.92	0.86	0.95	0.90
Balanced Accuracy	0.93	0.84	0.92	0.78	0.87	0.74	0.88	0.74
***In vivo* Balanced Accuracy**	0.81		0.89		0.82		0.79	

	LD50 values		LD50 values
	Train	Eval	***In Vivo***
R2	0.85	0.65	0.80
RMSE	0.30	0.49	0.42

- The consensus predictions perform just as well as replicate *in vivo* data do at predicting oral acute toxicity outcome

Acute Systemic Lethality: Mixtures and Mechanisms

Workshop

SHARE THIS:
https://ntp.niehs.nih.gov/go/atwksp-2019

Mind the Gaps: Prioritizing Activities to Meet Regulatory Needs for Acute Systemic Lethality

October 30-31, 2019
Porter Neuroscience Research Center
National Institutes of Health
Bethesda, Maryland, USA

- Co-organized by PCRM and NICEATM
- Participants included stakeholders from government, industry, NGOs
- Discussion topics included:
  - Estimating the LD50 of a chemical mixture/formulated product
  - Identifying gaps where model (or assay) development or optimization is needed
  - Pinpointing the types of mechanistic information that would be useful
  - Establishing the feasibility of using artificial intelligence in model development

Workshop Follow-up Activities

- Analysis of in vivo test variability – need it to establish confidence
  - Ideally focus the analysis on guideline-like studies (or in comparison to an overall analysis)
- Additivity – EPA-OPP pilot + existing publications
  - Can we identify non-toxics without in vivo testing?
- Explore adding biological/mechanistic information to complement in silico predictions
  - Critical to include metabolism
- Consider AOPs to organize available information (and identify where information gaps exist)
  - NOTE: can be very simple and don't require lengthy process
- **Critical to it all: transparency and training**

Workshop on Acute Inhalation Lethality

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report

Amy J. Clippinger[a,*], David Allen[b], Annie M. Jarabek[c], Marco Corvaro[d], Marianna Gaça[e], Sean Gehen[f], Jon A. Hotchkiss[g], Grace Patlewicz[h], Jodie Melbourne[a], Paul Hinderliter[i], Miyoung Yoon[j], Dongeun Huh[k], Anna Lowit[l], Barbara Buckley[c], Michael Bartels[m], Kelly BéruBé[n], Daniel M. Wilson[g], Ian Indans[o], Mathieu Vinken[p]

Toxicology in Vitro 52 (2018) 131–145

Contents lists available at ScienceDirect

Toxicology in Vitro

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

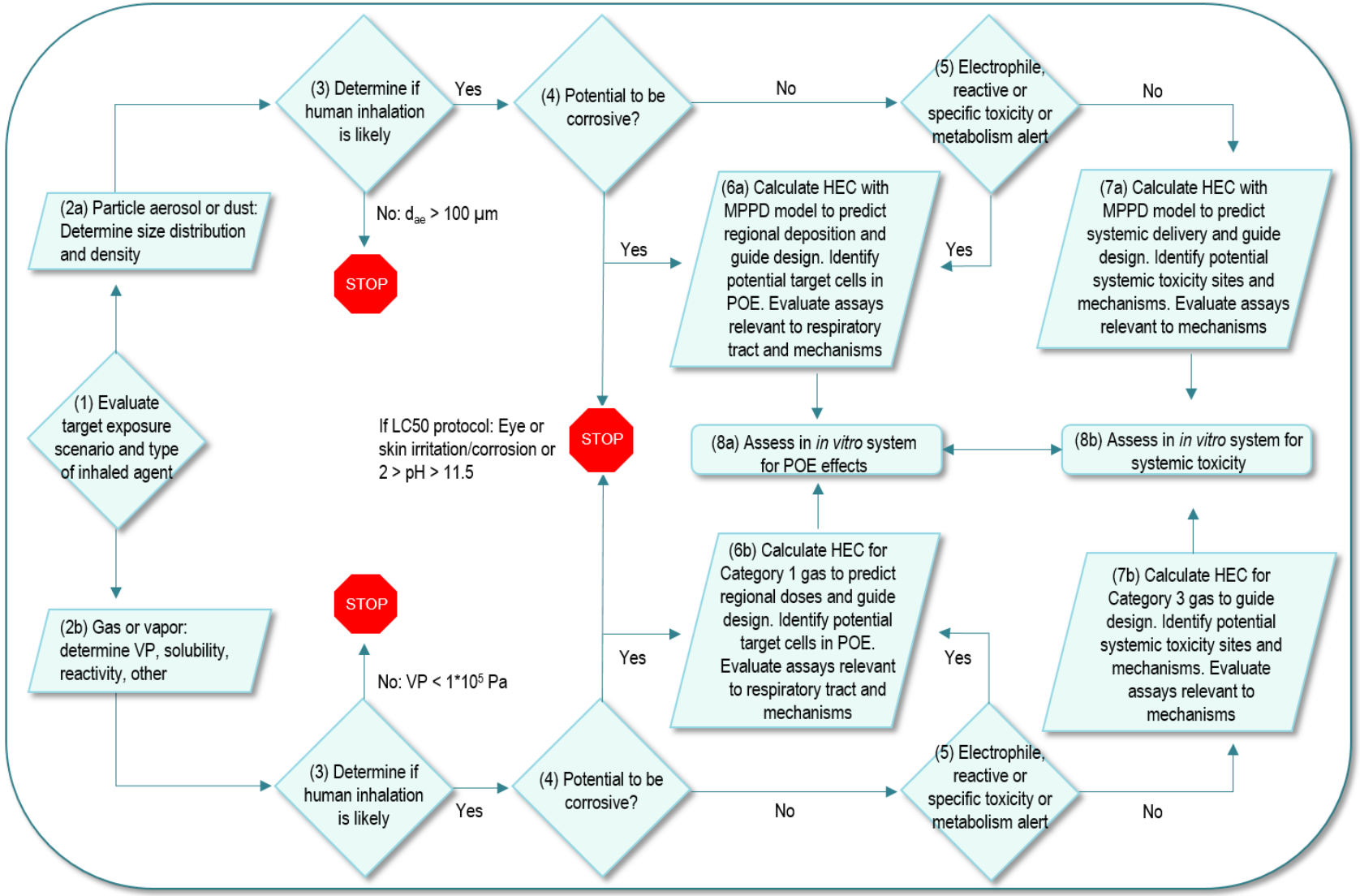
Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger[a,*], David Allen[b], Holger Behrsing[c], Kelly A. BéruBé[d], Michael B. Bolger[e], Warren Casey[f], Michael DeLorme[g], Marianna Gaça[h], Sean C. Gehen[i], Kyle Glover[j], Patrick Hayden[k], Paul Hinderliter[l], Jon A. Hotchkiss[m], Anita Iskandar[n], Brian Keyser[o], Karsta Luettich[n], Lan Ma-Hock[p], Anna G. Maione[k], Patrudu Makena[o], Jodie Melbourne[a], Lawrence Milchak[g], Sheung P. Ng[q], Alicia Paini[r], Kathryn Page[s], Grace Patlewicz[t], Pilar Prieto[r], Hans Raabe[c], Emily N. Reinke[u], Clive Roper[v], Jane Rose[w], Monita Sharma[a], Wayne Spoo[o], Peter S. Thorne[x], Daniel M. Wilson[m], Annie M. Jarabek[y]

- NIH, Porter Neuroscience Center
- Co-organized by the PETA International Science Consortium and NICEATM

Designing a Non-Animal Testing Approach



Clippinger, et al. *Toxicol In Vitro*. 2018;52:131-145

Adverse Outcome Pathways – Acute Exposures to Inhaled Materials

Target Site Exposure	Molecular Initiating Events	Cellular Key Events	Tissue / Organ Key Events	Organism / Population Responses

• Solubility
• Vapor pressure
• Particle size, density, distribution
• Chemical reactivity
	• Oxidation of cellular molecules
	• Acetylcholinesterase inhibition
	• Cytochrome C oxidase inhibition
	• DNA/protein alkylation
	• Modulation of ion channels
	• Receptor binding e.g.,
	  • *Activation of EGFR (via phosphorylation)*
	  • *Activation of TRPA1 receptor*
	  • *Activation of glucocorticoid receptor*
	  • *Activation/inhibition of G protein coupled receptors*
	  • *Inhibition of muscarinic acetylcholine receptors*
	  • *Inhibition of NMDA receptors*
	  • *Binding to hormone receptor*
		• ROS formation
		• Antioxidant (e.g., glutathione) depletion
		• Inhibition of energy (ATP) production
		• Cytotoxicity
		• Collagen deposition
		• Increased mucous production
		• Cytoskeleton disruption
		• Cytokine/chemokine production
		• Surfactant depletion
		• Modulation of signal transduction pathways
		• Inhibition of nucleotide synthesis
		• Protein modification
		• Modulation of protein synthesis
		• Effects on the blood
		• Vitamin interference
			• Cell proliferation
			• Inflammatory response
			• Cell transformation
			• Squamous cell metaplasia
			• Loss of epithelial barrier function
			• Reduced ciliary beat frequency
			• Goblet (mucous) cell hyperplasia, metaplasia, and proliferation
			• Respiratory failure
			• Tracheitis
			• Bronchiolitis
			• Alveolitis
			• Pulmonary edema
			• Bronchoconstriction
			• Alveolar distention
			• Smooth muscle remodeling
			• Change in lung mechanics (resistance, compliance, pressure-volume curves, FEV1)
				• Systemic toxicity
				• Acute lethality
				• Target organ effects (e.g., hepatotoxicity)
				• Airway hyperreactivity
				• Chemical narcosis

Clippinger et al. (2018)



BASF
The Chemical Company

RAI
Reynolds American

HSE
Health and Safety Executive

Penn
University of Pennsylvania

NTP
National Toxicology Program

British American Tobacco

Cardiff University
Prifysgol Caerdydd

ULB
Université Libre de Bruxelles

SciToVation
Innovative Cell Based Science

Universitas Friburgensis
Uni Freiburg

charles river

Corteva
agriscience

TNO
innovation for life

The University of Iowa

PETA International Science Consortium Ltd.

DuPont

The Clorox Company

SimulationsPlus
Science + Software = Success

EURL
ECVAM
European Union Reference Laboratory
for Alternatives to Animal Testing

U.S.
Dept of
Defense

MatTek
Corporation

IIVS
Institute for In Vitro Sciences
Advancing Science & Animal Welfare Together

Cultex
Laboratories

VITROCELL
SYSTEMS

Fraunhofer
ITEM

ARA

Dow

PM
Philip Morris International

Forskningscenter for Arbejdsmiljø

National Research Centre
for the Working Environment

MultiCASE

Unilever

syngenta

United States
Environmental Protection Agency

ToxStrategies

Epithelix


Health
Canada

P&G

3M

Luxembourg
Institute
of Science
and Technology
LIST

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AlveoliX
In-vitro models inspired by nature

Recent and Ongoing Efforts for Acute Exposures to Inhaled Materials

Toxicology in Vitro 58 (2019) 245–255

Contents lists available at ScienceDirect

Toxicology in Vitro

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

Validation of the CULTEX® Radial Flow System for the assessment of the acute inhalation toxicity of airborne particles

Amelie Tsoutsoulopoulos[a,*], Katrin Gohlsch[b], Niklas Möhle[c], Andreas Breit[b], Sebastian Hoffmann[e], Olaf Krischenowski[c,d], Harald Mückter[b], Thomas Gudermann[b], Horst Thiermann[a], Michaela Aufderheide[c,d], Dirk Steinritz[a,b]

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[c] Cultex® Laboratories GmbH, Hannover, Germany
[d] Cultex® Technology GmbH (formerly Cultex® Laboratories GmbH), Hannover, Germany
[e] seh consulting + services, Paderborn, Germany

ARTICLE INFO

Keywords:
CULTEX® RFS
Acute inhalation toxicity
In vitro
Air-liquid interface
Validation

ABSTRACT

The CULTEX® Radial Flow System (F
airborne particles at the air-liquid int
general applicability of the CULTEX®
results, the methodology was optimi
established. Cell viability of A549 cell
independent laboratories. Cytotoxicit
used as an indicator of toxicity. Subst
decreased below 50% (prediction mo
100 µg/cm²). Results were then comp
with a specificity of 83% and a sens
between-laboratory reproducibility ra
In summary, the CULTEX® RFS w
method for the qualitative assessmen

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2018.0004

Prevalidation of an Acute Inhalation Toxicity Test Using the EpiAirway *In Vitro* Human Airway Model

George R. Jackson, Jr., Anna G. Maione, Mitchell Klausner, and Patrick J. Hayden

Abstract

Introduction: Knowledge of acute inhalation toxicity potential is important for establishing safe use of chemicals and consumer products. Inhalation toxicity testing and classification procedures currently accepted within worldwide government regulatory systems rely primarily on tests conducted in animals. The goal of the current work was to develop and prevalidate a nonanimal (*in vitro*) test for determining acute inhalation toxicity using the EpiAirway™ *in vitro* human airway model as a potential alternative for currently accepted animal tests.
Materials and Methods: The *in vitro* test method exposes EpiAirway tissues to test chemicals for 3 hours, followed by measurement of tissue viability as the test endpoint. Fifty-nine chemicals covering a broad range of toxicity classes, chemical structures, and physical properties were evaluated. The *in vitro* toxicity data were utilized to establish a prediction model to classify the chemicals into categories corresponding to the currently accepted Globally Harmonized System (GHS) and the Environmental Protection Agency (EPA) system.
Results: The EpiAirway prediction model identified *in vivo* rat-based GHS Acute Inhalation Toxicity Category 1–2 and EPA Acute Inhalation Toxicity Category I–II chemicals with 100% sensitivity and specificity of 43.1% and 50.0%, for GHS and EPA acute inhalation toxicity systems, respectively. The sensitivity and specificity of the EpiAirway prediction model for identifying GHS specific target organ toxicity-single exposure (STOT-SE) Category 1 human toxicants were 75.0% and 56.5%, respectively. Corrosivity and electrophilic and oxidative reactivity appear to be the predominant mechanisms of toxicity for the most highly toxic chemicals.
Conclusions: These results indicate that the EpiAirway test is a promising alternative to the currently accepted animal tests for acute inhalation toxicity.

Toxicology Letters 316 (2019) 119–126

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

Exposure of 19 substances to lung A549 cells at the air liquid interface or under submerged conditions reveals high correlation between cytotoxicity *in vitro* and CLP classifications for acute lung toxicity

Katrin Gohlsch[a], Harald Mückter[a], Dirk Steinritz[a,b], Michaela Aufderheide[c], Sebastian Hoffmann[d], Thomas Gudermann[a], Andreas Breit[a,*]

[a] Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University, Munich, Germany
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[c] Cultex®Technology GmbH, Hannover, Germany
[d] SEH Consulting + Services, Paderborn, Germany

NFO

ABSTRACT

In vivo experiments are still widely used for the testing of lung toxicity but there is an ethical and legal obligation to replace, reduce and refine animal testing. Lung A549 cells could serve as an *in vitro* indicator for acute lung toxicity but little data about the correlation of the cytotoxicity in A549 cells and data leading to CLP classifications are available. We exposed A549 cells to 19 CLP-classified substances with doses of 25, 50, and 100 µg/cm² either under submerged (SME) condition or with aerosols at the air-liquid interface (ALIF) and determined accuracy, precision, sensitivity and the F1 score with the CLP classifications H330, H332, or H335. When data from both exposure methods were combined, we found accuracies of 0.84 ± 0.05, precisions of 0.74 ± 0.1, sensitivities of 0.93 ± 0.08 and F1 scores of 0.82 ± 0.04. Separated from each other, ALIF exposure was more sensitive at any dose but, at higher doses, also less accurate and precise compared to SME. Considering the 19 substances tested, our data suggest that cytotoxicity in A549 cells could be a reliable *in vitro* indicator for *in vivo* toxicity. Thus, we discuss how A549 could be integrated into validation test guidelines.

PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.

- Webinar Series (n=21)
- IATAs, In silico models, In vitro systems
- www.piscltd.org.uk/inhalation-webinars

Inhalation Lethality: In Search of a Modeling Dataset

- Preliminary inventory pulled from eChemPortal and EPA sources
  - Approximately 1500 CASRN; but not all have structure information (i.e., defined chemical)
  - Appears weighted towards less toxic classifications
  - Hoping for additional data from ECHA
- But…
  - Curation and clean up critical
  - Both electronic and manual
  - Can’t eliminate expert judgement
  - Transparency

Acute Inhalation Toxicity Data – 5 orders of magnitude difference?

CASRN	LC50	LC50 unit	source
79-11-8	1268	mg/L	eChemPortal
79-11-8	0.18	mg/L	ChemIDplus

Acute Inhalation Toxicity Data – Units matter…

Results and discussion

Effect levels

Sex:	male/female
Dose descriptor:	LC50
Effect level:	> 1 268 mg/L air (analytical)
Based on:	test mat.
Exp. duration:	4 h

Acute Toxicity: inhalation

Currently viewing: 001 Key | Experimental result

Administrative data | Data source | Materials and methods | Results and discussion | Ap

Duration of exposure:	ca. 4 h
Concentrations:	512 (± 150) mg/m3 and 1268 (± 77) mg/m3
No. of animals per sex per dose:	5 animals per sex per dose

CASRN	LC50	LC50 unit	source
79-11-8	1268	mg/L	eChemPortal
79-11-8	0.18	mg/L	ChemIDplus

NIH U.S. National Library of Medicine | TOXNET TOXICOLOGY DATA NETWORK

Help | FAQs | TOXNET Fact Sheet | Training Manual & Schedule

TOXNET > ChemIDplus > Substance

ChemIDplus
A TOXNET DATABASE
Lite • Browse • Advanced

Registry Number | equals | 79-11-8 | Search

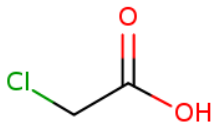
Download | Start New Query | Modify Query | Search History | Switch to Summary View

Substance Name: Chloroacetic acid [BSI:ISO]
RN: 79-11-8
UNII: 5GD84Y125G
InChIKey: FOCAUTSVDIKZOP-UHFFFAOYSA-N

Note
Urinary metabolite of vinyl chloride.

Molecular Formula
C2-H3-Cl-O2

Molecular Weight
94.4967



3D

All | Classifications | Links to Resources | Names & Synonyms | Registry Numbers | Structure Descriptors | Toxicity | Physical Properties

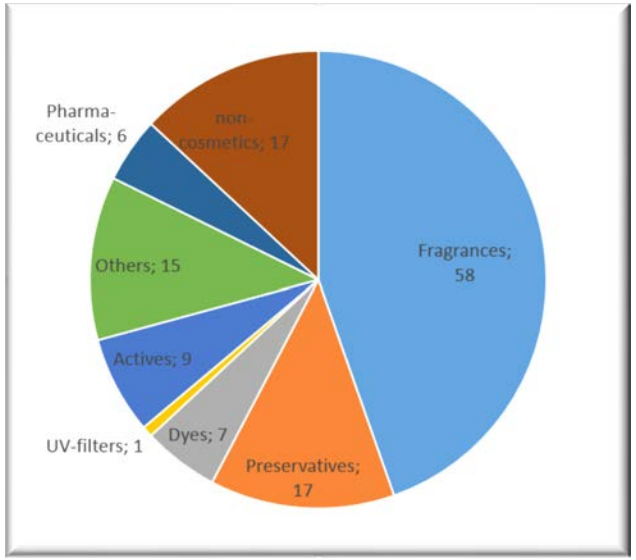
Toxicity

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Effect	Source
mouse	LD50	subcutaneous	250mg/kg (250mg/kg)		Archives Internationales de Pharmacodynamie et de Therapie. Vol. 116, Pg. 154, 1958.
rat	LC50	inhalation	180mg/m3 (180mg/m3)		Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. Vol. 18(9), Pg. 32, 1974.
rat	LD50	intraperitoneal	16600ug/kg (16.6mg/kg)		Russian Pharmacology and Toxicology Vol. 41, Pg. 113, 1978.
rat	LD50	oral	55mg/kg (55mg/kg)		Gigiena Truda i Professional'nye Zabolevaniya. Labor Hygiene and Occupational Diseases. Vol. 18(9), Pg. 32, 1974.
rat	LD50	subcutaneous	5mg/kg (5mg/kg)		Toxicology and Applied Pharmacology. Vol. 22, Pg. 303, 1972.

LC50 = 1.268 and 0.18 mg/L instead??

Global Skin Sensitization Project

- Objective: analysis of available non-animal defined approaches (DAs)
- Collaboration with Cosmetics Europe
  - 128 substance dataset
  - LLNA (mouse) and human data
  - Curation/generation of
    - *in vitro* cell-based data that maps to AOP
    - *in silico* computer predictions, chemical structural features & properties



Spectrum of 128 substances

- Analyze non-animal DAs in an open source and transparent way
- Evaluated performance against the LLNA and human hazard/potency categories
- All DAs analyzed had equivalent or superior performance to the LLNA when compared to human data

Kleinstreuer et al. 2018 Crit Rev Tox

ICCVAM Skin Sensitization Models

Research article

Journal of Applied Toxicology

Received: 13 October 2016, Revised: 26 October 2016, Accepted: 1 November 2016 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3424

Prediction of skin sensitization potency using machine learning approaches

Qingda Zang[a], Michael Paris[a], David M. Lehmann[b], Shannon Bell[a], Nicole Kleinstreuer[ ] Warren Casey[c] and

ABSTRACT: The replacement of agencies that use data from such out using animal data have been classified into potency categorie node assay (LLNA) and human o

Research article

Journal of Applied Toxicology

Received: 16 February 2016, Revised: 21 June 2016, Accepted: 21 June 2016 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3366

Multivariate models for prediction of human skin sensitization hazard

Judy Strickland[a]*, Qingda Zang[a], Michael Paris[a], David M. Lehmann[b], David Allen[a], Neepa Choksi[a], Joanna Matheson[d], Abigail Jacobs[e], Warren Casey[c] and Nicole Kleinstreuer[c]

ABSTRACT: One of the Interagency Coordinating Committee on the V the development and evaluation of non-animal approaches to ident events necessary to produce skin sensitization suggests that no single imal tests. ICCVAM is evaluating an integrated approach to testing a

Research article

Journal of Applied Toxicology

Received: 9 October 2015, Revised: 10 November 2015, Accepted: 2 December 2015 Published online in Wiley Online Library: 6 February 2016

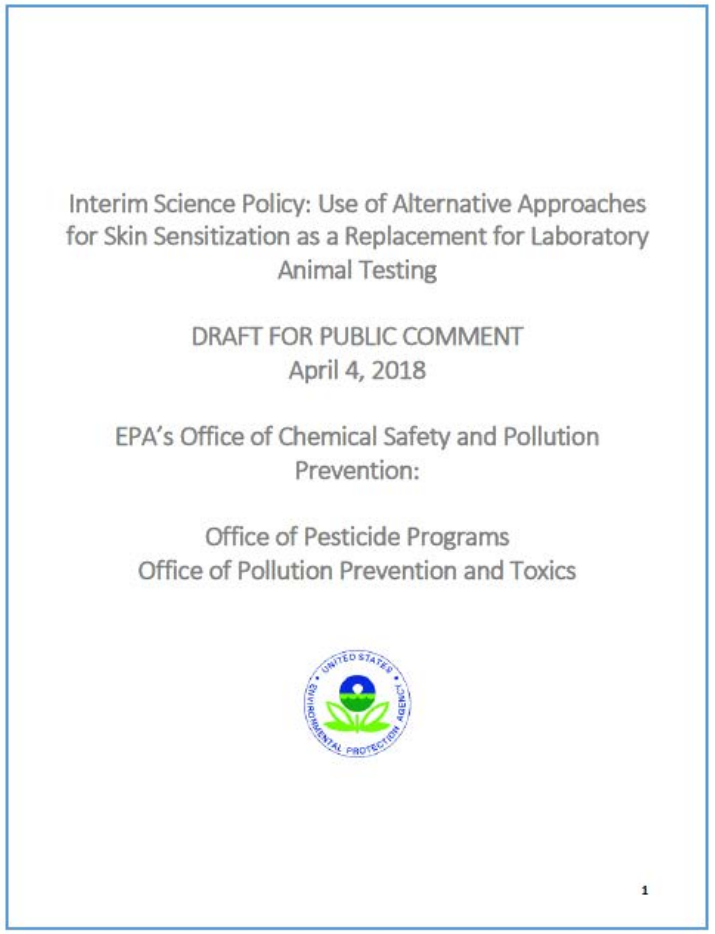
(wileyonlinelibrary.com) DOI 10.1002/jat.3281

Integrated decision strategies for skin sensitization hazard

Judy Strickland[a], Qingda Zang[a], Nicole Kleinstreuer[a], Michael Paris[a], David M. Lehmann[b], Neepa Choksi[a], Joanna Matheson[c], Abigail Jacobs[d], Anna Lowit[e], David Allen[a] and Warren Casey[f]*

ABSTRACT: One of the top priorities of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal alternatives for skin sensitization testing. Although skin sensitization is a complex process, the key biological events of the process have been well characterized in an adverse outcome pathway (AOP) proposed by the Organisation for Economic Co-operation and Development (OECD). Accordingly, ICCVAM is working to develop

Implementation of Alternative Approaches

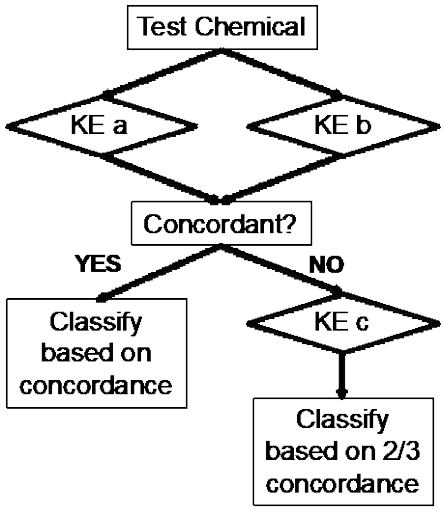

Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

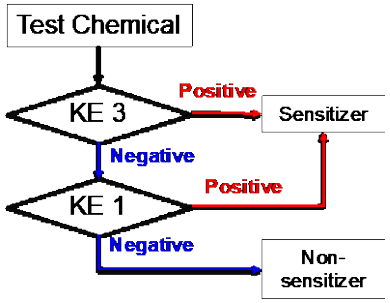
DRAFT FOR PUBLIC COMMENT
April 4, 2018

EPA's Office of Chemical Safety and Pollution Prevention:

Office of Pesticide Programs
Office of Pollution Prevention and Toxics

1

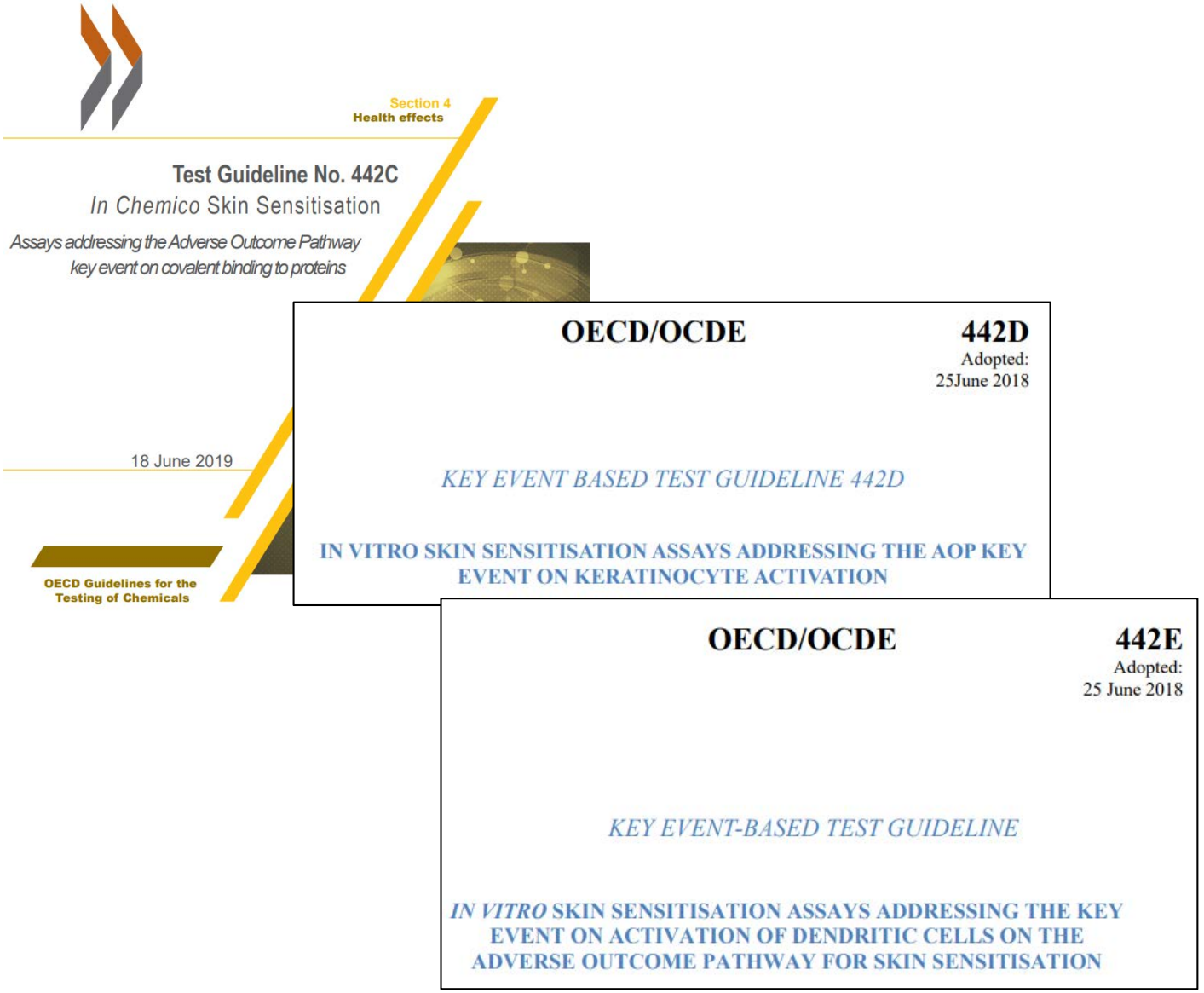




- Accepted by EPA based on comparison to LLNA data
- Used animal data reproducibility as threshold for performance

Expanding Coverage of Chemical Space

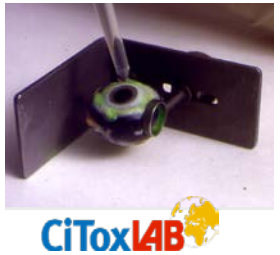
- NTP is supporting testing of a broad range of chemicals internationally adopted test methods: DPRA, KeratinoSens, hCLAT
  - Pesticide actives, agrochemical formulations, dermal excipients, personal care product ingredients, “challenge” chemicals
- Chemical nominations from multiple agencies
  - EPA OPP, OPPT, and ORD
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - NTP
- Testing began in late 2017 and will be completed in 2020



Prospective Testing: Agchems and Eye Irritation

BCOP

ICE



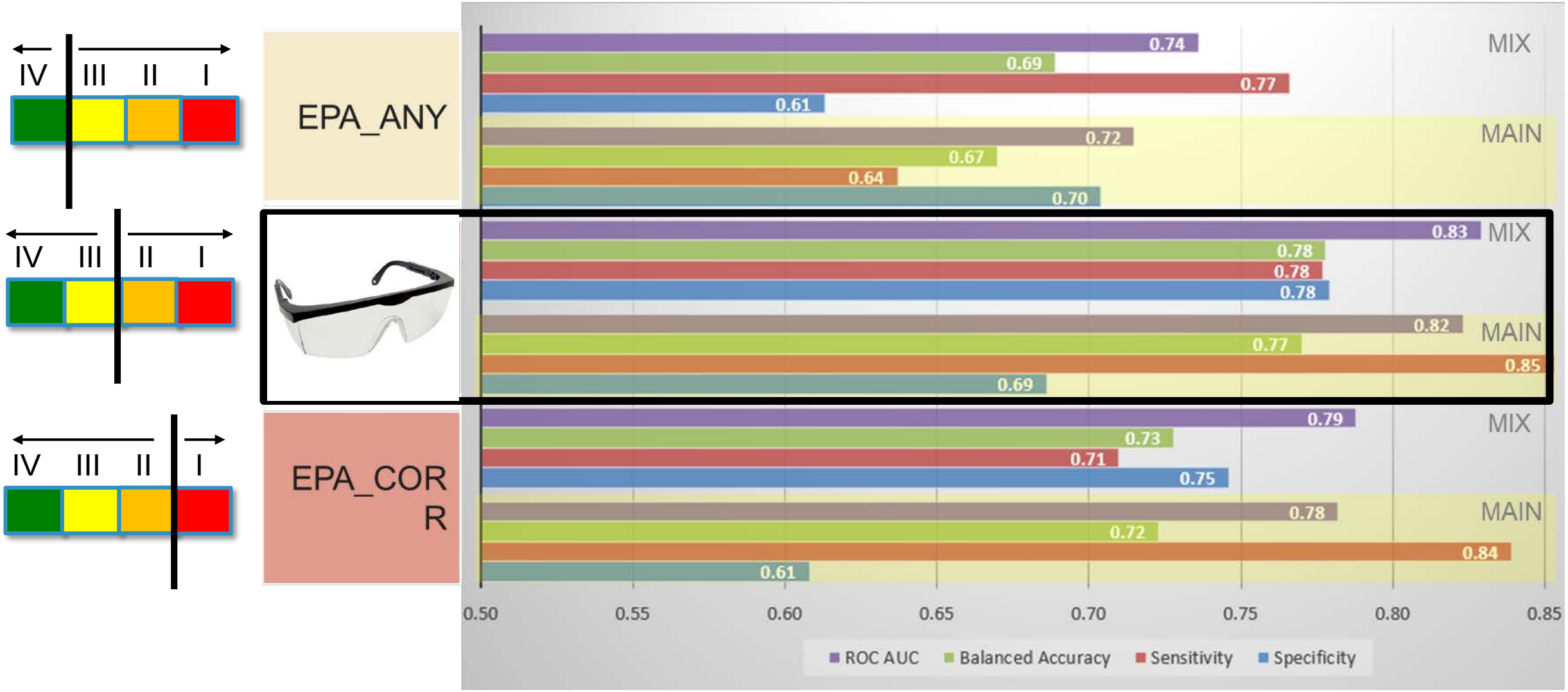
EpiOcular

NRR

PorCORA

- N=16 formulations (donated by companies) tested to date; No single test method correctly identified all 16 relative to their in vivo classifications.
- Combining results of multiple tests in an integrated approach may be useful in correct classification
- Results based on binary classification also explored
- Co-organized by NICEATM and the PETA International Science Consortium, with stakeholders from ICCVAM, EURL ECVAM, PMRA, and industry

Ocular QSAR Performance – Binary Models



Sedykh et al. SOT 2019

Skin Irritation: Private-Public Partnership

- Optimization of 3D skin model for testing agrochemicals and antimicrobial cleaning products (AMCPs)
- Companies donated agrochemical formulations and AMCPs
- Protocol optimization studies conducted at IIVS
- Regular stakeholder teleconferences to discuss updates, data needs, etc.
  - PISC, PCRM
  - EPA and NTP
  - Industry

Skin Data

Company	# Formulations
Church & Dwight	1
Clorox	9
Colgate	1
Ecolab	36
P&G	8
SCJ	10
Total	**65**

OECD Guidelines for Skin and Eye Irritation Testing

OECD/OCDE **430** Adopted: 28 July 2015

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

***In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)**

OECD/OCDE **435** Adopted: 28 July 2015

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

***In Vitro* Membrane Barrier Test Method for Skin Corrosion**

OECD/OCDE **491** Adopted: 25 June 2018

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

OECD/OCDE **438** Adopted: 25 June 2018

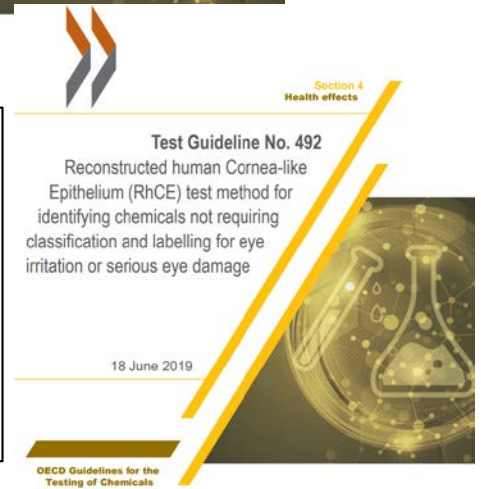
OECD GUIDELINE FOR THE TESTING OF CHEMICALS

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

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



Skin and Eye Irritation Analyses – A Way Forward

- Expanded and refined variability analyses of the rabbit tests using available data from repeat tests.
  - Will provide a metric for comparison
  - Will largely rely on ECHA data
  - Conditional probabilities assessment
- Continue to compile available data that can be used to develop a reference chemical set for prospective testing of alternative methods
  - Data requests to industry
- Establish human relevance
- EPA OPP-led regular stakeholder calls to provide updates and request input

Prior type	1	2A	2B	NC	Total
1	73%	16.1%	0.4%	10.4%	46
2A	4.2%	32.9%	3.5%	59.4%	138
2B	0.2%	4%	15.5%	80.2%	86
NC	1.1%	3.5%	1.5%	93.9%	400

Acute 6-Pack Status of Alternative Approaches

Dermal lethality	• Waiver guidance available
Oral lethality	• In silico approaches for single chemicals; additivity for formulations under consideration
Inhalation lethality	• 3D models being evaluated; LC50 database for model development being built
Eye irritation	• NAMs for Cat I and/or Cat IV? (TG 437, 438, 460, 491, 492, 494)
Skin irritation	• NAMs for Cat I or Cat IV? (TG 430, 431, 435, 439)
Skin sensitization	• Science policy for using DAs

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  - Alex Sedykh
  - Jason Phillips
  - Ruchir Shah

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