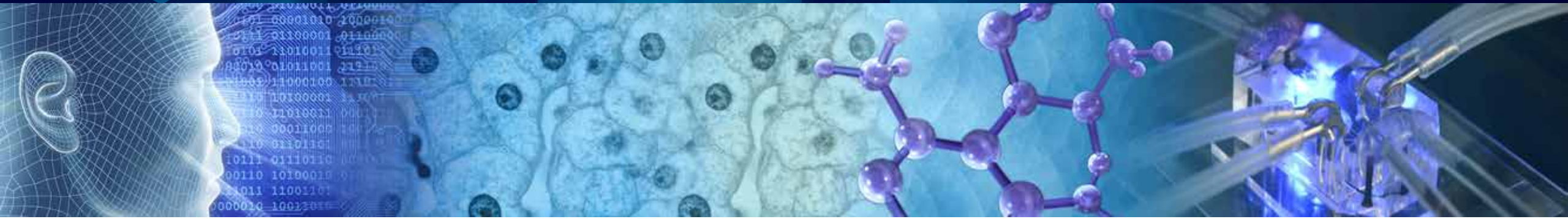
National Institute of Environmental Health Sciences
Division of Translational Toxicology



Roadmap Implementation Plans: Update on Each of the 6-pack Endpoints, How Close Are We to Replacement?

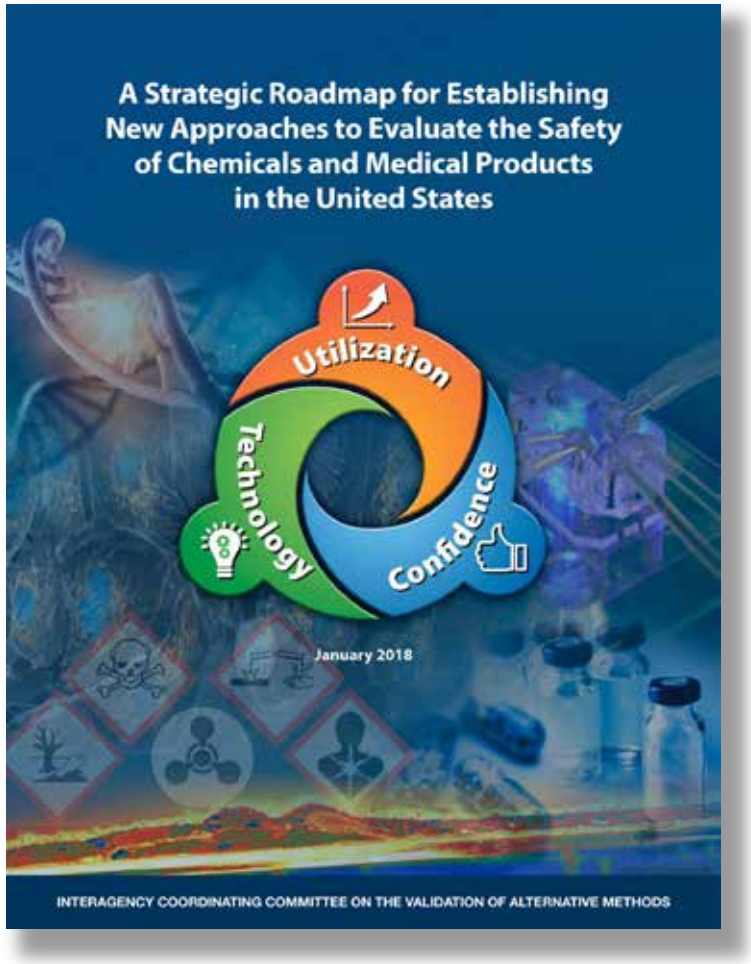
David Allen, PhD

Inotiv, contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods

SACATM Meeting
September 21, 2023

National Institutes of Health • U.S. Department of Health and Human Services

U.S. Strategy and Roadmap: January 2018

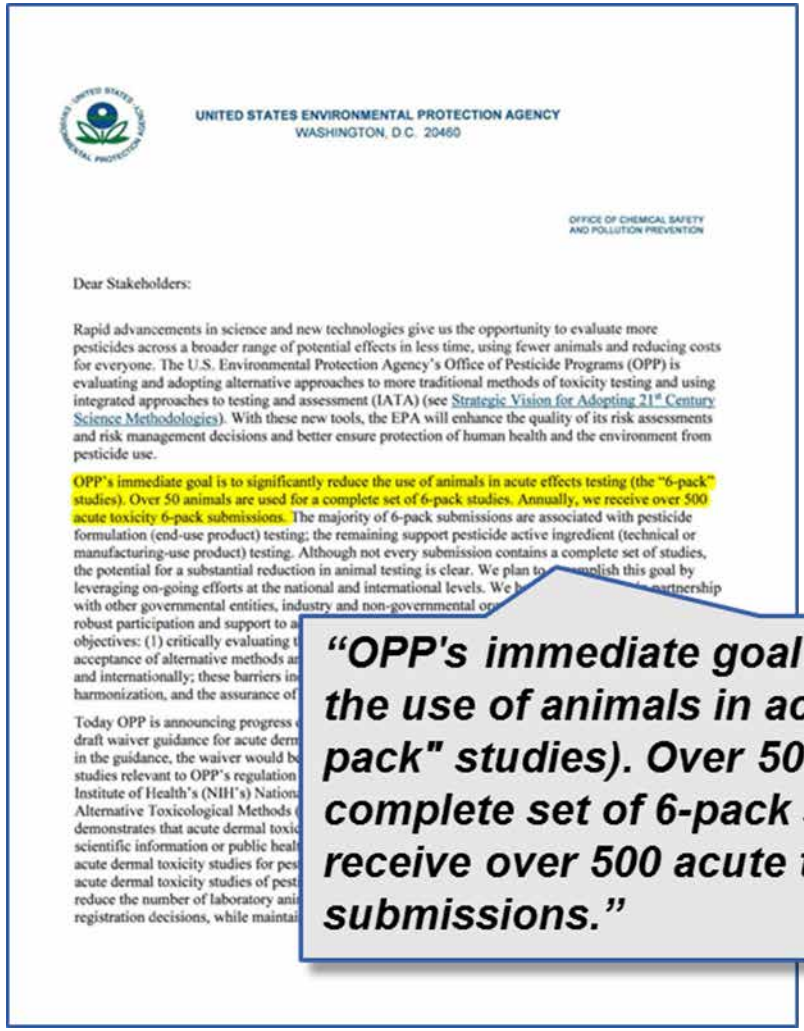


- **Connect end users with the developers of alternative methods**
- **Establish new validation approaches that are more flexible and efficient**
- **Ensure adoption and use of new methods by both regulators and industry**

More information: https://ntp.niehs.nih.gov/go/natl-strategy

The "Six Pack" of Acute Toxicity Studies

1. Acute dermal toxicity
2. Acute oral toxicity
3. Acute inhalation toxicity
4. Primary eye irritation
5. Primary skin irritation
6. Skin sensitization



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

Dear Stakeholders:

Rapid advancements in science and new technologies give us the opportunity to evaluate more pesticides across a broader range of potential effects in less time, using fewer animals and reducing costs for everyone. The U.S. Environmental Protection Agency's Office of Pesticide Programs (OPP) is evaluating and adopting alternative approaches to more traditional methods of toxicity testing and using integrated approaches to testing and assessment (IATA) (see Strategic Vision for Adopting 21st Century Science Methodologies). With these new tools, the EPA will enhance the quality of its risk assessments and risk management decisions and better ensure protection of human health and the environment from pesticide use.

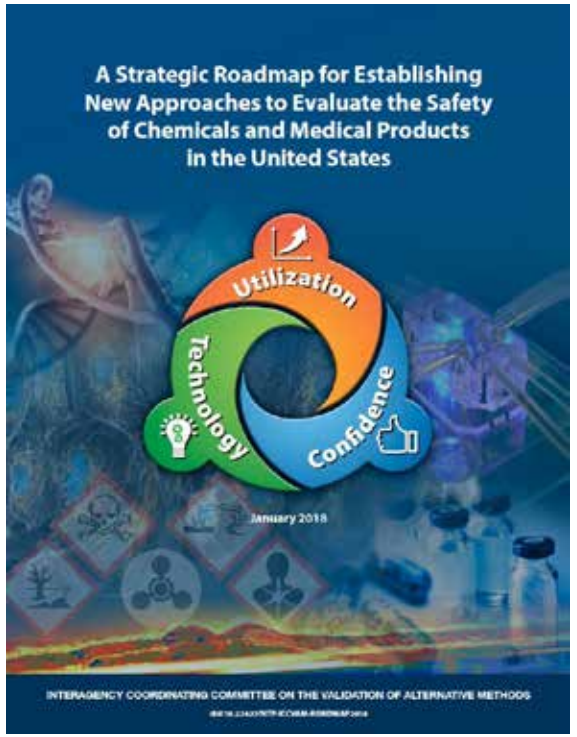
OPP's immediate goal is to significantly reduce the use of animals in acute effects testing (the "6-pack" studies). Over 50 animals are used for a complete set of 6-pack studies. Annually, we receive over 500 acute toxicity 6-pack submissions. The majority of 6-pack submissions are associated with pesticide formulation (end-use product) testing; the remaining support pesticide active ingredient (technical or manufacturing-use product) testing. Although not every submission contains a complete set of studies, the potential for a substantial reduction in animal testing is clear. We plan to accomplish this goal by leveraging on-going efforts at the national and international levels. We [illegible] partnership with other governmental entities, industry and non-governmental or[illegible] robust participation and support to a[illegible] objectives: (1) critically evaluating t[illegible] acceptance of alternative methods a[illegible] and internationally; these barriers in[illegible] harmonization, and the assurance of[illegible]

Today OPP is announcing progress [illegible] draft waiver guidance for acute derm[illegible] in the guidance, the waiver would b[illegible] studies relevant to OPP's regulation[illegible] Institute of Health's (NIH's) Nation[illegible] Alternative Toxicological Methods [illegible] demonstrates that acute dermal toxic[illegible] scientific information or public healt[illegible] acute dermal toxicity studies for pes[illegible] acute dermal toxicity studies of pest[illegible] reduce the number of laboratory ani[illegible] registration decisions, while maintai[illegible]

"OPP's immediate goal is to significantly reduce the use of animals in acute effects testing (the "6-pack" studies). Over 50 animals are used for a complete set of 6-pack studies. Annually, we receive over 500 acute toxicity 6-pack submissions."

Implementation Plan Outline

- Coordinate activities via ICCVAM Workgroups
- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts
- Coordinate efforts with stakeholders
- Identify, acquire, and curate high quality data from reference test methods
- Identify and evaluate new approach methodologies (NAMs)
- Gain regulatory acceptance and facilitate use of non-animal approaches



https://ntp.niehs.nih.gov/go/natl-strategy

Acute systemic toxicity: https://ntp.niehs.nih.gov/go/roadmap-acutetox

Skin and eye irritation: https://ntp.niehs.nih.gov/go/roadmap-irrit

Skin sensitization: https://ntp.niehs.nih.gov/go/roadmap-sensit

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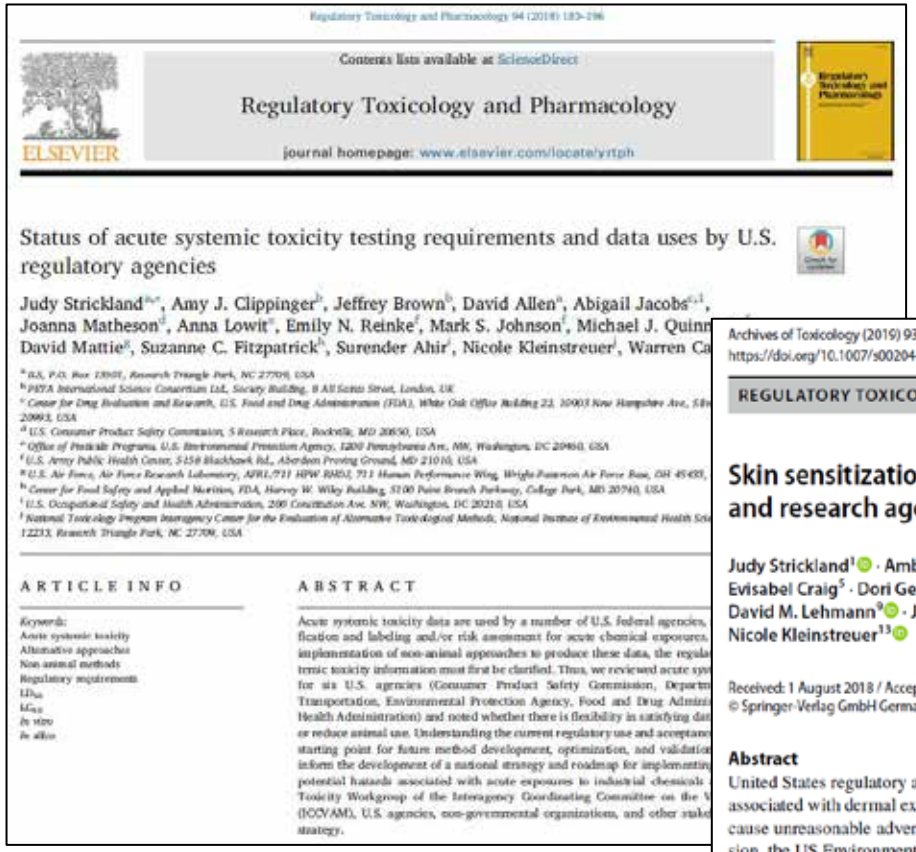
Start with the End User in Mind



Regulatory Toxicology and Pharmacology 94 (2018) 183–196

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

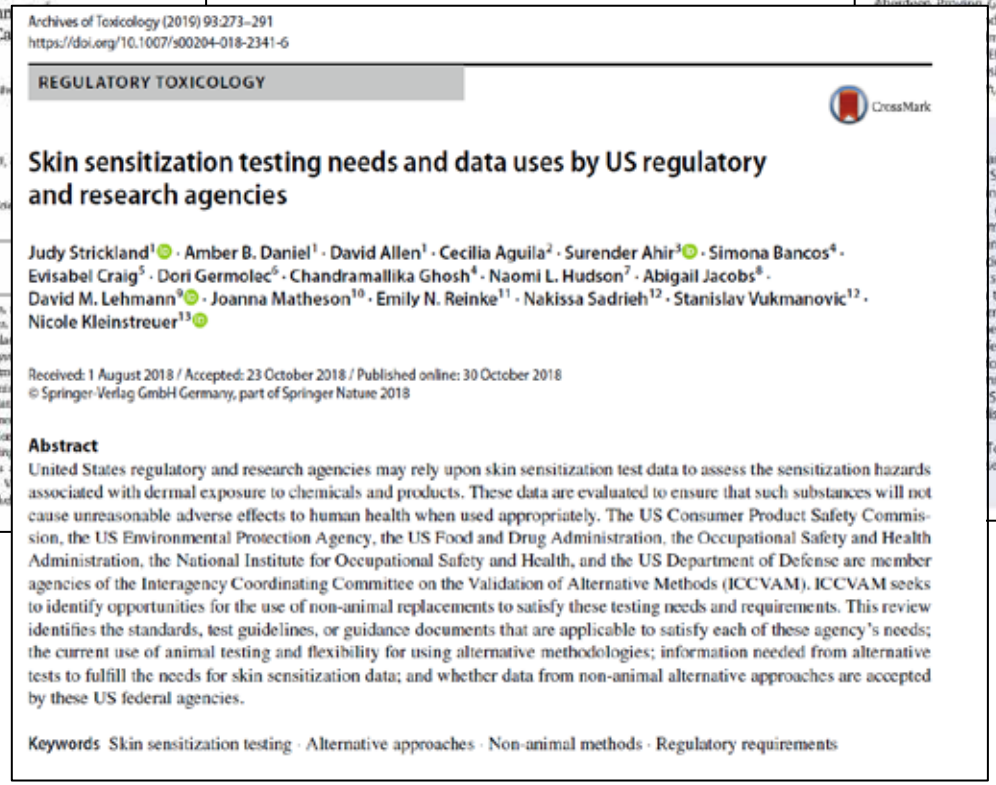
journal homepage: www.elsevier.com/locate/yrtph

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[g], David Mattie[g], Suzanne C. Fitzpatrick[h], Surender Ahir[i], Nicole Kleinstreuer[j], Warren Ca[k]

ARTICLE INFO

ABSTRACT



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]

Received: 1 August 2018 / Accepted: 23 October 2018 / Published online: 30 October 2018
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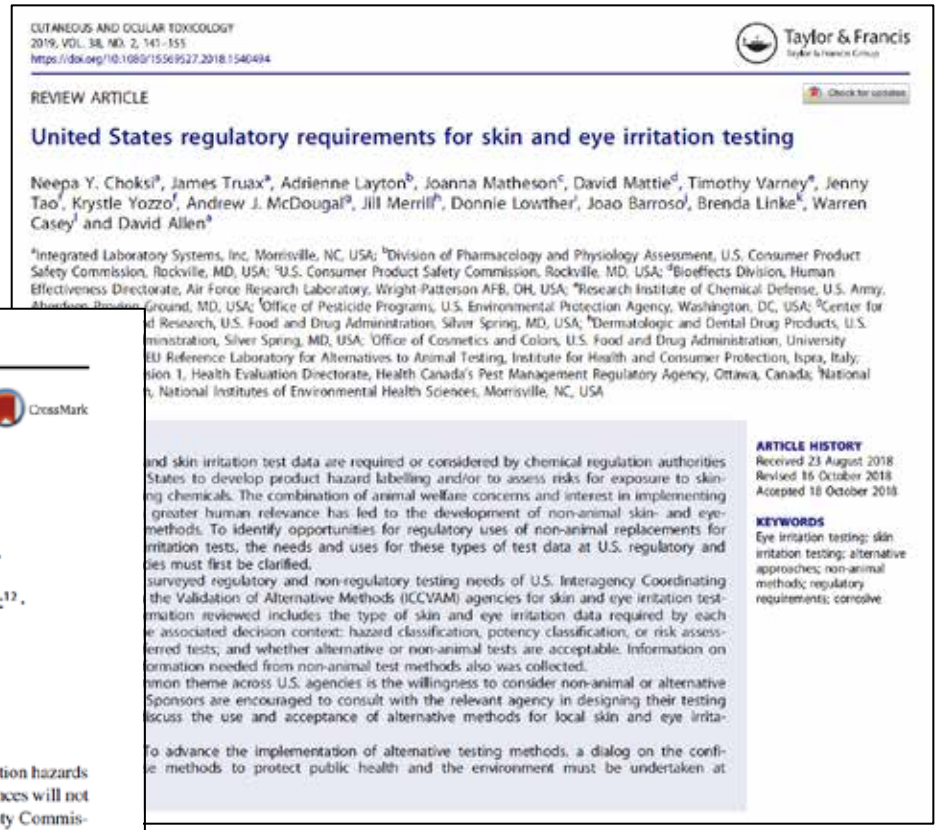
Abstract
United States regulatory and research agencies may rely upon skin sensitization test data to assess the sensitization hazards associated with dermal exposure to chemicals and products. These data are evaluated to ensure that such substances will not cause unreasonable adverse effects to human 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

REVIEW ARTICLE

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]

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

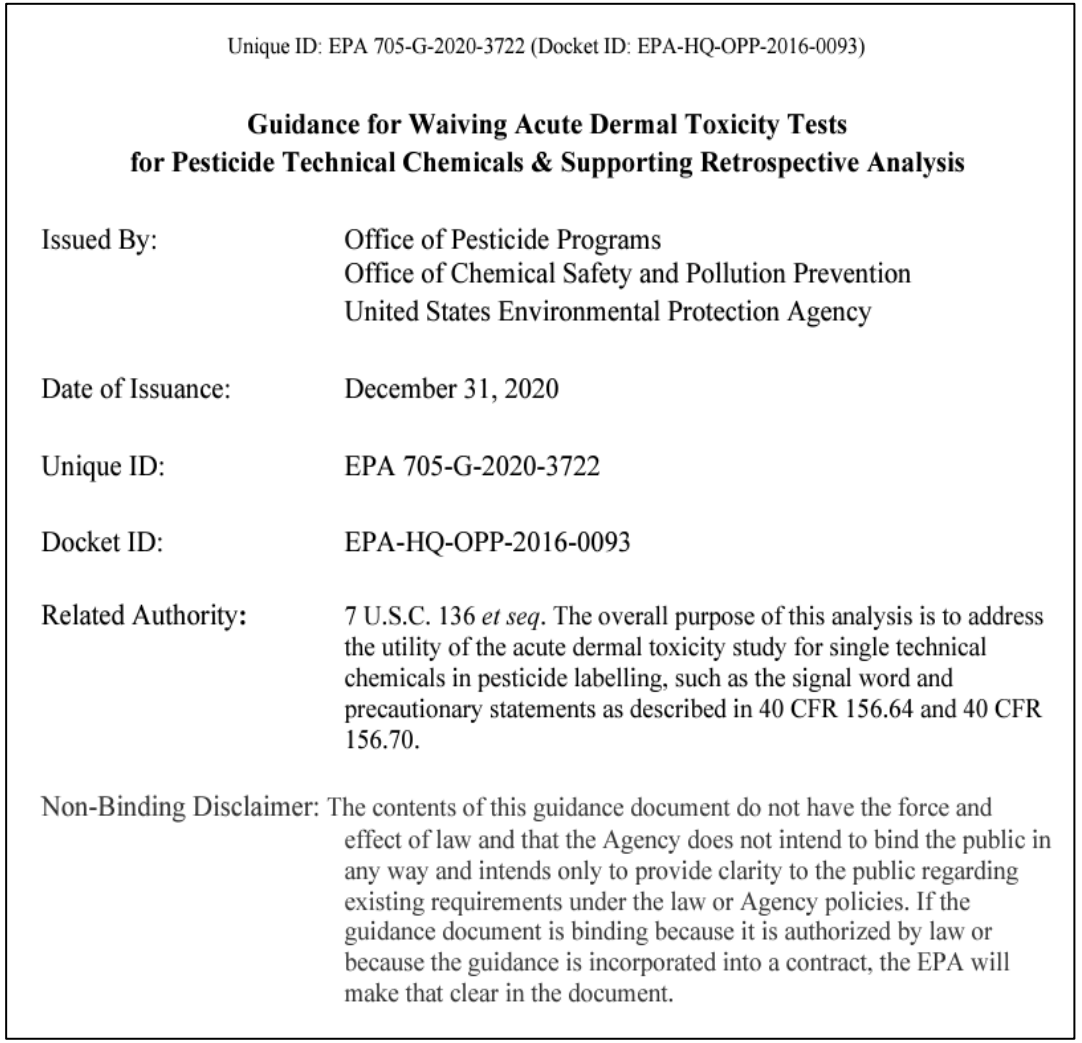
KEYWORDS
Eye irritation testing; skin irritation testing; alternative approaches; non-animal methods; regulatory requirements; corrosive

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

Acute Dermal Pesticide Toxicity Testing

- Collaboration between EPA & NICEATM
- Analyzed the relative contribution of data from acute oral and dermal toxicity tests to pesticide hazard classification and labelling
- Collected acute lethality dermal and oral toxicity data from rat studies with
  - pesticide formulations
  - technical ingredients

https://www.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements



Unique ID: EPA 705-G-2020-3722 (Docket ID: EPA-HQ-OPP-2016-0093)

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

Issued By:	Office of Pesticide Programs Office of Chemical Safety and Pollution Prevention United States Environmental Protection Agency
Date of Issuance:	December 31, 2020
Unique ID:	EPA 705-G-2020-3722
Docket ID:	EPA-HQ-OPP-2016-0093
Related Authority:	7 U.S.C. 136 *et seq*. The overall purpose of this analysis is to address the utility of the acute dermal toxicity study for single technical chemicals in pesticide labelling, such as the signal word and precautionary statements as described in 40 CFR 156.64 and 40 CFR 156.70.

Non-Binding Disclaimer: The contents of this guidance document do not have the force and effect of law and that the Agency does not intend to bind the public in any way and intends only to provide clarity to the public regarding existing requirements under the law or Agency policies. If the guidance document is binding because it is authorized by law or because the guidance is incorporated into a contract, the EPA will make that clear in the document.

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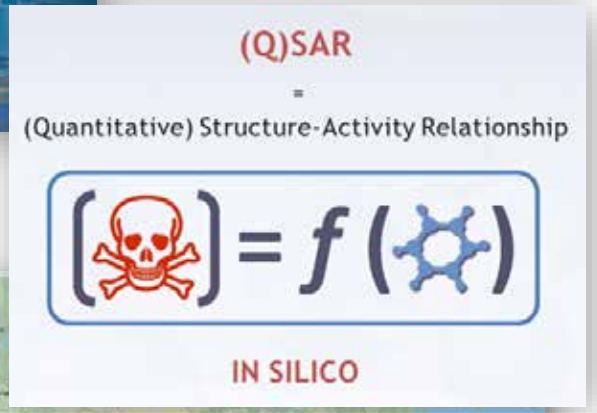
Acute Oral Toxicity: Global Crowdsourcing Predictive Models



- 35 Groups: academia, industry, govt
- Curate reference data to train & test models: >10k chemicals
- Use molecular structure and chemical properties to predict toxicity (e.g. endocrine disruption, acute systemic effects)
- Combine best models together into “ensemble” approaches
- Create open access AI/ML modeling suite

OPERA
OPEn (q)saR App

https://github.com/NIEHS/OPERA

Integrated Chemical Environment

https://ice.ntp.niehs.nih.gov/

Kleinstreuer et al. Comp Tox (2018); Mansouri et al. J Cheminform (2018), Env Health Persp (2020, 2022)

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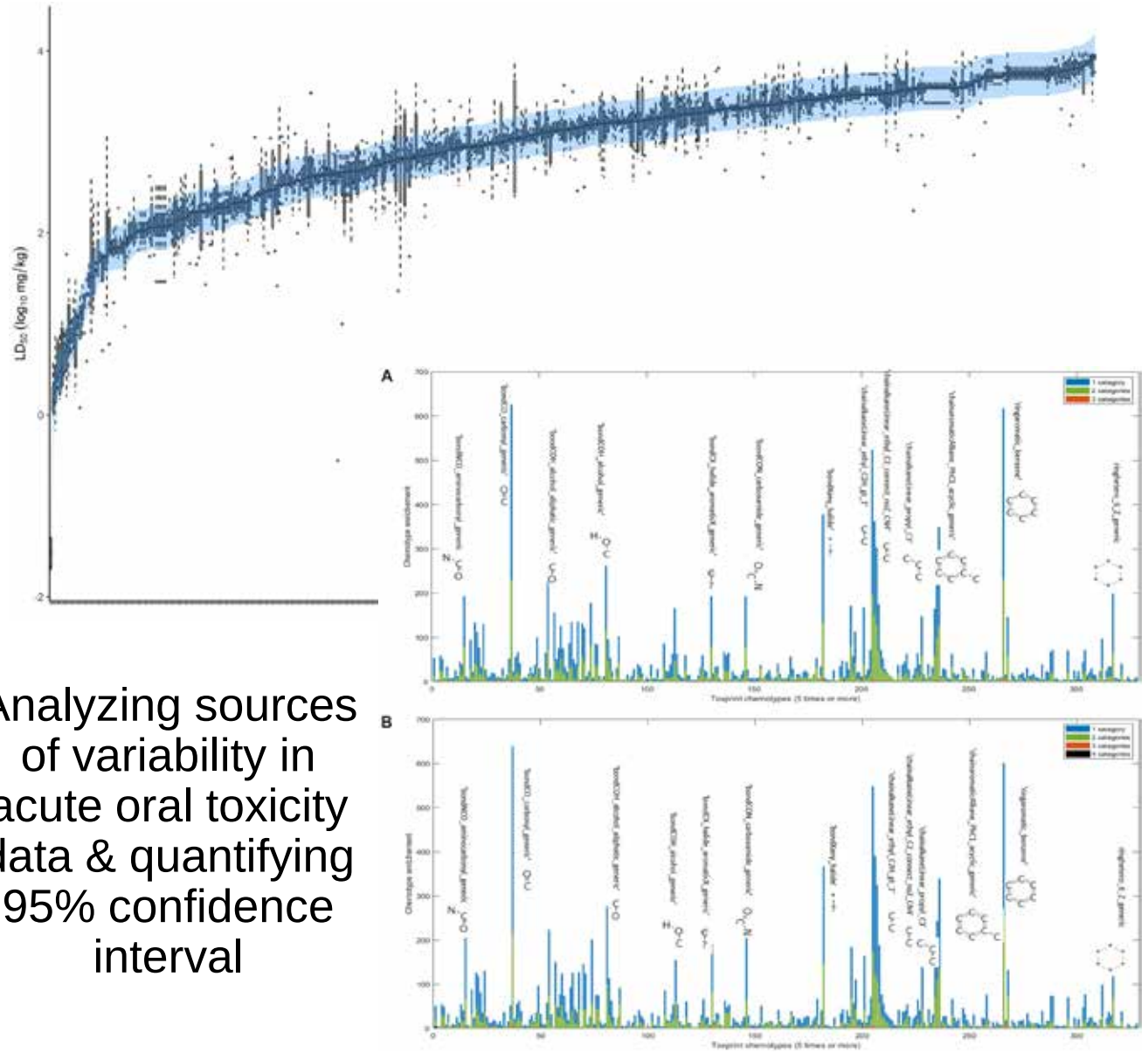
Characterizing Variability and Applying to Model Evaluation



Analyzing sources of variability in acute oral toxicity data & quantifying 95% confidence interval

Collaborative Acute Toxicity Modeling Suite (CATMoS) Performance

	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

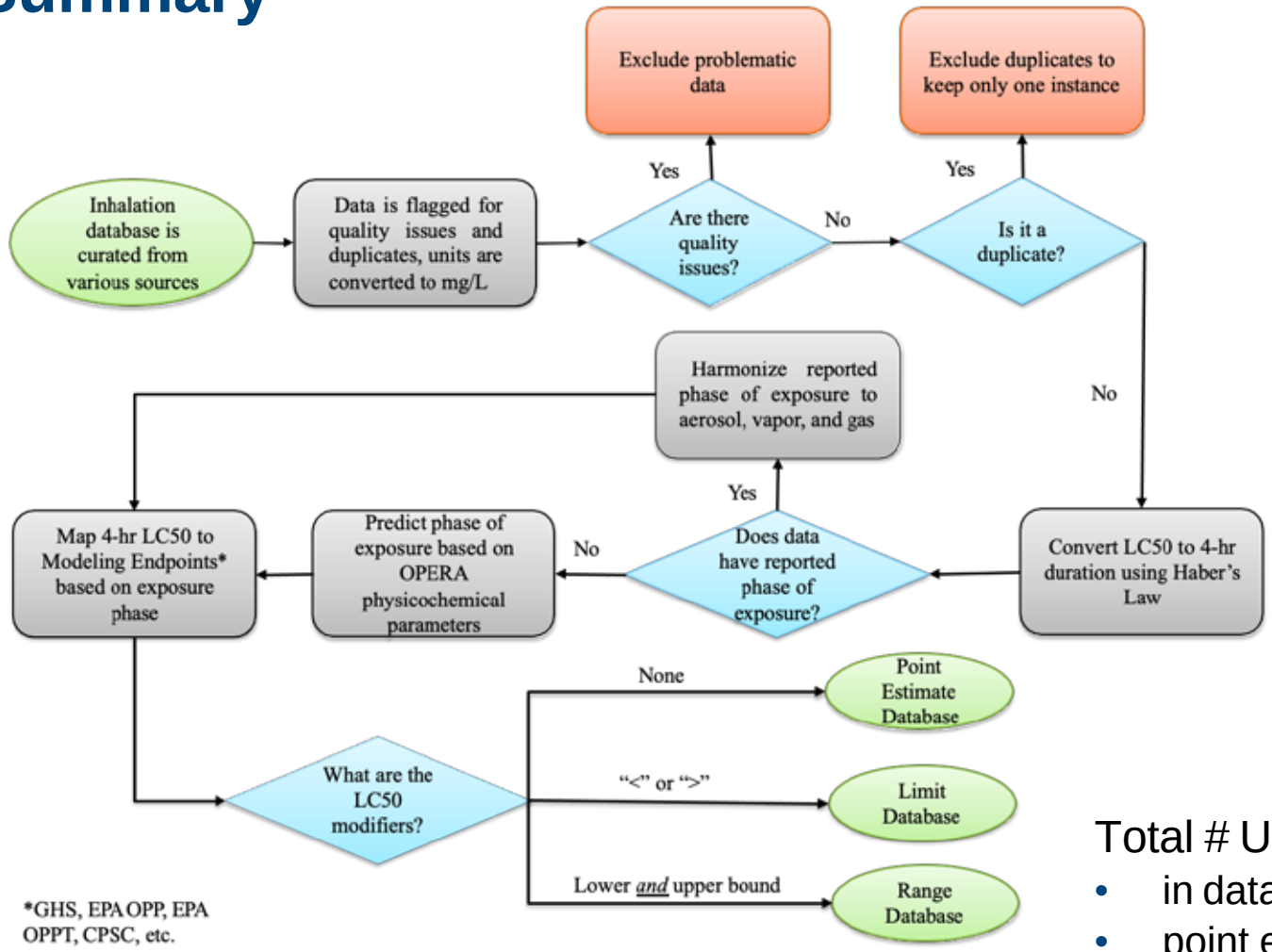
CATMoS QSAR predictions perform just as well as replicate *in vivo* data at predicting oral acute toxicity outcome

Karmaus et al. Toxicol Sci. 2022; Mansouri et al. EHP 2021

Extending to Acute Inhalation Toxicity

Inventory Sources and Summary

- **ECHA REACH Database**
  - Data Rows: 3016
  - Unique Substances: 611
- **ChemIDplus**
  - Data Rows: 2036
  - Unique Substances: 1249
- **Department of Defense**
  - Reports: 22
  - Unique Substances: 13
- **EPA AEGL**
  - Data Rows: 1682
  - Unique Substances: 271
- **NIOSH Pocket Guide**
  - Data Rows: 136
  - Unique Substances: 649



Total # Unique Chemicals
- in database: **1025**
- point estimate data: **780**
- limit data: **312**
- range data: **45**

Human Biology-Based Comparisons

Prior GHS category	1	2A	2B	NC
1 (serious eye damage)	73%	16%	0%	10%
2A (irritant)	4%	33%	4%	59%
2B (mild irritant)	0%	4%	16%	80%
NC (non-irritant)	1%	4%	2%	94%

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

Consider strengths and limitations of all available methods with respect to:

- their relevance to human ocular anatomy
- the mechanisms of eye irritation/corrosion in humans

Clippinger et al. 2021 Cut Ocu Tox

Assessing approaches for eye corrosion/irritation potential

- The rabbit test should not be used as a reference method to demonstrate the validity of *in vitro/ex vivo* assays
- *In vitro/ex vivo* methods are as or more reliable and relevant than the rabbit test

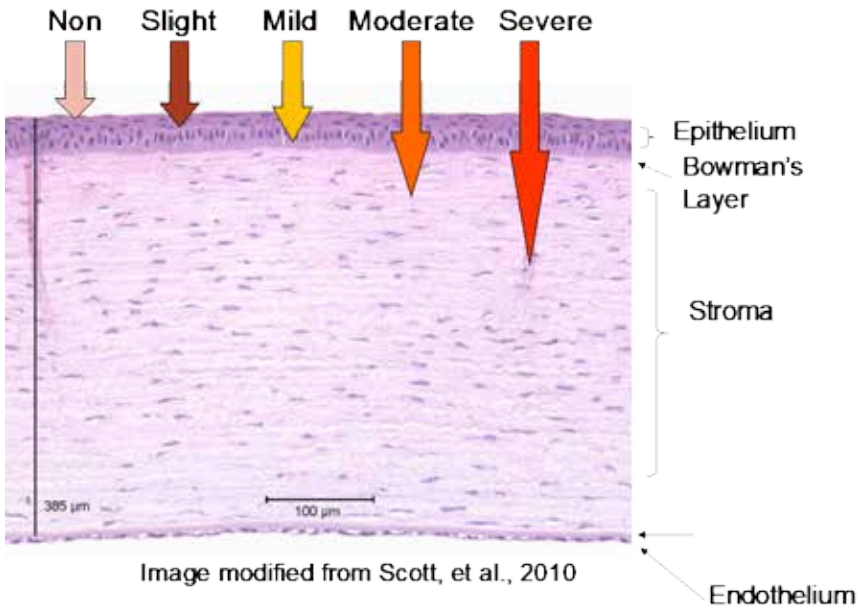


Image modified from Scott, et al., 2010

Prospective Testing: Agchems and Eye Irritation

Study Design: Test Phases/Test Methods

Phase 1

Assess validity of test methods

Six formulations were tested in eight methods/protocols
Cat. 1, n=3 | NC, n=3

- **BCOP**
  - Standard (IVIS w/histo)
  - Extended incubation (IVIS w/histo)
- **EpiOcular**
  - Standard
  - Time-to-toxicity neat
  - Time-to-toxicity diluted
- **Neutral red release**
- **Isolated chicken eye**
- **Porcine cornea reversibility assay**

Phase 2

Refine test methods for potential use in defined approaches

10 additional formulations were tested in eight methods/protocols
Cat. 1, n=4 | Cat. 2A, n=1 | NC, n=5

- **BCOP**
  - Standard (IVIS w/histo)
  - Extended incubation (IVIS w/histo)
- **EpiOcular**
  - Standard
  - Time-to-toxicity neat
  - Time-to-toxicity diluted
- **Neutral red release**
- **Isolated chicken eye**
- **Porcine cornea reversibility assay**

Phase 3

Expand the number of formulations classified as mild or moderate irritants based on the in vivo test

Two methods moved forward; 13 additional formulations were tested
Cat. 2A, n=5 | Cat. 2B, n=3 | NC, n=5

- **BCOP**
  - Standard (IVIS w/histo)
  - Extended incubation (IVIS w/histo)
- **EpiOcular**
  - Standard

All* formulations were tested in four additional methods/protocols
- **SkinEthic time-to-toxicity for liquids**
- **EyeIRR-IS**
- **In vitro depth of injury**
  - Neat
  - Diluted

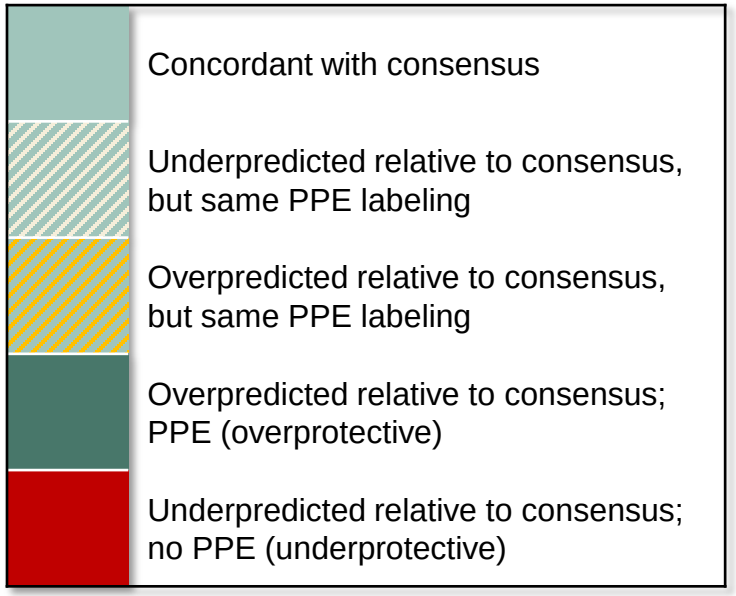
Abbreviations: BCOP = bovine corneal opacity and permeability; histo = histopathology
IVIS = OP-KIT opacitometer in vitro irritation score; LIS = laser light-based opacitometer irritation score

*Insufficient quantity of one formulation for testing in all additional methods

Over-/underprediction relative to consensus and PPE labeling

Formulation Information		GHS Predictions					
Code	Type	DA: BCOP/histo	DA: EO + BCOP/histo	DA: TTL + BCOP/histo	DA: EyeIRR-IS + BCOP/histo	Historical In Vivo	Consensus
A	EC/ME	NC	NC	NC	NC	NC	**NC**
B	SC	NC	NC	NC	NC	NC	**NC**
C	SC	NC	NC	NC	NC	NC	**NC**
D	EC	1	1	1	1	1	**1**
E	EC	2B	2B	2B	1	1	**1**
F	SL	1	1	1	1	1	**1**
G	EC	1	1	1	1	1	**1**
H	SL	1	1	1	1	1	**1**
I	SL	1	1	1	1	1	**1**
J	EC	1	1	1	1	1	**1**
K	SL	NC	2B	2B	2B	2A	**2A**
L	EC	NC	2B	2B	NC	NC	**NC**
M	SL	NC	NC	NC	NC	NC	**NC**
N	SC	NC	NC	NC	NC	NC	**NC**
O	SL	NC	2B	2B	NC	NC	**NC**
P	SC	NC	NC	NC	NC	NC	**NC**
Q	SL	**2A***	2A	2A	2A	NC	**2A**
R	SL	2A	2A	1	1	2A	**1**
S	SL	**2B***	2B	2B	2B	2B	**2B**
T	SC	**2B***	NC	2B	NC	NC	**NC**
U	EC	2A	2A	2A	1	2A	**2A**
V	SL	**1**†	**1**†	**1**†	**1**†	2B	**1**
W	SL	2B	2B	2B	2B	NC	**2B**
X	EC	2A	2A	2A	1	2A	**2A**
Y	EC	**2B***	2B	2B	2B	2A	**2B**
Z	EC	2B	NC	NC	NC	NC	**NC**
AA	EC	NC	2B	2B	2B	2A	**2A**
AB	EC	2A	2A	-	-	2B	**Inconclusive**
AC	EC	2B	2B	2B	NC	NC	**Inconclusive**

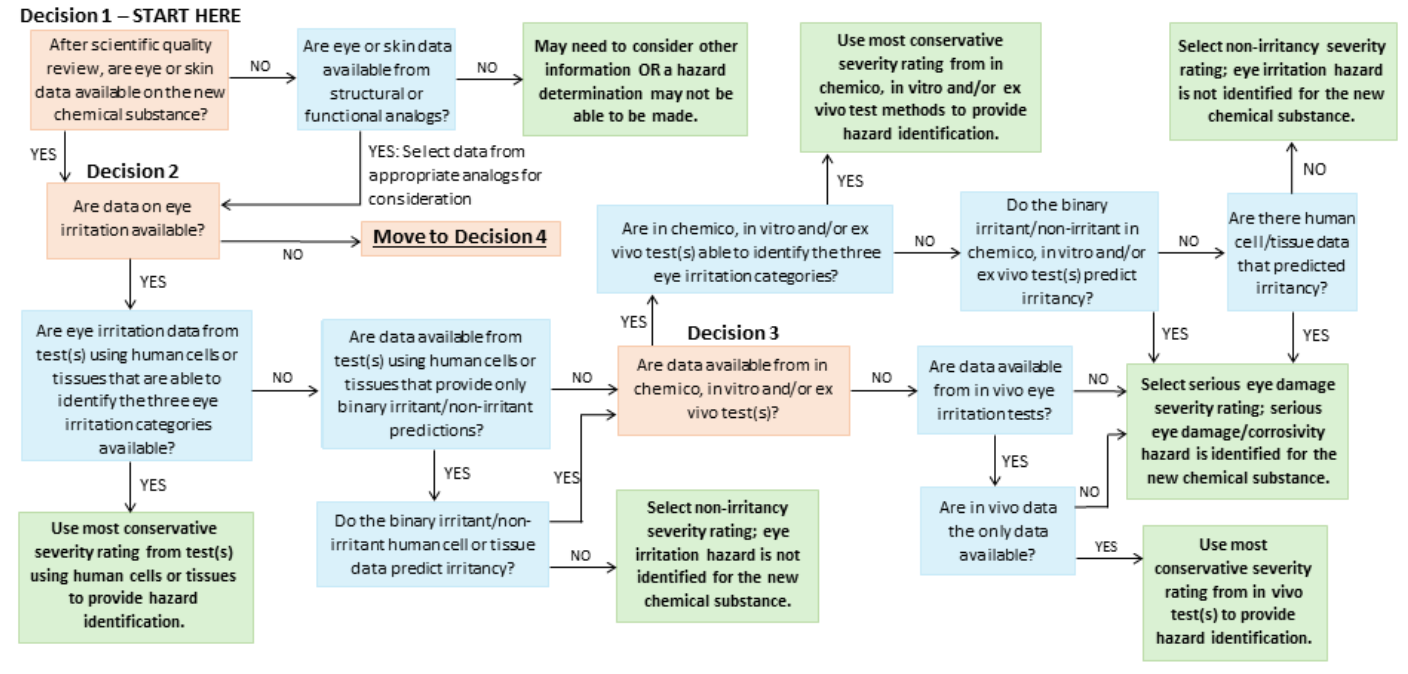
Effects	GHS Classification	PPE
Corrosive	Category 1	Eye protection
Moderate irritant	Category 2A	Eye protection
Mild irritant	Category 2B	Eye protection
Non-corrosive/ minimal irritant	Not Classified	None noted



***IVIS < 3 but histopathology analysis led to a more severe classification**
†Optional histopathology analysis would lead to a less severe classification (i.e., GHS Cat. 2A)

Informing Regulatory Strategies

Decision Tree



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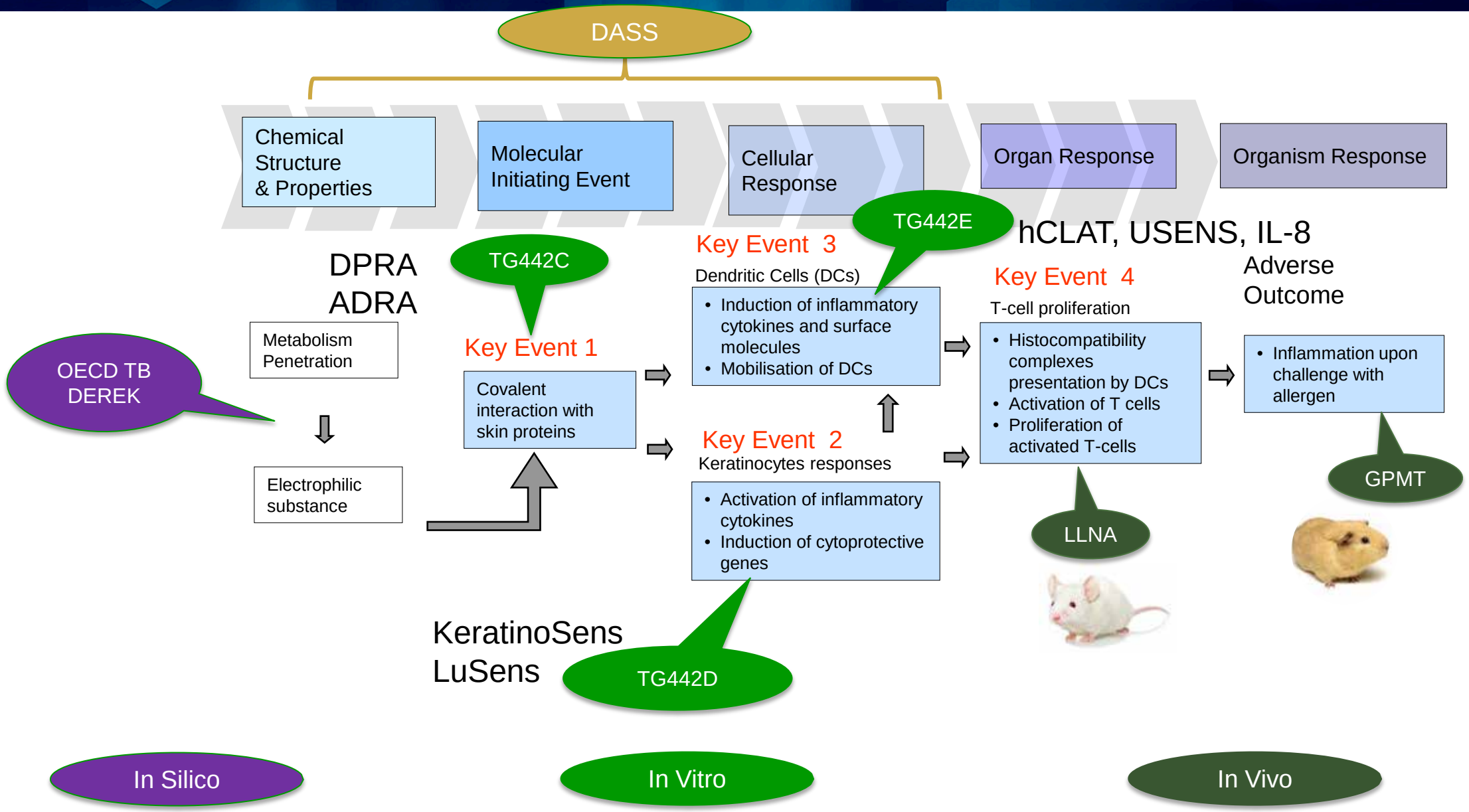
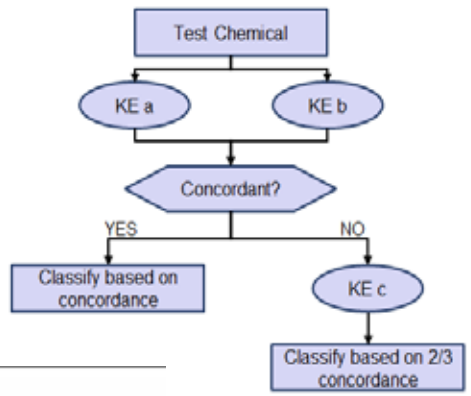
Test Methods Mapped to AOP

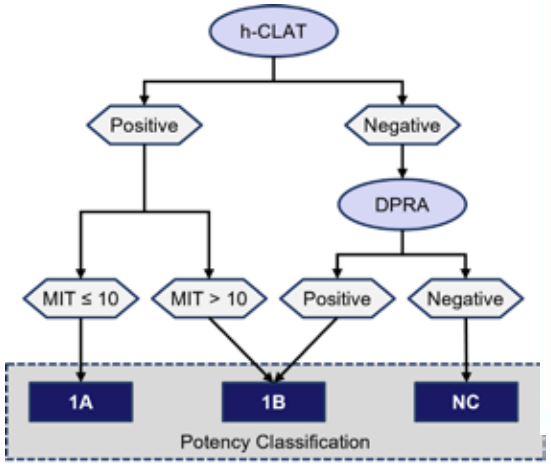


Defined Approaches for Skin Sensitization (DASS)

2 Out of 3



KE 3/1 STS




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

ITSv2

DPRA

Mean Cys and Lys Depletion (%)	Cys Depletion Only	Score
x ≥ 42.47	x ≥ 98.24	3
22.62 ≤ x < 42.47	23.09 ≤ x < 98.24	2
6.38 ≤ x < 22.62	13.89 ≤ x < 23.09	1
x < 6.38	x < 13.89	0

h-CLAT

MIT (µg/mL)	Score
≤ 10	3
10 < x ≤ 150	2
150 < x ≤ 5000	1
Negative	0

OECD Toolbox v4.5

Prediction	Score
Positive	1
Negative	0

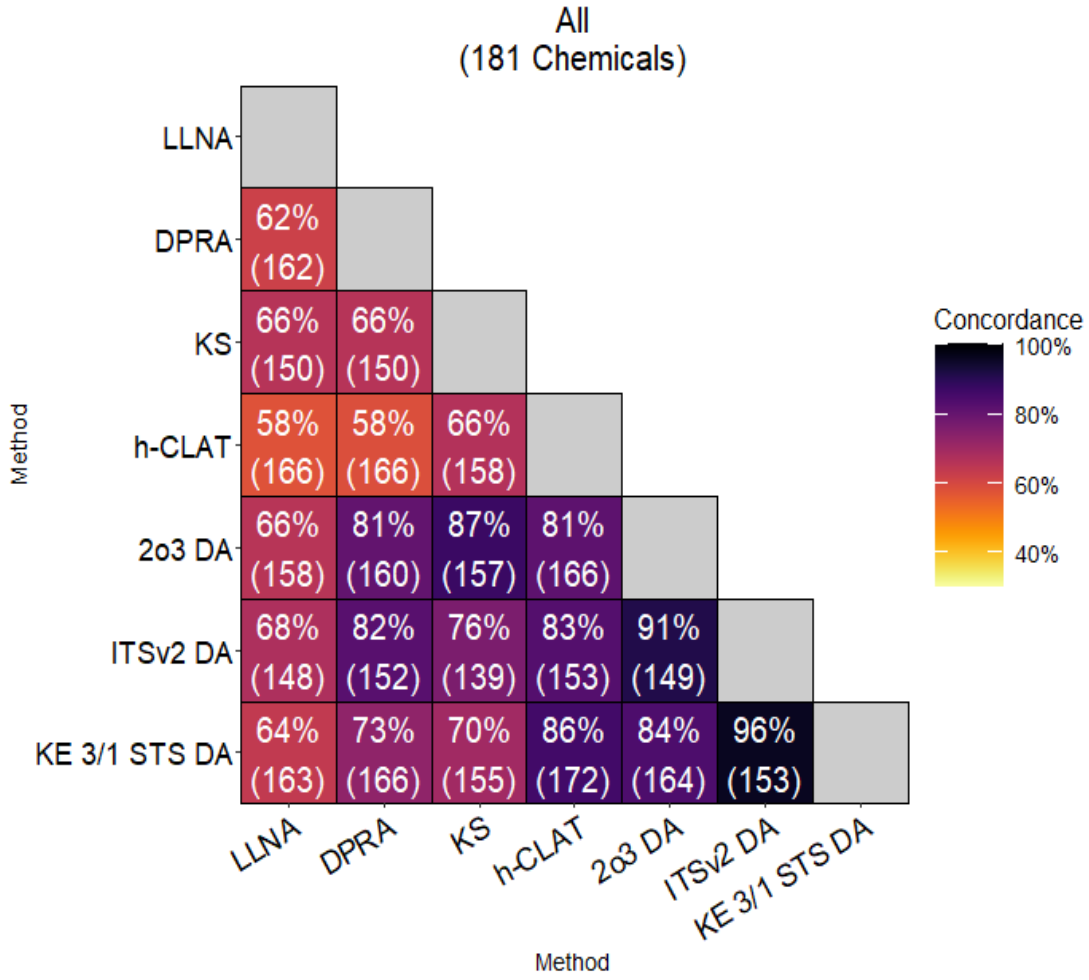
Sum scores

Total Score	Potency
6-7	Strong
2-5	Weak
0-1	NC

Cys, cysteine; Lys, lysine; MIT, minimum induction threshold

Evaluating DASS Applicability Domain

- Project evaluated three different DAs for skin sensitization (DASS):
  - 2 out of 3 (2o3) (OECD 2021a)
  - Integrated Testing Strategy (ITSv2) (OECD 2021a)
  - Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS) (EPA 2018)
- 181 substances relevant to programs within several US federal agencies were tested in NAMs that are information sources for the DASS
  - Nominating agencies: NIEHS, EPA, FDA, CPSC
  - Expands coverage of chemical space – pesticides, agrochemical formulations, dermal excipients, personal care product ingredients, “challenging chemicals”
  - NOTE: GARDskin, the first internationally harmonized test based on genomics and machine learning algorithms, was evaluated using a subset of 31 substances
    - Drop-in replacement for h-CLAT
    - Collaboration with SenzaGen and Burleson Research Technologies, Inc (BRT)



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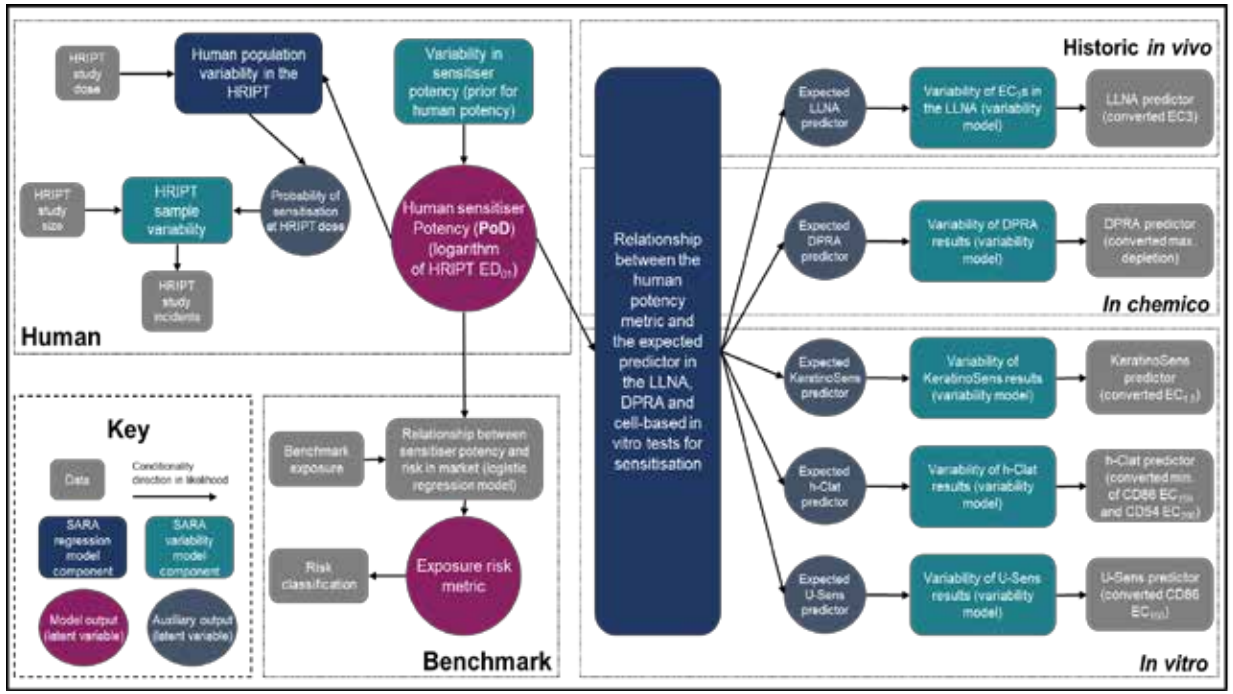
The Skin Allergy Risk Assessment (SARA) Model

- Developed by Unilever as a defined approach for skin allergy risk assessment, expanded using data from ICE and the OECD DASS project
- A Bayesian statistical model which estimates a human-relevant metric of sensitiser potency (termed ED_{01}), the dose with a 1% chance of human skin sensitisation
- Accounts for variability of the input data and explicitly quantifies uncertainty
- Utilises any combination of human predictive patch test (HPPT), LLNA, direct peptide reactivity assay (DPRA), KeratinoSens™, h-CLAT, U-SENS™ data
- The SARA-ICE Model was designed to be used within an NGRA Framework for decision making.
- On OECD workplan for TG497 evaluation



SARA Model overview: Reynolds et al 2022

Progress Towards a Six-Pack Replacement

Dermal lethality	• US EPA Waiver guidance available; Human (or rat) in vitro data for dermal absorption
Oral lethality	• In silico (CATMoS) for single chemicals; GHS additivity equation for formulations
Inhalation lethality	• 3D ALI models being evaluated; LC50 database evaluation for in silico model development ongoing
Eye irritation	• NAMs for Cat I and/or Cat IV (TG 437, 438, 460, 491, 492, 494, 496); Human-biology based DAs
Skin irritation	• NAMs for Cat I or Cat IV (TG 430, 431, 435, 439); Human-biology based DAs
Skin sensitization	• EPA science policy, draft risk assessment, and OECD international DASS guideline

Mansouri et al. 2021 EHP; Clippinger et al. 2021 Cut Ocu Tox; Rooney et al. 2021 Reg Tox Pharm; Allen et al. 2021 ALTEX; Hamm et al. 2021 Reg Tox Pharm

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