UNITED STATES
ICCVAM
Advancing Alternatives
to Animal Testing

**Interagency Coordinating Committee on
the Validation of Alternative Methods**

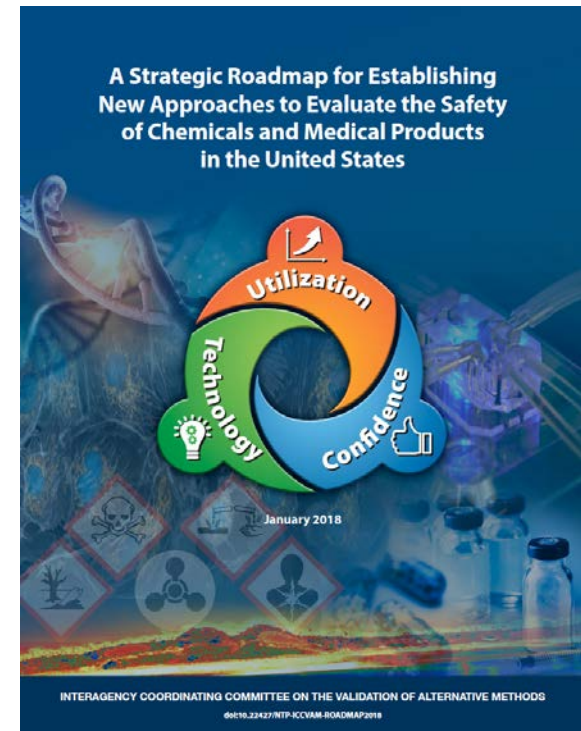# Skin Sensitization Implementation Plan

Nicole Kleinstreuer, Ph.D.
Deputy Director, NICEATM

ICCVAM Public Forum
May 24, 2018

Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture
Department of Defense • Department of Energy • Department of the Interior • Department of Transportation
Environmental Protection Agency • Food and Drug Administration • National Institute for Occupational Safety and Health
National Institutes of Health • National Cancer Institute • National Institute of Environmental Health Sciences
National Institute of Standards and Technology • National Library of Medicine • Occupational Safety and Health Administration

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 non-animal alternative approaches

- Gain regulatory acceptance and facilitate use of non-animal approaches

A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States

January 2018

INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS
doi:10.22427/NTP-ICCVAM-ROADMAP2018

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

Current ICCVAM SSWG Roster

- Moiz Mumtaz (ATSDR)
- Patricia Ruiz (ATSDR)
- John Gordon (CPSC)
- Joanna Matheson (CPSC)
- Emily N. Reinke (DOD)
- Evisabel Craig (EPA)
- David Lehmann (EPA)
- Anna Lowit (EPA)
- Timothy McMahon (EPA)
- Mamta Naidu (EPA)
- Todd Stedeford (EPA)
- Simona Bancos (FDA)
- Paul C. Brown (FDA)
- Rakhi M. Dalal-Panguluri (FDA)
- Wei Ding (FDA)
- Robert Heflich (FDA)
- Abigail C. Jacobs (FDA)

- Diego Rua (FDA)
- Nakissa Sadrieh (FDA)
- Stanislav Vukmanovic (FDA)
- Jeffrey Yourick (FDA)
- Warren Casey (NIEHS)
- Dori Germolec (NIEHS)
- Nicole Kleinstreuer (NIEHS)

ICATM Liaison Members
- Silvia Casati (EURL ECVAM)

NICEATM Support Staff (ILS)
- Michael Paris
- Judy Strickland
- David Allen

ICCVAM Skin Sensitization Models

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...

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

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 Va...
the development and evaluation of non-animal approaches to ident...
...in sensitization suggests that no single...
...g an integrated approach to testing an...

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

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 ...

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

Human Data Project

- Analyze existing human data and primary study references to understand uncertainty and sources of variability

  – Current database: 420 chemicals, 919 records

- Develop/apply transparent, reproducible system for human skin sensitization potency categorization

- Work with industry consortia to encourage data sharing of human skin sensitization data

  – Semi-automated extraction of data from Cosmetics Ingredient Review Reports

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

U.S. Agency Requirements/Needs

Accepted Animal Method

Evaluation Needs

Industrial chemicals — Not required — Hazard, risk

Pesticides — LLNA — NS / S — Hazard

Workplace chemicals — LLNA GPMT Buehler — NS / 1B / 1A — Potency

Non-animal alternatives considered on a case-by-case basis

8

U.S. Agency Requirements/Needs

Accepted Animal Method

Evaluation Needs

FDA — Dermatologic products — Unspecified

Potency*

FDA — Medical devices — GPMT

NS   S

Hazard

CPSC — Household products — LLNA GPMT Buehler

NS   S   SS

Potency

Non-animal alternatives considered on a case-by-case basis, except for medical devices.

9

Regulatory Requirements Publications

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

International regulatory requirements for skin sensitization testing

Amber B. Daniel[a], Judy Strickland[a,*], David Allen[a], Silvia Casati[b], Valérie Zuang[b], João Barroso[b], Maurice Whelan[b], M.J. Régimbald-Krnel[c], Hajime Kojima[d], Akiyoshi Nishikawa[d], Hye-Kyung Park[e], Jong Kwon Lee[e], Tae Sung Kim[e], Isabella Delgado[f], Ludmila Rios[g], Ying Yang[h], Gangli Wang[i], Nicole Kleinstreuer[j]

- US regulatory requirements paper (*Strickland et al.*) in final agency clearance

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

Expanding Substance Space Coverage

- NTP (*Tox Branch*/*D. Germolec*) is testing additional substances in three alternative test methods:

  - DPRA, KeratinoSens, hCLAT

- Expanded substance space includes:

  - pesticide/agrochemical formulations, dermal excipients, personal care product products, "challenge" chemicals

- Compiled nominations from multiple ICCVAM agencies/partners

  - EPA: Office of Pesticides, Office of Pollution Prevention and Toxics, Office of Research and Development
  - Consumer Product Safety Commission
  - Food and Drug Administration
  - National Toxicology Program
  - ICATM partners

Expanding Substance Space Coverage

- Total of 266 substances nominated

- NTP has procured 135 substances for initial testing phase (mostly nominations from the EPA)

- Testing began in late 2017

- Additional testing (~100 substances) to follow in mid-2018

- Coordinating with Dow Agro to test formulations already assessed in DPRA and KeratinoSens™ in the hCLAT assay

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

Skin Sensitization Data Collection

- Multiple conventional & antimicrobial registrants have kindly provided data to support our skin sensitization efforts

- We continue to collect additional, voluntary data submissions to expand current datasets

  – Paired *in vitro* & LLNA data that could increase coverage of various defined approaches

  – Other LLNA studies to help assess variability

  – Additional human data to assist in evaluating defined approaches

- NICEATM sent letters of request to industry consortia; data will be published in ICE

Accuracy Against Human Clinical Data (~150 chems)

LLNA   ## GPMT / Buehler

Hazard	Potency		Hazard	Potency
72%-82%	54% - 60%		~72%	~60%

Reproducibility of Multiple Tests (~100 chems)

Hazard	Potency
~78%	~62%

ICCVAM. 1999. NIH Publication No. 99-4494
ICCVAM. 2010. NIH Publication No. 11-7709
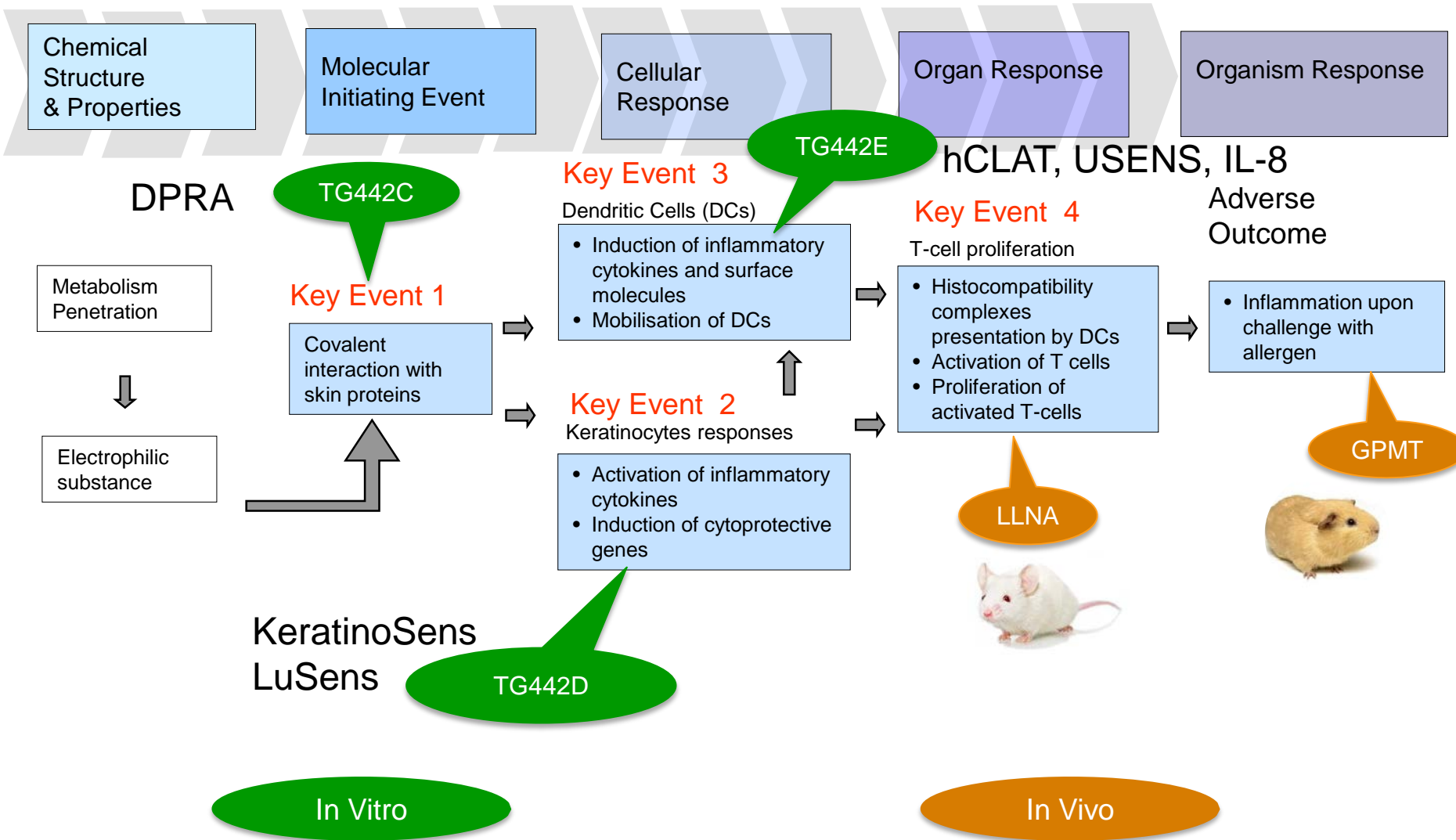*Urbisch et al. 2015. Reg Tox Pharm 71:337-351.*
Dumont et al. 2016. Tox In Vitro 34: 220-228
Hoffmann et al. 2018 Crit Rev Tox

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

Test Methods Mapped to AOP

Chemical Structure & Properties	Molecular Initiating Event	Cellular Response	Organ Response	Organism Response

DPRA

TG442C

hCLAT, USENS, IL-8

TG442E

Key Event 3

Dendritic Cells (DCs)

Key Event 1

Metabolism Penetration

Covalent interaction with skin proteins

- Induction of inflammatory cytokines and surface molecules
- Mobilisation of DCs

Key Event 4

T-cell proliferation

- Histocompatibility complexes presentation by DCs
- Activation of T cells
- Proliferation of activated T-cells

Adverse Outcome

- Inflammation upon challenge with allergen

Electrophilic substance

Key Event 2

Keratinocytes responses

- Activation of inflammatory cytokines
- Induction of cytoprotective genes

GPMT

LLNA

KeratinoSens
LuSens

TG442D

In Vitro

In Vivo

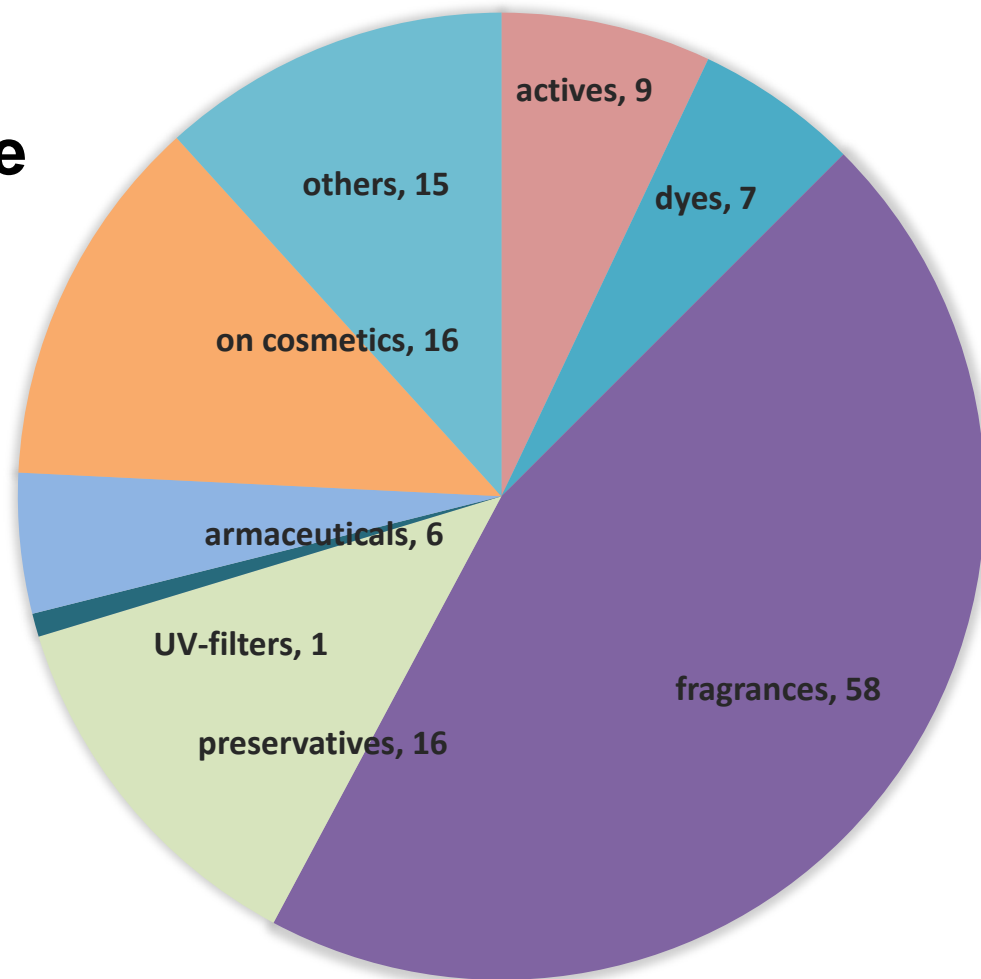# Global Skin Sensitization Project

- Objective: analysis of available non-animal defined approaches (DAs)

- Collaboration with Cosmetics Europe

  - Curation/generation of

    - *in vivo* LLNA and human data

    - *in vitro* cell-based data that maps to AOP

    - *in silico* computer predictions, chemical structural features & properties

- Qualitative and quantitative evaluation of OECD-submitted DAs

- Fully transparent approach (i.e., build open-source code packages)

- Evaluate performance against LLNA and human hazard/potency categories

Hoffmann et al. 2018 Crit Rev Tox
Kleinstreuer et al. 2018 Crit Rev Tox
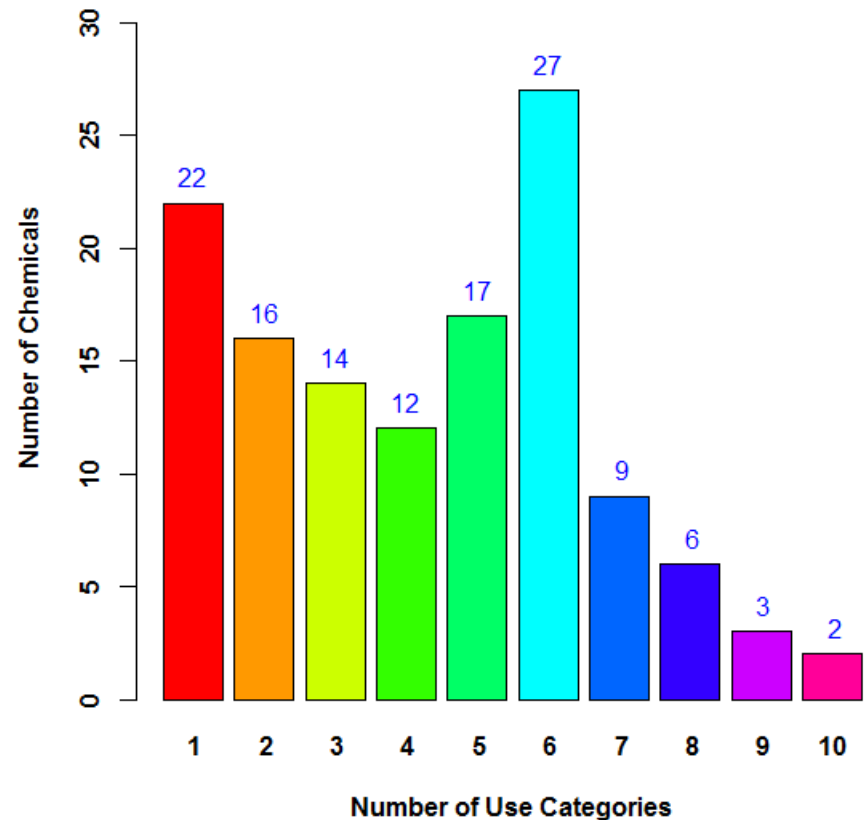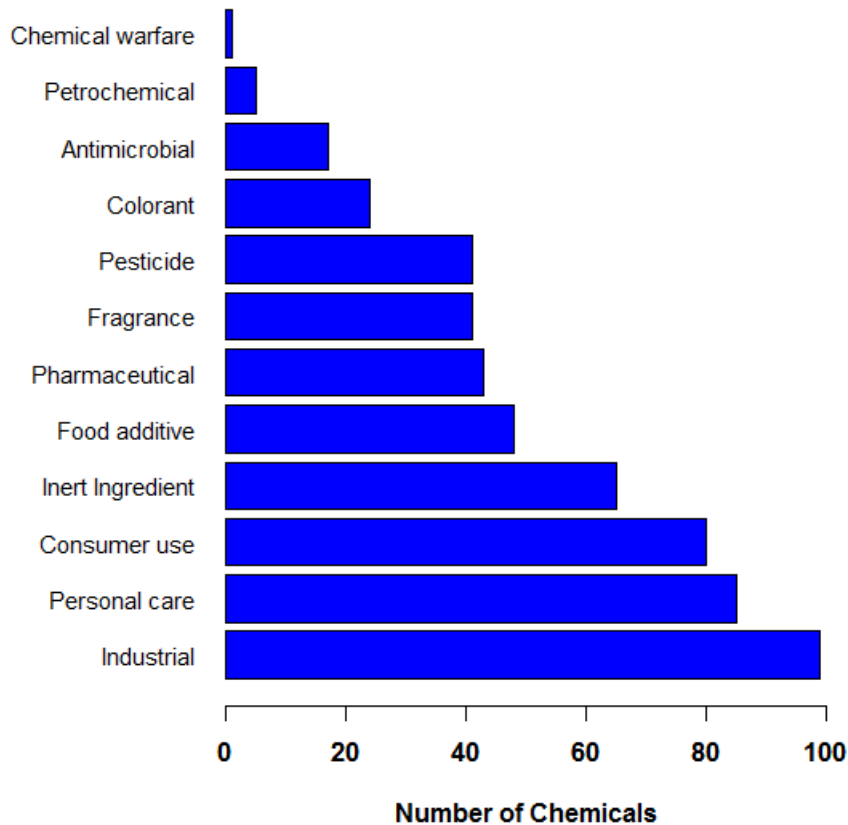
Compilation of a Reference Database: Substance Selection

- **human potency available**
  (Basketter et al. 2014, Api et al. 2017)

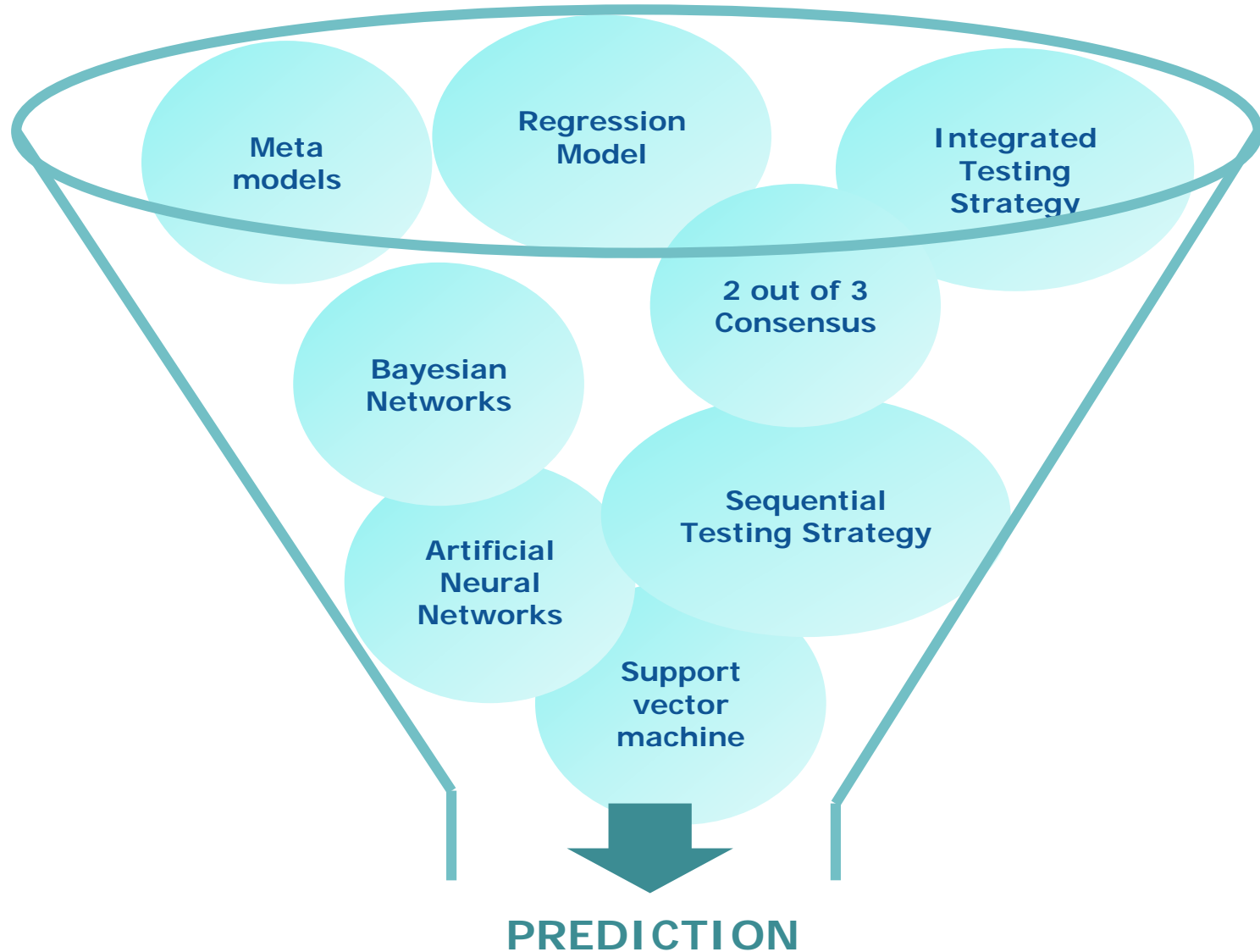- **LLNA data available**

➡ **128 substances**

Pie chart:
- actives, 9
- dyes, 7
- others, 15
- on cosmetics, 16
- armaceuticals, 6
- UV-filters, 1
- preservatives, 16
- fragrances, 58

Hoffmann et al. 2018 Crit Rev Tox

CE Chemical Use Space Coverage

U.S. EPA ACToR UseDB Categories

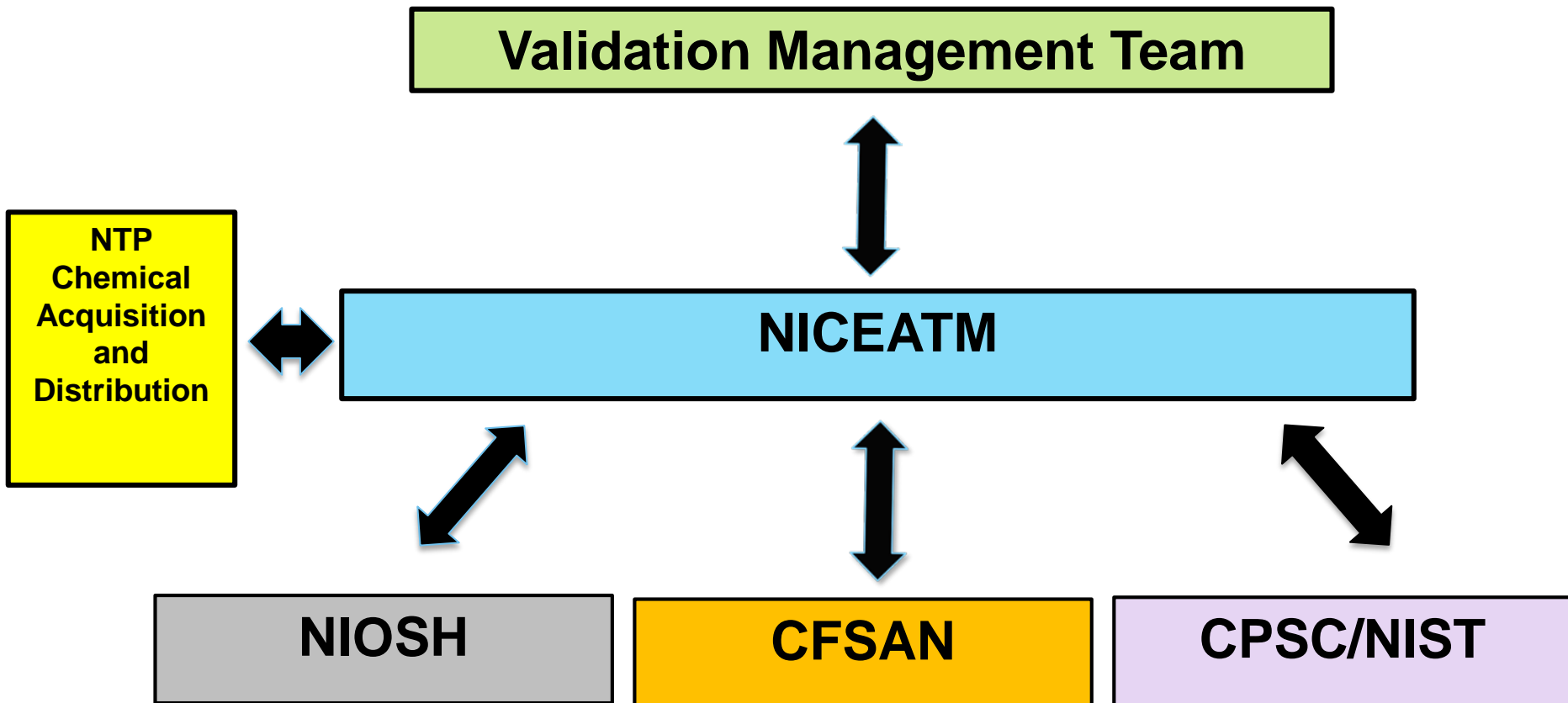Average of 4.3 use cases per substance

Non-Animal Approach Evaluation

Most non-animal testing strategies evaluated so far perform **better** than the LLNA at predicting human skin sensitization hazard and potency.

(And when compared to the LLNA, are equivalent in performance to the LLNA at predicting itself.)

Hoffmann et al. 2018 Crit Rev Tox
Kleinstreuer et al. 2018 Crit Rev Tox

Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)

- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data

- Coordinate efforts with stakeholders

- Identify, acquire, and curate high quality data from reference test methods

- Identify and evaluate non-animal alternative approaches to skin sensitization testing

- Gain regulatory acceptance and facilitate use of non-animal approaches

EPA Draft Science Policy

- EPA/OPP and OPPT now accept two non-animal defined approaches as alternatives to the LLNA

- Covers pesticide actives ingredients, inerts, and mono-constituent industrial chemicals regulated under TSCA

Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing
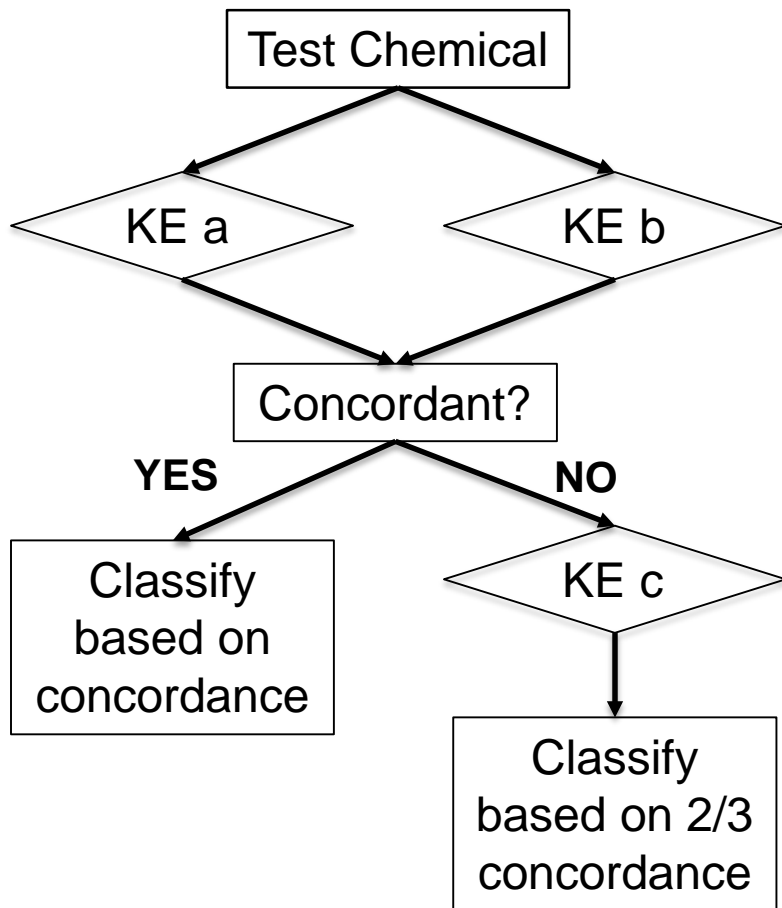
DRAFT FOR PUBLIC COMMENT
April 4, 2018

EPA's Office of Chemical Safety and Pollution Prevention:

Office of Pesticide Programs
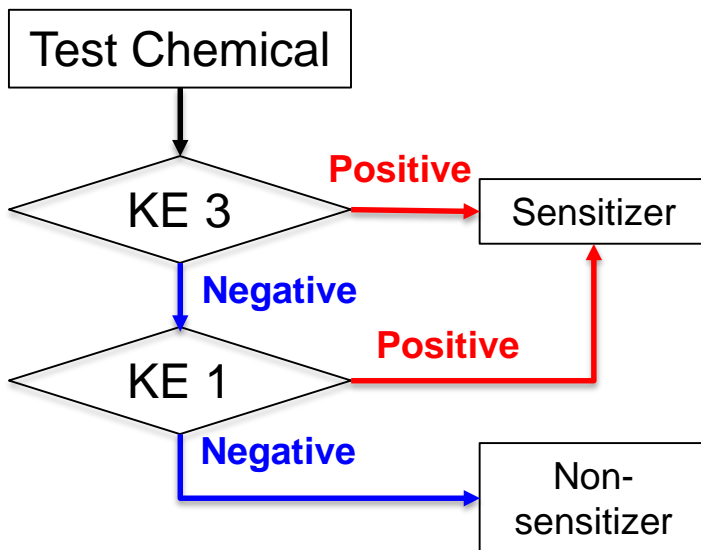Office of Pollution Prevention and Toxics

1

Defined Approaches: KE-based Hazard Prediction

AOP WoE: 2 out of 3 KE

- No differential weighting of individual test methods, or defined sequential order of testing
- Usually KE1 (e.g. DPRA) and KE2 (e.g. KeratinoSens) performed first since less expensive
- Third test is KE3 (e.g. hCLAT, U-SENS)

Test Chemical

KE a   KE b

Concordant?

YES   **NO**

Classify based on concordance

KE c

Classify based on 2/3 concordance

Defined Approaches: KE-based Hazard Prediction

KE 1 & 3 STS

- Prediction can be derived after first tier
- Depends on KE 3 (e.g. h-CLAT) and KE 1 (e.g. DPRA)

Test Chemical → KE 3

KE 3 — **Positive** → Sensitizer

KE 3 — **Negative** → KE 1

KE 1 — **Positive** → Sensitizer

KE 1 — **Negative** → Non-sensitizer

Cross-Partner Project Concept:

Development of High Throughput Screening Assays to Detect Chemicals that may Induce Skin Sensitization, and Skin or Eye irritation

- HTS versions of DPRA (OECD 442C), KeratinoSens (OECD 442D)
- HTS assays for irritation (~OECD TG491) using human primary keratinocytes and corneal epithelial cells

Acknowledgments

- Sebastian Hoffmann & Cosmetics Europe STTF

- Dori Germolec & NTP colleagues

- ICCVAM SSWG

- ILS/NICEATM

- US EPA/OPP & OPPT

- EURL ECVAM/JRC

- Health Canada

- ICATM partners