(R)evolution in Validation: Establishing Scientific Confidence in NAMs

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NICEATM and ICCVAM

National Institute of Environmental Health Sciences
Division of Translational Toxicology

UNITED STATES
ICCVAM
Advancing Alternatives
to Animal Testing

- National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (**NICEATM**), supporting the Interagency Coordinating Committee for the Validation of Alternative Methods (**ICCVAM**)

- ICCVAM Authorization Act of 2000: To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new and revised toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing (**3Rs**) animal tests and ensuring human safety and product effectiveness.
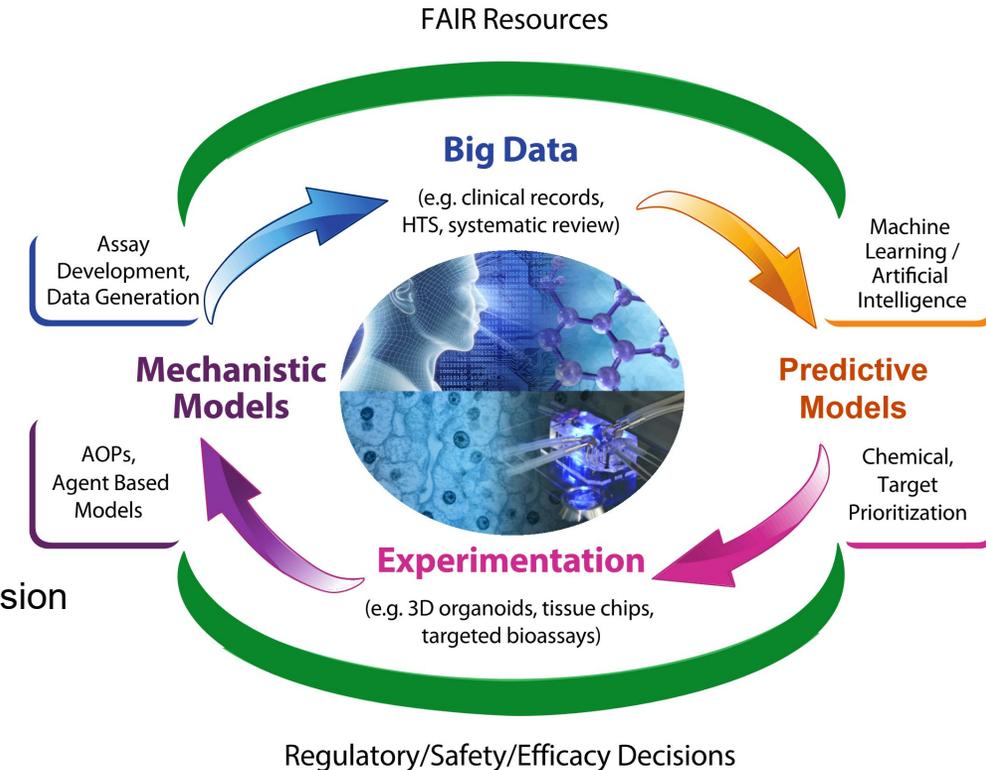
FAIR Resources

Big Data
(e.g. clinical records, HTS, systematic review)

Assay Development, Data Generation

Machine Learning / Artificial Intelligence

Mechanistic Models

Predictive Models

AOPs, Agent Based Models

Chemical, Target Prioritization

Experimentation
(e.g. 3D organoids, tissue chips, targeted bioassays)

Regulatory/Safety/Efficacy Decisions

10 Research Agencies

Agency for Toxic Substances and Disease Registry
National Institute for Occupational Safety and Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
National Institutes of Health
Department of Defense
Department of Energy
National Institute of Standards and Technology
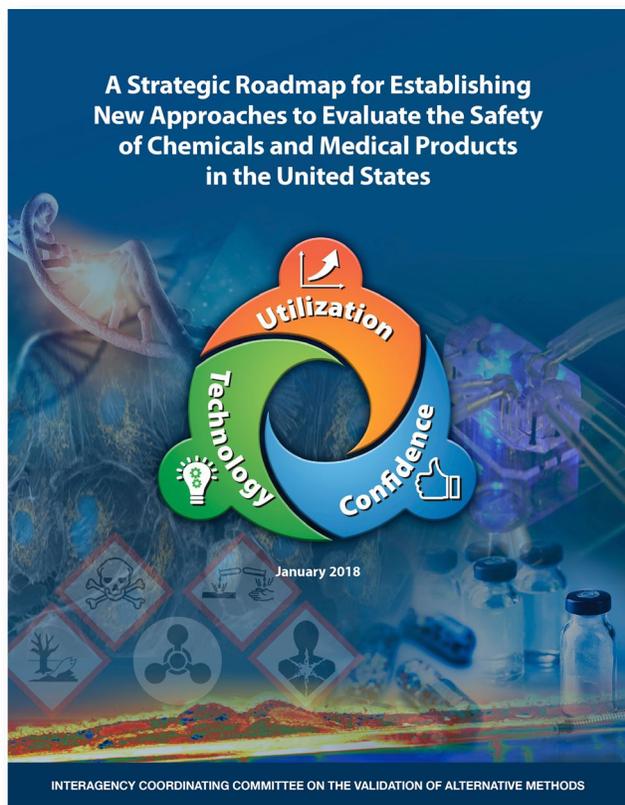Veterans Affairs Office of Research and Development

7 Regulatory Agencies

Consumer Product Safety Commission
Department of Agriculture
Department of the Interior
Department of Transportation
Environmental Protection Agency
Food and Drug Administration
Occupational Safety and Health Administration

*Other participants include: NCATS, Tox21

USDA   ATSDR   NIOSH   NLM   FDA   NATIONAL CANCER INSTITUTE

More information: https://ntp.niehs.nih.gov/go/iccvam

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"Advances in science and technology have not been effectively leveraged to predict adverse human health effects"

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

Utilization

Technology

Confidence

January 2018

INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS

Help end-users guide the development of the new methods
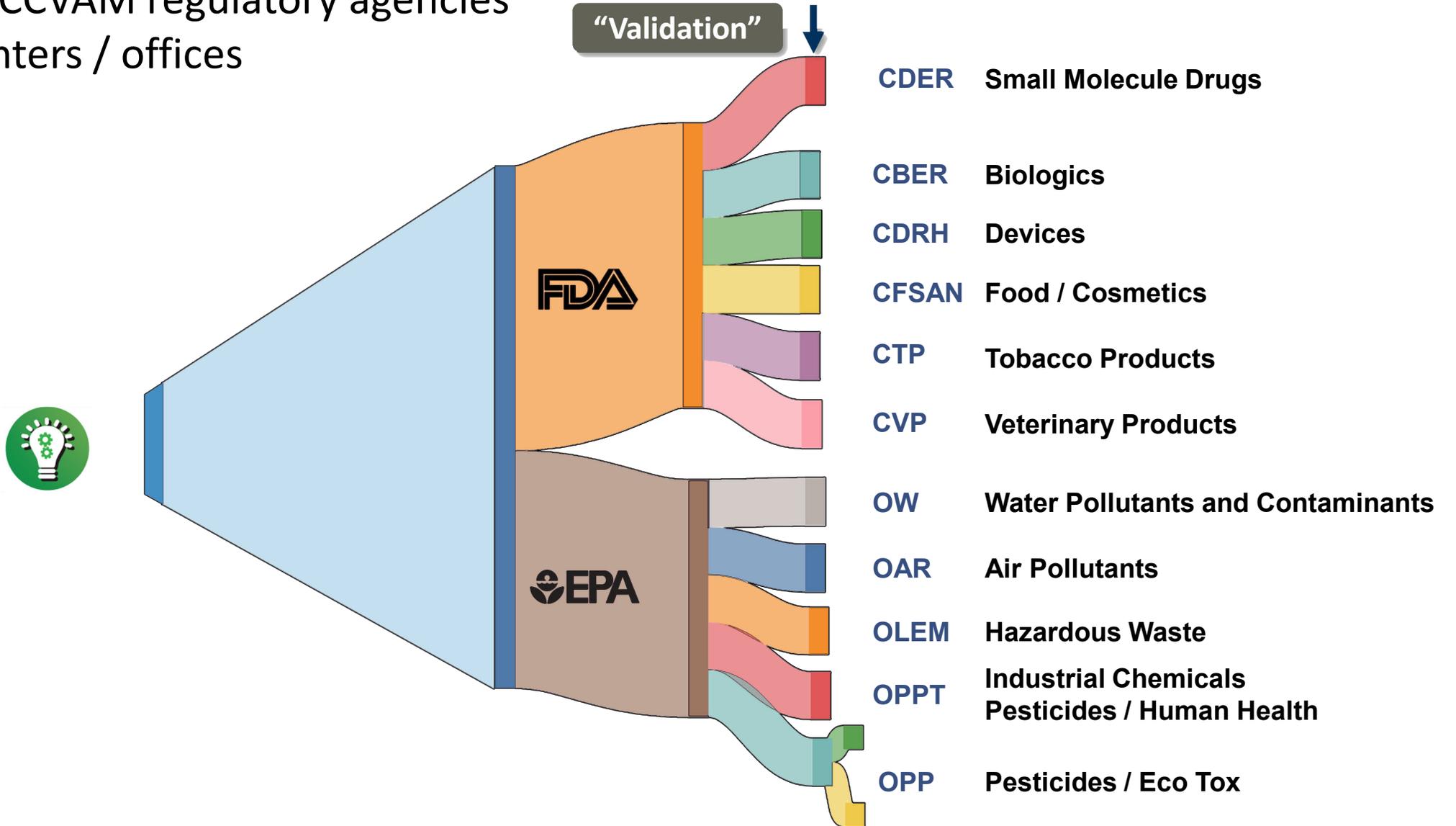
**Use efficient and flexible approaches to establish confidence in new methods**

Encourage the adoption of new methods by federal Agencies and regulated industries

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

Example of two ICCVAM regulatory agencies with multiple centers / offices

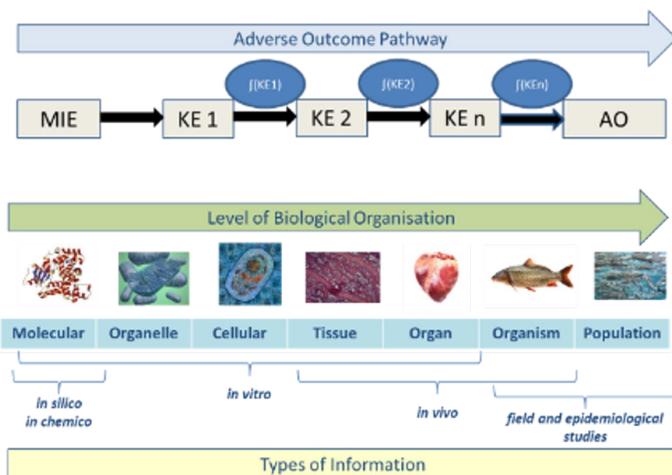# "Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies"
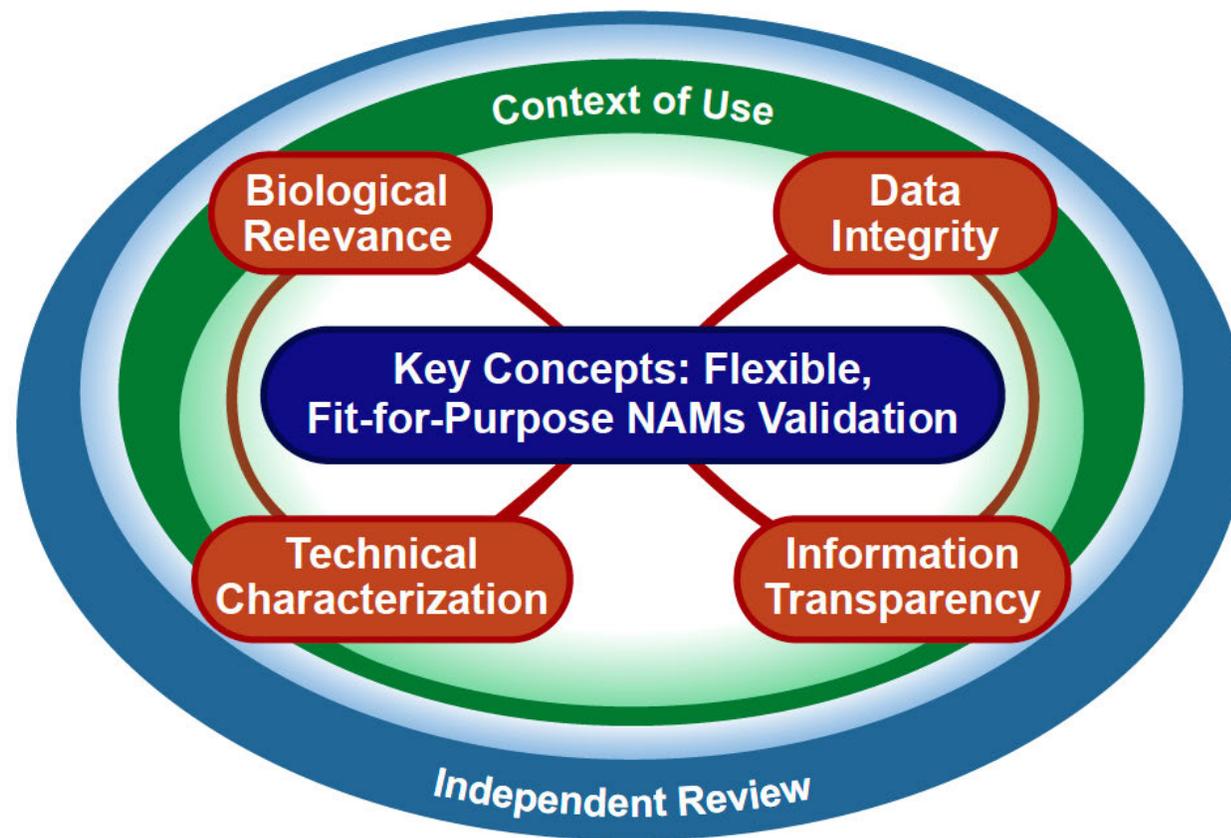
Public Comment by Sept. 5th

Draft ICCVAM Validation Report, Figure 1

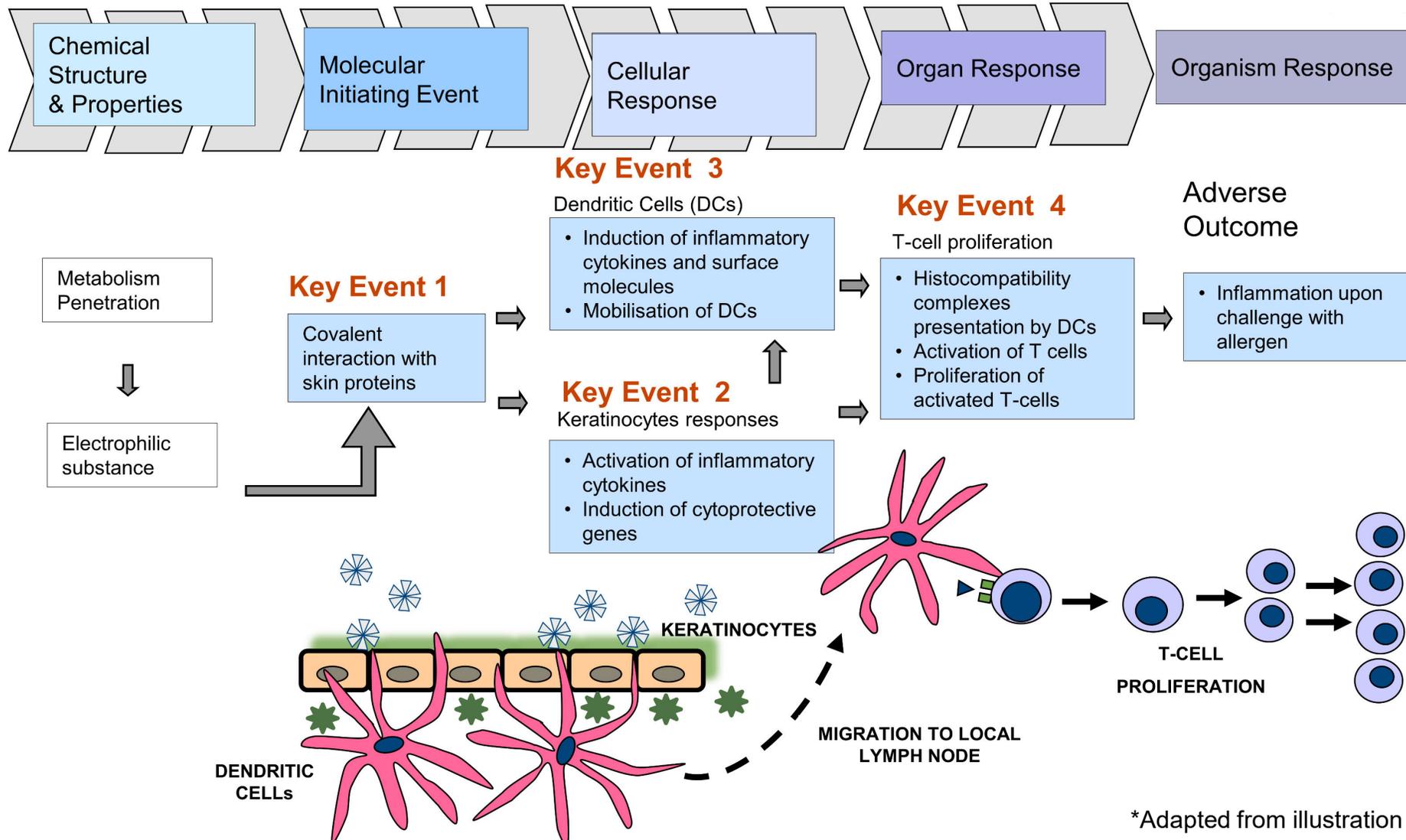https://ntp.niehs.nih.gov/go/ICCVAM-submit

Examples of Endpoints where Biological and Mechanistic Relevance of NAMs has been Demonstrated to Support Regulatory Applications

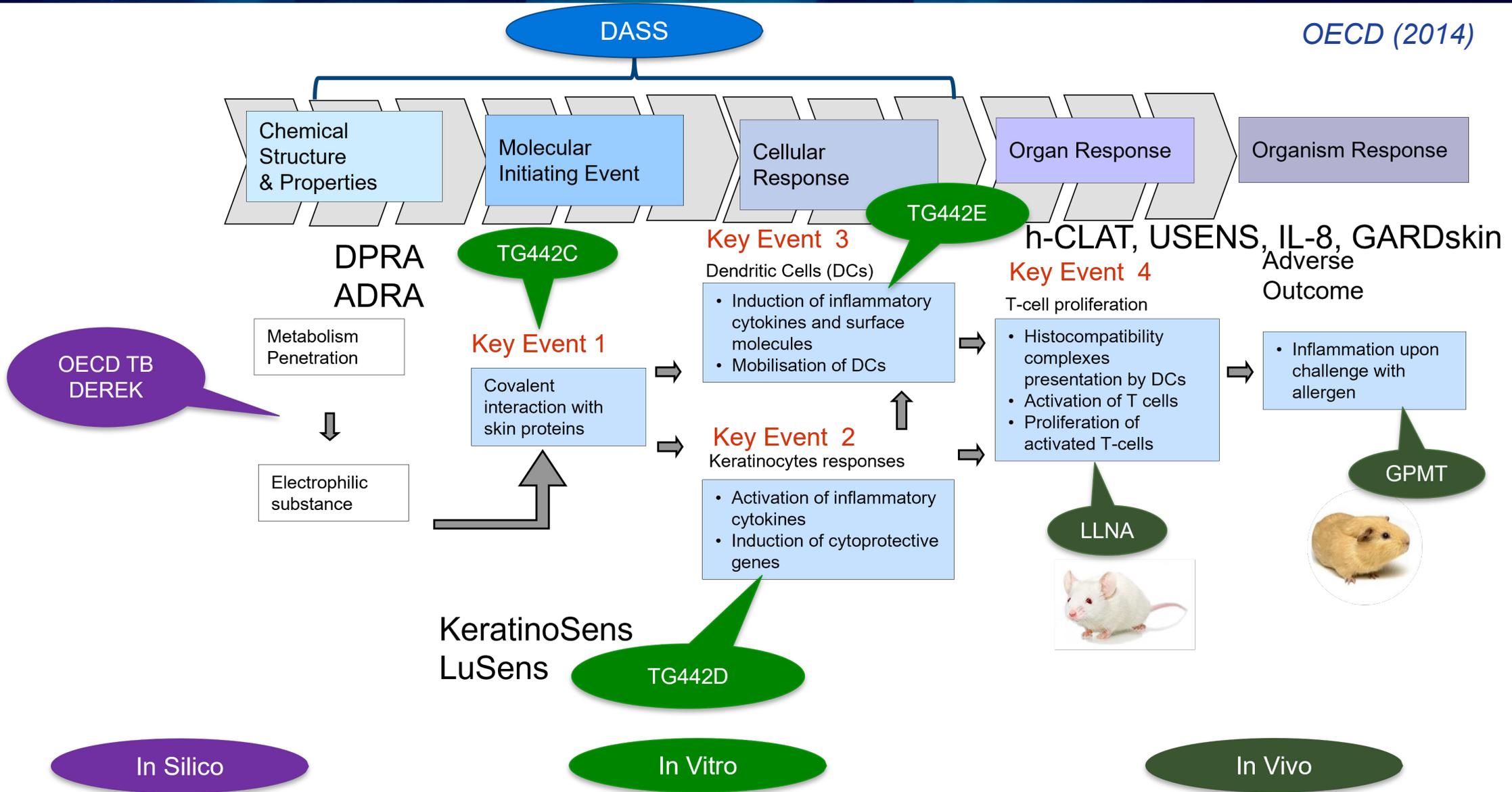| Endpoint	Summary	Reference
Skin sensitization	The endpoint has a well-developed human relevant AOP to which defined approaches combining several NAMs are mapped and described in OECD Guideline 497.	Kleinstreuer et al., 2018; OECD, 2021a
Endocrine disruption	Established pathway models using complementary NAMs as part of an integrated strategy are available for estrogen and androgen receptor activity. EPA accepts these NAMs for Tier 1 screening in the Endocrine Disruptor Screening Program.	Judson et al., 2015; Kleinstreuer et al., 2017; EPA, 2023
Developmental neurotoxicity	Limited AOPs exist for this complex endpoint. Instead, a battery of NAMs covering critical processes of human neurodevelopment has been developed. An OECD GD on the battery is available that includes integrated approaches to testing and assessment (IATA) case studies.	Crofton and Mundy, 2021; OECD, 2022a; OECD, 2023
Inhalation toxicity	An alternative approach using an in vitro human-cell based assay and computational modeling was used to characterize the hazard of chlorothalonil and derive a point of departure for use in EPA human health risk assessment. This approach was also published as an OECD IATA case study.	Corley et al., 2021; EPA, 2021c; OECD, 2022b

Draft ICCVAM Validation Report, Table 3

http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

Test Methods Mapped to AOP

OECD (2014)

DASS

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

DPRA
ADRA

TG442C

Metabolism Penetration

OECD TB DEREK

Electrophilic substance

Key Event 1

Covalent interaction with skin proteins

Key Event 3

Dendritic Cells (DCs)
- Induction of inflammatory cytokines and surface molecules
- Mobilisation of DCs

TG442E

h-CLAT, USENS, IL-8, GARDskin

Adverse Outcome

Key Event 4

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

- Inflammation upon challenge with allergen

GPMT

Key Event 2

Keratinocytes responses
- Activation of inflammatory cytokines
- Induction of cytoprotective genes

KeratinoSens
LuSens

TG442D

LLNA

In Silico

In Vitro

In Vivo

*Adapted from illustration by D. Sailstad

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Section 4
Health effects

Guideline No. 497

Guideline on Defined Approaches for Skin Sensitisation

4 July 2023

OECD Guidelines for the Testing of Chemicals
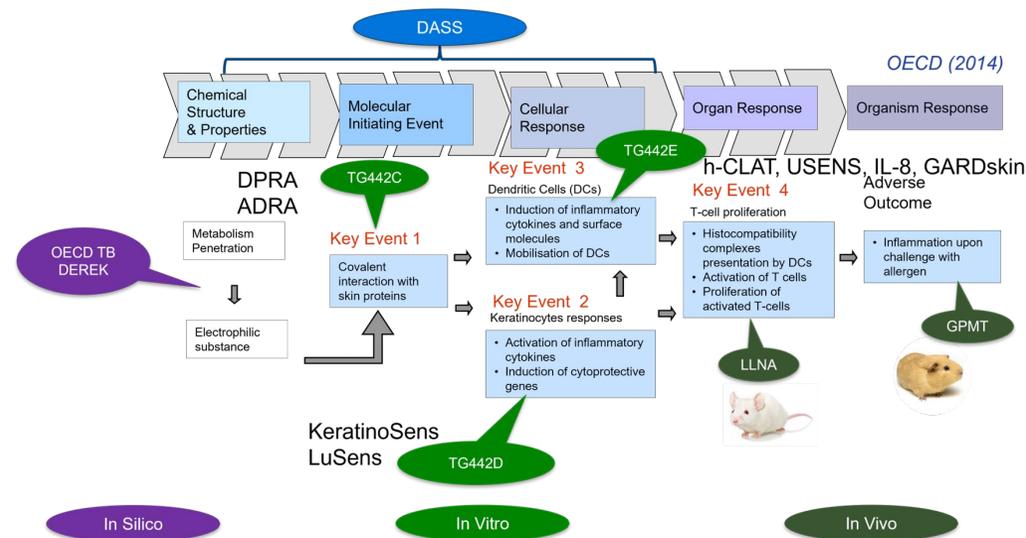
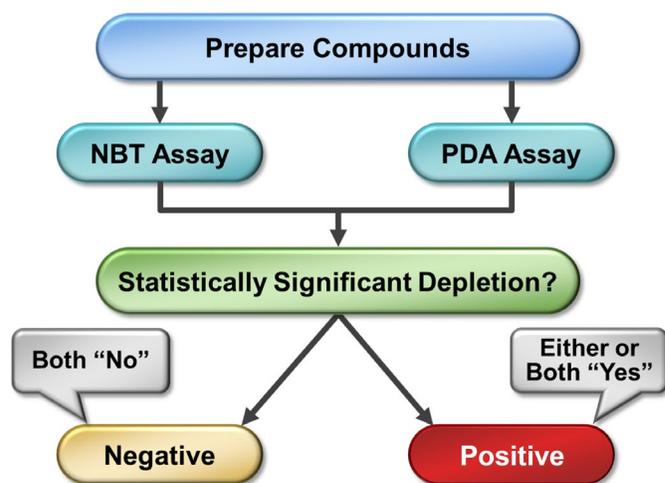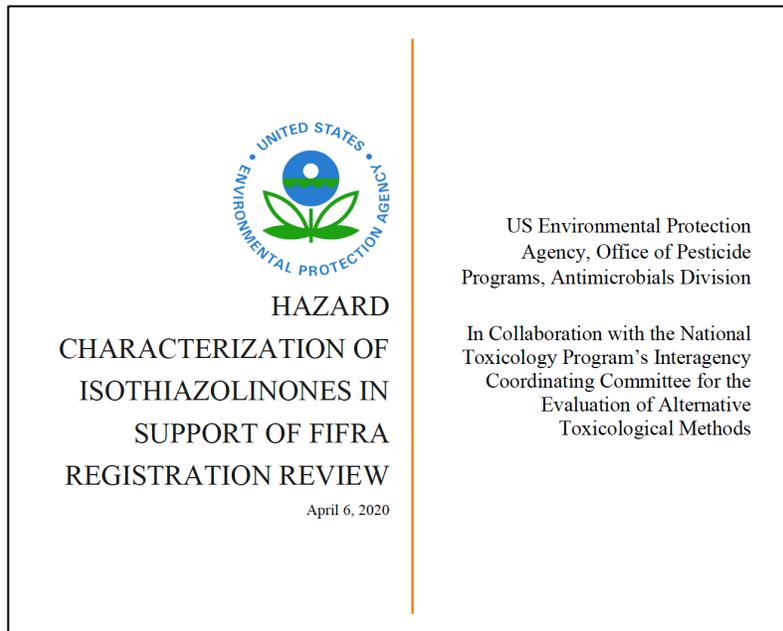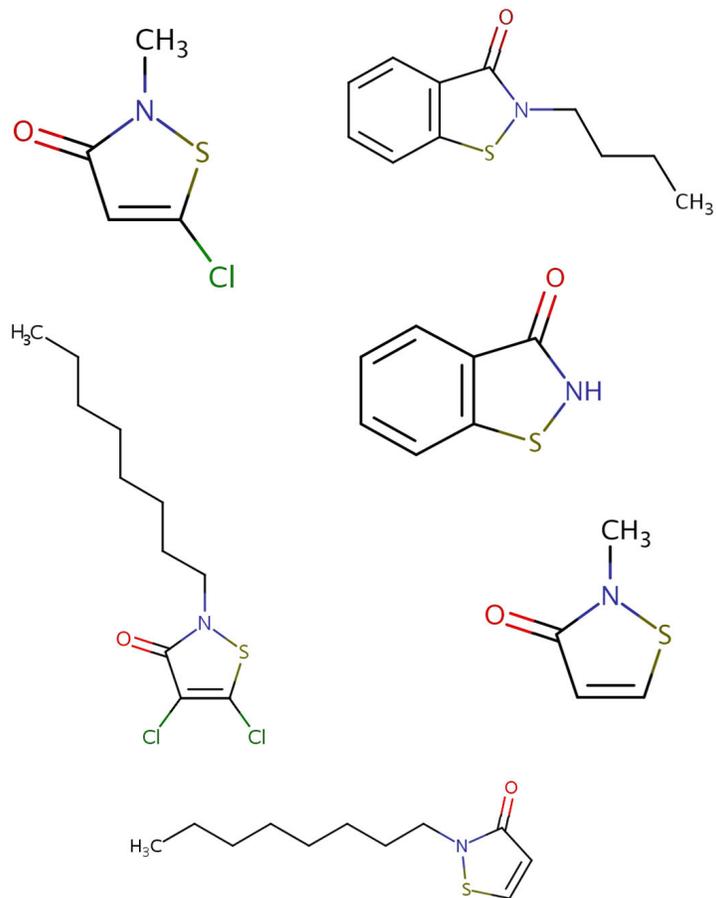| DA/Method	Information Sources	Capability (Hazard and/or Potency)	Hazard Performance vs. LLNA N~168	Hazard Performance vs. Human N~63	GHS Potency Performance vs. LLNA (Accuracy)	GHS Potency Performance vs. Human (Accuracy)
2o3 DA	DPRA, KeratinoSens™, h-CLAT	Hazard	84% BA, 82% Sens, 85% Spec	88% BA, 89% Sens, 88% Spec	-	-
ITSv1 DA	DPRA, h-CLAT, DEREK Nexus v6.1.0	Hazard, Potency (GHS)	81% BA, 92% Sens, 70% Spec	69% BA, 93% Sens, 44% Spec	70% NC, 71% 1B, 74% 1A	44% NC, 77% 1B, 65% 1A
ITSv2 DA	DPRA, h-CLAT, OECD QSAR Toolbox v4.5	Hazard, Potency (GHS)	80% BA, 93% Sens, 67% Spec	69% BA, 94% Sens, 44% Spec	67% NC, 72% 1B, 72% 1A	44% NC, 80% 1B, 67% 1A
LLNA (provided for comparison)	*in vivo*	Hazard, Potency	-	58% BA, 94% Sens, 22% Spec	-	25% NC, 74% 1B, 56% 1A

- Addresses KE1 in the Skin Sensitization AOP
- *In chemico* plate-based assay
  – Measures protein reactivity of a chemical via fluorescent or colorimetric probes
- Multi-lab validation study
  – Participating labs: U.S. FDA, DoD, CPSC/NIST, BRT, Inc.
  – Utilize 2019 OECD* Performance Standards for KE1-based assays for validation study
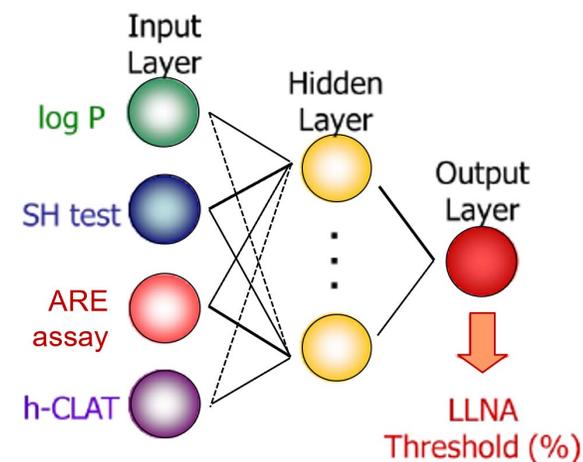 – Testing and data analysis are completed

Validation Study

Lab#	Balanced Accuracy	Sensitivity	Specificity	Within Lab Reproducibility	Between Lab Reproducibility
1	76%	85%	67%	94%	
2	82%	92%	71%	100%	
3	84%	85%	83%	97%	96%
4	84%	85%	83%	94%	
Mean	82%	87%	76%	96%	

*No. 303; Series on Testing and Assessment https://www.oecd.org/chemicalsafety/testing/performance-standards.htm
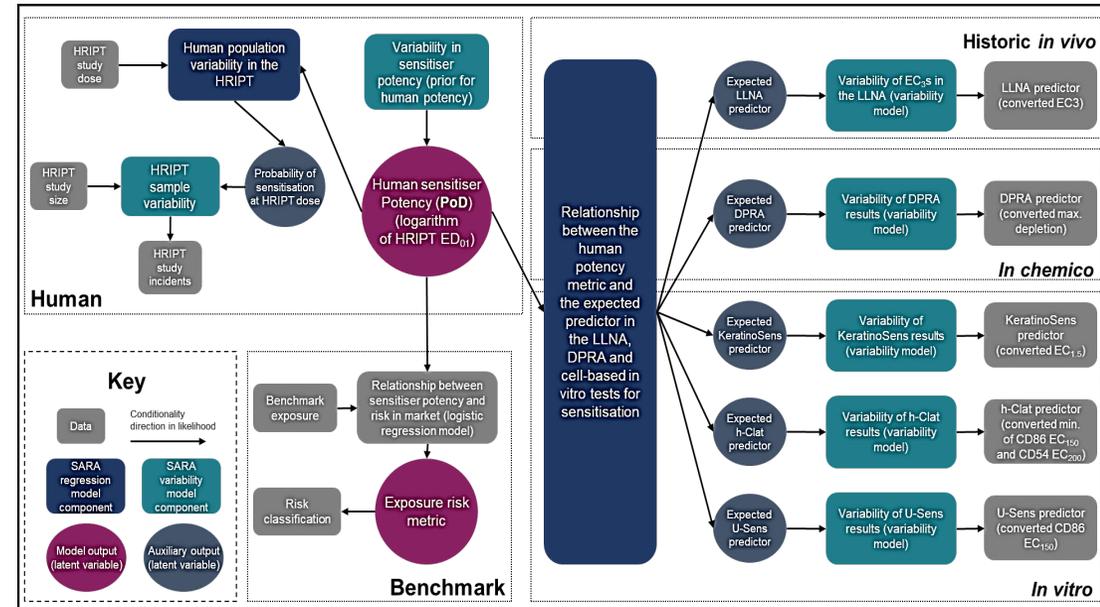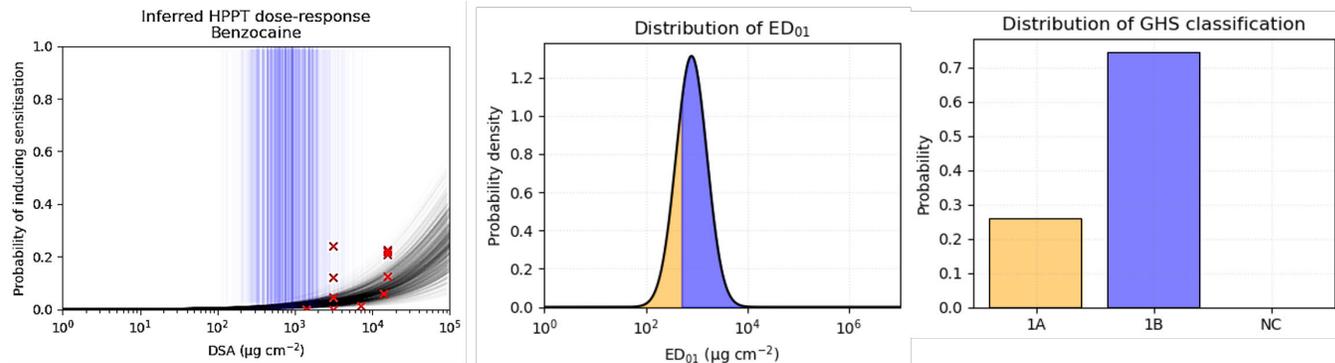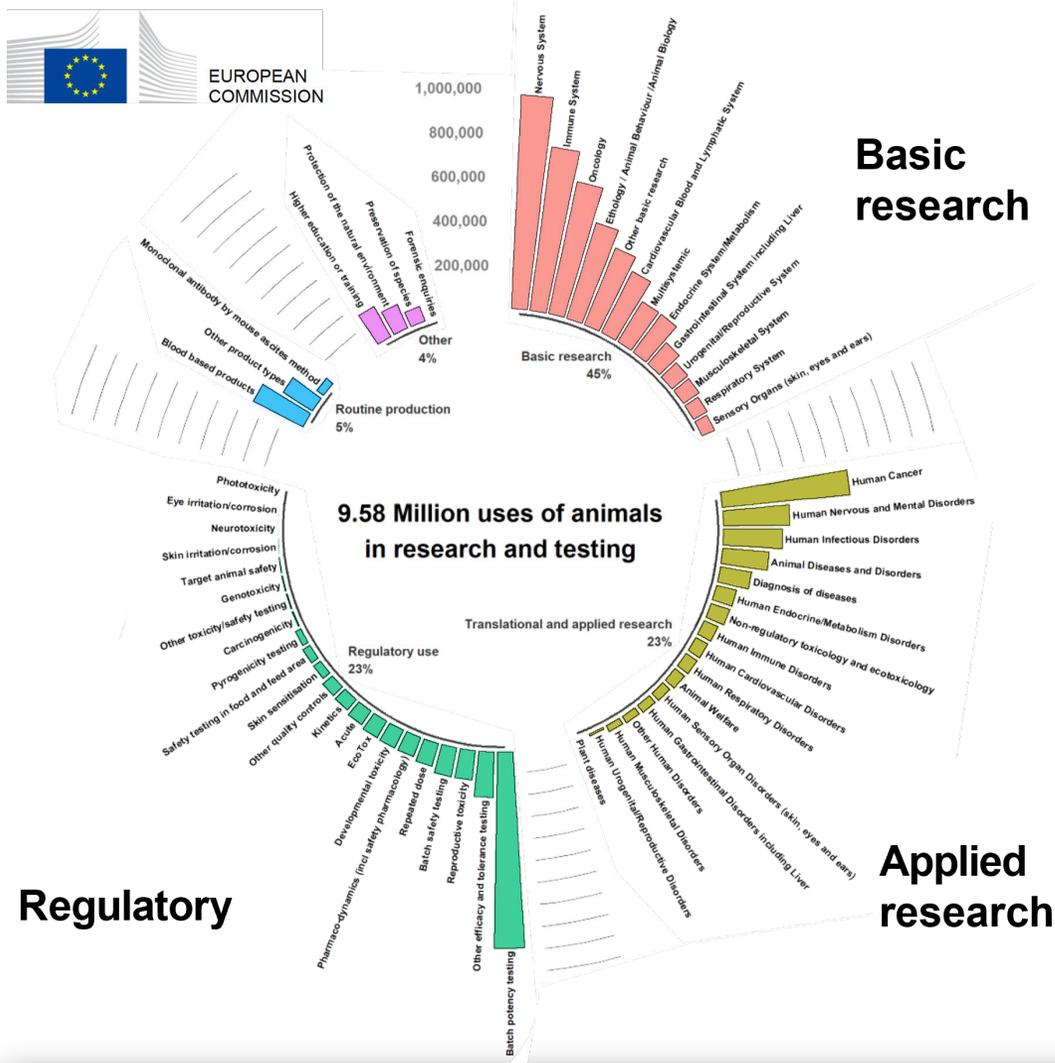
Artificial Neural Network Models

Isothiazolinone biocides are used as material preservatives to prevent the growth of microbial organisms and are used in industrial processes and consumer products

https://www.federalregister.gov/documents/2020/05/14/2020-10376/

pesticide-registration-review-draft-human-health-and-ecological-risk-assessments-for-several

Bayesian Probabilistic Modeling

Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) Model

- A Bayesian statistical model which infers a human-relevant metric of sensitizer 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.

- Uses various combinations of *in chemico*, *in vitro*, and *in vivo* data to yield a probability distribution that can be applied to regulatory decision making.

Gilmour et al., 2022 and Reynolds et al., 2022

On OECD Workplan for potential inclusion in TG497

9.58 Million uses of animals in research and testing

2019 report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017

- Effective regulatory decision making relies upon effective research to develop methods that will answer critical questions about human health and the environment.

- A shift in focus from a chemical-centric to a disease-centric approach requires developing a more in-depth mechanistic understanding of human diseases and their susceptibility to exogenous perturbations.

- NAMs are key to this understanding, and engaging the biomedical research community is foundational to successful NAM development, validation, and application.

Acknowledgments

The NICEATM Group

ICCVAM 2020-2021 Biennial Progress Report

https://ntp.niehs.nih.gov/go/2021iccvamreport

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Integrated Chemical Environment

ICCVAM VWG

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New Approach Methodologies to Address Population Variability and Susceptibility in Human Risk Assessment

Guest Editors:

Helena Hodberg-Durdock: The National Institutes of Health, USA
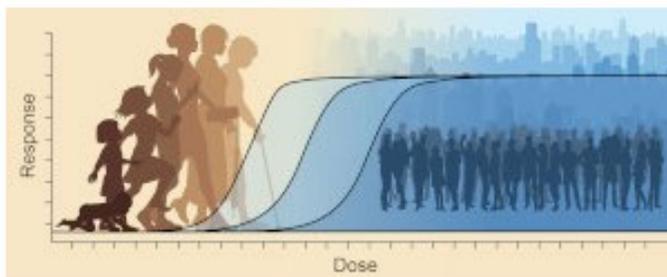
Nicole Kleinstreuer: The National Institutes of Health, USA

Kim To: Inotiv, USA

SPRINGER NATURE GROUP
SDG Programme
supporting the Sustainable Development Goals

Submission Status: Open | Submission Deadline: 31 December 2023

Human Genomics is calling for submissions to our Collection on "New Approach Methodologies to Address Population Variability and Susceptibility in Human Risk Assessment". This Collection supports and amplifies research related to SDG 3, Good Health and Well-Being.

https://www.biomedcentral.com/collections/NAMAPVS