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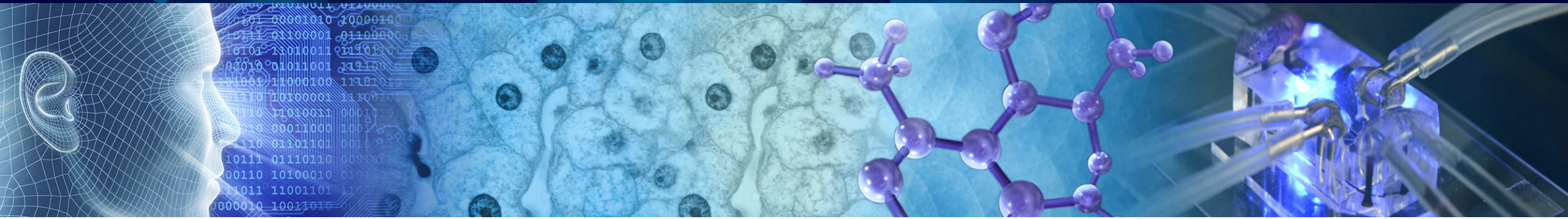

# Experiences from the US-Roadmap to regulatory acceptance of non-animal methods

**Nicole C. Kleinstreuer, PhD**

**Director, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods**

**Executive Director, Interagency Coordinating Committee for the Validation of Alternative Methods**

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

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# NICEATM and ICCVAM

- 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.


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

UNITED STATES ICCVAM *Advancing Alternatives to Animal Testing*

### 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

### 10 Research Agencies

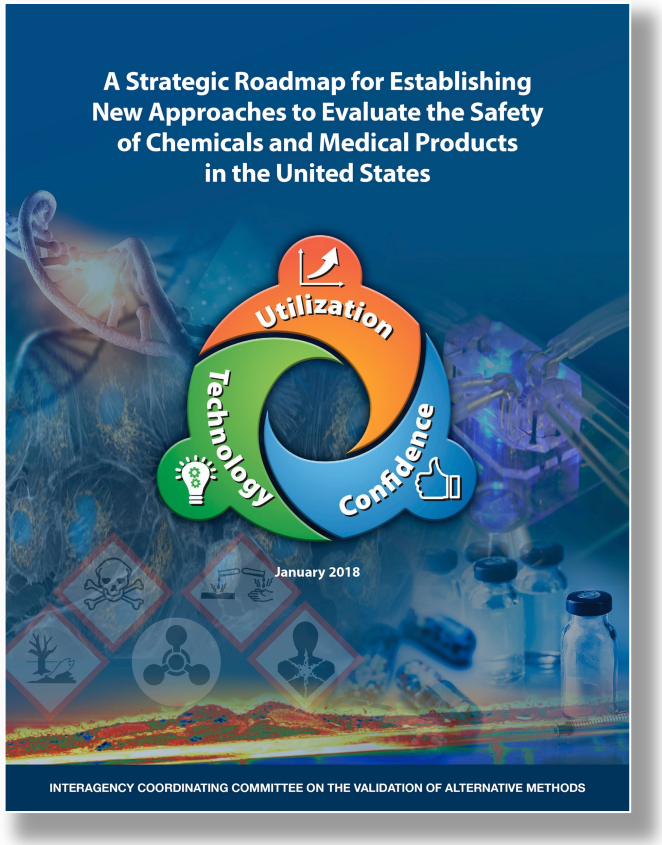
Agency for Toxic Substances and Disease Registry
National Institute for Occupational Safety and Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
National Institutes of Health
Department of Defense
Department of Energy
National Institute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

*Other participants include: NCATS, Tox21 Representatives

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

# U.S. Strategy and Roadmap

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

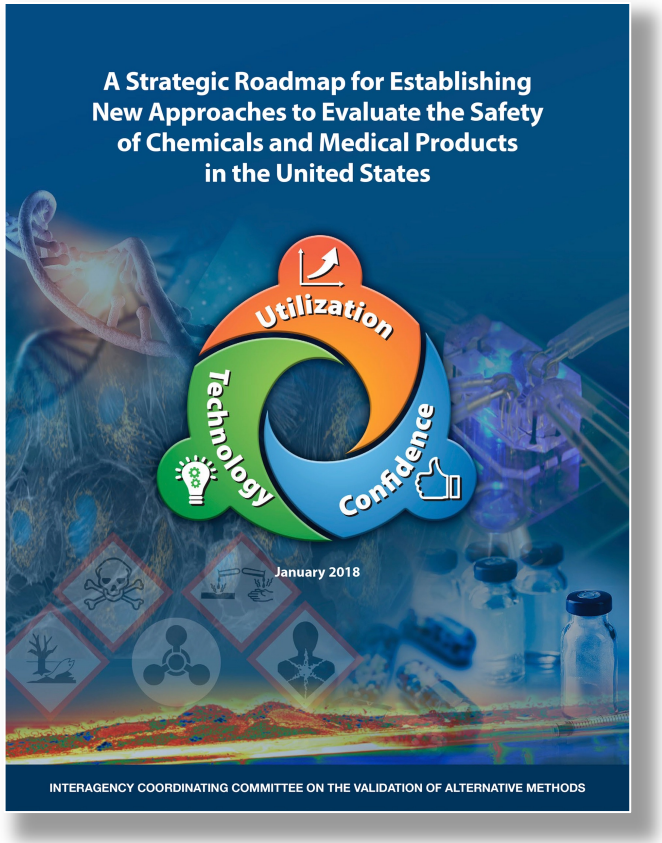



- **Help end-users guide the development of the new methods**
- **Use efficient and flexible approaches to establish confidence in new methods**
- **Encourage the adoption of new methods by federal Agencies and regulated industries**

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

# U.S. Strategy and Roadmap

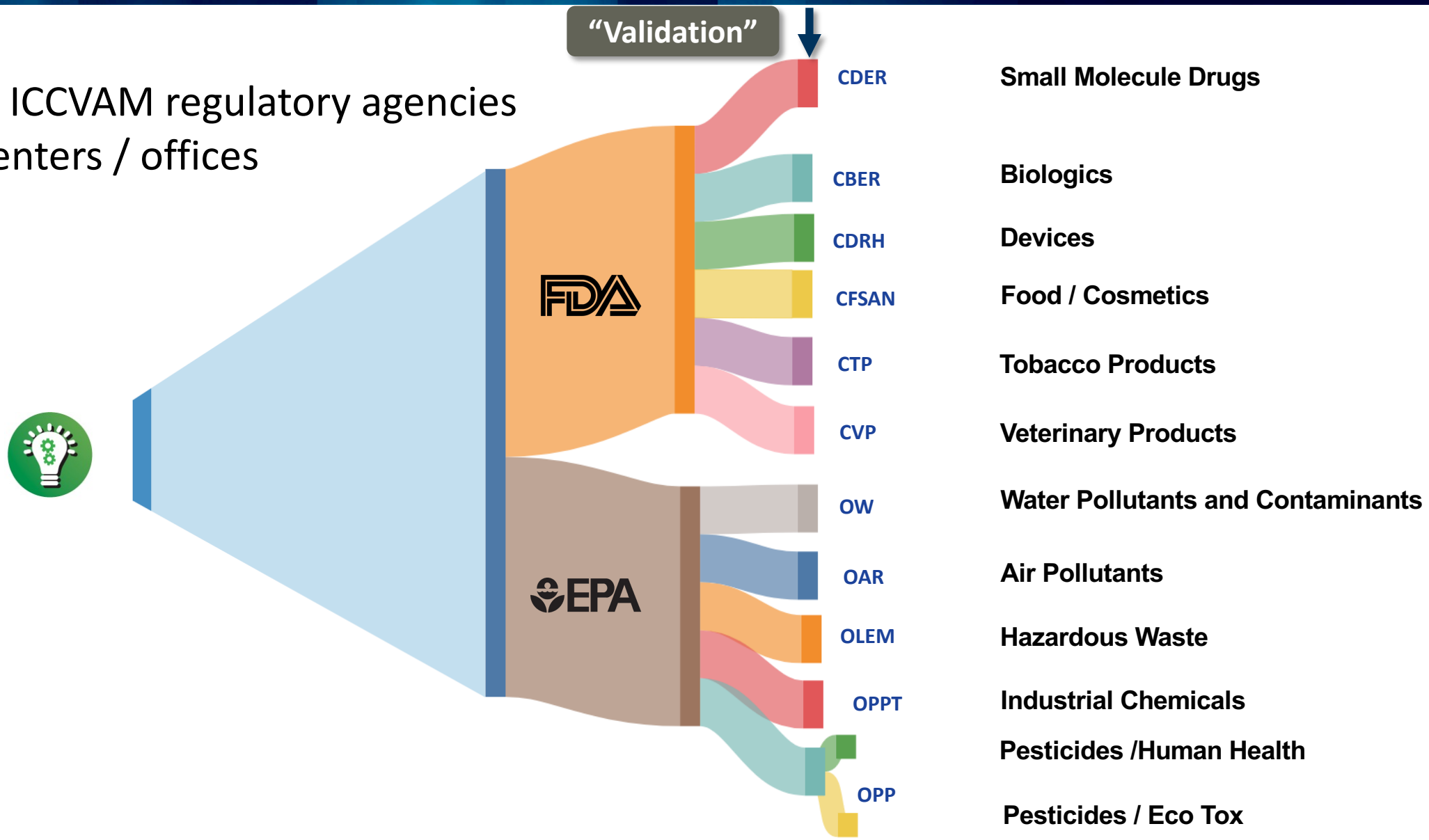
*"Advances in science and technology have not been effectively leveraged to predict adverse human health effects"*




- Help end-users [illegible] the development of the new m[illegible]
- Use eff[illegible]nt and [illegible]e ap[illegible]aches to establis[illegible]nce [illegible]new [illegible]thods
- Encourage t[illegible] new methods by federal Agencies and regulated industries

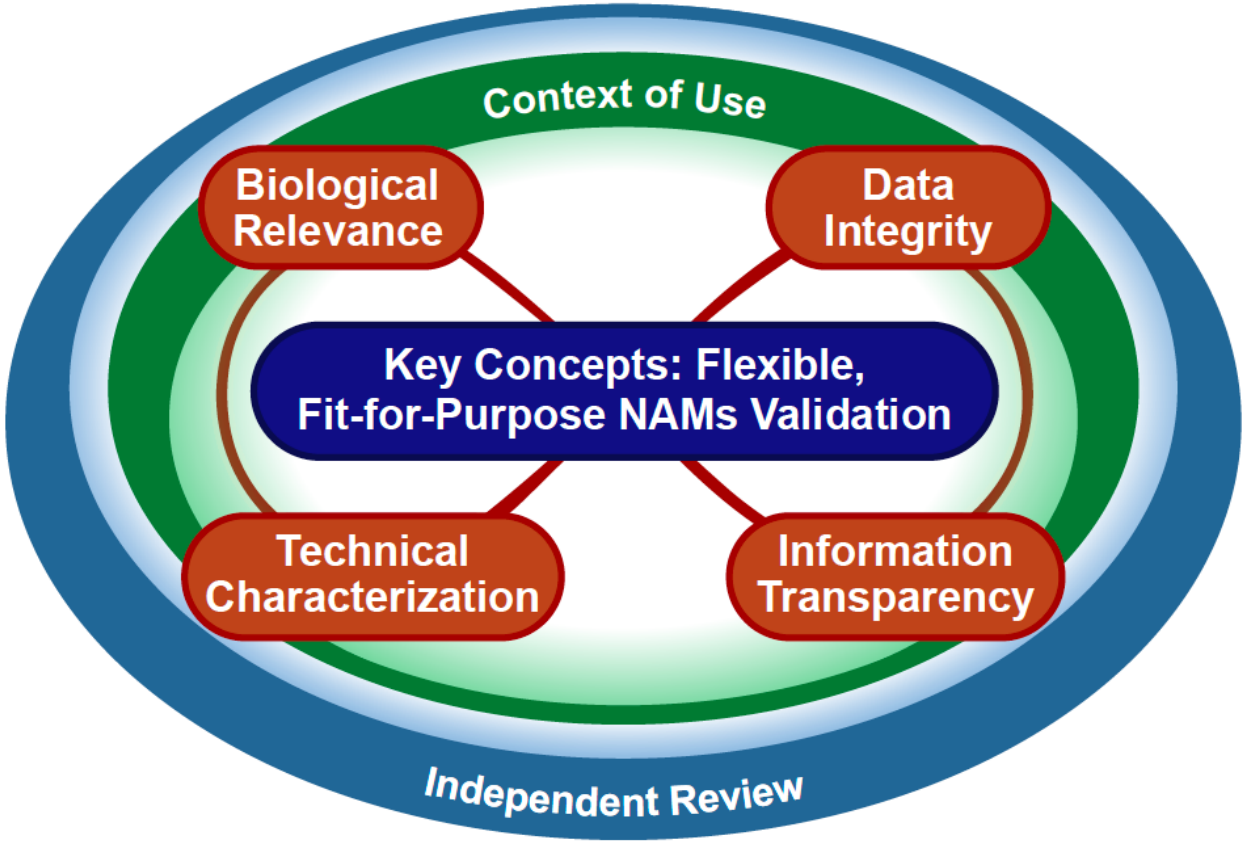

https://ntp.niehs.nih.gov/go/natl-strategy

# Example of two ICCVAM regulatory agencies with multiple centers / offices



# ICCVAM VWG Report: Validation, Qualification, and Regulatory Acceptance of NAMs

## Key Concepts to Consider During Development and Implementation of Flexible, Fit-for-Purpose NAMs Validation Strategies




*Draft ICCVAM Validation Workgroup Report, Figure 1*
*Adapted from van der Zalm et al. 2022*

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*

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# Acute Toxicity 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 for in silico model development ongoing |
| Eye irritation | • NAMs for Cat I and/or Cat IV (TG 437, 438, 460, 491, 492, 494, 496); Prospective testing ongoing; Human-biology based DAs |
| Skin irritation | • NAMs for Cat I or Cat IV (TG 430, 431, 435, 439); Prospective testing ongoing; 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; van der Zalm et al. 2023 Cut Ocu Tox*

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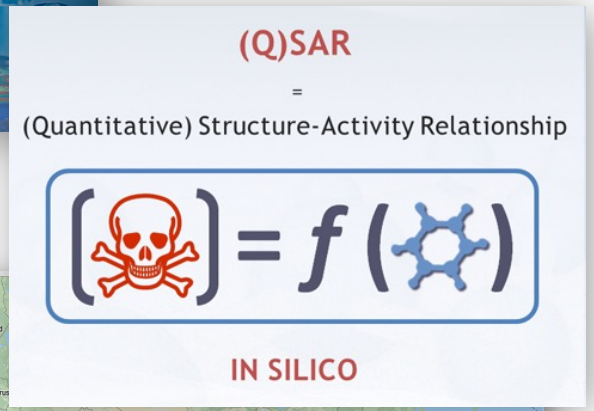
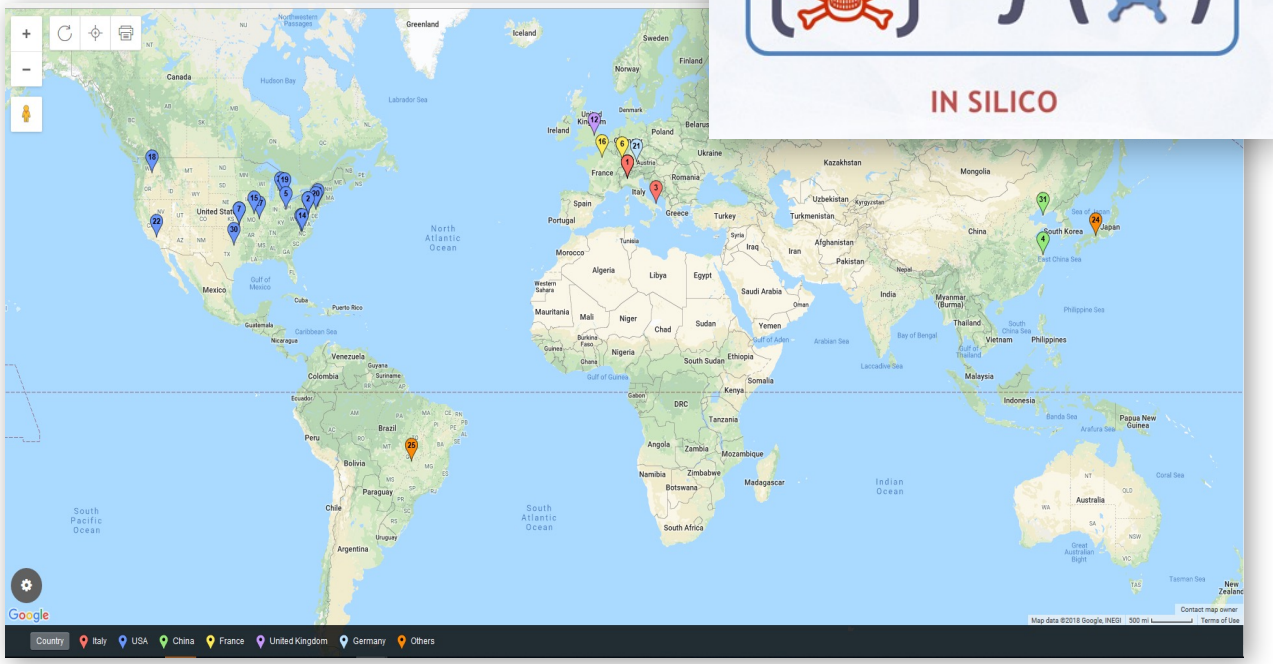
# Acute Oral Toxicity: Global Crowdsourcing Predictive Models




- 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

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

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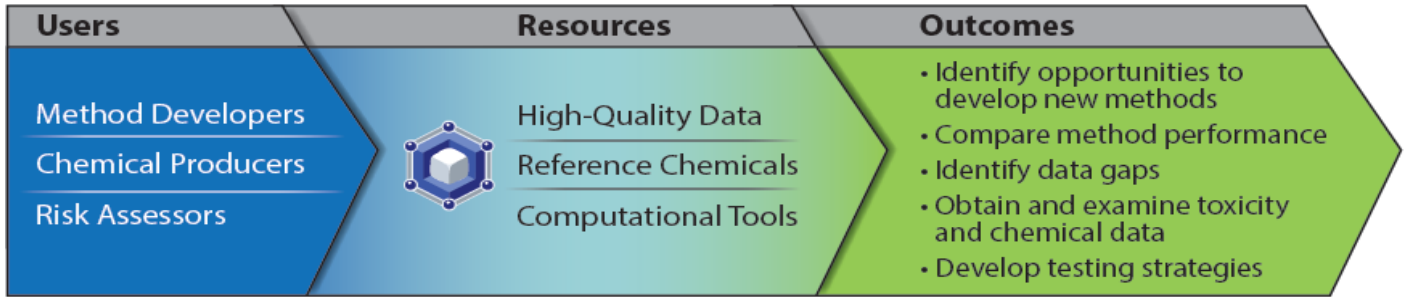
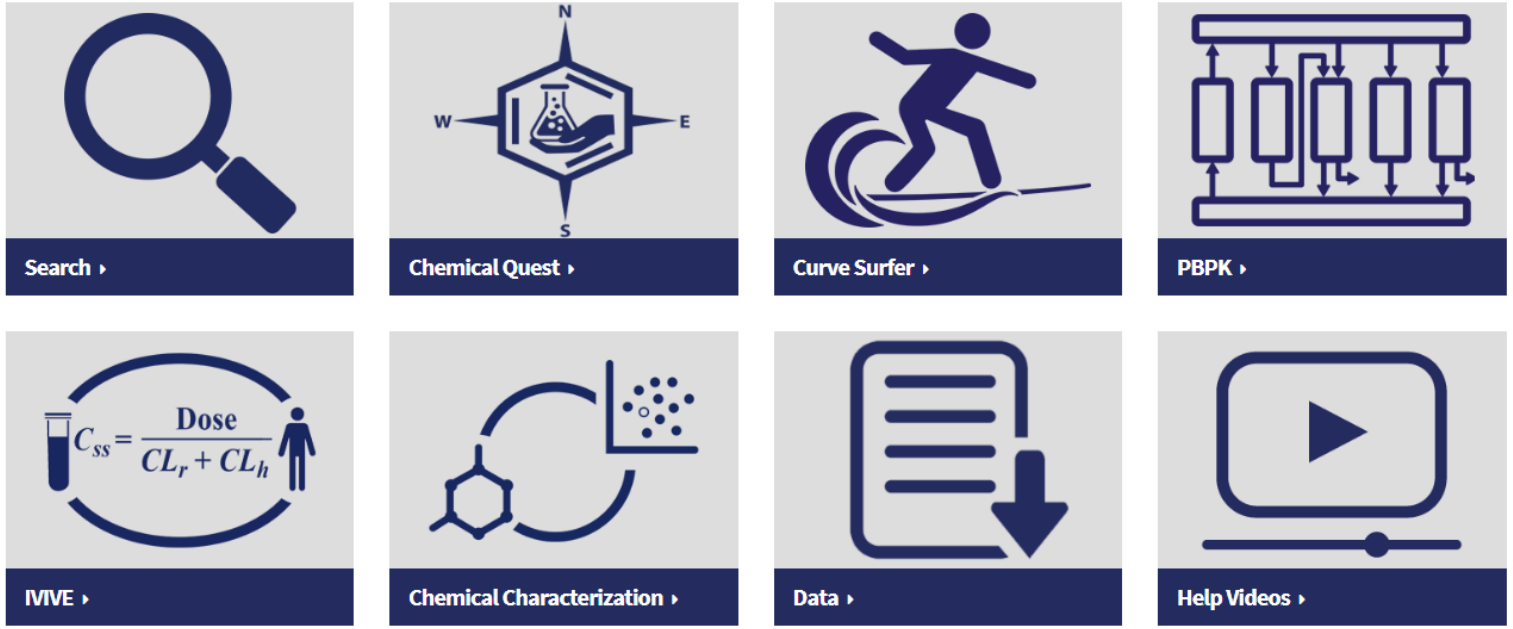
# ICE: The Integrated Chemical Environment

OPERA
OPEn (q)saR App




Integrated Chemical Environment




**ICE v4.0.1**
**August 2023**

Findable Accessible Interoperable Reusable

*Bell et al. 2017 EHP*
*Bell et al. 2020 Tox In Vitro*
*Abedini et al. 2021 Comp Tox*
*Daniel et al. 2022 Front Toxicol*

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

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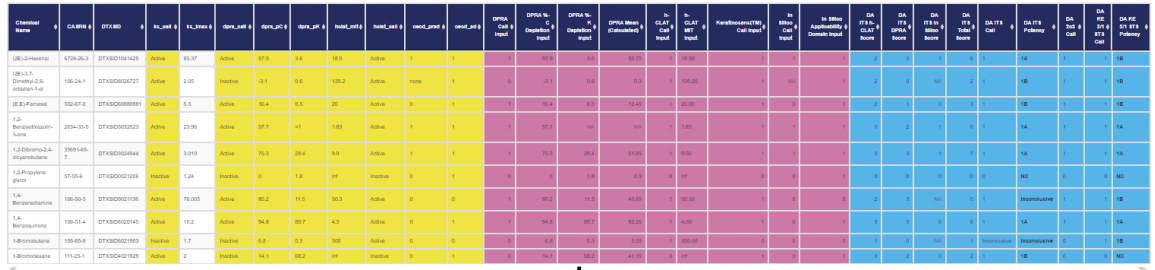
# User Friendly DASS App



**Color-coded results table**
**Yellow: User selected data columns**
**Pink: Translated user data, input for DASS algorithms**
**Blue: DASS predictions**

| DPRA Mean (Calculated) |
|---|
| 50.75 |
| 0.3 |
| 12.45 |
| NA |
| 51.85 |
| 0.9 |
| 45.85 |
| 92.25 |
| 3.55 |
| 41.15 |

**Values calculated by the app are shown to help the user understand derivation of predictions.**

**Translated input shows how the app interprets flagged data.**

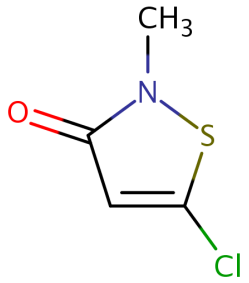
**Individual and combined scores from the ITS are provided.**

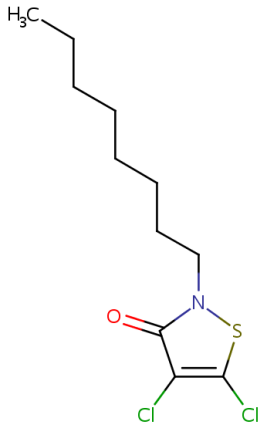
| dpra_pK | DPRA %-K Depletion Input |
|---|---|
| 3.6 | 3.6 |
| 0.6 | 0.6 |
| 8.5 | 8.5 |
| <1 | NA |
| 28.4 | 28.4 |
| 1.8 | 1.8 |
| 11.5 | 11.5 |
| 89.7 | 89.7 |
| 0.3 | 0.3 |
| 68.2 | 68.2 |

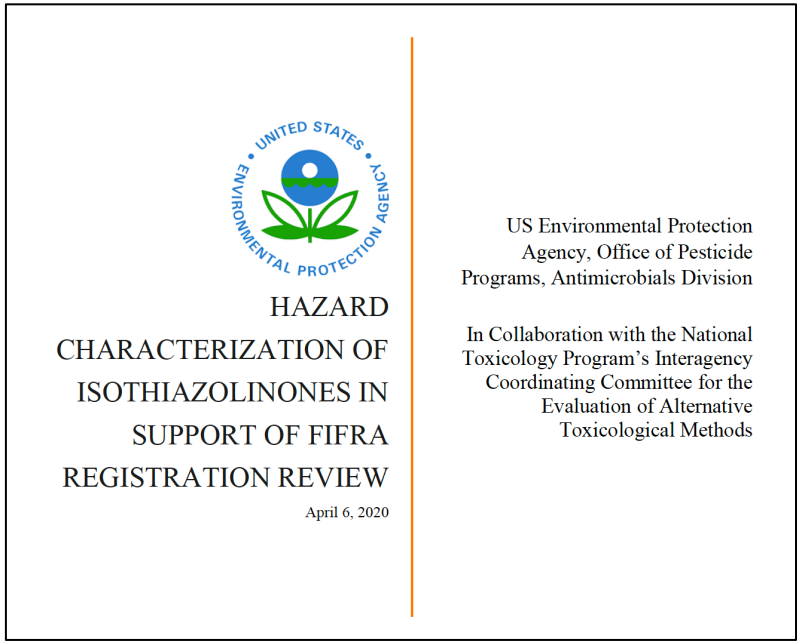
| DA ITS h-CLAT Score | DA ITS DPRA Score | DA ITS in Silico Score | DA ITS Total Score | DA ITS Call | DA ITS Potency | DA 2o3 Call | DA KE 3/1 STS Call | DA KE 3/1 STS Potency |
|---|---|---|---|---|---|---|---|---|
| 2 | 3 | 1 | 6 | 1 | 1A | 1 | 1 | 1B |
| 2 | 0 | NA | 2 | 1 | 1B | 1 | 1 | 1B |
| 2 | 1 | 0 | 3 | 1 | 1B | 1 | 1 | 1B |
| 3 | 2 | 1 | 6 | 1 | 1A | 1 | 1 | 1A |
| 3 | 3 | 1 | 7 | 1 | 1A | 1 | 1 | 1A |
| 0 | 0 | 0 | 0 | 0 | NC | 0 | 0 | NC |
| 2 | 3 | NA | 5 | 1 | Inconclusive | 1 | 1 | 1B |
| 3 | 3 | 0 | 6 | 1 | 1A | 1 | 1 | 1A |
| 1 | 0 | NA | 1 | Inconclusive | Inconclusive | 0 | 1 | 1B |
| 0 | 2 | 0 | 2 | 1 | 1B | 0 | 0 | NC |

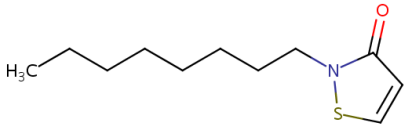
**Access the DASS App**
https://ntp.niehs.nih.gov/go/952311

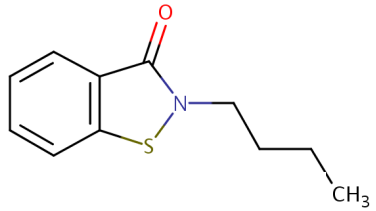
# Application of DASS to Risk Assessment

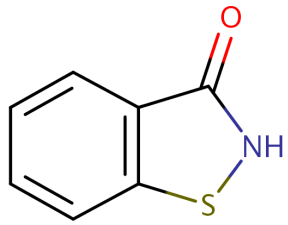


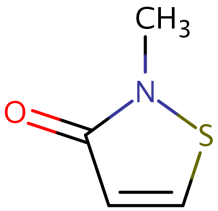




Isothiazolinone biocides are used as material preservatives to prevent the growth of microbial organisms and are used in industrial processes and consumer products

https://www.federalregister.gov/documents/2020/05/14/2020-10376/pesticide-registration-review-draft-human-health-and-ecological-risk-assessments-for-several

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# US EPA/OCSPP NAMs Metrics


https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-new-approach-0

## Strategic Vision for Adopting New Approach Methodologies – Metrics

| Fiscal year | Granted | Animal Reduction | Cost Savings* |
|---|---|---|---|
| 2018 | 62 | 16,500 | $8,900,000 |
| 2019 | 57 | 22,000 | $8,500,000 |
| 2020 | 36 | 11,800 | $3,500,000 |
| 2021 | 70 | 29,500 | $9,100,000 |

**Hazard and Science Policy Council (HASPOC) Metrics**

**Chemistry and Acute Toxicology Science Advisory Council (CATSAC) Metrics**

| Fiscal Year | Studies Saved | Animal Reduction | Cost Savings* |
|---|---|---|---|
| 2018 | 18 | 171-384 | $170,400 |
| 2019 | 24 | 255-590 | $284,900 |
| 2020 | 12 | 102-178 | $56,500 |
| 2021 | 18 | 165-410 | $221,700 |

**Non-animal Test Methods**

| Fiscal Year | Eye Irritation Tests | | Skin Irritation Tests | | Skin Sensitization Tests | |
|---|---|---|---|---|---|---|
| | OPP | OPPT | OPP | OPPT | OPP | OPPT |
| 2018 | 19 | 45 | 11 | 56 | 1 | 20 |
| 2019 | 12 | 40 | 7 | 49 | 0 | 19 |
| 2020 | 13 | 42 | 7 | 52 | 3 | 31 |
| 2021 | 32 | 39 | 28 | 54 | 12 | 23 |
| 2022 | 17 | 43 | 13 | 38 | 7 | 17 |
| Total | 93 | 209 | 66 | 249 | 23 | 110 |

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# Acknowledgments

## The NICEATM Group


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

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