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

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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

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

"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


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

Skin sensitization: [https://ntp.niehs.nih.gov/go/roadmap-sensit](https://ntp.niehs.nih.gov/go/roadmap-sensit)

Start with the End User in Mind

- 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
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


Characterizing Variability and Applying to Model Evaluation

Collaborative Acute Toxicity Modeling Suite (CATMoS) Performance

<table>
<thead>
<tr>
<th></th>
<th>Very Toxic</th>
<th>Non-Toxic</th>
<th>EPA</th>
<th>GHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Train</td>
<td>Eval</td>
<td>Train</td>
<td>Eval</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.87</td>
<td>0.70</td>
<td>0.88</td>
<td>0.67</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.99</td>
<td>0.97</td>
<td>0.97</td>
<td>0.90</td>
</tr>
<tr>
<td>Balanced</td>
<td>0.93</td>
<td>0.84</td>
<td>0.92</td>
<td>0.78</td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>In vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanced</td>
<td>0.81</td>
<td>0.89</td>
<td>0.82</td>
<td>0.79</td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
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</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Prior GHS category</th>
<th>1 (serious eye damage)</th>
<th>2A (irritant)</th>
<th>2B (mild irritant)</th>
<th>NC (non-irritant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73%</td>
<td>16%</td>
<td>0%</td>
<td>10%</td>
</tr>
<tr>
<td>2A (irritant)</td>
<td>4%</td>
<td>33%</td>
<td>4%</td>
<td>59%</td>
</tr>
<tr>
<td>2B (mild irritant)</td>
<td>0%</td>
<td>4%</td>
<td>16%</td>
<td>80%</td>
</tr>
<tr>
<td>NC (non-irritant)</td>
<td>1%</td>
<td>4%</td>
<td>2%</td>
<td>94%</td>
</tr>
</tbody>
</table>

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
Study Design: Test Phases/Test Methods

Phase 1
Assess validity of test methods

Phase 2
Refine test methods for potential use in defined approaches

Phase 3
Expand the number of formulations classified as mild or moderate irritants based on the in vivo test

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

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

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-LS
- In vitro depth of injury
  - Neat
  - Diluted

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

*Insufficient quantity of one formulation for testing in all additional methods
Over-/underprediction relative to consensus and PPE labeling

<table>
<thead>
<tr>
<th>Formulation Information</th>
<th>GHS Predictions</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
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<td>D</td>
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<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Z</td>
</tr>
<tr>
<td></td>
<td>AB</td>
</tr>
</tbody>
</table>

*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)

Consensus

<table>
<thead>
<tr>
<th>Effects</th>
<th>GHS Classification</th>
<th>PPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosive</td>
<td>Category 1</td>
<td>Eye protection</td>
</tr>
<tr>
<td>Moderate irritant</td>
<td>Category 2A</td>
<td>Eye protection</td>
</tr>
<tr>
<td>Mild irritant</td>
<td>Category 2B</td>
<td>Eye protection</td>
</tr>
<tr>
<td>Non-corrosive/ minimal irritant</td>
<td>Not Classified</td>
<td>None noted</td>
</tr>
</tbody>
</table>

**Effects**

- Concordant with consensus
- Underpredicted relative to consensus, but same PPE labeling
- Overpredicted relative to consensus; PPE (overprotective)
- Underpredicted relative to consensus; no PPE (underprotective)
Test Methods Mapped to AOP

Key Event 1: Covalent interaction with skin proteins
- Induction of inflammatory cytokines
- Mobilisation of DCs

Key Event 2: Keratinocytes responses
- Activation of inflammatory cytokines
- Induction of cytoprotective genes

Key Event 3: Dendritic Cells (DCs)
- 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

Organism Response: Inflammation upon challenge with allergen

In Silico
- OECD TB
- DEREK

In Vivo
- LLNA
- GPMT
- DPRA
- ADRA
- KeratinoSens
- LuSens

In Vitro
- TG442C
- TG442D
- TG442E
- hCLAT, USENS, IL-8

DASS

Chemical Structure & Properties
- Metabolism
- Penetration
- Electrophilic substance

Molecular Initiating Event
- Covalent interaction with skin proteins

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

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

Organism Response
- Inflammation upon challenge with allergen

Keratinocytes responses
- Key Event 1
- Key Event 2
- Key Event 3
- Key Event 4

Adverse Outcome

Defined Approaches for Skin Sensitization (DASS)

2 Out of 3

Test Chemical

KE a

KE b

Concordant?

YES

NO

Concordance

Concordance

Ke class

Classify based on concordance

Classify based on 95% concordance

KE 3/1 STS

h-CLAT

Positive

Negative

DPPRAL

MIT ≤ 10

MIT > 10

Positive

Negative

Potency Classification

1A

1B

NC

ITSv2

DPRA

h-CLAT

OECD Toolbox v4.5

Mean Cys and Lys Depletion (%) Cys Depletion Only Score

x ≥ 42.47 x ≥ 98.24 3

29.02 ≤ x < 42.47 25.09 ≤ x < 98.24 2

6.38 ≤ x < 22.62 13.89 ≤ x < 23.09 1

x < 6.38 x < 13.89 0

MIT (μg/mL) Score Prediction Score

≤ 10 3 Positive 1

10 < x ≤ 150 2 Negative 0

150 < x ≤ 5000 1

Negative 0

Total Score Potency

6-7 Strong

2-6 Week

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)
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
Acknowledgments

The NICEATM Group