DNTP/NICEATM Update

ICCVAM Public Forum
May 27, 2021

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Acting NICEATM Director
Each assessment in the pipeline qualifies the previous one.

Build confidence in the relationship between MOA and in vivo health effect.
DNTP Strategic Areas of Focus

Collaboratively address public health challenges

Generate trusted scientific information to support decision-making

Develop and apply innovative tools and strategies

- Cardiovascular
- Carcinogenesis
- Developmental Neurotox
Resources to make existing information on carcinogens FAIR

- **Curated data and search tools**
  - Organized by toxicity endpoints
  - Standardized terminology, units, and formatting

- **Curated chemical lists**
  - Reference lists with classifications and bioactivity
  - In vitro assays linked with defined terminology

- **Computational models**
  - In vitro to in vivo extrapolation (IVIVE)
  - Quantitative structure-activity relationship (QSAR) models

**Chemical Effects in Biological Systems (CEBS)**
https://manticore.niehs.nih.gov/cebssearch

**Integrated Chemical Environment (ICE)**
https://ice.ntp.niehs.nih.gov/

Slide courtesy: Carci HEI PMT
Integrated Chemical Environment (ICE) database

Tox21 HTS assays mapped to Key Characteristics of Carcinogens (KCC)

- genotoxicity data
- highest dose tested
- dose and tissue used for level of evidence call
- type of lesion

Additional information on each chemical:

EPA OPP to be added 2Q2021

Slide courtesy: Carci HEI PMT
Implement a DNT screening battery that covers key neurodevelopmental events

DNTP DNT HEI Objectives

Human-derived + rodent + zebrafish

2-D assays 3D- Neurospheres Zebrafish

DNTP’s Proposed Battery: Initial Assay Selection
Compare activity of compounds/classes across multiple assays

Individual dose-response curves

Plate and well level information

Control variability in assay

DNT- Data Integration and Visualization Enabling Resource

(DNT-DIVER)

Example: Flame Retardants

Compare activity of compounds/classes within an assay

https://sandbox.ntp.niehs.nih.gov/neurotox/

Slide courtesy: DNT HEI PMT
In process/complete

- Define testing framework (CV failure modes)
- CV hazard identification
- CV QSAR screening tool (build)
- Predictive transcriptomics (build)
- Suite of in vitro CV testing platforms
- In vivo CV assessment (capability/paradigm dev’t)
- CVD in U3 populations (gap analysis)

Ongoing

- Evidence map of the literature
- Evidence map of the literature
- Predictive transcriptomics (test)
- Suite of in vitro CV testing platforms
- CV in vivo pilot studies
- CVD in U3 populations (capability build/disease screening application)

Future

- CV QSAR screening tool (test)
- CV In vivo integration into testing paradigm
- CV implementation strategy/decision framework

Slide courtesy: CV HEI PMT
CardioToxPi: Using Tox21 qHTS Data and AI

Krishna et al. 2020, Chem Res Tox

Self Organizing Map: structural clusters enriched for CV activity

- Special Issue Cover: **Computational Toxicology**
- Published February 2021
  - QSAR and other in silico studies
  - IVIVE methods
  - Application of NextGen sequencing and HTS data
  - Use of artificial intelligence and machine learning to model critical in vivo toxicity endpoints.

Slide courtesy: CV HEI PMT
Ongoing NICEATM and ICCVAM Projects

- Integrated Chemical Environment
- OPERA (QSAR/QSPR)
- Reference data curation
- Variability of in vivo data
- Acute Systemic Toxicity
- Dermal absorption
- Eye and skin irritation
- Skin sensitization
- ZF models (SEAZIT)
- Acute Fish Retrospective
- Carcinogenesis
- Cardiovascular toxicity
- Developmental Toxicity
- Animal-free affinity reagents
- Microphysiological Systems
- Evolving Process of Validation

- Summarizes US agency activities to promote alternatives or reduce animal use
  - Contributions from every ICCVAM member agency

- Subscribe to NICEATM News email list
  [https://ntp.niehs.nih.gov/go/niceatm](https://ntp.niehs.nih.gov/go/niceatm)
Acute 6-Pack Alternatives

- **Dermal lethality**
  - US EPA Waiver guidance available

- **Oral lethality**
  - In silico (CATMoS) for single chemicals; additivity for formulations under consideration

- **Inhalation lethality**
  - 3D models being evaluated; LC50 database for in silico model development being built

- **Eye irritation**
  - NAMs for Cat I and/or Cat IV (TG 437, 438, 460, 491, 492, 494); Prospective testing ongoing

- **Skin irritation**
  - NAMs for Cat I or Cat IV (TG 430, 431, 435, 439); Prospective testing ongoing

- **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 under review
Collaborative Modeling Project for Predicting Acute Oral Toxicity (CATMoS)

CATMoS implementation in OPERA

OPERA suite of models:
• Free, open-source, and open-data
• Command line and GUI
• Single chemical and batch mode
• Windows OS and Linux
• Embeddable wrapper libraries in Java, C, C++, and Python

Collaboration with ATWG partners and ICCVAM agencies

<table>
<thead>
<tr>
<th>Agency</th>
<th>No. Substances</th>
<th>Agency</th>
<th>No. Substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Force</td>
<td>421</td>
<td>EPA OPP</td>
<td>36</td>
</tr>
<tr>
<td>Army Public Health Command</td>
<td>18</td>
<td>EPA OPPT</td>
<td>8</td>
</tr>
<tr>
<td>Army Edgewood Chemical Biological Center</td>
<td>42</td>
<td>EPA NCCT</td>
<td>4815</td>
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<tr>
<td>CPSC</td>
<td>110</td>
<td>EPA EFED</td>
<td>160</td>
</tr>
<tr>
<td>DOT</td>
<td>3671</td>
<td>FDA CFSAN</td>
<td>22</td>
</tr>
</tbody>
</table>

Progress made with EPA EFED

• Compare CATMoS predictions to acute oral toxicity data on 160 pesticides registered in the last 25 years.

• Determine impact on risk assessments, leading to additional curation and characterizing confidence in predictions.
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

<table>
<thead>
<tr>
<th>Prior GHS category</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (serious eye damage)</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.

• 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
Skin Irritation Variability: EPA Regulatory Categories

- Curated data for chemicals with multiple study reports
- Calculated PDII (avg. erythema + avg. edema)

**Curated EPA Dataset**

- I, 207, 19%
- II, 35, 3%
- III, 133, 13%
- IV, 690, 65%

**Data quality flags (methodological differences) applied**

- All Data 2624 ESRs 990 Substances
- Full Dataset 2431 ESRs 797 Substances
- Clean Data 1212 ESRs 669 Substances
- Clean Dataset 837 ESRs 294 Substances
- Curated Data 1834 ESRs 867 Substances
- Curated Dataset 1065 ESRs 425 Substances
### Skin Irritation Conditional Probability Tables

<table>
<thead>
<tr>
<th>EPA</th>
<th>Category I</th>
<th>Category II</th>
<th>Category III</th>
<th>Category IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDII</td>
<td>Corrosive</td>
<td>&gt;5.0</td>
<td>2.1-5.0</td>
<td>0-2.0</td>
</tr>
<tr>
<td>Signal Word</td>
<td>DANGER</td>
<td>WARNING</td>
<td>CAUTION</td>
<td>CAUTION</td>
</tr>
<tr>
<td>PPE Required</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrosive</td>
<td>Coveralls worn over long-sleeved shirt and long pants</td>
<td>Coveralls worn over short-sleeved shirt and short pants</td>
<td>Long-sleeved shirt and long pants</td>
<td>Long-sleeved shirt and long pants</td>
</tr>
<tr>
<td>socks</td>
<td>socks</td>
<td>socks</td>
<td>socks</td>
<td>socks</td>
</tr>
<tr>
<td>Chemical-resistant footwear</td>
<td>Chemical-resistant footwear</td>
<td>Shoes</td>
<td>Shoes</td>
<td></td>
</tr>
<tr>
<td>Waterproof or chemical resistant gloves</td>
<td>Waterproof or chemical resistant gloves</td>
<td>Waterproof or chemical resistant gloves</td>
<td>No minimum</td>
<td></td>
</tr>
</tbody>
</table>

#### Curated Dataset with Binary Approach

<table>
<thead>
<tr>
<th>Prior Result</th>
<th>Irritant (Cat I or II)</th>
<th>Non-irritant (Cat III or IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritant (Cat I or II)</td>
<td>75.6%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Non-irritant (Cat III or IV)</td>
<td>3.9%</td>
<td>96.1%</td>
</tr>
</tbody>
</table>

#### Curated Dataset

<table>
<thead>
<tr>
<th>Prior Result</th>
<th>COR</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>COR</td>
<td>86.3%</td>
<td>4.2%</td>
<td>7.1%</td>
<td>2.5%</td>
</tr>
<tr>
<td>II</td>
<td>14.1%</td>
<td>44.9%</td>
<td>20.5%</td>
<td>20.5%</td>
</tr>
<tr>
<td>III</td>
<td>6.9%</td>
<td>5.2%</td>
<td>53.6%</td>
<td>34.3%</td>
</tr>
<tr>
<td>IV</td>
<td>0.9%</td>
<td>2.0%</td>
<td>9.1%</td>
<td>88.0%</td>
</tr>
</tbody>
</table>
• Absorption through in vitro human skin was found to be similar to, or less than, that observed in rat skin (in vitro and in vivo) for all formulations.

• The human in vitro assay provided a similar or higher estimate of dermal absorption than the triple pack.

• For human health risk assessment, in vitro assays using human skin would be preferable. Such tests would be directly relevant to the species of interest (humans) and avoid any overestimation of dermal absorption using rat models.

• However, rat in vitro studies would still have utility if human in vitro data were not available.

• In vitro rat data provide estimates of dermal absorption that are at least as protective as in vivo rat data, and thus could also be considered adequate for use in establishing dermal absorption factors.

\[
\text{triple pack DAF} = \text{rat in vivo} \times \left( \frac{\text{human in vitro}}{\text{rat in vitro}} \right)
\]
• GHS Mixtures Equation - mathematical approach to calculating toxicity of mixtures based on components

• Compare LD50s predicted for formulations based on the GHS Mixtures Equation to those determined from in vivo results with the complete formulation.

• Data set consisted of 671 formulations produced by eight companies:
  – 51 antimicrobial cleaning products (AMCPs), 620 agrochemical formulations

<table>
<thead>
<tr>
<th>In vivo Classification</th>
<th>EPA Additivity Classification</th>
<th>Within-class Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In vivo Classification</th>
<th>GHS Additivity Classification</th>
<th>Within-class Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5/NC</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

• 79% (128/163) of “discordant” substances (EPA Cat III predicted as Cat IV, yellow highlight) had in vivo LD50 values measured between 2000 and 5000 mg/kg or a limit test LD50 > 2000 mg/kg.
Supplementary Analysis & Conclusions

• Precautionary statements and associated PPE are much more stringent with LD50 < 500 mg/kg; supplementary analysis combined all substances with LD50 > 500 mg/kg together.

<table>
<thead>
<tr>
<th>In vivo LD₅₀</th>
<th>Additivity LD₅₀ Prediction (mg/kg)</th>
<th>Within-class Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤50</td>
<td>&gt;50 to ≤500</td>
</tr>
<tr>
<td>≤50</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>&gt;50 to ≤500</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>&gt;500</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>66</td>
</tr>
</tbody>
</table>

- Most “discordant” substances had in vivo LD50s values measured between 2000 and 5000 mg/kg or a limit test LD50 > 2000 mg/kg.
- When considering formulations with LD50 >500 mg/kg together, overall concordance increased from 55% to 82%.
- Within-class concordance for less toxic substances was consistently over 85% regardless of classification system.
- Animal tests are inherently variable. Similar underclassification could also be observed following a repetition of the animal test.
- Our results suggest the mixtures equation is promising for identifying substances that would not be expected to induce toxicity.
- However, the lack of more toxic formulations in the dataset preclude us from reaching definitive conclusions across the spectrum of hazard categories.
SEAZIT: Systematic Evaluation of the Application of Zebrafish in Toxicology

SEAZIT Goals:

• Provide the scientific basis on which to make a programmatic decision on the further routine use of zebrafish in toxicological evaluation of chemicals

• Provide fundamental knowledge on the use of zebrafish in toxicology, which will support further research endeavors by the academic community

SEAZIT Activities:

• Inter-laboratory study to compare the impact of chorion-on v. chorion-off and single v. repeat exposure

• Reference phenotype atlas for zebrafish screening assays along with a means of differentiating abnormal from normal

Establish organ-on-chip technology at the NIAID Integrated Research Facility

Joint working group to support global COVID-19 tissue chip research activities
Partnership with UK NC3Rs, DoD, NIAID, NCATS.

https://ntp.niehs.nih.gov/go/mps

Development of a COVID-19 Disease Portal in the Microphysiology Systems (MPS) Database
(University of Pittsburgh)
Curation to assist meaningful assay selection and model building

- Curated high-throughput screening data (cHTS) starts with EPA invitrodb and incorporates chemical QC information and technology-specific flags
- Assays are grouped by biological process, mechanistic target, and MoA, and linked to ontologies
Curve Surfer is an interactive concentration response visualization tool for cHTS data
- Select/filter assays based on Mechanistic Target
- View specific assays/chemicals
- Filter on activity call, AC50
PBPK tool allows users to calculate internal chemical concentrations using PBPK models from the EPA httk R package and in-house code.

- Tissue level concentrations
- View individual chemical curves
- View overall distribution in different tissue compartments for all query chemicals.
The IVIVE tool uses pharmacokinetic models to predict the equivalent administered dose (EAD) from the activity concentration of selected assays.

Transparency and annotation to help guide use and interpretation.
The Chemical Characterization tool allows you to view and compare one or two chemical lists based on their physicochemical properties. Comparisons are available in tabular format along with principal component analysis plots of list against subsets of the ICE chemical inventory.

Chemical Characterization tool allows users to explore one or two chemical lists:
- Physicochemical property distributions
- Interactive PCA plots of chemical space coverage
- Presence in consumer products (EPA CPDat)
Questions?