21st Century Science: Recent Activities

USEPA, Office of Pesticide Programs

Eleventh Antimicrobial Workshop  March 10-11, 2015
Mission Statement

- Sound regulatory decisions that are protective of public health and environment
- High quality, transparent risk assessments based on best available scientific information
EPA OPP’s 21st Century Vision

• EPA OPP’s Strategic Direction for New Pesticide Testing and Assessment Approaches

• Two elements of the strategic direction are:
  – Improved approaches to more traditional toxicity tests to minimize the number of animals used while expanding the amount of information obtained,
  – Improved understanding of toxicity pathways to allow development and use of non-animal tests to better relate pesticide exposures to adverse effects
Stepwise Transition to New Predictive Methods in a Transparent Manner

- **Short Term Goals**
  - Expand the capabilities of *in silico* & *in vitro* methods to strengthen predictive screening and hazard assessment methods
  - Maximize value obtained from each *in vivo* study
  - Transition away from chemical-by-chemical approaches though formation of chemical categories with shared biological and structured properties for read across
Stepwise Transition to New Predictive Methods in a Transparent Manner

- Long Term Goals
  - Use exposure and toxicity data (including in silico, in vitro, omics, etc) to determine if chemicals potentially trigger AOPs
  - Use key events for read across to other chemicals, both qualitatively and quantitatively.
  - Use key events themselves for points of departure

Use AOP knowledge to link the results of new methods to toxicologically relevant outcomes to focus data generation
Benefits

- Use best science to ensure safety
- Generate relevant information
- Reduce costs and animal usage
- Increase certainty, predictive ability and timeliness
- Harmonize requirements
- Provide more robust and informed risk management decisions
Because the emphasis of toxicology testing is shifting from in vivo testing results in animals to mechanism-based biological outcomes in vitro, NICEATM will expand its scope and concentrate its resources on providing bioinformatic and computational toxicology support to NIEHS Tox21 projects.

Two projects sponsored by EPA were identified as priorities:
- Acute oral / dermal toxicity testing (EPA-OPP)
- Skin sensitization (EPA, FDA, CPSC)
While several published studies have investigated comparability between oral and dermal acute hazard classifications to assess whether tests for both routes are needed (Creton et al. (2010); Seidle et al. (2011); Moore et al. (2013)), these studies focused on technical active ingredients & have not used the OPP categorization system.
Can animal use be reduced by waiving the dermal acute toxicity study for pesticide formulations?

Retrospective analysis of existing data

In late 2013-2014, data from oral & dermal acute toxicity studies submitted to EPA for registration were compiled.

Potential animal savings comes from formulation studies

- There are 1000’s of end use products registered by EPA & 100’s of studies submitted each year.
- Typically, <20 technical active ingredient studies submitted to EPA each year.
Oral-Dermal LD$_{50}$ Data Evaluation Project

- Dataset contains > 600 paired studies:
  - Conventionals, antimicrobials, biopesticides
  - PC Code, CAS #, formulation name, active ingredient(s), species, route, LD50, Tox Category, acceptability
  - 12 different formulation types
  - Toxicity categories I, II, III, IV
  - >300 different combinations of active ingredients (single ai’s, multiple ai’s in various combinations)

- Draft statistical analysis with dataset will be available for public comment soon.
In 2013, the skin sensitization working group (SSWG) was convened under the reorganized ICCVAM for the purpose of supporting ICCVAM’s interest in alternative test methods for skin sensitization, including the numerous development and evaluation activities in Europe.

The SSWG also provides expertise in the design and examination of the predictive value of a battery of *in vitro* and *in silico* methods for skin sensitization.

In 2014, the SSWG began to focus on development of an integrated decision strategy for skin sensitization using non-animal methods.
**OECD Adverse Outcome Pathway (AOP) for Skin Sensitization**

<table>
<thead>
<tr>
<th>Chemical Structure &amp; Properties</th>
<th>Molecular Initiating Event</th>
<th>Cellular Response</th>
<th>Organ Response</th>
<th>Organism Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism Penetration</td>
<td>Covalent interaction with skin proteins</td>
<td>• Induction of inflammatory cytokines and surface molecules</td>
<td>• Histocompatibility complexes presentation by DCs</td>
<td>• Inflammation upon challenge with allergen</td>
</tr>
<tr>
<td>Electrophilic substance</td>
<td>Keratinocytes responses</td>
<td>• Mobilisation of DCs</td>
<td>• Activation of T cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Activation of inflammatory cytokines</td>
<td>• Proliferation of activated T-cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Induction of cytoprotective genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Key Event 1</td>
<td>Key Event 2</td>
<td>Key Event 3</td>
<td>Key Event 4</td>
<td>Adverse Outcome</td>
</tr>
</tbody>
</table>

For sensitization that is initiated by covalent binding to proteins.

Non-Animal Methods are Aligned to the AOP Key Events

1. Skin Penetration

2. Electrophilic substance: Directly or via auto-oxidation or metabolism

3-4. Haptenation: Covalent modification of epidermal proteins
   - DPRA
   - PPRA [P&G]
   - KeratinoSens [Givaudan]
   - LuSens [BASF]
   - Sensi-DERM [Proteome Sciences]
   - SENS-IS [Immunosearch]
   - SensCeeTox [CeeTox]
   - NCTC 2544 IL-18 [Corsini; Univ. Milan]
   - Tiered testing approach [Corsini/Gibbs; Univ. Milan/VUMC]

5-6. Activation of epidermal keratinocytes & Dendritic cells
   - AREc322 [CXR Bio.]

7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells
   - Human T cell priming [Martin; Univ. Freiburg]
   - Human T cell proliferation (hTCPA) [Nicholas; Univ. Lyon]

8-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death
   - PBMDC [Beiersdorf]
   - DPRA
   - PPRA [P&G]
   - In silico Toxicokinetic model [Kasting; Univ. Cincinnati]
   - Q (SAR)s [Various]
   - h-CLAT [KAO/Shiseido]
   - KeratinoSens [Givaudan]
   - LuSens [BASF]
   - Sensi-DERM [Proteome Sciences]
   - SENS-IS [Immunosearch]
   - SensCeeTox [CeeTox]
   - NCTC 2544 IL-18 [Corsini; Univ. Milan]
   - Tiered testing approach [Corsini/Gibbs; Univ. Milan/VUMC]
   - VITOSens [VITO]
   - h-CLAT [KAO/Shiseido]
   - mMUSST [BASF]
   - MUSST [L’Oreal]
   - PBMD [Beiersdorf]
   - GARD [Borrebaeck; Univ.Lund]
   - PBMDC [Beiersdorf]
   - DPRA
   - PPRA [P&G]
   - In silico Toxicokinetic model [Kasting; Univ. Cincinnati]
   - Q (SAR)s [Various]
   - h-CLAT [KAO/Shiseido]
   - KeratinoSens [Givaudan]
   - LuSens [BASF]
   - Sensi-DERM [Proteome Sciences]
   - SENS-IS [Immunosearch]
   - SensCeeTox [CeeTox]
   - NCTC 2544 IL-18 [Corsini; Univ. Milan]
   - Tiered testing approach [Corsini/Gibbs; Univ. Milan/VUMC]
   - VITOSens [VITO]
   - h-CLAT [KAO/Shiseido]
   - mMUSST [BASF]
   - MUSST [L’Oreal]
   - PBMD [Beiersdorf]
   - GARD [Borrebaeck; Univ.Lund]

*Slide courtesy of Gavin Maxwell (Unilever/Cosmetics Europe)*
Produce and test an integrated decision strategy for skin sensitization using:

- three *in chemico* or *in vitro* assays validated by EURL ECVAM or have OECD guidelines
- physicochemical properties: Measured when available or predicted when not
- *in silico* read across prediction using OECD Toolbox

NICEATM has collected a database for over 100 substances evaluated with DPRA, h-CLAT, KeratinoSens™, and LLNA that have been characterized by physicochemical properties.

EURL ECVAM has also collected published data for DPRA, h-CLAT, and KeratinoSens™.
Skin Sensitization

- **Initial Phase:** Focus analysis on predictability of the integrative decision strategies for LLNA related to “yes/no” regulatory needs.

- **Next Phase(s):** Focus analysis on predictability of the integrative decision strategies to assess potency.
Skin Sensitization

- Modeling efforts to predict the LLNA outcome (yes/no) and human outcomes (yes/no) are complete.

- Modeling efforts to predict potency (GHS classes) will begin soon. Data collection and QC for that part are almost complete.
Skin Sensitization

- Work is on-going to use the database to evaluate other approaches to development of an ITS.
  - Machine learning: Artificial Neural Network, Naïve Bayes Network, Classification and Regression Tree, Linear Discriminant Analysis, Logistic Regression, Support Vector Machines
  - Test battery approaches
Alternative Assays Guidance

- **PROCESS FOR ESTABLISHING & IMPLEMENTING ALTERNATIVE APPROACHES TO TRADITIONAL IN VIVO ACUTE TOXICITY STUDIES**
  - Comment period just ended (March 10, 2015)
    - schlosser.christopher@epa.gov
  - This draft document describes a transparent, stepwise process for evaluating and implementing alternative methods of testing for acute oral, dermal, inhalation toxicity, along with skin and eye irritation and skin sensitization (often referred to as the “six pack studies”).

- Three phases of this process and the implications for reporting information under section 6(a)(2) of FIFRA.
  - Evaluation
  - Transition
  - Implementation
Stepwise Process For Evaluating & Implementing Alternative Methods

- **Evaluation phase**: determine whether an alternative method(s) could be used for regulatory purposes.

- **Transition phase**: OPP will accept data generated from the alternative method(s), which were conducted following the draft policy document, and submitted along with corresponding data from its *in vivo* method counterpart (or citation to previously submitted *in vivo* studies).

- **Implementation phase**: After addressing any potential issues identified through public comments, a final science policy will be completed and released. Subsequently, data generated from the accepted alternative approach may be submitted to fulfill the requirement of its standard *in vivo* counterpart.
Alternative Assays: Implementation

- Early stages of collaborative project with multiple stakeholders & NICEATM
- Goal: Integrated testing strategies for skin sensitization, dermal irritation, skin irritation that apply to pesticides
  - EPA OPP, Canada PMRA, animal welfare groups, & industry
  - Current activities: some CLA members entering eye irritation data into spreadsheet
    - Face to face meeting planned for San Diego during SOT
- Science & policy issues for consideration:
  - single agents vs. mixtures (formulations)
  - GHS vs. US/Canada categorization schemes
  - Training of staff
  - Implications for labeling
21st Century Science: Other Activities

- **Guiding Principles for Data Requirements**
  - Purpose: provide consistency in the identification of data needs, promote and optimize full use of existing knowledge, and focus on the critical data needed for risk assessment.

- **Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies**
  - Purpose: use a WoE evaluation to determine data needs or to review a waiver justification

- OPP’s guidance documents on the use of open literature studies in ecological and human health risk assessments.
  - [http://www.epa.gov/pesticides/science/literature-studies.html](http://www.epa.gov/pesticides/science/literature-studies.html)

- Alternate testing framework for classifying eye irritation potential for labeling antimicrobial pesticide products with cleaning claims
  - [http://www.epa.gov/pesticides/science/eye-irritation.html](http://www.epa.gov/pesticides/science/eye-irritation.html)

- Advances in Genetic Toxicology and Integration of in vivo Testing into Standard Repeat Dose Studies
Threshold of Toxicological Concern for Antimicrobial Pesticides project

- A principle which refers to the establishment of human exposure threshold values for categories of chemicals, below which there would be no appreciable risk to human health

- Purpose: Development of a TTC-decision tree tool to aid in prioritization of toxicity testing needs for antimicrobial pesticides

- Draft manuscript has been prepared and is under peer review
International Collaboration

- OECD Test Guidelines program - provides a mechanism for the international evaluation and adoption of alternative methods by its 34 member countries.

- International Cooperation on Alternative Test Methods (ICATM) - provides means to coordinate the validation and adoption of alternative test methods among its member countries: United States, Canada, Europe Union, Japan, and Ko.
International Collaboration

- European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM):
  - conducts validation study review and approval processes in a manner that meets the needs of US regulatory agencies

- ICCVAM and NICEATM are working with EURL-ECVAM on a process that will enable US scientists to participate actively in the EURL-ECVAM test method evaluation of the usefulness and limitations of relevant test method and that will increase the transparency of the evaluation
Summary

- Rapid advances to implementing the 3R’s into regulatory testing and alternative approaches but there is more work to do.

- Lessons learned so far...
  - Mining existing data provides a robust source of information for assessing data which are needed.
  - Granting of data waivers through OPP committees (e.g. HASPOC) for data that are not needed
  - Collaborative approaches working with investigators across sectors is most effective approach
  - Harmonization and coordination across state, federal, and international regulatory agencies is important