Toxicity Testing in the 21st Century: Updates & Progress

Anna B. Lowit, Ph.D.
Senior Scientist
Health Effects Division
Co-Chair, ICCVAM
lowit.anna@epa.gov
(w) 703-308-4135

Jennifer McLain , Ph.D.
Deputy Director
Antimicrobials Division
Chair, PPDC 21st C Workgroup
mclain.jennifer@epa.gov
(w) 703-308-0293

OPP 21st Century Vision

- OPP Vision
  - Integrative (Tiered)
  - Hypothesis-driven
  - Efficient & effective
- Transition Strategy
  - Based on sound science and risk management needs
  - Research in concert with regulatory dialogue
  - Incremental application to decision making
  - Expert peer review and stakeholder involvement

Focus resources on risks of greatest concern

http://www.epa.gov/opp00001/science/testing-assessment.html

Partnerships

- Stakeholder engagement
  - Transparency
  - Public trust
- PPDC 21st Century Toxicology/ New Integrated Testing Strategies Workgroup
- Collaboration with industry, Federal agencies, international governments

PPDC 21st Century Workgroup

- Objective: Focus on communication & transition issues as EPA phases in new molecular and computational tools
- Activities: Stakeholder workshops, biomonitoring tools, program metrics
- 2013 Recommendation for OPP Goals and Metrics for Acute Toxicity Studies

http://epa.gov/pesticides/ppdc/testing/index.html

Recommendation for OPP Metrics

- GENERAL GOALS
  - Phase out animal testing for acute "6-pack" endpoints (acute oral, dermal, inhalation; dermal and eye irritation; dermal sensitization)
  - Consistent and regular reductions in the numbers of animals used for acute tests
  - Consistent and regular increases in the use of non-animal methods and existing information used to make regulatory decisions

Recommendation for OPP Metrics

- SPECIFIC GOALS
  - Allow OECD-approved *in vitro* skin irritation method for registration for all chemistries during 2015 Calendar Year
  - Accept suite of *in vitro* tests for skin sensitization within 6 months of acceptance at the OECD level
  - Phase out multiple routes of exposure (by developing reliable route-to-route extrapolation principles or other comprehensive waiving policies)
    - Phase out acute dermal test for majority of registrations within 3 years

Method Acceptance Status

			REGULATORY STATUS			
ANIMAL TEST	NUMBER OF ANIMALS	ALTERNATIVE TEST	OECD	US	OPP	OTHER
Skin Irritation (severe)	3 rabbits	Reconstructed Human Epidermis models (various)	OECD TG 431 (2004)	ICCVAM 2002	Accepted	
Skin Irritation (mild)		Reconstructed Human Epidermis models (various)	OECD TG 439 (2010)			
Eye Irritation (severe)	3 rabbits	Bovine corneal opacity permeability (BCOP) test	OECD TG 437 (2009)	ICCVAM (2007)	Replaces rabbit for antimicrobial cleaning products	Now validated for "non-irritants" for EPA and GHS classification systems
		Cytosensor Microphysiometer modified (cytotoxicity/cell-based assay)		ICCVAM (2010)*	Replaces rabbit for antimicrobial cleaning products	
		Fluorescein Leakage (cytotoxicity/cell-based assay)	OECD TG 460 (2011)			
		Isolated chicken eye (ICE) test	OECD TG 438 (2009)	ICCVAM (2007)		Now validated for "non-irritants" for GHS classification system
Eye Irritation (mild)		Cytosensor Microphysiometer modified		ICCVAM (2010)*	Replaces rabbit for antimicrobial cleaning products	
		EpiOcular (MatTek)			Replaces rabbit for antimicrobial cleaning products	
Sensitisation	32 guinea pigs or 16-33 mice	Direct Peptide Reactivity Assay (DPRA)	OECD TG drafting			ECVAM validation pending
		Keratinosens assay	OECD TG drafting			ECVAM validation pending
		Myeloid U937 Skin Sensitization Test (MUSST)				ECVAM validation pending
		Human Cell Line Activation Test (h-CLAT)				ECVAM validation pending
	16 or 33 mice	Local Lymph Node Assay (LLNA) or reduced LLNA (rLLNA)	OECD 429 (2002/2010)	ICCVAM (2008)	Accepted	
Acute Dermal Toxicity	20 rabbits					Opportunities for bridging or waiving exist.
Acute Oral Toxicity	7 rats (average)					Opportunities for bridging or waiving exist.
Acute Inhalation Toxicity	20 rats					Opportunities for bridging or waiving exist.

*Only for certain chemicals and in a WOE approach

Recommendation for OPP Metrics

- Workgroup Proposed Near-Term Metrics
  - Number of *in vitro* tests submitted per endpoint per year
  - Number acute animal tests submitted per endpoint per year
  - Estimate of animals used in acute tests per year
  - Number of dossiers with "alternative approaches" submitted per year
- OPP Metrics Workgroup
  - Goal: Develop an OPP process for measuring and reporting progress towards 21st C goals
  - Charged with developing metrics, criteria, tracking & reporting methodologies
  - Participants from multiple divisions. Report to Under OPP's Science Policy Council (SciPoc) & OPP's Risk Management Forum (RMF).

Federal Collaboration

- In 2000, Congress passed the ICCVAM Authorization Act and established Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
  - Comprised of 15 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information.
- NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) of the NIEHS provides scientific and operational support for ICCVAM technical evaluations and related activities.

Background

- ICCVAM and NICEATM work together to promote the development, validation, and regulatory acceptance of new and revised regulatory test methods and integrated testing and decision strategies that replace, reduce, and refine the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment.

- In 2013, Director of NIEHS and ICCVAM announced that ICCVAM and NICEATM will be making significant changes to the focus and priorities of both organizations (http://iccvam.niehs.nih.gov/announcements/ICCVAM-all/2013-02-06-EHP.htm).

Introduction & Outline of the Presentation

- The 2013 draft document, "A New Vision and Direction for ICCVAM," describes the initial steps towards a new strategic direction for ICCVAM and NICEATM.
- Draft covers three areas:
  - ICCVAM priority setting and science focus areas for immediate ICCVAM resource investment;
  - Plans to improve communications with stakeholders and the public;
  - Exploring new paradigms for the validation and utilization of alternative toxicological methods.

New Vision & Direction for ICCVAM

- ICCVAM priority setting & current science focus areas:
  - Member agencies are taking a more active role in priority setting and operations of the Committee.
  - Change in approach:
    - Streamline the number of active projects where the science has advanced
      - There is a reasonable likelihood of success with a reasonable timeframe (1-5 years) for implementing into regulatory use.
    - Maintain flexibility to reorient efforts to maximize potential progress towards use of alternative approaches

New Vision & Direction for ICCVAM

- ICCVAM is developing revised procedures for the submission/ nomination of new assays or projects.
  - These revised procedures will be provided to the public for comment in the future.
  - Key change to the process, however, will be the need for documented support by at least one federal agency.
    - This federal agency will take the role of 'sponsor' for the proposed project, thereby ensuring that work done by ICCVAM is aligned with the needs of the agencies.
- Recently, there have discussions with ECVAM about better international coordination
  - Proposals for collaboration/coordination will be announced as part of SACATM (September, 2014)

New Vision & Direction for ICCVAM

- Short-term: Initially three projects were identified
  - Biologics: *Leptospira* vaccine potency (USDA)
  - *Acute oral and dermal toxicity testing (EPA-OPP)*
  - Skin sensitization (EPA, FDA, CPSC)

New Vision & Direction for ICCVAM

- Acute oral and dermal toxicity testing (EPA-OPP)
  - Step 1: Compile dataset(s) of oral & dermal LD50 studies
  - Step 2: Comparison analysis---How do the results of acute and dermal Lethal Dose (LD50) tests compare?
  - Step 3: Implication---Are both the oral & dermal LD50 tests needed for labelling?

Guideline	Study Type	Food Use	Non-Food Use
870.1100	Acute oral toxicity – Rat	R	R
870.1200	Acute dermal toxicity – Rat /Rabbit	R	R
870.1300	Acute inhalation toxicity – Rat	R	R
870.2400	Primary eye irritation – Rabbit	R	R
870.2500	Primary dermal irritation – Rabbit	R	R
870.2600	Dermal sensitization – Guinea Pig	R	R
870.6200	Acute neurotoxicity – Rat	R	R

Example of OPP's use of Acute Dermal LD50 Data: Pesticide Handlers

- Pesticide handlers are those who mix, load and apply pesticides
- Pesticide labeling requirements describe how protective clothing, respiratory protection and engineering controls are assigned to products based on toxicity of the end use product
- Risk assessment is also used to assign protective equipment to labels in addition to these criteria

Table 1. Handler PPE for WPS Products

Route of Exposure	Toxicity Category by Route of Exposure of End-Use Product			
	I DANGER	II WARNING	III CAUTION	IV CAUTION
Dermal Toxicity or Skin Irritation Potential[1]	Coveralls worn over long-sleeved shirt and long pants	Coveralls worn over short-sleeved shirt and short pants	Long-sleeved shirt and long pants	Long-sleeved shirt and long pants
	Socks	Socks	Socks	Socks
	Chemical-resistant footwear	Chemical-resistant footwear	Shoes	Shoes
	Chemical-resistant Gloves[2]	Chemical-resistant Gloves[2]	Chemical-resistant Gloves[2]	No minimum[4]
Inhalation Toxicity	Respiratory protection device[3]	Respiratory protection device[3]	No minimum[4]	No minimum[4]
Eye Irritation Potential	Protective eyewear[5]	Protective eyewear[5]	No minimum[4]	No minimum[4]

From Chapter 10, Label Review Manual. http://www.epa.gov/oppfead1/labeling/lrm/chap-10.pdf

Oral-Dermal Hazard Classification Analyses in the Literature

- 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) reported on 240 pesticide actives and 438 industrial chemicals
  - Seidle et al. (2011) reported on 1569 industrial substances and 337 pesticide actives
  - Moore et al. (2013) reported on 225 substances from the European Chemicals Agency (ECHA) database and 110 pesticide actives from Creton et al. (2010)
- *These have focused on technical active ingredients & have not used the OPP categorization system*.

NICEATM Oral-Dermal LD50 Data Evaluations

- In 2012, NICEATM presented a poster at SOT, "Analysis to Determine if Acute Oral Systemic Toxicity Data Can Be Used to Estimate and Avoid Acute Dermal Systemic Toxicity Testing."
  - This initial analysis concluded that acute oral toxicity data could not be used to determine acute dermal hazard,
    - 346 Substances with rat oral and rabbit dermal data
    - 81 Substances with rat oral and rat dermal data

- In 2013, a re-evaluation was initiated with collaboration from EPA's Office of Pesticide Programs
  - Reconsider data analysis strategy with limit test data
  - Improved QA/QC of data set
  - Focus on dermal data from rats only for more appropriate comparison to oral rat data

NICEATM Oral-Dermal LD50 Data Evaluations

- From 2013 to now, work continues
  - Studies compiled for both formulations & technical active ingredients.
  - However, the focus of current efforts is on the formulations:
    - Formulation LD50 studies are used for determining PPE for pesticide handlers.
    - Potential animal savings comes primarily from formulation acute studies
      - There are 1000's of end use products registered by EPA
    - The dermal LD50 data for the technical active ingredients are often used in eco assessments.

Oral-Dermal LD50 Data Evaluation Project

- A draft dataset of acute & dermal LD50 data for formulations is close to completion
  - QA/QC is still on-going
  - Evaluation of 'chemical-space' coverage
- Current draft version includes:
  - Conventionals, antimicrobials, biopesticides
  - PC Code, CAS #, formulation name, active ingredient(s), species, route, LD50, Tox Category, acceptability
  - 12 different formulation types
    - Toxicity (particularly absorption) can be influenced by the nature of the exposure
  - Toxicity categories I, II, III, IV
  - >400 different combinations of active ingredients (single ai's, multiple ai's in various combinations)

Oral-Dermal LD50 Data Evaluation Project

- Next Steps:
  - Finish compiling & QA/QC of the dataset
  - NICEAM will be conducting statistical analysis
  - Followed by….
    - Discussions between NICEATM & EPA-OPP on the findings
    - Write up the project for public comment
      - Will include the dataset & the statistical analysis
  - Timeline: Goal is to have the draft analysis & summary for public comment by end of September, 2014

New Vision & Direction for ICCVAM

- Short-term: Initially three projects were identified
  - Biologics: Leptospira vaccine potency (USDA)
  - Acute oral and dermal toxicity testing (EPA-OPP)
  - *Skin sensitization (EPA, FDA, CPSC)*

- OECD documents on the AOP for skin sensitization
- Significant worldwide progress in the development of *in vitro/in chemico/in silico* assays which do not use intact animals.
- ICCVAM is developing a strategy for how it plans to evaluate skin sensitization test methods and for making progress towards skin sensitization testing without the use of intact animals.
  - In 2013, developed & received public comments on "ICCVAM's Proposed Activities on Alternative Skin Sensitization Test Methods and Testing Strategies"

Skin Sensitization

- Within ICCVAM, developing a multi-phase project plan to develop integrated testing strategy
  - Initial Phase: Focus analysis on predictability of alternative assays for LLNA related to *"yes/no"* regulatory needs
  - Next Phase(s): Focus analysis on predictability of alternative assays for to assess *potency*

Alternative Assays

- EPA-OPP is in the early stages of collaborative project with multiple stakeholders & NICEATM
- Alternative batteries for skin sensitization, dermal irritation, skin irritation
  - Canada PMRA, animal welfare groups, & industry
  - Meeting held on May 20

New Vision & Direction for ICCVAM

- "New vision" draft covers three areas:
  - ICCVAM priority setting and science focus areas for immediate ICCVAM resource investment;
  - *Plans to improve communications with stakeholders and the public;*
  - Exploring new paradigms for the validation and utilization of alternative toxicological methods.

Improving the Communications Role of ICCVAM

- Achieve broader engagement with the scientific community and stakeholders (e.g., the regulated community, public interest groups) though a number of different mechanisms, such as:
  - Elicit public input on on-going & planned activities
  - Face-to-face forums:
    - Stakeholder public forum meeting scheduled for June 25 at NIH's Natcher Center
    - To register: http://ntp.niehs.nih.gov/?objectid=6195B370-C53D-6057-A12B38F1679E9222
  - Focused workshops with well-defined objectives,
  - Community of practice webinars,
  - Web-based questionnaires and comment forms.

Improving the Communications Role of ICCVAM

- Increase agency awareness of international 3R efforts.
  - The US national coordinator for OECD has become an ad hoc member of ICCVAM.
  - The national coordinator is providing frequent updates on topics of interest to federal agencies and is using ICCVAM as a forum to provide feedback on those activities to OECD.
  - Improved coordination with ECVAM & ICATM
- In order to improve communications, ICCVAM is making plans to:
  - Improve the ICCVAM website
    - Agency-specific content on scientific efforts which are consistent with the 3Rs;
    - Update for easier navigation and to provide a venue for more transparency.

New Vision & Direction for ICCVAM

- The draft document, "A New Vision and Direction for ICCVAM," describes the *initial* steps towards a new strategic direction for ICCVAM and NICEATM.
- Draft covers three areas:
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  - Plans to improve communications with stakeholders and the public;
  - *Exploring new paradigms for the validation and utilization of alternative toxicological methods.*

Exploring New Paradigms

- ICCVAM has identified a long-term goal---to work towards a new definition/concept of "validation" in order to speed up acceptance of methods & to be more responsive to on-going paradigm shifts in toxicity testing.
  - Better alignment with the vision laid out by the National Academy of Sciences in the 2007 NRC Report on Toxicity Testing in the 21st Century (NRC, 2007) while simultaneously fulfilling the mission of ICCVAM to implement the 3Rs of toxicity.
- Fit for purpose validation:
  - Screening/prioritization vs. replacement of guidelines
  - Single assays vs. test batteries

In Summary

- ICCVAM & NICEATM are making changes :
  - Member agencies are taking a more active role in priority setting and operations of the Committee.
  - Change in approach:
    - Streamline the number of active projects in where the science has advanced there is a reasonable likelihood of success with a reasonable timeframe (1-5 years) for implementing into regulatory use.
    - Maintain flexibility to reorient efforts to maximize potential progress towards use of alternative approaches.
    - Long-term goal to work towards a new definition/concept of "validation" in order to speed up acceptance of methods & to be more responsive to on-going paradigm shifts in toxicity testing.
  - Improve communications & more engagement with the scientific community, the public & stakeholders.
  - These are initial steps & there is more work to do….

Backpocket Slides

- Creton et al. 2010. Acute toxicity testing of chemicals-Opportunities to avoid redundant testing and use alternative approaches. Crit Rev Toxicol 40: 50-83.
- Seidle et al. 2011. Examining the regulatory value of multi-route mammalian acute systemic toxicity studies. ALTEX 28:95-102.
- Moore et al. 2013. Can acute dermal systemic toxicity tests be replaced with oral tests? Regul Toxicol Pharmacol 66:30-7.