

SOT IVAM Webinar: IATA in Regulatory Toxicology & Risk Assessment

Anna B. Lowit, Ph.D. lowit.anna@epa.gov
703-308-4135 (work); 703-258-4209 (cell)
U.S. Environmental Protection Agency
Office of Pesticide Programs
Washington, DC



Outline of Presentation

- Regulatory context & background
- Example using *N*-methyl carbamates
- Example using skin sensitization



Integrated Approach to Testing and Assessment (IATA)



- IATA integrates and weighs all relevant existing evidence and guides the targeted generation of new data, where required, to inform regulatory decision-making regarding potential hazard and/or risk
- The overall assessment within an IATA is performed on the basis of a weight-of-evidence approach

Systematic Review



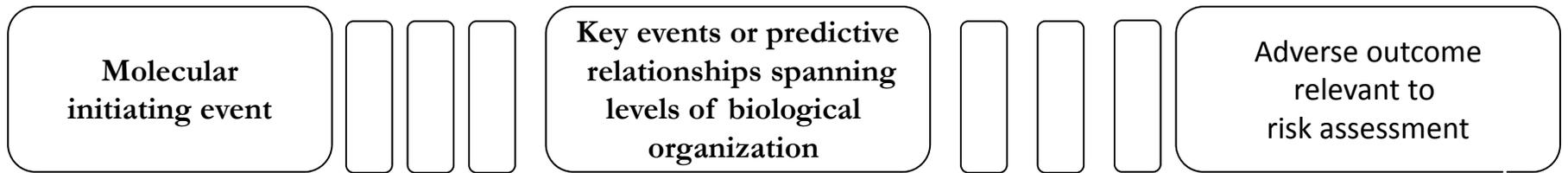
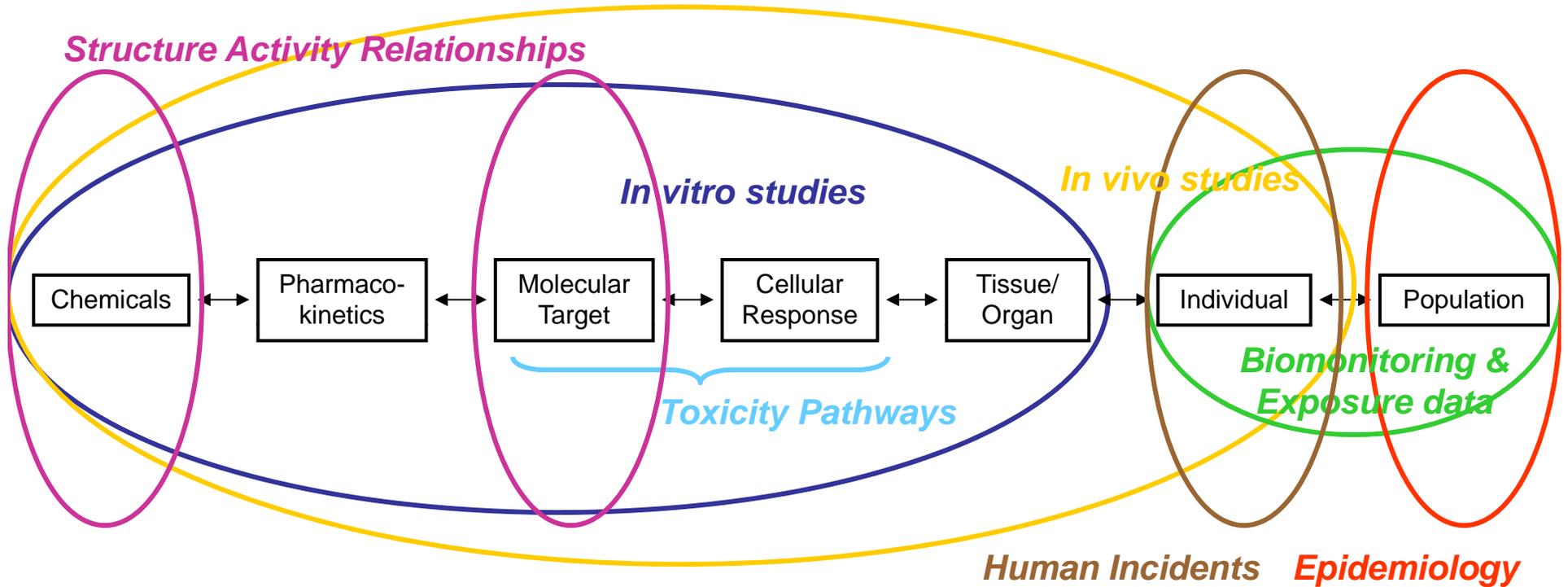
- Integration across lines of evidence starts with transparent & objective review of data: *through systematic review*
- NRC is define systematic review as "a scientific investigation that focuses on a specific question and uses explicit, prespecified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies."
 - <http://dels.nas.edu/Report/Review-Integrated-Risk/18764>
- Several common elements of systematic review:
 - transparent and explicitly documented methods,
 - consistent and critical evaluation of all relevant literature,
 - application of a standardized approach for grading the strength of evidence,
 - and clear and consistent summative language.

Systematic Review



- OPP's guidance document on the use of open literature studies in ecological and human health risk assessments.
 - <http://www.epa.gov/pesticides/science/literature-studies.html>
- EPA is developing systematic review approaches and policies for use in chemical and pesticide risk assessments & is updating this guidance.

Adverse Outcome Pathway

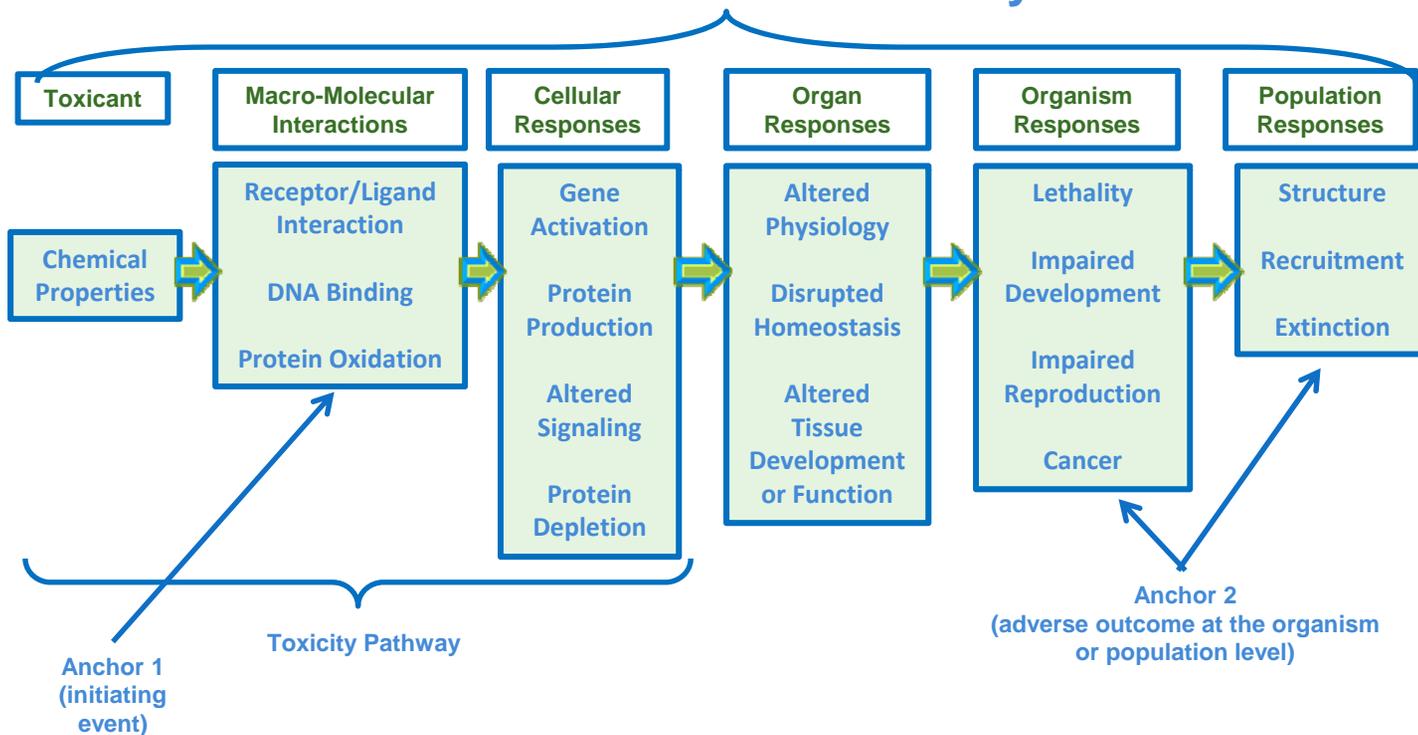


Greater Toxicological Understanding → Greater Risk Relevance

Adverse Outcome Pathway



Adverse Outcome Pathway



Background



- EPA's 2009 Strategic Plan for Evaluating the Toxicity of Chemicals
 - Characterizing or predicting potential human exposures;
 - Estimating the resulting chemical dosimetry (magnitude, frequency, and duration) for target pathways, tissues or organs;
 - Measuring toxicity pathway response at doses consistent with human exposures;
 - Predicting the *in vivo* human response resulting from pathway perturbations;
 - Quantifying the range of human variability and susceptibility; and
 - Validating predictions utilizing *in vivo* systems (e.g., laboratory animals, human data).

Background



- EPA's Office of Pesticide Programs has developed a Strategic Direction for New Pesticide Testing and Assessment Approaches
 - <http://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science>
 - A broader suite of computer-aided methods to better predict potential hazards and exposures, and to focus testing on likely risks of concern;
 - 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 of non-animal tests that better predict how exposures relate to adverse effects;
 - Improved diagnostic biomonitoring and surveillance methods to detect chemical exposures and identify causes of toxic effects;
 - A suite of spatial databases and geographic information tools, which will aid in developing more spatially explicit risk assessments that identify geographic areas of concern for both human health and ecological exposure.

Background



- USEPA's Office of Pesticide Programs is a licensing program regulating pesticide products in the U.S.
 - Review effects of pesticides on human and ecological health
- Federal Insecticide, Fungicide & Rodenticide Act (FIFRA)
 - Requires registration of new products and uses
 - Requires review of older pesticides
 - Includes ability to issue data call-ins

Guiding Principles for Data Needs



- 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.
 - <http://www.epa.gov/pesticide-registration/guiding-principles-data-requirements>
- Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies
 - Purpose: use a weight of evidence evaluation to determine data needs or to review a waiver justification
 - <http://www.epa.gov/pesticide-registration/determining-toxicology-data-requirements>

Guiding Principles for Data Needs



- “...ensure there is sufficient information to reliably support registration decisions that are protective of public health and the environment while avoiding the generation and evaluation of data that does not materially influence the scientific certainty of a regulatory decision....”
- “It is important to only require data that adequately inform regulatory decision making and thereby avoid unnecessary use of time and resources, data generation costs, and animal testing.”

Data Waivers



- Waiver guidance document covers:
 - Subchronic Inhalation, subchronic dermal, acute and subchronic neurotoxicity, and immunotoxicity
- Although not specifically covered by the guidance, we still consider other guideline studies using the same principles.....
 - Replace: Alternate testing framework for classifying eye irritation potential for labeling antimicrobial pesticide products with cleaning claims
 - <http://www.epa.gov/pesticide-registration/alternate-testing-framework-classification-eye-irritation-potential-epa>
 - Reduce: Waivers for developmental, reproductive, DNT, chronic/carcinogenicity toxicity
 - Refine: Special protocol studies (e.g., acute inhalation for fumigants, CCA studies, shorter duration) instead of standard guideline protocols
 - Refine: Pharmacokinetic studies in lieu of toxicity study

Data Waivers



- Weight of evidence approach:
 - Physical chemical properties
 - Use & exposure pattern
 - Hazard characterization:
 - Toxicity profile,
 - Information on MOA/AOP,
 - Read across (other pesticides in the class)
- Risk assessment implications

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Introduction

- Food Quality Protection Act (FQPA, 1996)
 - Requires EPA to take into account when setting pesticide tolerances:
 - “available evidence concerning the cumulative effects on infants and children of such residues and other substances
➔ that have a *common mechanism of toxicity*.”
- Under FQPA (1996), cumulative risk is defined as:
 - The risk associated with a group of chemicals that are toxic by a common mechanism from all pathways
 - Multi-chemical & Multi-pathway
 - Food, drinking water, consumer uses
 - Routes of exposure (oral, dermal, inhalation)

IATA in Pesticide Testing



- Highlight how AOP knowledge can be used to design a testing strategy to focus on potential for life susceptibility for an entire class of pesticides.
- Developing an IATA case study for OECD
 - OECD case study will cover organophosphates (OPs) & *N*-methyl carbamates (NMCs).
- NMCs for this webinar



Time-Frame Considerations

Important to consider *biological time*

- What is the nature of the toxicity?
 - Long-term, chronic (cancer) →
 - Intermediate-term (developmental) →
 - Short-term or acute →
 - How does the key event compare to the outcome?
- What is the time scale of the toxicity?
 - Months to years?
 - Days to months?
 - Minutes to hours to days?



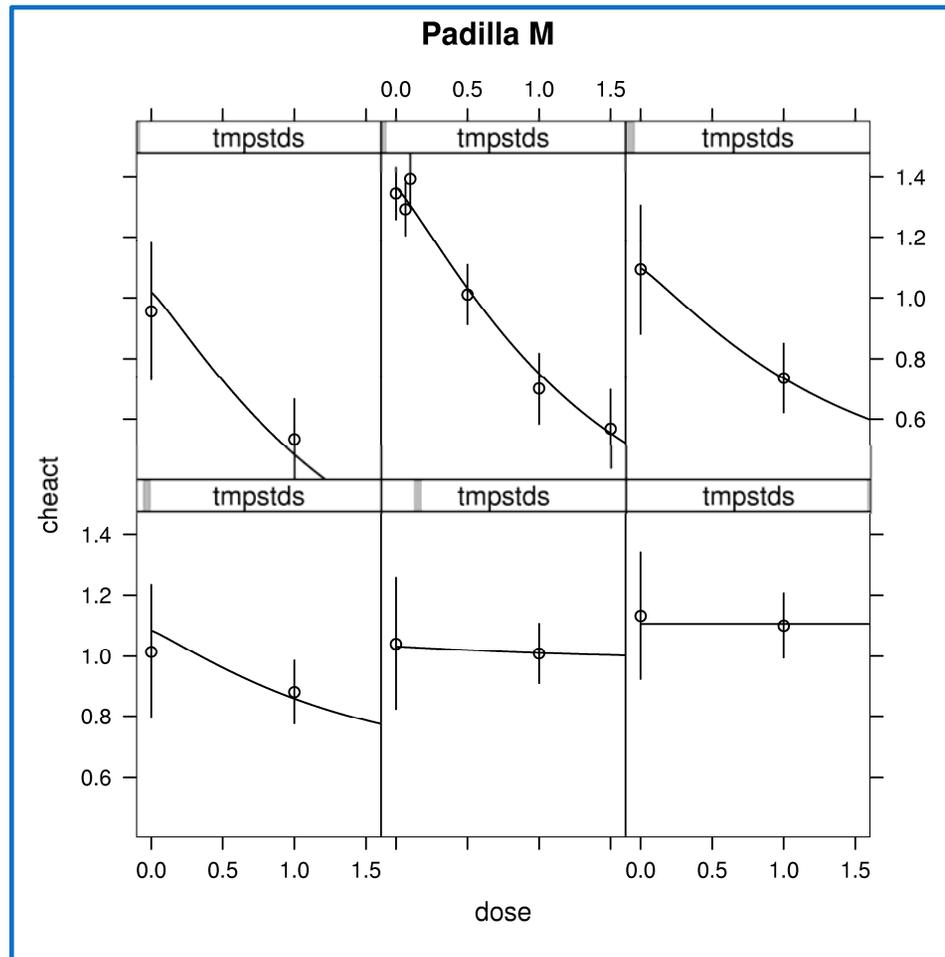
Time-Frame Considerations

- Integrating *toxicology* & *exposure*
 - What is known about the pathway of toxicity and/or the mode/mechanism of action?
 - Key events leading to toxicity
 - Dose metric (AUC, peak)
 - Time course information
 - Steady state or fast acting (toxicokinetic/toxicodynamic)?
 - Time to peak effect? Time to recovery?
- Example:
 - *N*-methyl carbamates: inhibition of acetylcholinesterase via carbamylation
 - Toxicity is characterized by rapid onset & rapid recovery



Example: *N*-methyl carbamates (oxamyl): inhibition of acetylcholinesterase via carbamylation

Toxicity is characterized by rapid onset & rapid recovery



Comparative Cholinesterase Study (CCA)



- Acute testing in young adult & PND11 rats
 - Time course study to define ½ life of AChE inhibition
 - Range finding & definitive dose response studies
 - Table extracted from 2007 NMC CRA

Chemical	PND11 Brain		Adult Brain
	BMD ₁₀ (mg/kg)	Half-Life (hrs.)	Adult BMD (mg/kg)
Aldicarb ¹	0.017	NA	0.033
Carbaryl	1.459	5.4 ³	2.627
Carbofuran	0.039	3.0	0.109
Formetanate	0.188	9.5	0.382
Methomyl	0.104	0.4	0.317
Oxamyl	0.051	1.5	0.177

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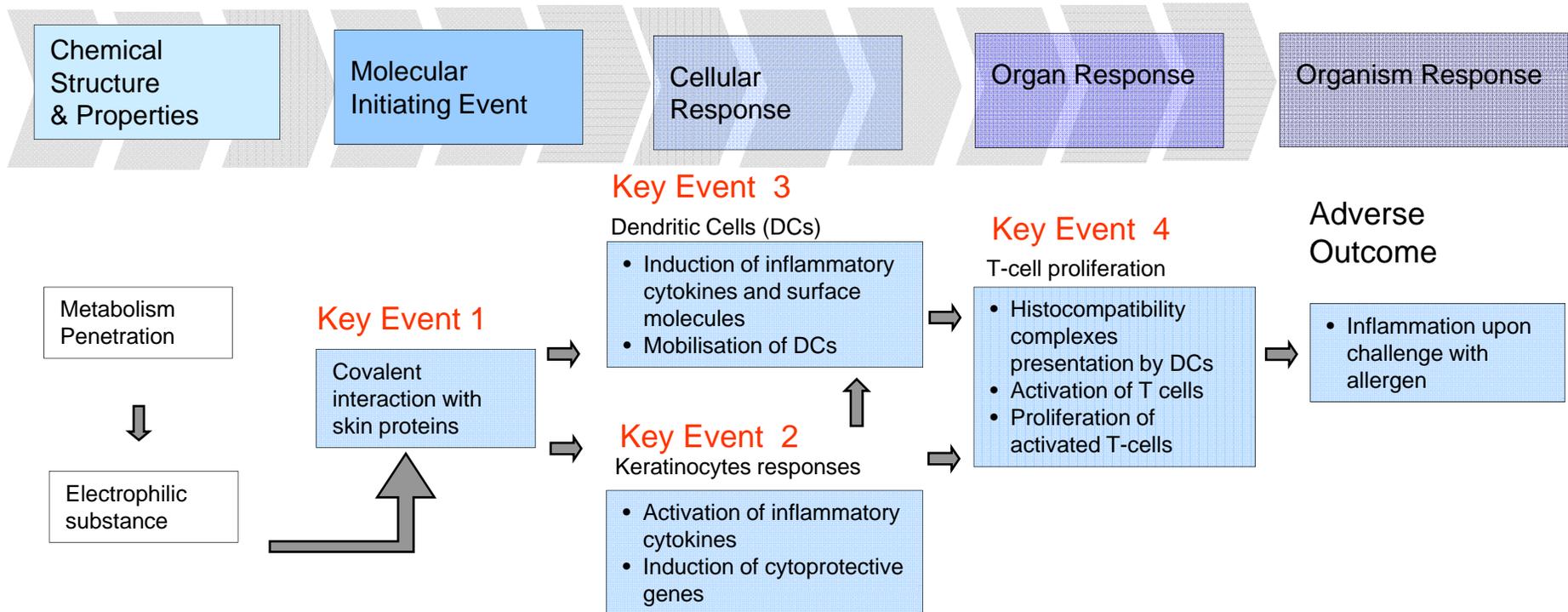
U.S. Federal Collaboration

- 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.

ICCVAM Skin Sensitization Workgroup

- Allergic contact dermatitis (ACD) is a skin reaction, characterized by localized redness, swelling, blistering, or itching, that can develop after repeated direct contact with a skin allergen.
- U.S. regulatory agencies establish hazard categories to determine appropriate labeling to warn consumers and workers of potential skin sensitization hazards. Data used to assign substances to appropriate hazard categories are generated using animal tests.
- Since its inception, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has given a high priority to replacing, reducing, and refining the use of animals for skin sensitization testing.
- Skin sensitization is a complex process, and it is likely that no single non-animal test can replace animal use for this testing. A more promising approach involves integrating data from several non-animal methods using an integrated decision strategy (IDS).

OECD Adverse Outcome Pathway (AOP) for Skin Sensitization¹



¹ For sensitization that is initiated by covalent binding to proteins.

OECD 2012. Guidance Document No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins: Part 1, Part 2.

<http://www.oecd.org/chemicalsafety/testing/seriesontestingandassessmentpublicationsbynumber.htm>

ICCVAM Skin Sensitization Workgroup

- Produce and test an IATA for skin sensitization using
 - Physicochemical parameters
 - QSAR/in silico methods (OECD Toolbox)
 - The three in chemico or in vitro assays validated by EURL ECVAM
 - DPRA, KeratinoSens, and h-CLAT
- Initial goal is to predict skin sensitization (yes/no) based on LLNA results
 - On-going activities (not presented here) on prediction of potency and/or human outcomes



In Vitro Methods

- DPRA
 - Assesses protein reactivity of a test substance
 - Mimics the ability of a test substance to bind to skin proteins to produce the hapten-protein complex
- KeratinoSens™
 - Assesses the activation of the AKR1C2-ARE element
 - Caused by electrophilic agents, which tend to be skin sensitizers
 - Measures fold-induction of luciferase activity
 - Uses KeratinoSens cells, a reporter cell line that contains a stable insertion of a luciferase gene under control of the ARE-element of AKR1C2 gene
 - Derived from HaCaT keratinocytes



In Vitro Methods

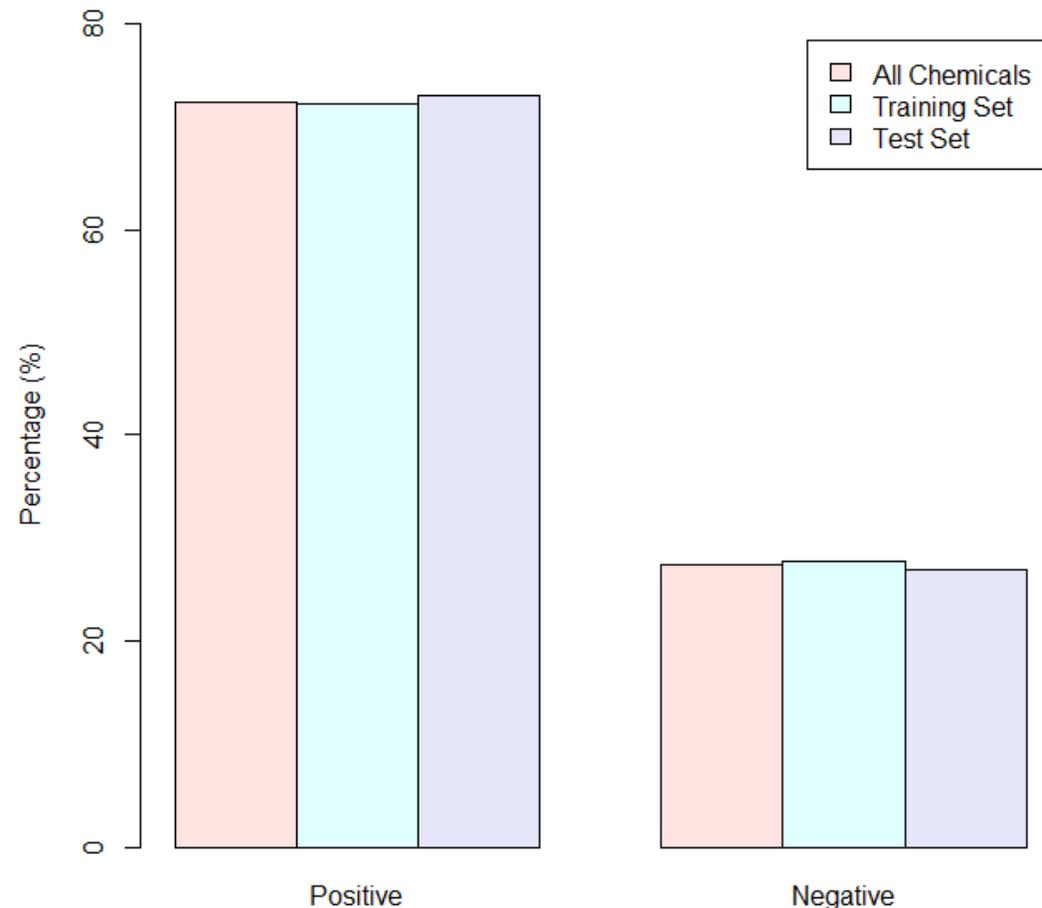
- h-CLAT
 - Measures 2 cell surface markers, CD86 and CD54, on dendritic cell surrogates
 - Assesses the maturation process of dendritic cells as they transform from antigen processing cells to antigen presenting cells
 - Uses THP-1 cells, an immortalized human monocytic leukemia cell line, as the dendritic cell surrogate

Chemical Database

- 120 chemicals from published sources
 - Collected DPRA, KeratinoSens, and h-CLAT categorical data [yes/no]
 - DPRA, KeratinoSens, and h-CLAT quantitative data collection underway
 - Performed OECD QSAR Toolbox predictions for sensitizer/nonsensitizer prediction
 - Collected physicochemical data
 - Collected skin penetration coefficient data

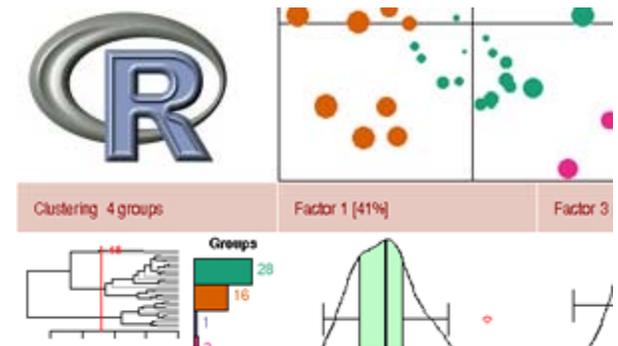
Summary of LLNA Data

- **Total:** 120 chemicals
 - 87 (73%) positive
 - 33 (27%) negative
- **Training set:** 94 (78%)
 - 68 positive (72%)
 - 26 negative (28%)
- **Test set:** 26 (22%)
 - 19 positive (73%)
 - 7 negative (27%)



Machine Learning Approaches (Binary Classification)

- Artificial Neural Network (ANN)
- Bayesian Network (BN)
- Classification and Regression Tree (CART)
- Linear Discriminant Analysis (LDA)
- Logistic Regression (LR)
- Support Vector Machines (SVM)



Based on overall accuracy for predicting LLNA outcomes, the modeling approaches ranked as follows: SVM > ANN > LR > LDA > CART = NB.

Individual Assays Compared to LLNA

hCLAT vs LLNA

	NEG	POS
NEG	21	14
POS	12	73

DPRA vs LLNA

	NEG	POS
NEG	23	15
POS	10	72

Keratino vs LLNA

	NEG	POS
NEG	21	21
POS	12	66

OECD vs LLNA

	NEG	POS
NEG	25	20
POS	8	67

Sensitivity %: 83.9

82.8

75.9

77.0

Specificity %: 63.6

69.7

63.6

75.8

Accuracy %: 78.3

79.2

72.5

76.7

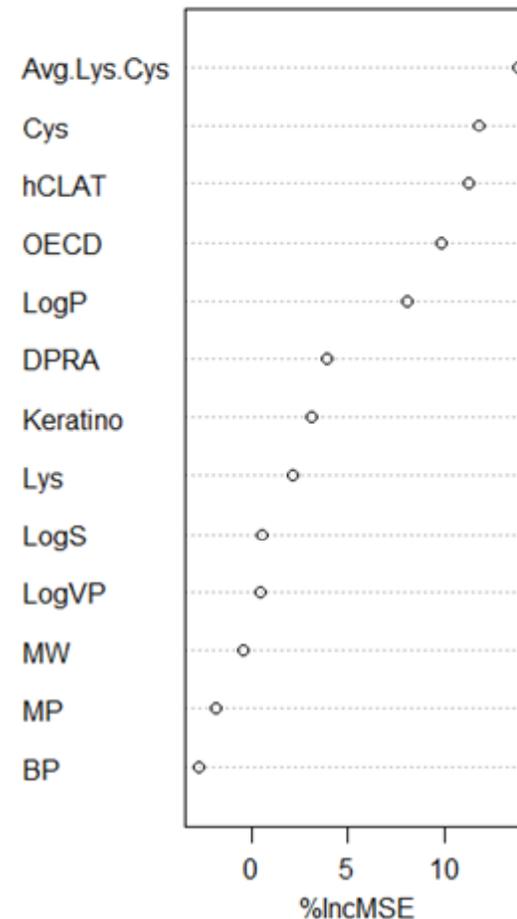
Variable Importance

Dependent variable: Overall LLNA Call (Majority)

13 independent variables:

Ranked by Random Forest:

- hCLAT:** hCLAT majority call [0/1]
- DPRA:** DPRA majority call [0/1]
- Keratino:** Keratino majority call [0/1]
- OECD:** QSAR Toolbox [0/1]
- Avg.Lys.Cys:** avg Depletion Lys & Cys [-1.9, 95.0]
- Lys:** avg % Depletion Lys [-5.6, 91.0]
- Cys:** avg % Depletion Cys [-0.9, 100]
- LogP:** partition coefficient [-8.28, 6.46]
- LogS:** water solubility [-5.94, 3.00]
- LogVP:** vapor pressure [-28.47, 5.89]
- MW:** molecular weight [30.03, 581.57]
- MP:** melting point [-148.50, 288.00]
- BP:** boiling point [-19.1, 932.2]



Skin Sensitization

The variable set that included h-CLAT, QSAR Toolbox, and the six physicochemical properties achieved the highest average accuracy for the test and training sets (97%).

Variable Set	Data Set ^b	Sensitivity (%)	Specificity (%)	Accuracy (%)
h-CLAT + Toolbox + 6 properties	Training	97.1	96.2	96.8
	Test	94.7	100	96.2
Avg.Lys.Cys + Toolbox + 6 properties	Training	91.2	100	93.6
	Test	78.9	100	84.6

Conclusions

- Machine learning approaches and integrated testing strategies yield higher predictivity than individual assays.
- Further QSAR/HTS/in vitro hybrid models are being developed, as well as models to predict potency.
- These methods could be readily applied to in vitro data on formulations.
- Formulation data would facilitate computational approaches to predict mixtures results from individual chemical data.

Summary & Thoughts

- Rapid advances to implementing the 3R's into regulatory testing and alternative approaches but there is more work to do.
- AOPs provide strong foundation for considering data needs & designing toxicity studies
- Lessons learned so far...
 - Collaborative approaches working with investigators across sectors is most effective approach
 - Harmonization and coordination across state, federal, and international regulatory agencies is important

