Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM
Skin Sensitization Projects

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SACATM Meeting
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National Institute of Environmental Health Sciences
Durham, North Carolina

Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture
Department of Defense • Department of Energy • Department of the Interior • Department of Transportation
Environmental Protection Agency • Food and Drug Administration • National Institute for Occupational Safety and Health
National Institutes of Health • National Cancer Institute • National Institute of Environmental Health Sciences
National Library of Medicine • Occupational Safety and Health Administration

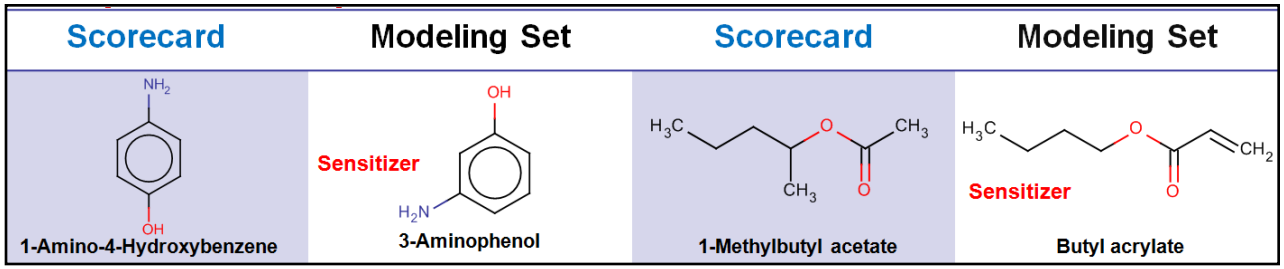
NICEATM Efforts: Skin Sensitization

3 R's

- Collaborations to develop and evaluate chemical structure-activity relationship (SAR) models for predicting skin sensitization
- Develop an open-source Bayesian network that uses multiple physicochemical, *in silico*, *in chemico*, and *in vitro* inputs to predict skin sensitization
- Coordinate with the OECD AOP program for skin sensitization to guide development of an integrated testing strategy (ITS)
- Evaluate high throughput screening (HTS) assays from ToxCast/Tox21 program for relevance to skin sensitization

QSAR Model of Skin Sensitization

- After NICEATM data curation, 262 compounds retained for modeling (multiple 2D chemical descriptors and Random Forests)
  - 134 sensitizers and 128 non-sensitizers
  - Consensus model (75% coverage): 80% BA (5-fold cross val.)
- External validation on Scorecard dataset using QSAR models and similarity search



- Benchmarking with OECD QSAR Toolbox on 153 external compounds

	Sensitivity	Specificity	Coverage
Consensus	**73%**	**91%**	**84%**
OECD Toolbox	69%	20%	97%

Courtesy of Prof. Alexander Tropsha (UNC-CH)

Creating an Open Source Model for Probabilistic Skin Sensitization Hazard Prediction

Research Article

Journal of Applied Toxicology

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(wileyonlinelibrary.com) DOI 10.1002/jat.2869

Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice

Joanna Jaworska[a]*, Yuri Dancik[a], Petra Kern[a], Frank Gerberick[b] and Andreas Natsch[c]

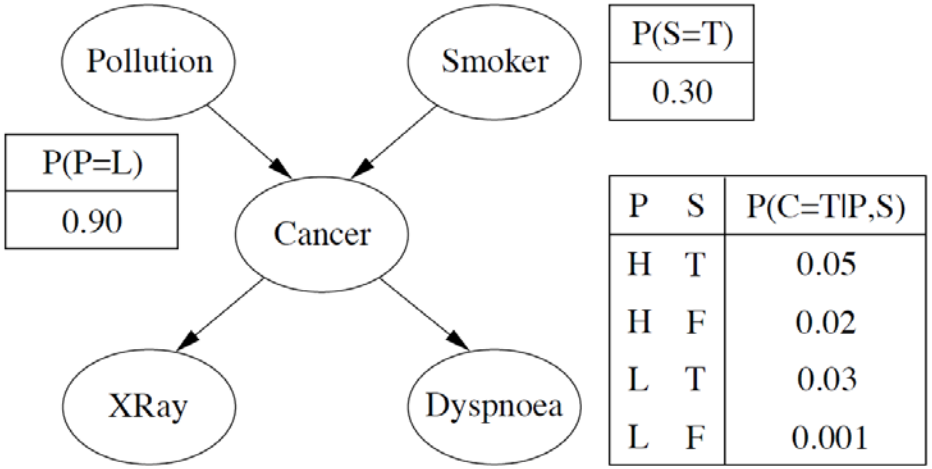
ABSTRACT: Frameworks to predict *in vivo* effects by integration of *in vitro*, *in silico* and *in chemico* information using mechanistic insight are needed to meet the challenges of 21st century toxicology. Expert-based approaches that qualitatively integrate multifaceted data are practiced under the term 'we ereas quantitative approaches remain rare. To address this gap we previously developed a methodology ated Testing Strategy (ITS) in the form of a Bayesian Network (BN). This study follows up on our proof presents an updated ITS to assess skin sensitization

Open source software

http://www.r-project.org/

Bayesian Networks (BNs):

- Probabilistic graphical models
- Can be used to represent knowledge about a domain of interest and facilitate reasoning involving uncertain evidence



P(S=T)
0.30

P(P=L)
0.90

P	S	P(C=T|P,S)
H	T	0.05
H	F	0.02
L	T	0.03
L	F	0.001

C	P(X=pos|C)
T	0.90
F	0.20

C	P(D=T|C)
T	0.65
F	0.30

Figure 1. Simple Lung Cancer BN

Korb and Nicholson. 2010

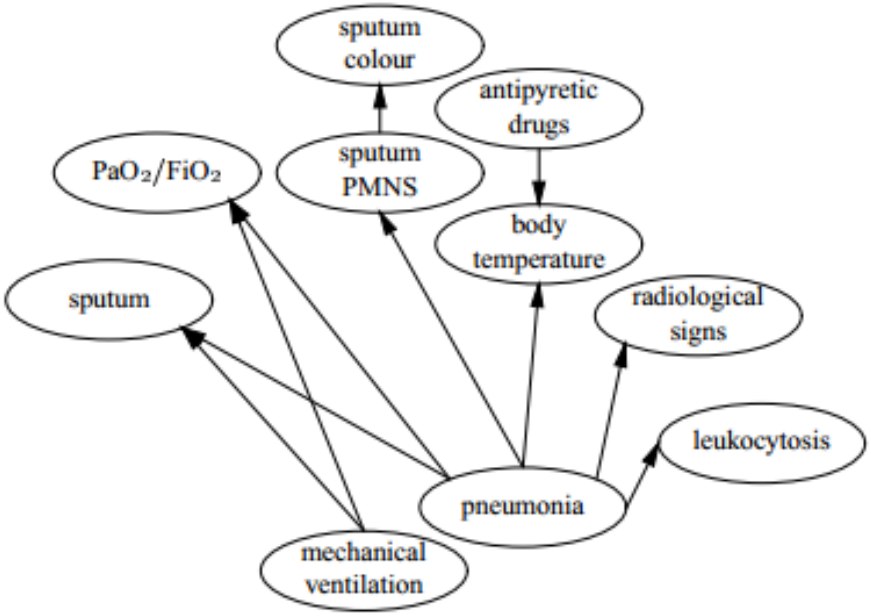
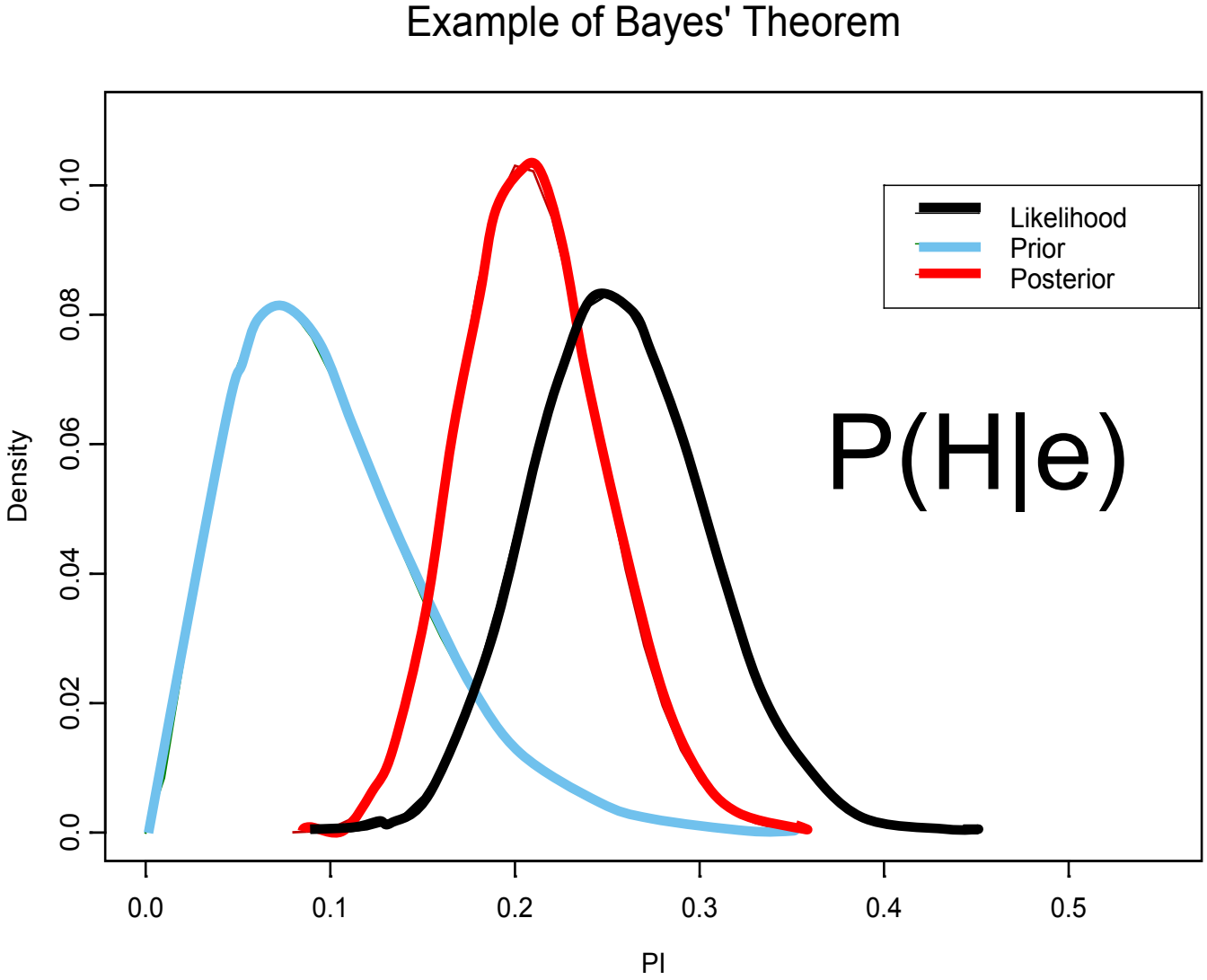


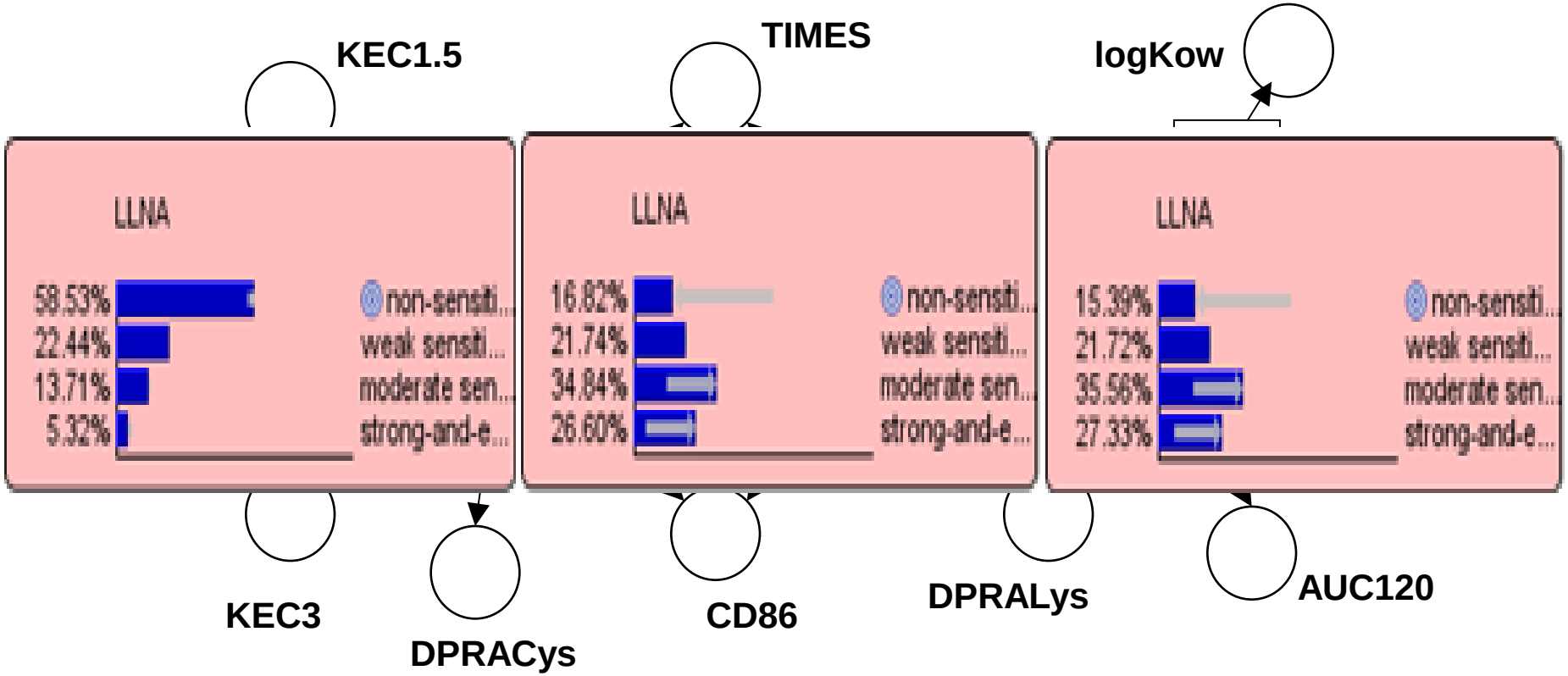
Figure 2. BN of Signs and Symptoms of Pneumonia

Charitos et al. 2007

Hypothesis (prior) X Evidence (likelihood) = Revised Hypothesis (posterior)

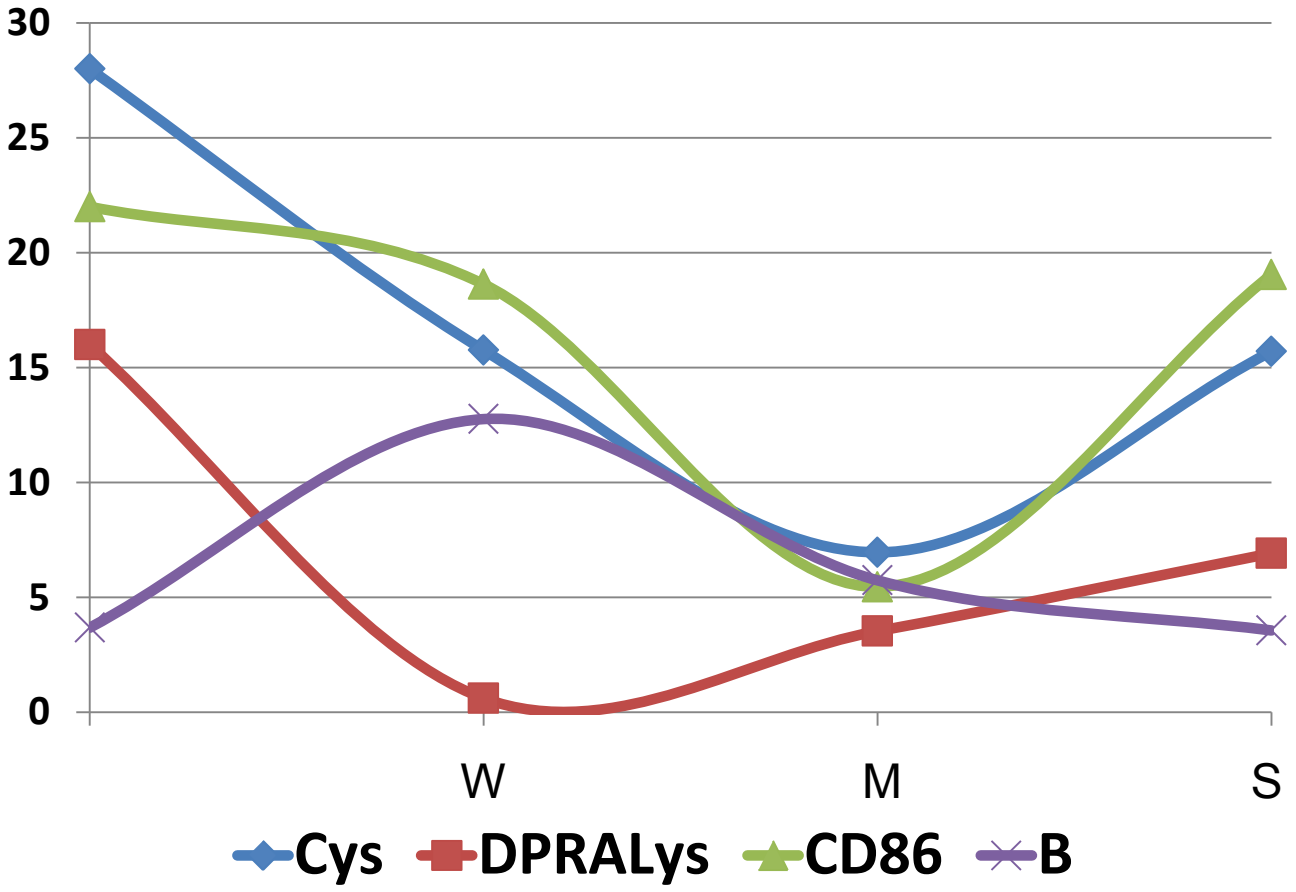


Jaworska et al. 2013
ITS-2
P(LLNA=NS, W, M, S| evidence)



Data set n=145: Training set n=121, Test set n=21

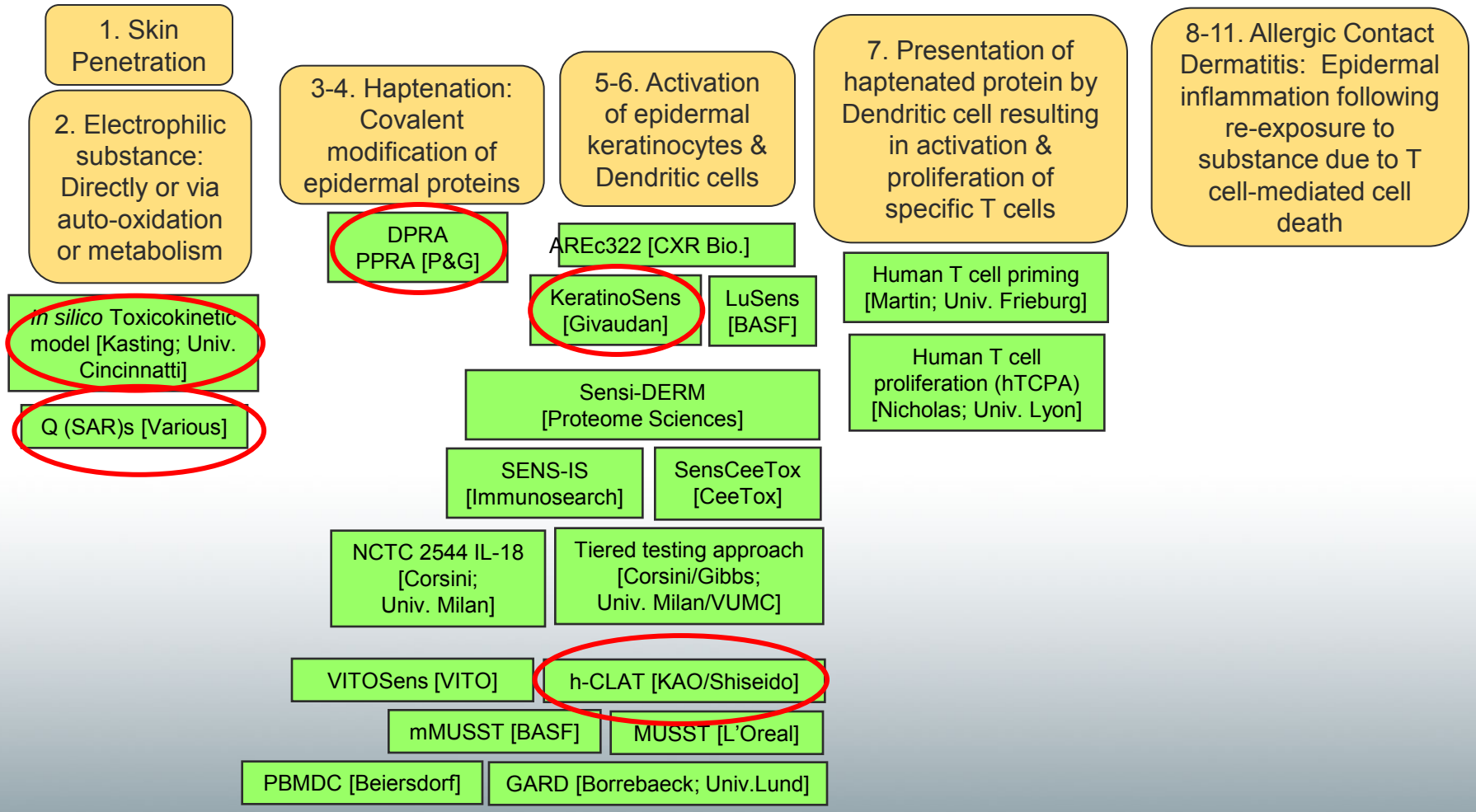
Mutual Information
Assays that Help Predict LLNA Potency Class



Courtesy of Joanna Jaworska

Non-animal Methods for Skin Sensitisation: Aligned to AOP Key Events

Cosmetics Europe
the personal care association



Slide courtesy of Gavin Maxwell (Unilever/Cosmetics Europe)

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Tox21 Assays: Aligned to AOP Key Events

1. Skin Penetration

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

QSAR Model of skin permeability and penetration *(Tropsha, et al.)*

3-4. Haptenation: Covalent modification of epidermal proteins

Novascreen enzyme activity biochemical cell-free assays *(HDACs, EGFR, etc.)*

5-6. Activation of epidermal keratinocytes & Dendritic cells

BSK_hDF3CGF Primary human dermal fibroblasts

BSK_KF3CT Primary human keratinocytes and fibroblasts

Attagene reporter gene assays HepG2 *(Nrf2, LXR, RXR etc.)*

Odyssey Thera oxid. stress in U2OS *(H2AFX)*

Apredica oxidative stress in HepG2 *(H2AFX, MitoMem)*

Tox21 assay HepG2 bla *(Nrf2/ARE)*

7. Presentation of haptenated protein by Dendritic cell resulting in activation & proliferation of specific T cells

BSK_SAg, 3C, 4H and BSK_LPS Primary human monocytes and endothelial cells

8-11. Allergic Contact Dermatitis: Epidermal inflammation following re-exposure to substance due to T cell-mediated cell death

QSAR Model built off NICEATM LLNA database *(Tropsha, et al.)*

Local Lymph Node Assay (LLNA)

Random Forest Model for predicting LLNA with ToxCast *in vitro* HTS data: 5-fold Cross Validation (n=64 chemicals)

Model Run					BA
1					0.92
2					0.94
3					0.69
4					0.67
5	0.71	0.8	0.83	0.67	0.76
AVG	0.75			0.74	**0.79**

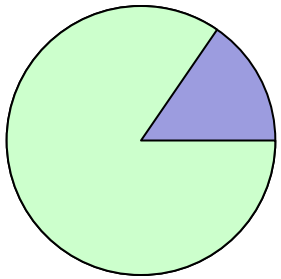
Primary human dermal fibroblasts
Collagen III
Proliferation

Activated monocytes
Prostaglandin
IL-8

Transactivation assays
Nrf2/ARE
RXRb

Oxidative Stress
Mitochondrial membrane potential

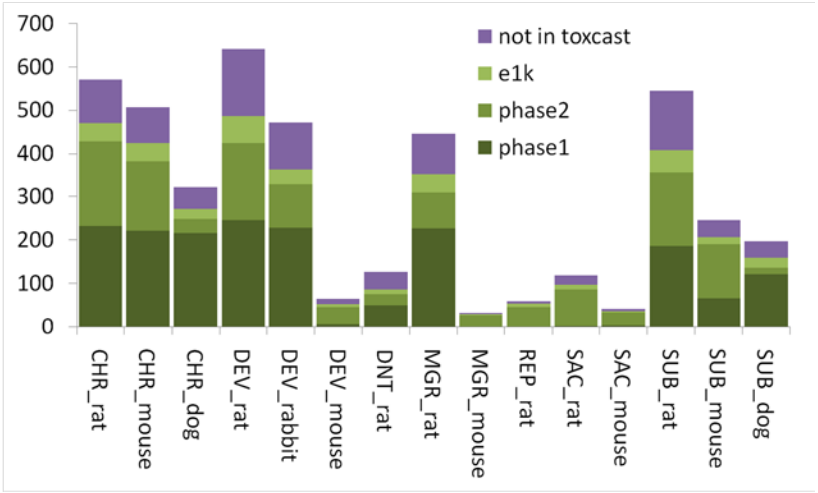
Assay targets that map to AOP



80% Training Set
20% Test Set

Expand ToxRefDB: *in vivo* Study Data

- ImmunoTox initiative (led by NTP/NICEATM)
  - Develop ontology for entering study data
    - Including LLNA and other skin sensitization studies
  - Enter NTP/EPA studies first, then open lit search
- Skin sensitization data exist for ALL pesticides
  - NO skin sens data currently in ToxRefDB
  - Studies requested from EPA
    - Via FOIA in 2012
    - Via personal request (May 2013)
    - Recent data evaluation records (DERs) may be available soon



http://actor.epa.gov/toxrefdb

Summary

- NICEATM supports efforts to create probabilistic frameworks for inference and testing strategy development
- Open source ITS Bayesian Network structure that follows mechanistic steps of skin sensitization induction process
  - BN ITS topology and AOP are very similar
  - External validation: 86% correct for potency, 95% for hazard
  - QSAR models under development may improve ITS
- Well characterized AOPs like skin sensitization provide opportunities to use HTS data (ToxCast, Tox21)
  - Mapping *in vitro* assays to AOP based on biological knowledge
  - Building statistical models on training sets using random forest and other multivariate techniques

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Questions?