



**Interagency Coordinating Committee on
the Validation of Alternative Methods**

Implementation: Alternatives for Skin Sensitization Testing

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Consumer Product Safety Commission
Co-Chair, ICCVAM Skin Sensitization Workgroup

SACATM Meeting
September 18-19, 2017

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 Institute of Standards and Technology • National Library of Medicine • Occupational Safety and Health Administration



Skin Sensitization Implementation Plan:

- Coordinate activities via the ICCVAM Skin Sensitization Workgroup (SSWG)
- Draft a scoping document to identify U.S. agency requirements, needs, and decision contexts for skin sensitization data
- Coordinate efforts with stakeholders
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Current ICCVAM SSWG Roster

- Moiz Mumtaz (ATSDR)
- Patricia Ruiz (ATSDR)
- John Gordon (CPSC)
- Joanna Matheson (CPSC)
- Emily N. Reinke (DOD)
- Evisabel Craig (EPA)
- David Lehmann (EPA)
- Anna Lowit (EPA)
- Timothy McMahon (EPA)
- Mamta Naidu (EPA)
- Todd Stedeford (EPA)
- Simona Bancos (FDA)
- Paul C. Brown (FDA)
- Rakhi M. Dalal-Panguluri (FDA)
- Wei Ding (FDA)
- Robert Heflich (FDA)
- Abigail C. Jacobs (FDA)

- Diego Rua (FDA)
- Nakissa Sadrieh (FDA)
- Stanislav Vukmanovic (FDA)
- Jeffrey Yourick (FDA)
- Warren Casey (NIEHS)
- Dori Germolec (NIEHS)
- Nicole Kleinstreuer (NIEHS)

ICATM Liaison Members

- Silvia Casati (EURL ECVAM)

NICEATM Support Staff (ILS)

- Michael Paris
- Judy Strickland
- David Allen



Interagency Coordinating Committee on the Validation of Alternative Methods

ICCVAM Skin Sensitization Models

Research article

Journal of Applied Toxicology

Received: 13 October 2016, Revised: 26 October 2016, Accepted: 1 November 2016, Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3424

Prediction of skin sensitization potency using machine learning approaches

Qingda Zang^a, Michael Paris^a, David M. Lehmann^b, Shannon Bell^a, Nicole Kleinstreuer^c, Warren Casey^c and

ABSTRACT: The replacement of agencies that use data from such out using animal data have been classified into potency categories: mouse assay (LLNA) and human or



Research article

Journal of Applied Toxicology

Received: 16 February 2016, Revised: 21 June 2016, Accepted: 21 June 2016, Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.3366

Multivariate models for prediction of human skin sensitization hazard

Judy Strickland^{a*}, Qingda Zang^a, Michael Paris^a, David M. Lehmann^b, David Allen^a, Neepa Choksi^a, Joanna Matheson^d, Abigail Jacobs^e, Warren Casey^c and Nicole Kleinstreuer^c

ABSTRACT: One of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal approaches to identify events necessary to produce skin sensitization suggests that no single animal tests. ICCVAM is evaluating an integrated approach to testing animal and human data to predict human skin sensitization hazard.



Research article

Journal of Applied Toxicology

Received: 9 October 2015, Revised: 10 November 2015, Accepted: 2 December 2015, Published online in Wiley Online Library: 6 February 2016

(wileyonlinelibrary.com) DOI 10.1002/jat.3281

Integrated decision strategies for skin sensitization hazard

Judy Strickland^a, Qingda Zang^a, Nicole Kleinstreuer^a, Michael Paris^a, David M. Lehmann^b, Neepa Choksi^a, Joanna Matheson^c, Abigail Jacobs^d, Anna Lowit^e, David Allen^a and Warren Casey^{f*}

ABSTRACT: One of the top priorities of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) is the identification and evaluation of non-animal alternatives for skin sensitization testing. Although skin sensitization is a complex process, the key biological events of the process have been well characterized in an adverse outcome pathway (AOP) proposed by the Organisation for Economic Co-operation and Development (OECD). Accordingly, ICCVAM is working to develop





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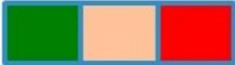
U.S. Statutes and Regulations

US Statute/Regulations	Agency
Federal Hazardous Substances Act (FHSA) (1964): 16 CFR 1500.3: Consumer Products	CPSC
Labeling of Hazardous Art Materials Act (LHAMA) (1988): 16 CFR 1500.14: Art Materials	CPSC
Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6): 40 CFR 156, 40 CFR 158.2230: Antimicrobials	EPA
Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6): 40 CFR 156, 40 CFR 158.230, 40 CFR 158.2050, CFR 158.2230: Pesticides	EPA
Toxic Substances Control Act (TSCA; 1976): 40 CFR 700-799: Industrial Chemicals	EPA
Federal Food, Drug, and Cosmetic Act (1938): Cosmetics	FDA
Federal Food, Drug, and Cosmetic Act (1938): Pharmaceuticals	FDA
Occupational Safety and Health Act (1970): 29 CFR 1910.1200: Workplace Chemicals	OSHA

Strickland et al. 2017 in prep



U.S. Agency Requirements/Considerations

		Reference Animal Method	Classification Criteria
	Pesticides Industrial chem	 LLNA	NS S  Hazard
	Household Products	 LLNA	NS S SS  Potency
	Dermatological Products	 GPMT*	 Potency*

*human data preferred



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International Cooperation on Alternative Test Methods (ICATM)

- First ever ICATM Workshop: “International regulatory applicability and acceptance of alternative non-animal approaches to skin sensitization assessment of chemicals used in a variety of sectors”
- Convened by EURL ECVAM on October 4-5, 2016, in Ispra, Italy





ICATM Workshop Outcomes

- White paper characterizing international regulatory requirements for skin sensitization testing (**final draft**)
- Position paper authored by ICATM partners covering workshop outcomes and ICATM recommendations (**final draft**)
 - Including proposed acceptance criteria for defined approaches to testing and assessment of skin sensitization
- OECD SPSF for “development of a performance based test guideline for defined approaches to testing and assessment of skin sensitization” (**submitted**)
 - Develop framework detailing performance standards and acceptance criteria for the assessment of defined approaches as replacements for the LLNA
 - Apply performance standards and acceptance criteria to OECD case studies
- Annual ICATM workshop (e.g. performance standards, validation approaches, respiratory sensitization, computational approaches...)



Skin Sensitization Data Collection: Ongoing Efforts

- Multiple conventional & antimicrobial registrants have kindly provided data to support our skin sensitization efforts
- We continue to collect additional, voluntary data submissions to expand current datasets
 - Paired *in vitro* & LLNA data that could increase coverage of various defined approaches
 - Other LLNA studies to help assess variability
 - Additional human data to assist in evaluating defined approaches



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Accuracy Against Human Clinical Data (~150 chems)

LLNA



Hazard

72%-82%

Potency

54% - 60%

GPMT / Buehler



Hazard

~72%

Potency

~60%

Reproducibility of Multiple Tests (~100 chems)

Hazard

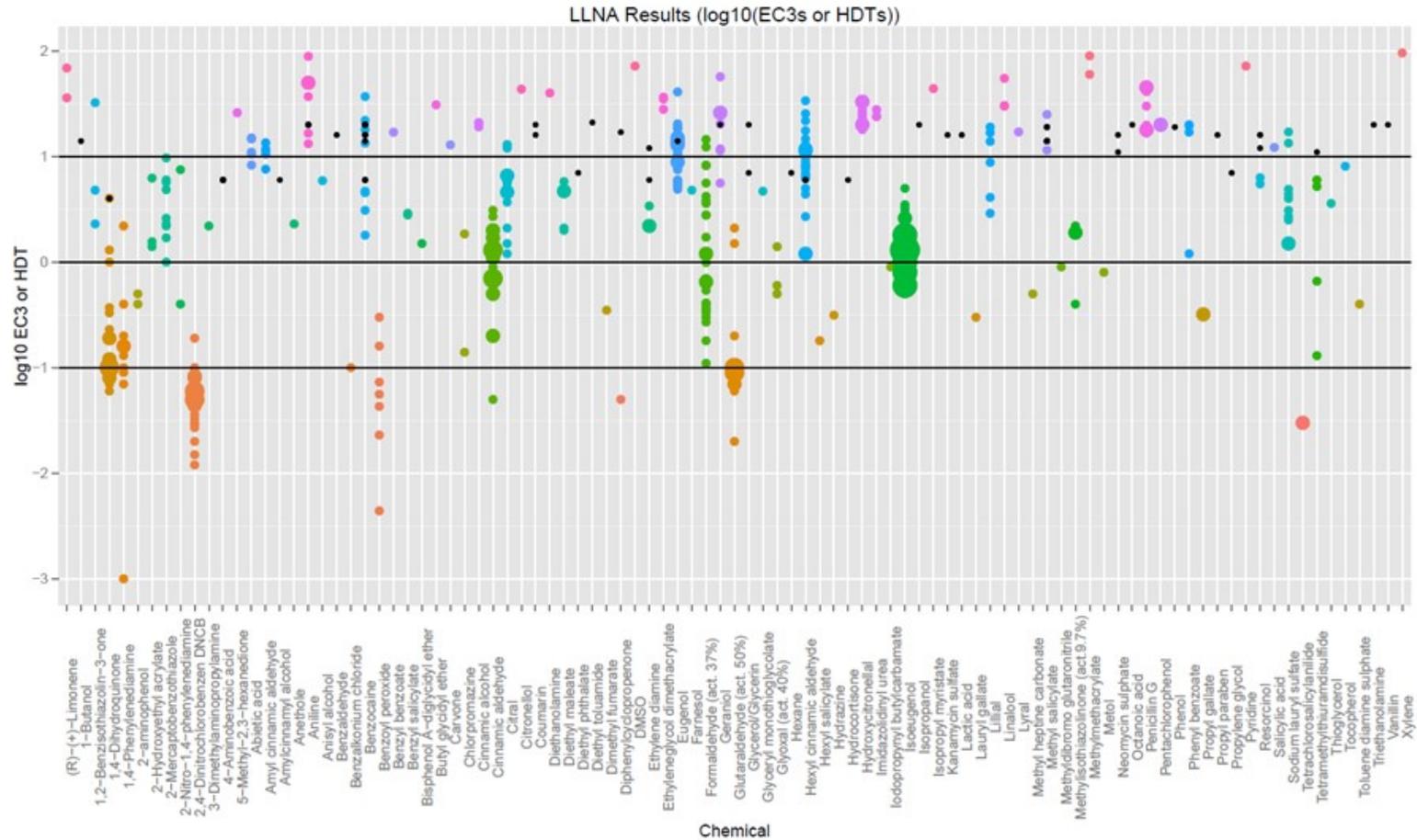
~78%

Potency

~62%

*ICCVAM. 1999. NIH Publication No. 99-4494
 ICCVAM. 2010. NIH Publication No. 11-7709
 Urbisch et al. 2015. Reg Tox Pharm 71:337-351.
 Dumont et al. 2016. Tox In Vitro 34: 220-228
 Hoffmann et al. 2017 submitted*

95 chemicals with multiple LLNA results (541 total tests)



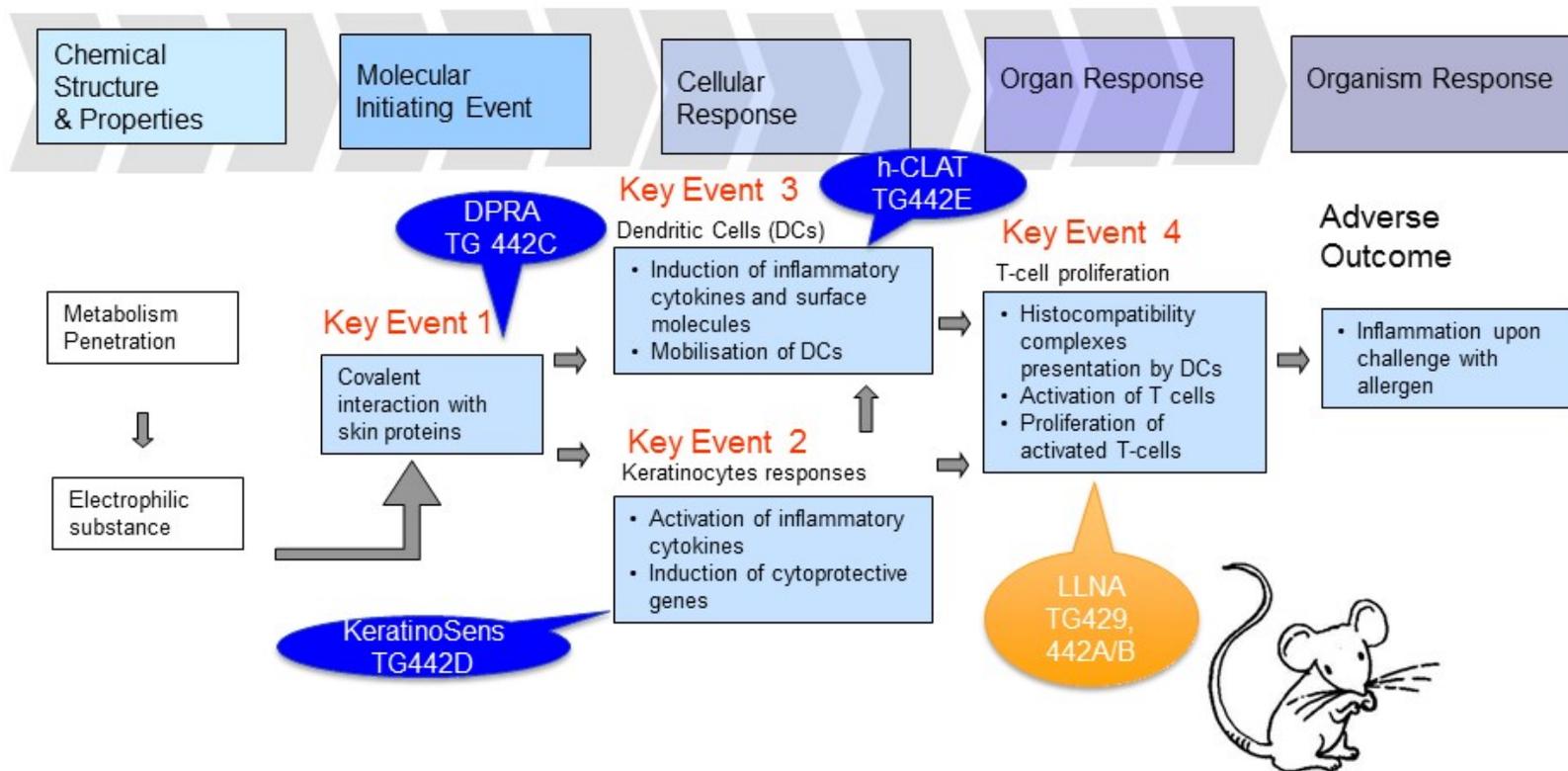


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AOP for Skin Sensitization: Available Methods

For sensitization that is initiated by covalent binding to proteins.

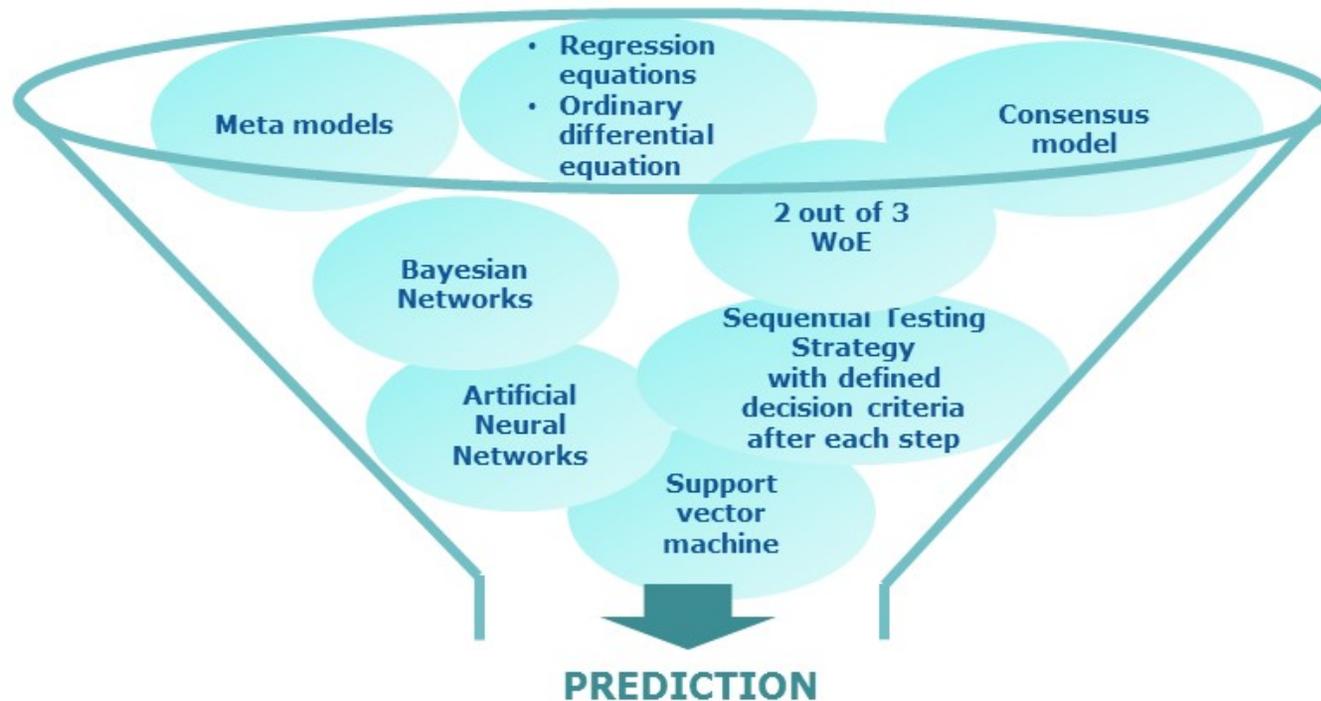


OECD 2012. Guidance Document No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins: Part 1, Part 2.

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

Global Skin Sensitization Project

- Collaboration with Cosmetics Europe – Analyze OECD-submitted modeling approaches
 - 128 substance dataset
 - Evaluate performance against mouse and human hazard/potency categories





Non-Animal Approach Evaluation

Most non-animal testing strategies evaluated so far perform **better** than the LLNA at predicting human skin sensitization hazard and potency.

(And when compared to the LLNA, are equivalent in performance to the LLNA at predicting itself.)

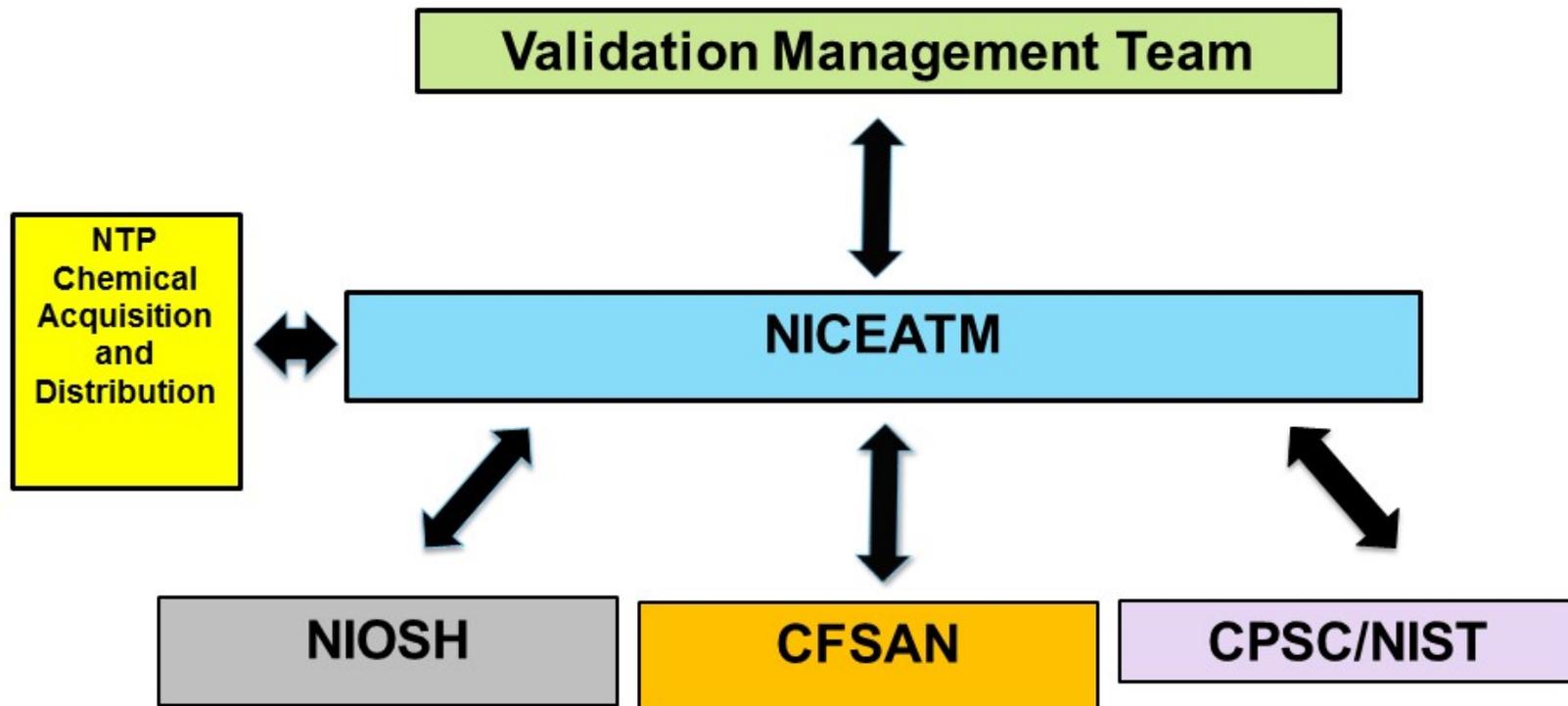


Validation Study: Electrophilic Allergen Screening Assay (EASA)

- To characterize the usefulness and limitations of a non-animal *in chemico* test method (EASA) to classify the allergic contact dermatitis (ACD) hazard of products and chemicals
 - Optimize and standardize the test method protocol
 - Assess intra- and inter-laboratory reproducibility
 - Assess accuracy for the classification of ACD hazard



EASA Validation Study Organization





NIST/CPSC collaboration with EASA assay

- Participate as one of the three laboratories (i.e. instrument sharing) in an interlaboratory comparison
- Collaboration provides the opportunity to assess the robustness and reproducibility of the assay and potential protocol modifications to provide evidence for measurement assurance
- Evaluate usage of this assay with challenging test compounds such as nanomaterials





Some key preliminary findings

- Hazard assessment of laboratory and protocol is critical due to skin sensitization reagents! Resulted in approximately 10 pages Hazard Review.
- NBT assay is light sensitive and steps need to be taken to minimize decrease in signal of negative control
- There are substantial variations among suppliers for the NBT reagent with regards to reproducibility of negative control readings
- Discussions about dosing concentrations to use for positive controls in the assay
- Key impact of cuvette design with some cuvettes potentially leading to cross-contamination among samples or risk of exposure to skin sensitizers on gloves

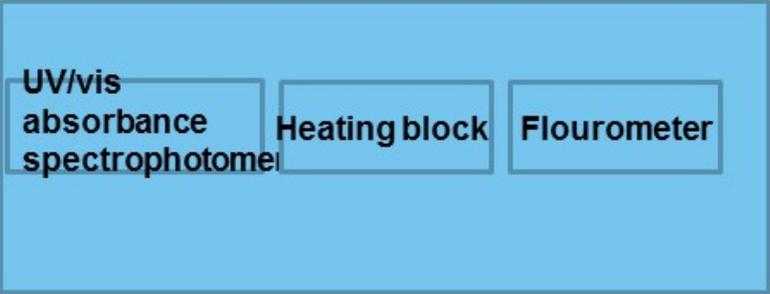




NIST/CPSC laboratory setup to minimize hazard and improve measurement precision within laboratory



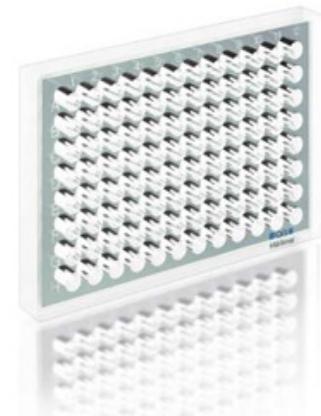
Fume hood





Future efforts

- Calibration standard for absorbance and fluorescence instruments between laboratories
- Identification of intermediate process measurements to provide troubleshooting and in-line controls for measurement assurance
- Translation of assay to 96-well plate format with a quartz microplate
- Evaluation and protocol modifications for use with challenging substances (i.e. nanomaterials)





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International Harmonization

- OECD proposal (SPSF) submitted November 2016
 - Co-led by U.S., EU, and Canada
 - Create an international performance based test guideline for non-animal defined approaches to skin sensitization testing
 - Achieve widespread replacement of mouse test
- Comments from OECD member countries received January 2017, revised SPSF submitted March 2017
- National coordinators from 35 member countries voted unanimously to approve the project on April 27, 2017





Scientific and Non-scientific Challenges

- Animal methods currently provide the reference data for evaluating alternatives
 - Results are variable
 - Many testing strategies outperform the LLNA in predicting human outcomes
- Data requirements vary across U.S. and global regulatory authorities, and are often ambiguous/subjective
- Coverage of chemical space
- Limited commercial availability of alternatives
- Overcoming regulatory and institutional inertia
 - Education and training



Expanding Chemical Space Coverage

- Prospective *in vitro* testing supported by NTP (D. Germolec)
- Chemicals with existing LLNA data nominated by ICCVAM agencies
 - NTP, EPA (OPP, OPPT, ORD), CPSC, FDA
 - Pesticides, formulations, excipients, industrial chemicals, etc.
- NTP Contractor (BRT) running:
 - LuSens (me-too method under OECD TG442D)
 - DPRA (OECD TG442C)
 - h-CLAT (OECD TG442E)
- Screening of 47 chemicals underway
- Procurement of ~150 additional test chemicals is ongoing
- Results will expand defined approach evaluations