Review of Alternative Test Methods and Integrated Strategies for Allergic Contact Dermatitis Hazard Assessments

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ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Allergic Contact Dermatitis

January 20, 2011

William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD

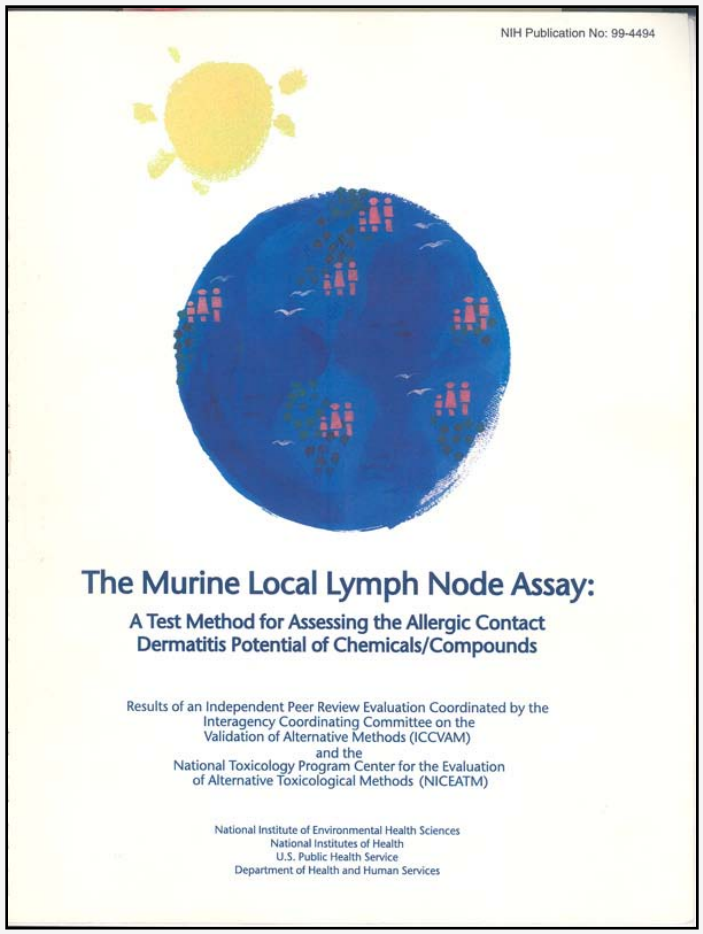
Outline

- Workshop Goals
- LLNA Introduction
- Overview of ICCVAM evaluation, recommendations, and agency responses for available alternative methods for allergic contact dermatitis (ACD) hazard testing
  - LLNA performance standards, including updated LLNA protocol
  - Reduced LLNA (rLLNA)
  - LLNA applicability domain
  - Nonradioactive LLNA
    - LLNA: DA
    - LLNA: BrdU-ELISA
  - LLNA for skin potency categorization
- Integrated decision strategies for ACD hazard assessments

ACD Regulatory Safety Testing Workshop Goals

- Provide an overview of available methods
- Provide information for conducting and interpreting data in accordance with regulatory testing requirements and guidelines
- Become familiar with data generated by each test method
- Provide a forum for scientists to share information on the appropriate use of results in regulatory safety testing
- Discuss challenges of incorporating alternative test methods into regulatory safety testing guidelines
- Identify and discuss new methods in the development and validation pipeline

Validation and Regulatory Acceptance of the LLNA



- Submitted to ICCVAM, 1997
  - Dr. F. Gerberick, P&G
  - Dr. D. Basketter, Unilever
  - Dr. I. Kimber, Zeneca
- ICCVAM International Peer Review Panel Meeting
  - September 1998
  - *Valid substitute for the traditional guinea pig tests*
  - *A reduction and refinement success*
- Regulatory Acceptance
  - U.S. EPA, FDA, CPSC
    - October 1999
  - OECD TG 429: 2002
  - ISO 10993-10: 2002
  - EPA OPPTS 870.2600: 2003

ICCVAM. 1999. NIH Publication No. 99-4494. RTP, NC: NTP. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel98.htm

LLNA Advantages

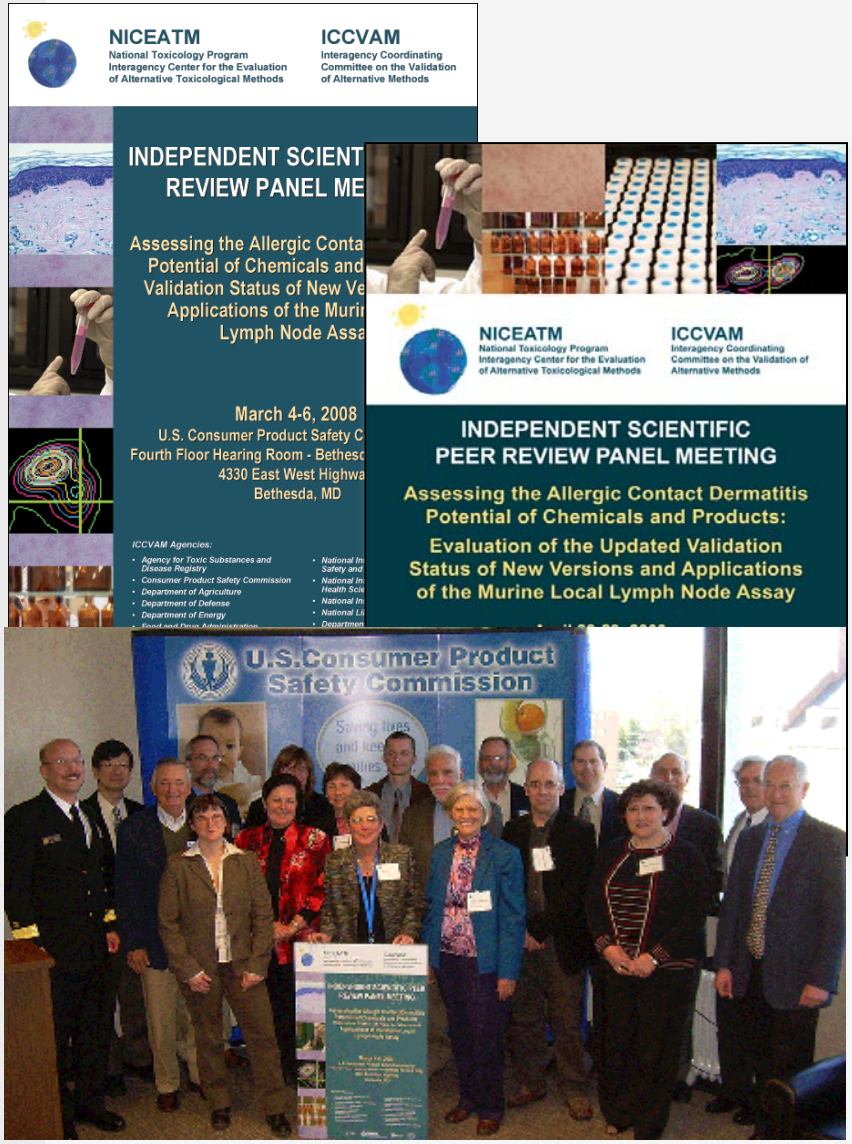
	GPMT[1]	LLNA
■ Time to perform:	22+ days	7 days
■ Number of animals:	30	12-20
■ Dermatitis induced:	Yes	No
■ Adjuvant required:	Yes	No

Advantages over guinea pig test methods

- Elimination of potential pain and distress
- 33-60% fewer animals
- 30% of the time required to perform
- Dose-response information

[1] guinea pig maximization test

ICCVAM 2008-10 Evaluations: New Versions and Applications of the LLNA to Assess ACD Potential



- International Public Peer Review Panel meetings in 2008 and 2009
  - 19 experts from 8 countries
  - Considered draft background review documents and ICCVAM recommendations
- Evaluation topics:
  - LLNA performance standards and updated LLNA protocol
  - rLLNA protocol
  - 3 nonradioactive LLNA versions:
    - LLNA: DA – Dr. Kenji Idehara
    - LLNA: BrdU-ELISA – Dr. Masahiro Takeyoshi
    - LLNA: BrdU-FC – MB Labs
  - Updated LLNA applicability domain
  - Use of the LLNA for skin potency categorization

ICCVAM Reports and Recommendations: rLLNA and LLNA Performance Standards

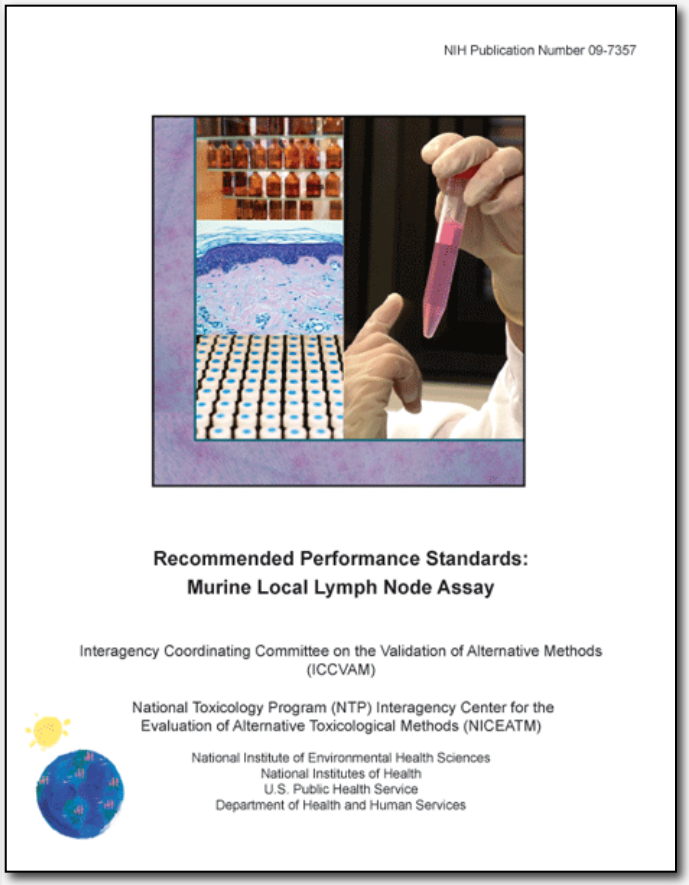
- Published 2009; accepted by U.S. agencies March 2010
  - Both documents include an updated LLNA protocol
    - 20% reduction in animal use
    - Guidance on selection of the highest dose
    - Collection of individual animal data
  - rLLNA procedure
    - 40% reduction in animal use

Updated OECD TG 429 adopted July 22, 2010

ICCVAM. 2009. NIH Publication No. 09-7357. RTP, NC NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/PerfStds/llna-ps.htm

ICCVAM. 2009. NIH Publication No. 09-6439. RTP, NC:NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/TMER.htm

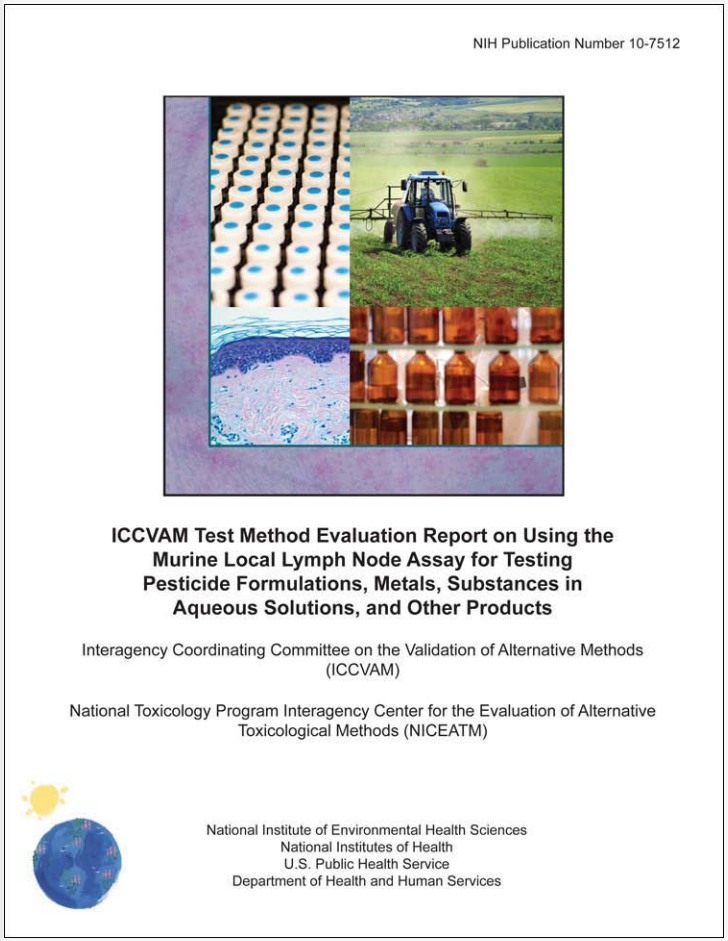
ICCVAM Recommendations: LLNA Performance Standards



- Provide basis for validation of proposed methods that are mechanistically and functionally similar to the LLNA
- Enables rapid evaluation of new LLNA versions
- Essential test method components based on LLNA
- 18 required reference chemicals, plus 4 optional
  - 13 positives covering a wide range of potency
  - 5 negatives
- Performance criteria for accuracy and reproducibility using reference chemicals

ICCVAM. 2009. NIH Publication No. 09-7357. RTP, NC NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/PerfStds/llna-ps.htm

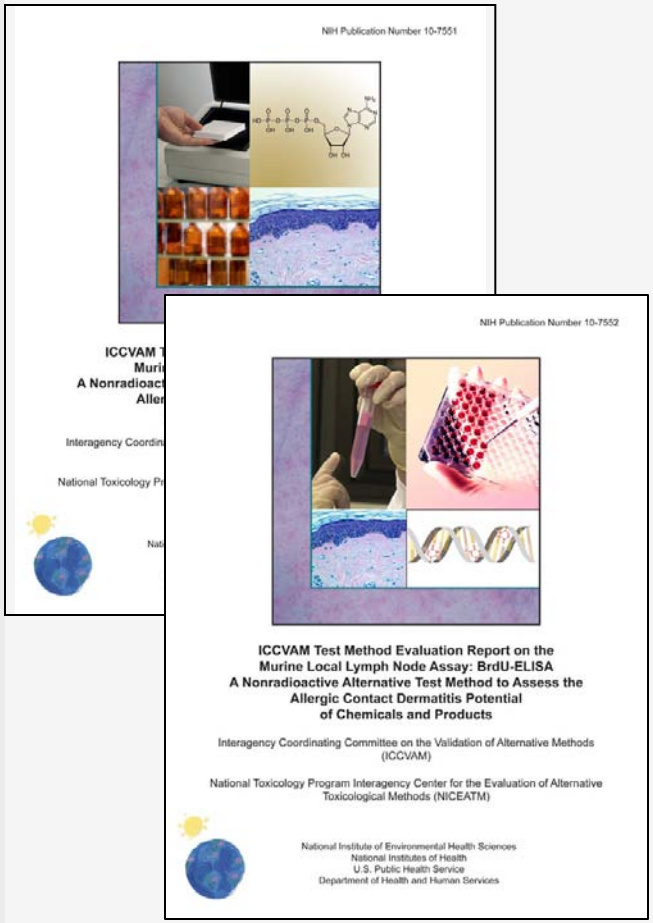
ICCVAM Report and Recommendations: Updated LLNA Applicability Domain



- Updates 1999 ICCVAM recommendations
- Performance of LLNA supports its use for testing
  - Pesticide formulations and other products
  - Metals, except nickel
  - Substances in aqueous solutions
  - Other substances/products unless physicochemical properties interfere with the ability of LLNA to detect sensitizers
- Transmitted to U.S. agencies June 14, 2010
- *Updated OECD TG 429 adopted July 22, 2010*

ICCVAM. 2010. NIH Publication No. 10-7512. RTP, NC:NIEHS.
Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-app.htm

ICCVAM Reports and Recommendations: Nonradioactive LLNA Methods

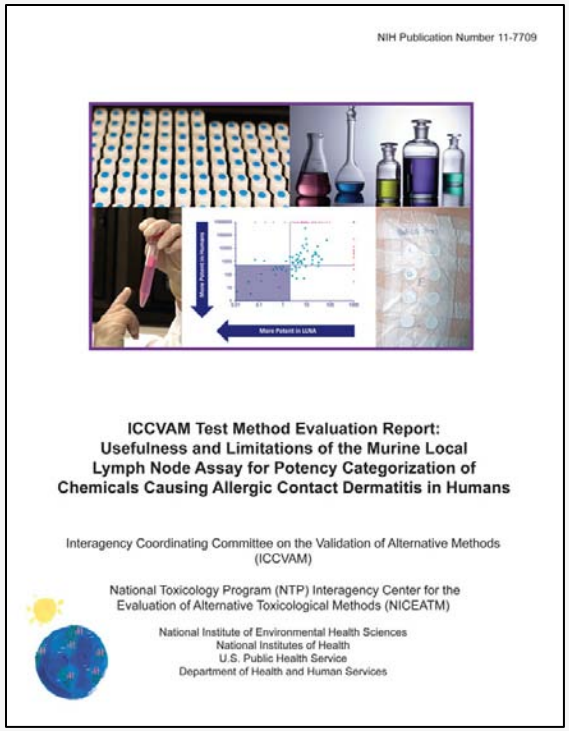


- LLNA: DA (Daicel-ATP)
  - Dr. Kenji Idehara at Daicel Industries, Hyogo, Japan
- LLNA: BrdU-ELISA
  - Dr. Masahiro Takeyoshi, Chemicals Evaluation Research Institute, Saitama, Japan
- Validation studies performed in collaboration with JaCVAM
- ICCVAM considered discussions from OECD expert consultation meeting and member countries
- Transmitted to U.S. agencies June 14, 2010
- *New OECD TGs adopted July 22, 2010*
  - *TG 442A, LLNA: DA*
  - *TG 442B, LLNA: BrdU-ELISA*

ICCVAM. 2010. NIH Publication No. 10-7551. RTP, NC:NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm

ICCVAM. 2010. NIH Publication No. 10-7552. RTP, NC:NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm

ICCVAM Report and Recommendations: LLNA for Skin Potency Categorization



- Evaluation of usefulness and limitations of the LLNA for potency categorization of chemicals causing ACD in humans
- Recommendations endorsed by ICCVAM October 2010:
  - The LLNA can be used to further categorize some substances/products as strong sensitizers when the estimated concentration that produces a positive LLNA result (i.e., EC3) is ≤2%
  - However, since this EC3 criterion only identified about half (48% [13/27]) of the known strong human skin sensitizers evaluated, the LLNA cannot be considered a stand-alone assay to determine skin sensitization potency categories
- Test method evaluation report to be published Spring 2011

ICCVAM. 2010. NIH Publication No. 11-7709. RTP, NC:NIEHS. Available: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-pot/TMER.htm

Summary

- The LLNA has gained widespread adoption and use internationally in the past 10+ years, providing for significant reduction and refinement
- Updated LLNA protocol reduces animal use by 20% and rLLNA can further reduce animal use by 40%
- Nonradioactive LLNA methods now allow for broad use, with reduced hazards for the environment and lab workers
- Appropriate use of the newly adopted and updated LLNA protocols are expected to support both continued protection of people and improved animal welfare

Use of Alternative Methods in Integrated Strategies for ACD Hazard Assessments

- Some alternative methods may have a range of responses that are associated with an unacceptable level of uncertainty and that cannot, therefore, be used alone for hazard decisions
- Additional information or data could be used to reduce the uncertainty associated with these results using an integrated strategy to reach a hazard decision
- Integrated strategies using multiple sources of data and information can increase the certainty of hazard decisions beyond the certainty associated with only a single source of data or information
- Important to include test methods that incorporate key events in skin sensitization (next slide)

Source: Stokes WS, Wind M. 2010. Validation of innovative technologies and strategies for regulatory safety assessment methods: challenges and opportunities. *ALTEX* 27:87-95.

Integrated Strategies: Consideration of Key Events in Skin Sensitization

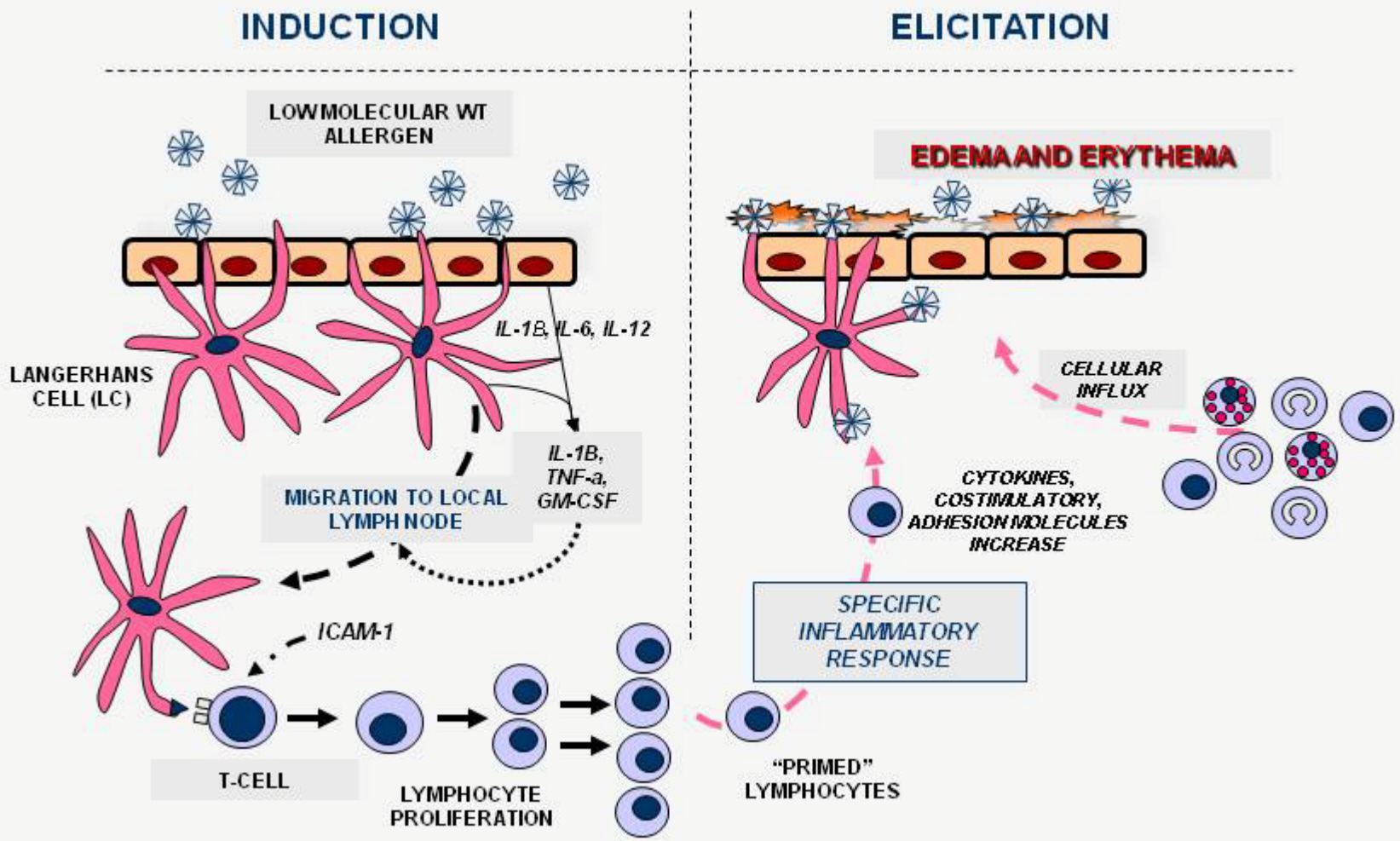


*Illustration by D. Sailstad

Integrated Strategies: Consideration of Key Events in Skin Sensitization Induction

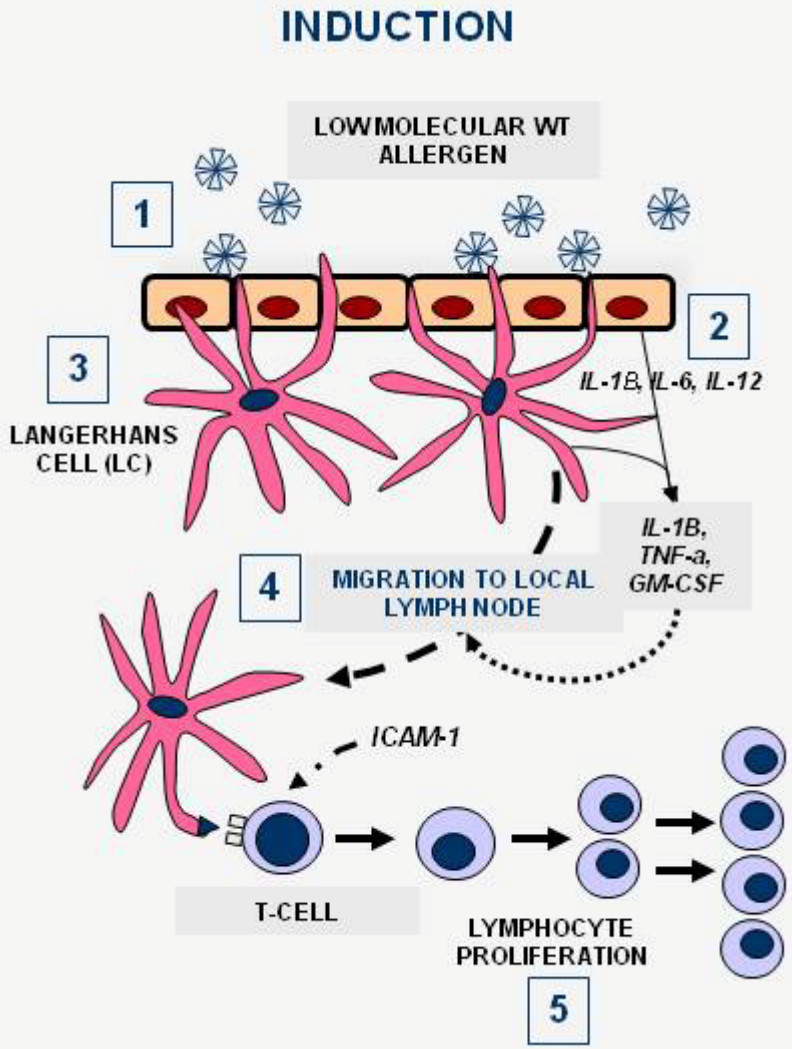


*Illustration by D. Sailstad

1. Haptenation: attachment of allergen to skin
2. Epidermal inflammation: release of pro-inflammatory signals by epidermal keratinocytes
3. Dendritic cell (DC) activation and maturation
4. DC migration: movement of DC bearing hapten-protein complex from skin to draining local lymph node
5. T-cell proliferation: clonal expansion of hapten-peptide specific T-cells

Integrated Strategies for ACD Hazard Assessments: Summary

- *In vitro* and *in silico* methods can be used as screens to identify substances with ACD hazard potential
  - Those needing further evaluation can be tested using the rLLNA, thereby contributing to reduced animal use
- New methods in the validation pipeline will further improve integrated strategies and are expected to increasingly replace the use of animals for ACD hazard testing
  - Myeloid U937 skin sensitization test (MUSST)
  - Direct peptide reactivity assay (DPRA)
  - Human cell line activation test (h-CLAT)
  - *More on these methods later today*

Acknowledgements

Independent Scientific Peer Review Panel

- Back row: Takahiko Yoshida, M.D., Ph.D., Asahikawa Medical College, Hokkaido, Japan; Michael Olson Ph.D., GSK, RTP, NC; Kim Headrick, B.Admin., B.Sc., Health Canada, Ottawa, Ontario, Canada; Thomas Gebel, Ph.D., Federal Institute for Occupational Safety & Health, Dortmund, Germany; James McDougal, Ph.D., Wright State Univ., Dayton, OH; Michael Woolhiser, Ph.D., Dow Chemical, Midland, MI; Howard Maibach, M.D., Univ. of California–San Francisco, San Francisco, CA; Steven Ullrich, Ph.D. M.D. Anderson Cancer Center, Houston, TX
- Middle row: William Stokes, D.V.M., DACLAM, NIEHS, RTP, NC, (ICCVAM Executive Director, NICEATM Director); Peter Theran, V.M.D., Consultant, Massachusetts Society for the Prevention of Cruelty to Animals, Novato, CA; Dagmar Jirová, M.D., Ph.D., National Institute of Public Health, Prague, Czech Republic; Jean Regal, Ph.D., Univ. of Minnesota Medical School, Duluth, MN; Michael Luster, Ph.D., Senior Consultant to NIOSH, Morgantown, WV, (Panel Chair); Raymond Pieters, Ph.D., Utrecht Univ., Utrecht, The Netherlands
- Front row: Nathalie Alépée, Ph.D., L'Oréal Research & Development, Aulnay sous Bois, France; Marilyn Wind, Ph.D., U.S. CPSC (ICCVAM Chair); Nancy Flournoy, M.S., Ph.D., Univ. of Missouri – Columbia, Columbia, MO; Anne Marie Api, Ph.D., Research Institute for Fragrance Materials, Woodcliff Lake, NJ; David Lovell, Ph.D., Univ. of Surrey, Guildford, Surrey, U.K.
- Not pictured: Sidney Green, Ph.D., Howard Univ., Washington, D.C.; Jonathan Richmond, MB, ChB, FRCSEd, Home Office, London, U.K.

Additional Acknowledgements

- ICCVAM
- ICCVAM Interagency Immunotoxicity Working Group
- NICEATM Staff

This presentation reflects the views of the author, has not been reviewed or approved by, and may not necessarily reflect the views of the U.S. Consumer Product Safety Commission