ICCVAM Evaluation of the Local Lymph Node Assay (LLNA) for Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

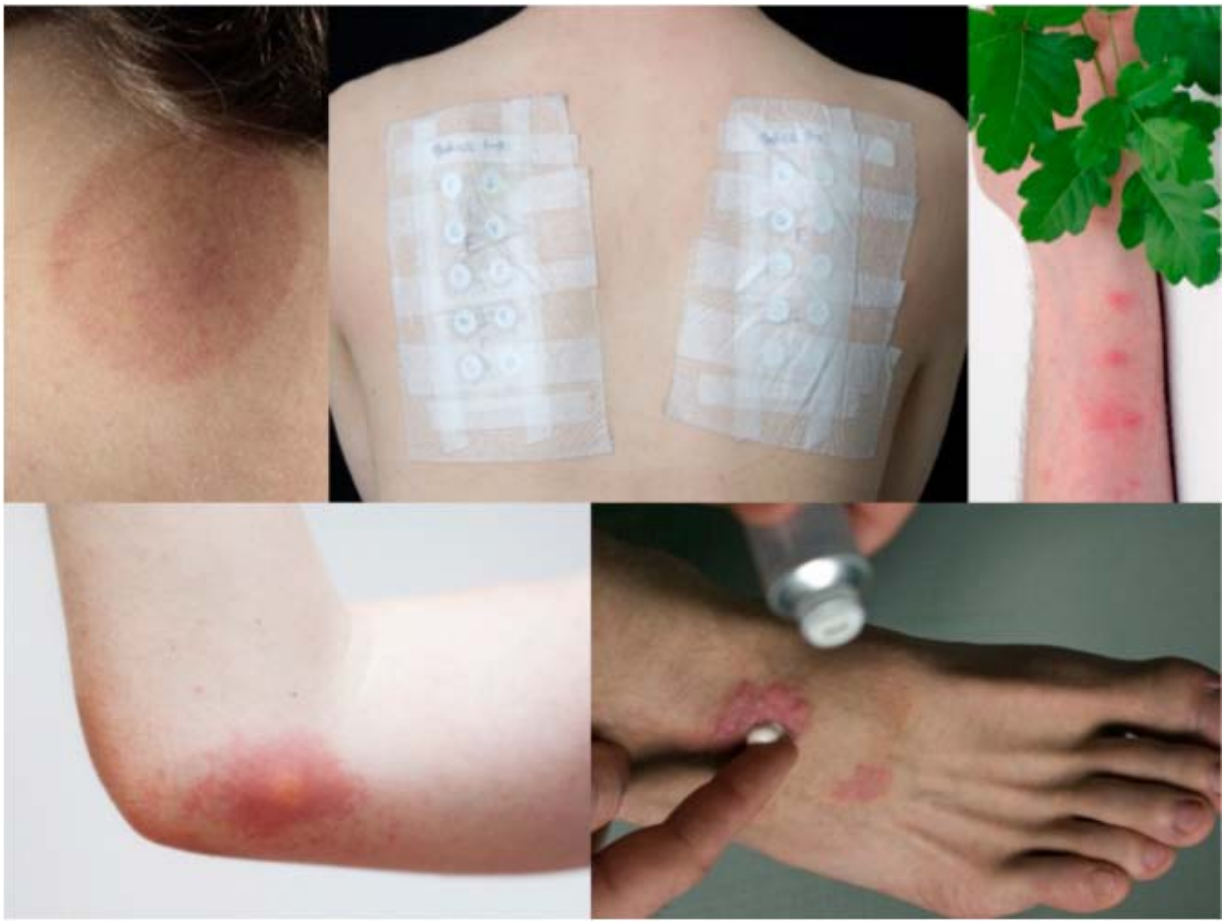
P Brown[1], J Matheson[2], A Jacobs[1], T McMahon[3], D Germolec[4], M Wind[2], W Stokes[5]
[1]US FDA, Silver Spring, MD; [2]US CPSC, Bethesda, MD; [3]US EPA, Washington, DC; [4]NIEHS, RTP, NC; [5]NICEATM/NTP/NIEHS/NIH/DHHS, RTP, NC

Abstract

ICCVAM evaluated the LLNA as a stand-alone test method to determine potency categorization of chemicals that may cause allergic contact dermatitis (i.e., potential skin sensitizers). The dose per unit skin area that induces a 5% positive response rate (i.e., DSA_{05}) in the human maximization test (HMT) or human repeat-insult patch test (HRIPT) was used as the human induction threshold. Substances with induction thresholds ≤500 μg/cm^2 were classified as "strong" human sensitizers. The extent to which the LLNA EC3 (estimated concentration needed to produce a stimulation index of 3, the threshold value for a positive response) correctly categorizes strong human sensitizers was evaluated by examining 136 substances with both LLNA and human data. Using EC3 ≤ 2%, a criterion recently adopted by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals, correctly categorized 52% (14/27) of the strong human sensitizers. However, nearly half (48% [13/27]) of the strong human sensitizers had an EC3 > 2% (11/27) or were negative in the LLNA (2/27). Of the 11 strong human sensitizers with an EC3 > 2%, 91% (10/11) had an LLNA EC3 value between 2% and 10%. ICCVAM concludes that the LLNA can be used to categorize substances as strong sensitizers when EC3 ≤ 2% but cannot be used as a stand-alone assay to predict sensitization potency categories. Substances producing an LLNA EC3 between 2% and 10% will require additional information to determine that the substance should not be categorized as a strong sensitizer. To improve the accuracy of the LLNA for identifying strong sensitizers, ICCVAM encourages the development and evaluation of integrated decision strategies that consider other types of relevant information such as quantitative structure-activity relationships, structural alerts, peptide reactivity, *in vitro* testing data, human data or experience, and existing data from similar chemical entities.

Introduction

- The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is charged with evaluating the scientific validity of new, revised, and alternative toxicological test methods applicable to U.S. Federal agency safety testing requirements.[1]
  - ICCVAM forwards recommendations to Federal agencies.
  - Agencies must respond to ICCVAM within 180 days.[1]
- After a 2007 nomination by the U.S. Consumer Product Safety Commission (CPSC), ICCVAM evaluated the murine local lymph node assay (LLNA) as a stand-alone test method to determine potency categorization of chemicals that may cause allergic contact dermatitis (ACD) in humans.
  - ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeat contact with a sensitizer.



[1] ICCVAM Authorization Act. 2000. Public Law 106-545. 42 U.S.C. § 2851-2, 2851-5. Available: http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf.

- The United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) was revised in 2009 to include the option of subdividing potential skin sensitizers into “strong” (1A) and “other” (1B) categories.
  - Classification criteria for human and LLNA data are based on
    - Induction concentrations in the human repeat-insult patch test (HRIPT) or the human maximization test (HMT) of ≤500 $\mu g/cm^2$ for strong skin sensitizers and >500 $\mu g/cm^2$ for other skin sensitizers
    - LLNA EC3 values (estimated substance concentration that produces a stimulation index of 3) of ≤2% for strong skin sensitizers and >2% for other skin sensitizers
- This poster summarizes the ICCVAM evaluation and recommendations for the LLNA as a stand-alone test method to determine potency categorization of chemicals that may cause ACD in humans.
  - Usefulness and limitations
  - Test method protocol
  - Future studies

Figure 1 Timeline for Evaluation of Potency Categorization of Chemicals Causing Allergic Contact Dermatitis in Humans

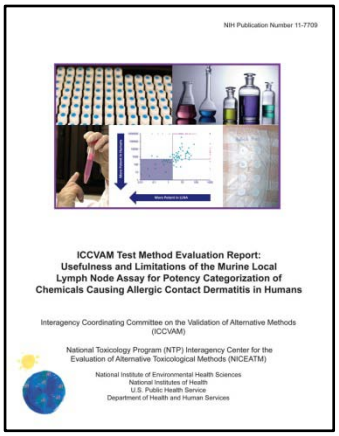
January 10, 2007	ICCVAM receives nomination from CPSC for several LLNA review activities,[1] including the use of the LLNA to determine skin sensitization potency categories.
March 4–-6, 2008	NICEATM-ICCVAM convene an Independent Peer Review Panel Meeting on LLNA review activities (public meeting with opportunity for oral public comments).[2]
March 10–11, 2008	The OECD convenes an Expert Meeting on Sensitization, which includes a discussion of GHS hazard categories for skin sensitizers.
July 2009	Publication of third revised edition of the GHS, which includes revised criteria for hazard classification and subcategories for skin sensitizers.
July 2009–August 2010	NICEATM performs additional analyses to evaluate the use of the LLNA for skin sensitization potency determinations based on comments from the independent scientific peer review panel, the public, and SACATM.
Spring 2011	*Federal Register* notice announces availability of the ICCVAM test method evaluation report on the usefulness and limitations of the LLNA for potency categorization of chemicals causing allergic contact dermatitis in humans.

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; GHS = United Nations Globally Harmonized System of Classification and Labeling of Chemicals; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; LLNA = murine local lymph node assay; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OECD = Organisation for Economic Co-operation and Development; SACATM = Scientific Advisory Committee on Alternative Toxicological Methods.

[1] The CPSC nomination may be viewed on the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

[2] The report of the 2008 Peer Review Panel meeting is available at: http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf

Validation Status of the LLNA to Classify Substances into Skin Sensitization Potency Categories



Accuracy

- Based on 136 substances with LLNA and human data
  - LLNA data from positive tests were expressed as EC3 values.
  - Human data from positive HMT or HRIPT were expressed as DSA_{05} values – the induction dose per skin area (DSA) that produces a positive response in 5% of the tested population.
  - Both LLNA EC3 and human DSA_{05} values represent a threshold positive response.
  - Substances with multiple LLNA EC3 or human DSA_{05} values were assigned geometric mean values.

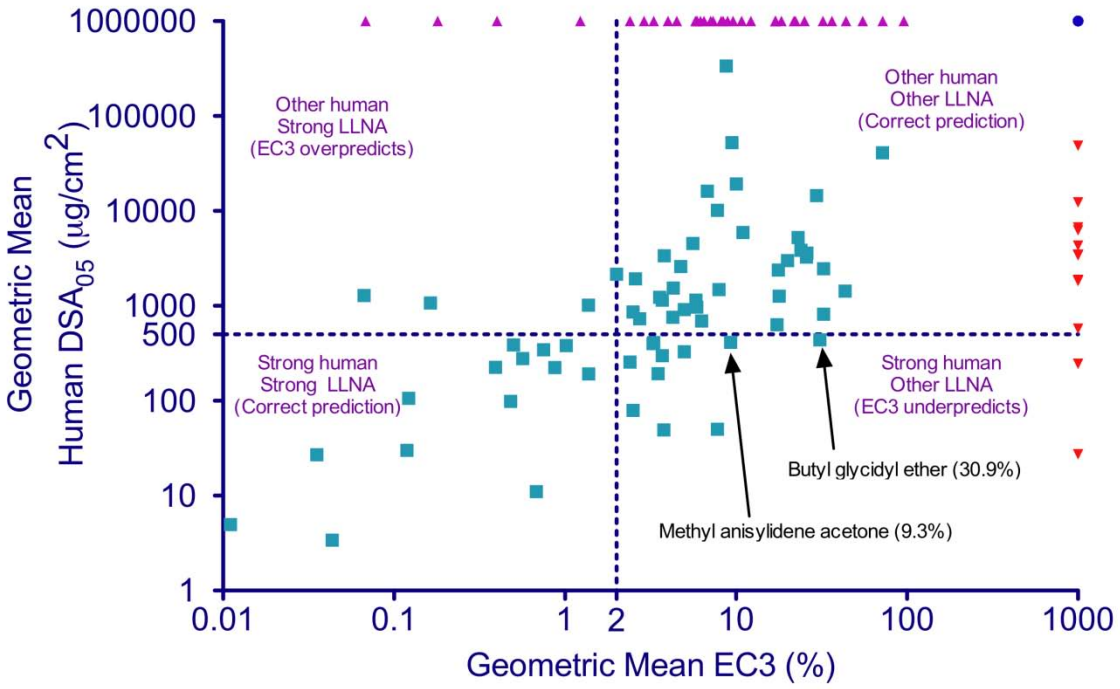
- **Figure 2** shows the distribution of the 136 substances among the GHS skin sensitization potency categories.
  - 76 total human skin sensitizers
  - 27 strong human skin sensitizers
    - 14 with LLNA EC3 ≤ 2%
    - 11 with LLNA EC3 > 2%
    - 2 with negative LLNA results
  - 49 other than strong human skin sensitizers
    - 3 with LLNA EC3 ≤ 2%

        - 35 with LLNA EC3 > 2%
        - 11 with negative LLNA results
    - 60 human nonsensitizers
        - 35 were LLNA sensitizers (4 with LLNA EC3 ≤ 2% and 31 with LLNA EC3 > 2%)
        - 25 were LLNA nonsensitizers
    - **Figure 2** shows geometric mean LLNA EC3 values plotted against the geometric mean human DSA_{05} values for 63 LLNA and human skin sensitizers.
        - Concordant LLNA and human nonsensitizers, LLNA false positives, and LLNA false negatives are shown on the edges of the graph.
        - GHS cutoffs, LLNA EC3 ≤ 2% and human DSA_{05} ≤ 500 $\mu g/cm^2$, are marked to show the correspondence of the data with the GHS classification criteria.
- The LLNA EC3 ≤ 2% correctly classified 52% (14/27) of the strong human skin sensitizers.
    - 48% (13/27) of strong human skin sensitizers were underclassified as either other than strong skin sensitizers (11 substances produced LLNA EC3 > 2%) or as nonsensitizers (2 substances).
- **Figure 3** shows the rates of correct and underclassification by the LLNA for the 27 strong human skin sensitizers.
    - The correct potency classification rate for strong human skin sensitizers increases and the underclassification rate decreases as the LLNA EC3 increases.
    - The correct classification rate plateaus, however, because the two strong human skin sensitizers that yielded negative results in the LLNA will not be correctly classified by any EC3 cutoff.
    - 14% (11/77) of substances with LLNA EC3 > 2% are strong human skin sensitizers (DSA_{05} ≤ 500 $\mu g/cm^2$).
    - 5% (2/38) of the LLNA negative substances were strong human skin sensitizers.
- Most substances with 10% ≥ EC3 ≥ 2% should be considered as potential strong skin sensitizers unless additional data support categorization as other than strong skin sensitizers.

- 37% (10/27) of the strong human skin sensitizers in this database produced LLNA EC3 values between 2% and 10%.
    - This accounts for 76% (10/13) of the strong human skin sensitizers underclassified by the LLNA.
    - Therefore, it is likely that a considerable number of strong human skin sensitizers within the broader population of chemicals may produce LLNA EC3 values within this range.

- Using LLNA EC3 ≤ 10% to classify substances as strong human skin sensitizers
    - Correctly classified 89% (24/27) of the strong human skin sensitizers
    - Underclassified only 11% (3/27) of the strong human skin sensitizers

Figure 2 LLNA EC3 and Human DSA_{05} by GHS Potency Category for 136 Substances



Legend: ■ Human/LLNA sensitizers (n = 63); ▲ LLNA false positive (n = 35); ▼ LLNA false negative (n = 13); ● Concordant negative (n = 25)

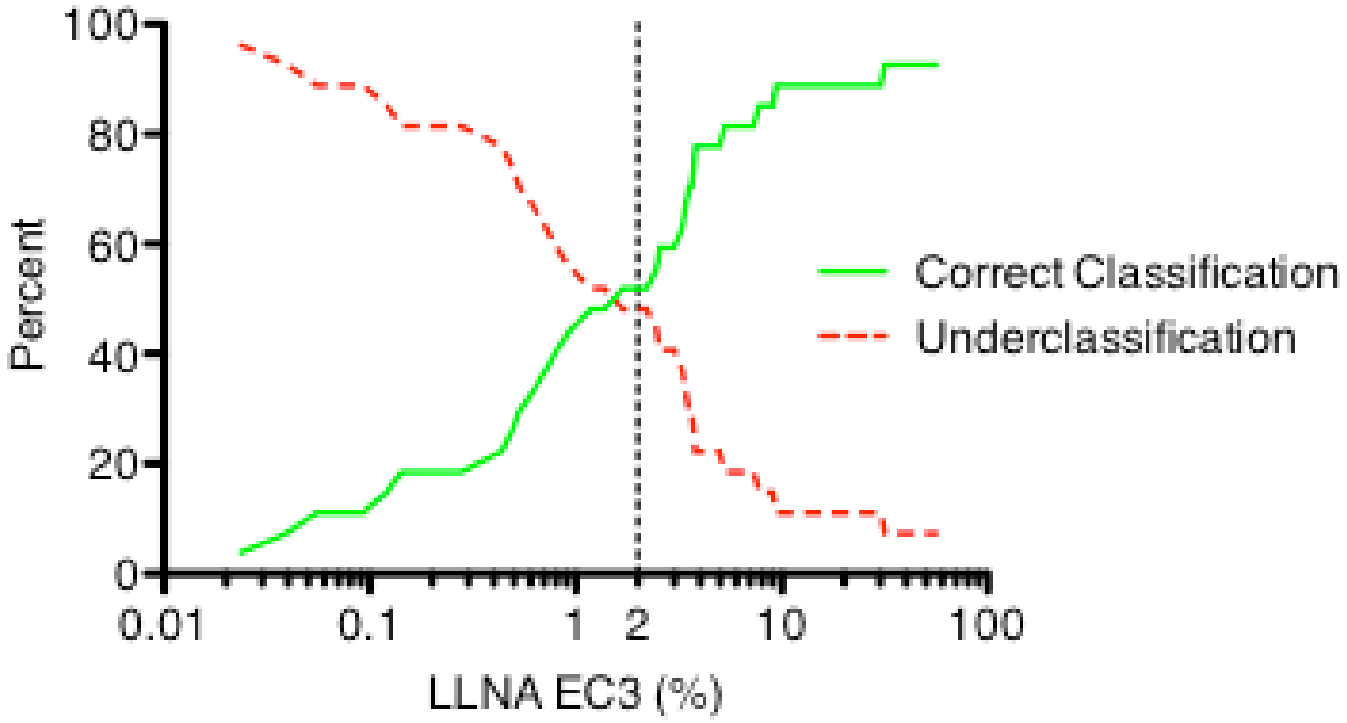
Abbreviations: DSA_{05} = induction dose per skin area, in µg/cm^2, in a human repeat-insult patch test or human maximization test that produces a positive response in 5% of the tested population; EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; GHS = Globally Harmonized System of Classification and Labelling of Chemicals (UN 2009); LLNA = murine local lymph node assay.

63 of the 136 substances had human DSA and LLNA values which were not false positive, false negative or classified as a nonsensitizer.

Note that concordant LLNA and human nonsensitizers, LLNA false positives, and LLNA false negatives are shown on the edges of the graph.

GHS cutoffs, LLNA EC3 ≤ 2% and human DSA_{05} ≤ 500 µg/cm^2, are marked to show the correspondence of the data with the GHS classification criteria.

Figure 3 LLNA EC3 Classification of 27 Strong Human Skin Sensitizers



Abbreviations: EC3 = estimated concentration of a substance expected to produce a stimulation index of 3, the threshold value for a substance to be considered a sensitizer in the LLNA; LLNA = murine local lymph node assay.

Analysis was based on 27 substances identified as strong skin sensitizers in humans using the human maximization test and/or the human repeat-insult patch test because the induction dose per skin area that produced a positive response in 5% of the tested population was ≤500 μg/cm^2.

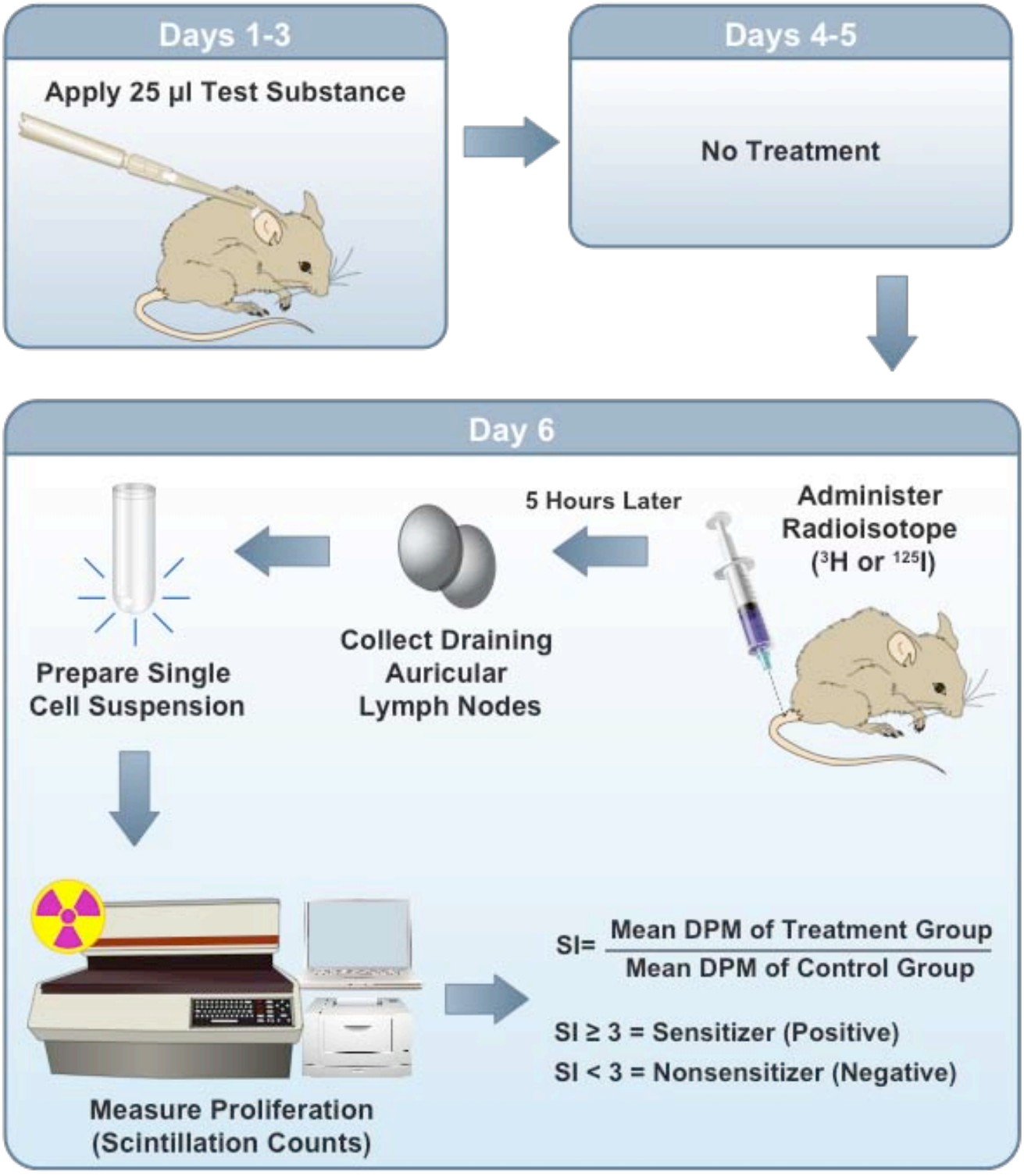
ICCVAM Recommendations: Test Method Usefulness and Limitations

- ICCVAM concludes that the LLNA, using the GHS classification criteria, can be used to categorize substances as strong human skin sensitizers (Subcategory 1A) when the EC3 ≤ 2%.
  - However, because almost half of the known strong human skin sensitizers have an EC3 > 2%, the LLNA cannot be considered a stand-alone assay to determine skin sensitization potency categories.
  - Additional information is required to categorize a substance as other than a strong human skin sensitizer (Subcategory 1B) when the substance produces an LLNA EC3 > 2%.

ICCVAM Recommendations: Test Method Protocol

- ICCVAM recommends use of the recently updated LLNA test method protocol, a schematic of which is shown in **Figure 4** (ICCVAM 2010). The updated LLNA protocol:
  - Includes improved dose selection procedures to guide selection of the highest dose that will help minimize false negatives
  - Provides for a 20% reduction in the required number of animals (reduces the number of required animals per group from five to four) compared to the previously recommended LLNA protocol
  - Recommends collection of individual animal data
  - Recommends inclusion of both a concurrent vehicle and a positive control in each study
  - Provides procedures for calculating the LLNA EC3, which are necessary for potency comparisons between substances

Figure 4 LLNA Test Method Protocol



Abbreviations: DPM = disintegrations per minute; LLNA = murine local lymph node assay; SI = stimulation index

ICCVAM Recommendations: Future Studies

- Efforts should be made to identify additional high-quality human test data and human experience for substances with LLNA data for comparison.
  - Emphasis should be placed on identifying substances that are classified as strong skin sensitizers based on a human threshold induction concentration of <500 $\mu g/cm^2$ to better evaluate the LLNA EC3 value that will best distinguish strong from other than strong human skin sensitizers.
- In order to develop a more accurate assessment of strong human skin sensitizers using LLNA results, especially for substances that produce an EC3 value between 2% and 10%, ICCVAM encourages the development, validation, and evaluation of integrated decision strategies that consider other types of relevant information such as:
  - Quantitative structure-activity relationships
  - Structural alerts
  - Peptide reactivity
  - *In vitro* testing data
  - Human test data or experience
  - Existing data from similar chemical entities

ICCVAM

Interagency Immunotoxicity Working Group

Consumer Product Safety Commission

Joanna Matheson, Ph.D. (Working Group Co-chair)
Marilyn Wind, Ph.D. (through July 2010)

Environmental Protection Agency

Office of Pesticide Programs

Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Marianne Lewis
Deborah McCall
Timothy McMahon, Ph.D.
John Redden
Jenny Tao, Ph.D.

Office of Pollution Prevention and Toxics

Elizabeth Margosches, Ph.D.
Ronald Ward, Ph.D.

Office of Research and Development

Marsha Ward, Ph.D.

Office of Science Coordination and Policy

Karen Hamernik, Ph.D.

Food and Drug Administration

Center for Devices and Radiological Health

Vasant G. Malshet, Ph.D., DABT
Jeffrey Toy, Ph.D.

Center for Drug Evaluation and Research

Ruth Barratt, Ph.D., D.V.M.
Paul Brown, Ph.D.
Abigail Jacobs, Ph.D. (Working Group Co-chair)
Jiaqin Yao, Ph.D.

Center for Food Safety and Applied Nutrition

Donnie Lowther
Neil Wilcox, D.V.M., M.P.H.

Office of the Commissioner

Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences

Warren Casey, Ph.D., DABT
Dori Germolec, Ph.D.
William Stokes, D.V.M., DACLAM

National Institute for Occupational Safety and Health

B. Jean Meade, D.V.M., Ph.D.
Paul D. Siegel, Ph.D.

National Library of Medicine

Pertti Hakkinen, Ph.D.

European Centre for the Validation of Alternative Methods - Liaison

Silvia Casati, Ph.D.
Alexandre Angers, Ph.D.

Japanese Center for the Validation of Alternative Methods - Liaison

Hajime Kojima, Ph.D.

Interagency Coordinating Committee on the Validation of Alternative Methods: Designated Agency Representatives

Agency for Toxic Substances and Disease Registry
*Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
*Joanna Matheson, Ph.D. (Vice Chair)
+Kristina Hatlelid, Ph.D., MPH

Department of Agriculture
*Jodie Kulpa-Eddy, D.V.M. (Chair)
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Department of Defense
*David Honey, Ph.D.
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Department of Transportation
+Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
*John R. "Jack" Fowle III, Ph.D., DABT
+Vicki Dellarco, Ph.D.
+Tina Levine, Ph.D.
Christine Augustyniak, Ph.D.
Deborah McCall

Food and Drug Administration
Office of the Commissioner
*Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Ying Huang, Ph.D.
Richard McFarland, Ph.D., M.D.
Center for Devices and Radiological Health
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Neil L. Wilcox, D.V.M., MPH
Center for Veterinary Medicine
M. Cecilia Aguila, D.V.M.
Devaraya Jagannath, Ph.D.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.

National Cancer Institute
*T. Kevin Howcroft, Ph.D.
+Chand Khanna, D.V.M., Ph.D.

National Institute of Environmental Health Sciences
*William S. Stokes, D.V.M., DACLAM
+Warren Casey, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
*Paul Nicolaysen, V.M.D.

National Institutes of Health
*Margaret D. Snyder, Ph.D.

National Library of Medicine
*Pertti (Bert) Hakkinen, Ph.D.
+Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
*Surender Ahir, Ph.D.

* Principal agency representative
\+ Alternate principal agency representative

LLNA Peer Review Panel Meetings

- Public meetings of an international independent scientific peer review panel (Panel) organized by ICCVAM and NICEATM were held at the CPSC in Bethesda, MD, on March 4-6, 2008, and at the National Institutes of Health in Bethesda, MD, on April 28-29, 2009 (see **Figure 1**).

Independent Scientific Peer Review Panel

Left to right: Back row: Takahiko Yoshida, M.D., Ph.D., Asahikawa Medical College, Hokkaido, Japan; Michael Olson, Ph.D., A.T.S., GlaxoSmithKline, Research Triangle Park, 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 University, Dayton, OH; Michael Woolhiser, Ph.D., Dow Chemical, Midland, MI; Howard Maibach, M.D., University 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., D.A.C.L.A.M., National Institute of Environmental Health Sciences, Research Triangle Park, 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., University of Minnesota Medical School, Duluth, MN; Michael Luster, Ph.D., Senior Consultant to the National Institute for Occupational Safety and Health, Morgantown, WV (Panel Chair); Raymond Pieters, Ph.D., Utrecht University, Utrecht, The Netherlands

Front row: Nathalie Alépée, Ph.D., L'Oréal Research and Development**,** Aulnay sous Bois, France**;** Marilyn Wind**,** Ph.D., U.S. Consumer Product Safety Commission, Bethesda, MD (ICCVAM Chair through July 2010); Nancy Flournoy, M.S., Ph.D., University of Missouri–Columbia, Columbia, MO; Anne Marie Api, Ph.D., Research Institute for Fragrance Materials, Woodcliff Lake, NJ; David Lovell, Ph.D., FIBiol, CStat, CBiol, University of Surrey, Guildford, Surrey, U.K.

Not pictured: Sidney Green, Ph.D., Howard University, Washington, DC; Jonathan Richmond, MB ChB, FRCSEd, Home Office, London, U.K.

Charge to the Peer Review Panel

- Review the draft background review document (BRD) for errors and omissions
- Provide conclusions and recommendations on the current validation status of the LLNA as a stand-alone test method for determining skin sensitization potency
- Comment on whether the draft BRD supports ICCVAM's draft test method recommendations

Peer Review Panel Conclusions

- Agreed with the ICCVAM draft recommendation made in January 2008 that the LLNA should not be considered as a stand-alone test method for determining skin sensitization potency, but could be used as part of a weight-of-evidence evaluation
- Suggested that additional analyses might improve the correlation between the LLNA EC3 values and the human threshold values (NOTE: This poster shows those analyses)
- Concurred with ICCVAM's recommendations for future studies
- The complete LLNA Peer Review Panel Reports can be accessed at:
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

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UN. 2009. Globally Harmonized System for Classification and Labelling of Chemicals. 3rd rev. ed. New York:United Nations. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html [accessed 21 October 2010].

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Acknowledgments

The Intramural Research Program of the NIH, National Institute of Environmental Health Sciences supported this poster. Technical support was provided by ILS, Inc., under NIEHS contract N01-ES 35504. The views expressed above do not necessarily represent the official positions of any Federal agency.

This poster reflects the views of the authors. Since the poster was written as part of the official duties of the authors, it can be freely copied.

NICEATM and ICCVAM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM used for this evaluation.

Anne Marie Api, Ph.D.
Research Institute for Fragrance Materials
Woodlake, NJ

Dori Germolec, Ph.D.
National Toxicology Program
Research Triangle Park, NC

David Basketter, Ph.D.[2]
Unilever Safety and Environmental Assurance Centre
Sharnbrook, U.K.

Ian Kimber, Ph.D.[3]
Syngenta Central Toxicology Laboratory
Macclesfield, U.K.

Phil Botham, Ph.D.
European Crop Protection Association
Brussels, Belgium

Heidi Ott
Federal Institute for Occupational Safety and Health
Dortmund, Germany

Eric Debruyne, Ph.D.
Bayer CropScience SA, Sophia Antipolis Cedex, France

Kirill Skirda, Ph.D.
TNO Quality of Life
Delft, The Netherlands

G. Frank Gerberick, Ph.D.
The Procter & Gamble Company
Cincinnati, OH

Peter Ungeheuer, Ph.D.
European Federation for Cosmetic Ingredients
Frankfurt, Germany

[2] Present affiliation: DABMEB Consultancy, Ltd., Sharnbrook, U.K.
[3] Present affiliation: The University of Manchester, Manchester, U.K.