ICCVAM Evaluation and Recommendations on the Nonradioactive LLNA: DA for Evaluating Allergic Contact Dermatitis Hazards

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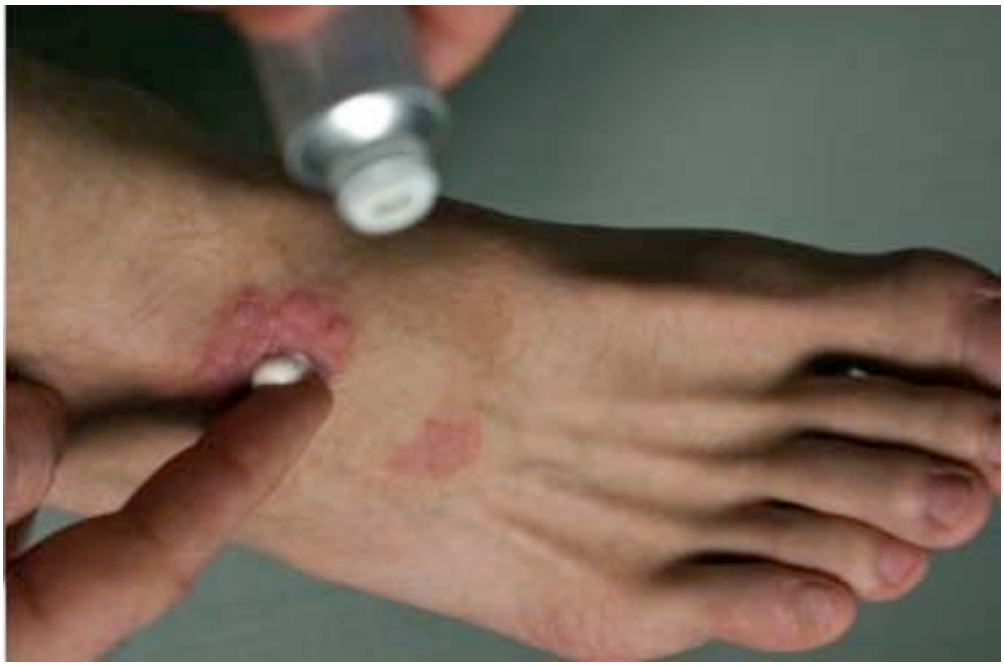
Abstract

ICCVAM assessed the usefulness and limitations of the LLNA: DA, a nonradioactive murine local lymph node assay (LLNA) that measures ATP content as an indicator of lymphocyte proliferation and, in turn, potential allergic contact dermatitis (ACD) hazards. Accuracy when compared to the traditional LLNA was assessed based on data generated with 44 substances and using several different stimulation indices (SI) as decision criteria. Optimal performance was achieved using SI ≥ 1.8; the LLNA: DA correctly identified all 32 LLNA sensitizers (0% [0/32] false negatives), and 9/12 LLNA nonsensitizers (25% [3/12] false positives). The 3 false positives had maximum SI between 1.8 and 2.5. There were 14 substances with repeat tests; results for 80% (8/10) of the LLNA sensitizers and 75% (3/4) of the LLNA nonsensitizers were 100% concordant among the repeat LLNA: DA tests. ICCVAM concludes that accuracy and reproducibility of the LLNA: DA support its use to identify potential skin sensitizers and nonsensitizers. ICCVAM recommends SI ≥ 1.8 to identify ACD hazards since there were no false negatives relative to the LLNA. In testing situations where dose-response information is not required, or negative results are anticipated, ICCVAM recommends that the single-dose reduced LLNA: DA should be considered and used, thereby reducing animal use by up to 40%. The ICCVAM-recommended protocol formed the basis for the recently adopted OECD Test Guideline 442A. Because the LLNA: DA does not require radioactive reagents more institutions can take advantage of the reduction and refinement benefits afforded by the LLNA compared to traditional guinea pig methods for ACD testing. The LLNA: DA will also eliminate the environmental hazard associated with use and disposal of radioactive materials used in the LLNA.

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 nonradioactive LLNA: DA (**Figure 1**) to assess the allergic contact dermatitis (ACD) hazard potential of substances.
  - ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeat contact with a sensitizer.

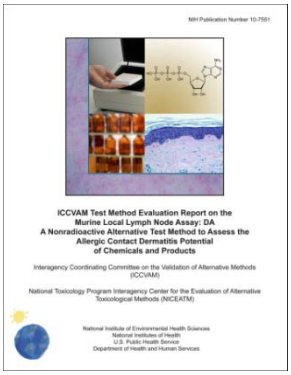


ACD Rash

- Daicel Chemical Industries Ltd., developed the LLNA: DA (Yamashita et al. 2005; Idehara et al. 2008).
  - Measures ATP content in draining auricular lymph nodes as an estimate of cell number at the end of cell proliferation.

[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.

- This poster summarizes the ICCVAM evaluation and recommendations for the LLNA: DA:
  - Usefulness and limitations
  - Test method protocol
  - Future studies
  - Performance standards



Validation Status of the LLNA: DA

Accuracy

- The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) analyzed accuracy of the LLNA: DA based on 44 substances with LLNA: DA and traditional LLNA data.
  - Idehara et al. 2008
  - Idehara, unpublished data
  - Omori et al. 2008 (interlaboratory validation study)
- Several decision criteria were evaluated to determine the optimum threshold for a positive LLNA: DA response (**Figure 2**).
- A stimulation index (SI) ≥ 1.8 produced optimum results with the highest accuracy and no false negatives.
  - Accuracy = 93% (41/44)
  - False positive rate = 25% (3/12)
    - Chlorobenzene, hexane and salicylic acid: all 1.8 < SI < 2.5
  - False negative rate = 0% (0/32)

Reliability

- Intra- and interlaboratory reproducibility were assessed.
- Quantitative: NICEATM did an intralaboratory coefficient of variation (CV) analysis of estimated concentrations expected to produce an SI of 1.8 (EC1.8 values) for isoeugenol and eugenol tested in three different experiments (Idehara et al. 2008).

- The mean EC1.8 values and corresponding CVs for isoeugenol and eugenol were 0.87% ± 0.31% (CV = 36%), and 3.38% ± 0.79% (CV = 23%), respectively.

- Qualitative: Concordance analysis of sensitizer/nonsensitizer results.
  - **Table 1** shows multiple tests of 14 substances (10 LLNA sensitizers and 4 nonsensitizers) across two phases of an interlaboratory validation study.
  - Concordance for 80% (8/10) of the sensitizer outcomes.
    - The two discordant LLNA sensitizers were 3-aminophenol and nickel (II) sulfate hexahydrate. Both substances had LLNA: DA test results in all three categories: Maximum SI < 1.8, 1.8 < maximum SI < 2.5, and maximum SI ≥ 1.8.
  - Concordance was observed for 75% (3/4) of the nonsensitizer outcomes.
    - The discordant LLNA nonsensitizer was isopropanol (91% concordance).

Figure 1 LLNA: DA Test Method Protocol

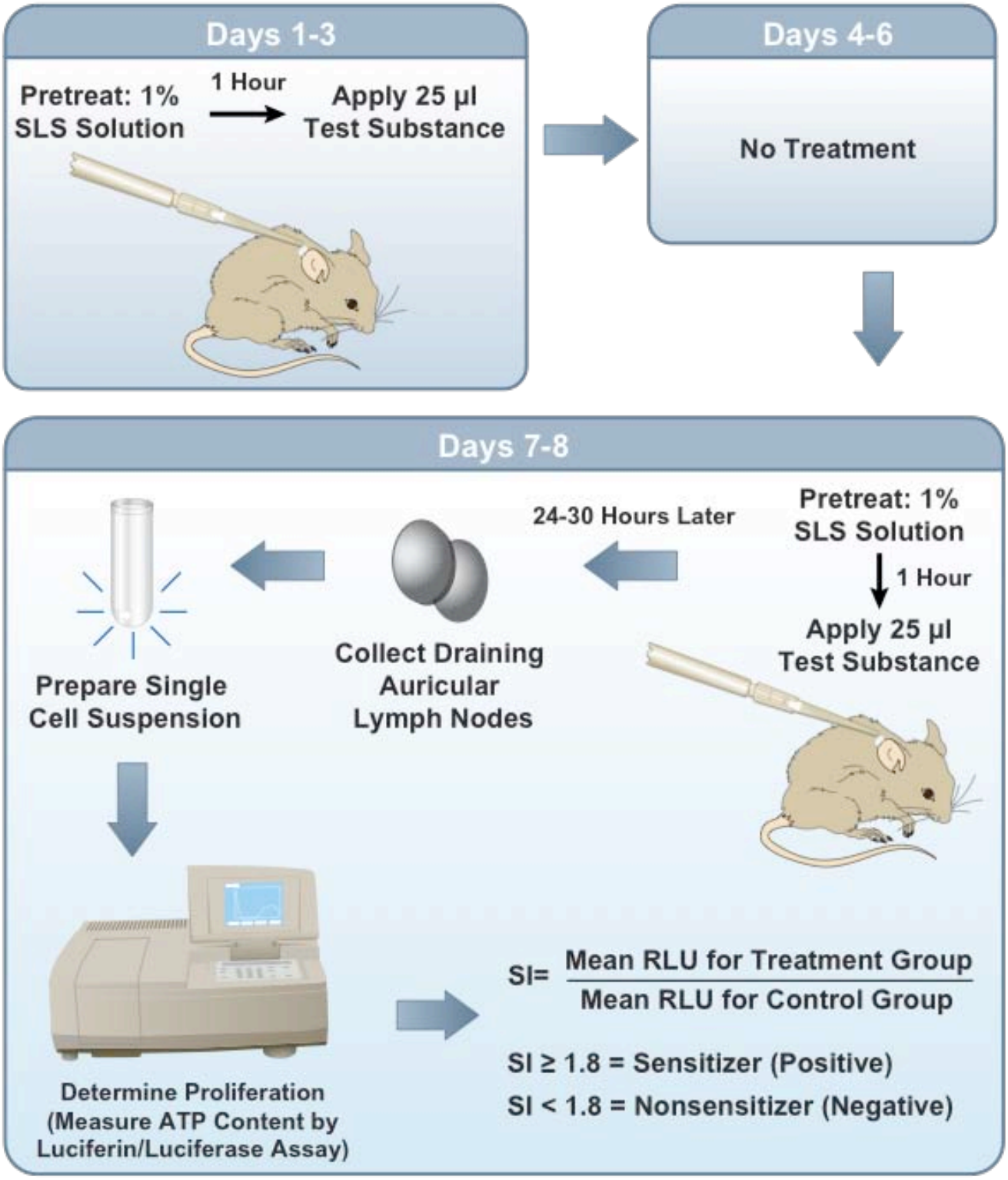


Table 1 Concordance of LLNA: DA Tests Across Maximum SI Categories

Substance Name	LLNA: DA Nonsensitizers (Maximum SI < 1.8)[1]	LLNA: DA Sensitizers (SI ≥ 1.8)		Total Tests
		1.8 < Maximum SI < 2.5[1]	Maximum SI ≥ 2.5[1]	
Sensitizers[2]				
Abietic acid	0 (0%)	0 (0%)	4 (100%)	4
3-Aminophenol	1 (33.3%)	1 (33.3%)	1 (33.3%)	3
Cobalt chloride	0 (0%)	1 (12.5%)	7 (87.5%)	8
2,4-Dinitrochlorobenzene	0 (0%)	0 (0%)	11 (100%)	11
Formaldehyde	0 (0%)	0 (0%)	4 (100%)	4
Glutaraldehyde	0 (0%)	0 (0%)	4 (100%)	4
Hexyl cinnamic aldehyde	0 (0%)	0 (0%)	18 (100%)	18
Isoeugenol	0 (0%)	0 (0%)	4 (100%)	4
Nickel (II) sulfate hexahydrate	4 (50%)	2 (25%)	2 (25%)	8
Potassium dichromate	0 (0%)	0 (0%)	5 (100%)	5
Nonsensitizers[2]				
Dimethyl isophthalate	4 (100%)	0 (0%)	0 (0%)	4
Isopropanol	10 (91%)	1 (9%)	0 (0%)	11
Lactic acid	5 (100%)	0 (0%)	0 (0%)	5
Methyl salicylate	4 (100%)	0 (0%)	0 (0%)	4

Abbreviations: LLNA: DA = murine local lymph node assay modified by Daicel Chemical Industries, Ltd., based on ATP content; SI = stimulation index.

[1] Numbers shown reflect number of tests. Percentage in parentheses reflects percentage of the total number of tests for each substance.

[2] Categorization is based on traditional LLNA test results.

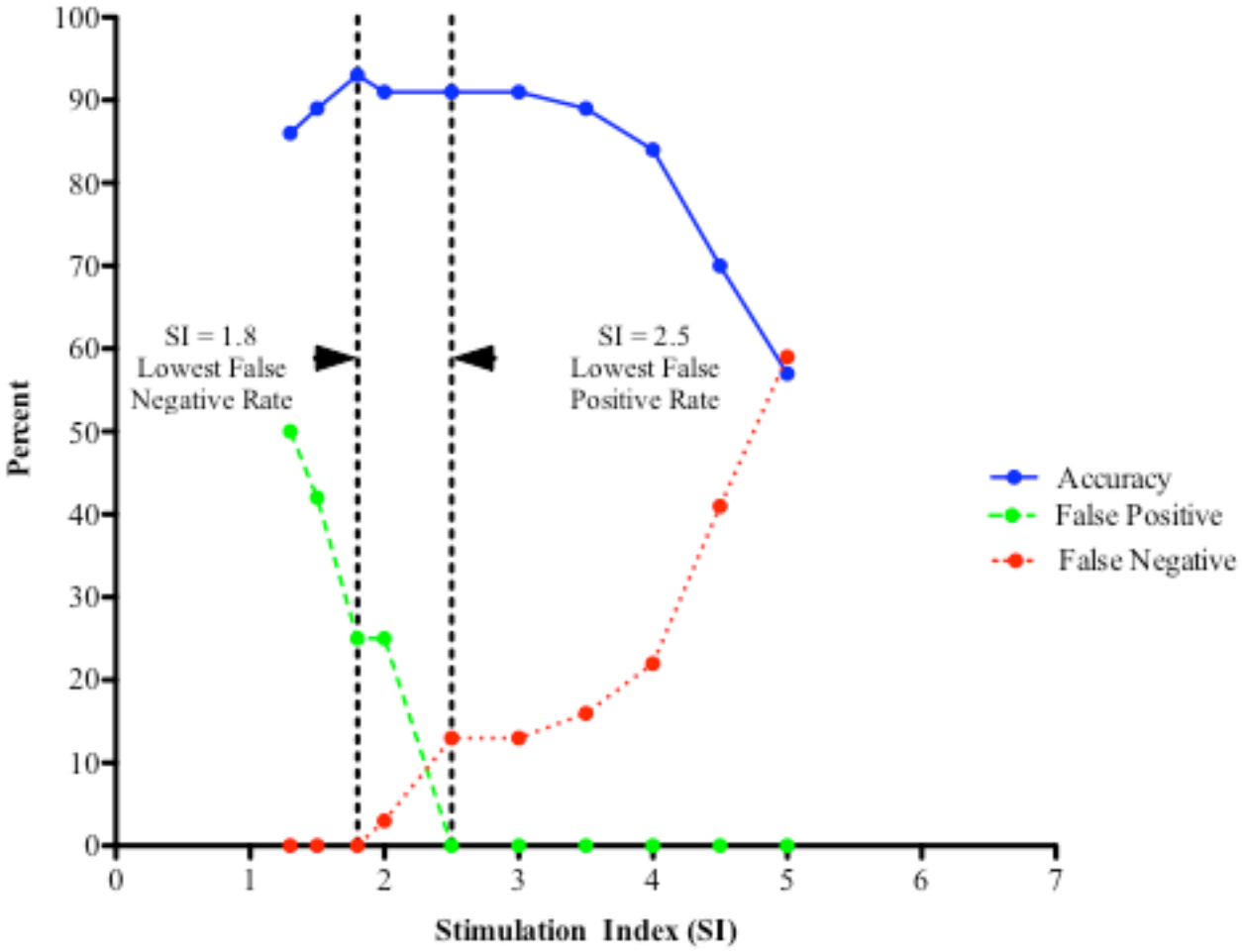
ICCVAM Recommendations: Test Method Usefulness and Limitations

- The LLNA: DA can be used to identify potential skin sensitizers or nonsensitizers.
  - Use SI ≥ 1.8 to identify potential skin sensitizers.
  - SI ≥ 1.8 produced no false negatives relative to the traditional LLNA.
- There is a slight potential for false positives with borderline weak positive responses (1.8 < SI < 2.5).
  - Consider additional information such as the strength of the dose-response relationship, statistical significance, evidence of systemic toxicity and/or excessive skin irritation together with SI values.
- The LLNA: DA might not be appropriate for testing certain classes of materials with properties that interfere with the assay. Consider if test substance might affect:
  - ATP levels (e.g., ATP inhibitors)
  - Accurate intracellular measurement of ATP levels (e.g., ATP-degrading enzymes or extracellular ATP in the lymph node)
- These limitations, as well as the expertise and equipment of the testing laboratory, should be considered when deciding whether this assay is appropriate for the intended use.

ICCVAM Recommendations: Test Method Protocol

- The LLNA: DA protocol incorporates all aspects of the traditional LLNA protocol except for those procedures unique to the conduct of the LLNA: DA.
- The reduced LLNA: DA (rLLNA: DA) should be used routinely to determine the ACD hazard potential of chemicals and products.
  - Like the reduced LLNA (Kimber et al. 2006; ESAC 2007; ICCVAM 2009a), the rLLNA: DA protocol uses only the high dose and reduces animal use by up to 40%.
  - Using a high dose group only and SI ≥ 1.8 to identify skin sensitizers, the accuracy of the rLLNA: DA was 98% (121/123), with a false positive rate of 0% (0/33) and a false negative rate of 2% (2/90). The two false negative rLLNA: DA studies were:
    - A test of 10%, 25% and 50% isopropanol that produced a maximum SI = 1.97 at the lowest dose tested
    - A test of 10%, 25% and 50% 2-mercaptobenzothiazole that produced a maximum SI = 2.00 at the lowest dose tested
  - If existing information suggests a substance might have ACD hazard potential *and* dose-response information is needed, consider testing in the multi-dose LLNA: DA.

Figure 2 SI Decision Criteria Performance of the LLNA: DA Compared with the Traditional LLNA Using 44 Substances



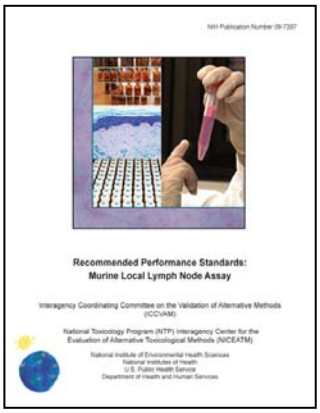
Abbreviations: LLNA: DA = murine local lymph node assay modified by Daicel Chemical Industries, Ltd., based on ATP content; SI = stimulation index.

Compared to traditional LLNA results, the lines show the change in performance characteristics for the LLNA: DA with the SI used to identify skin sensitizers. This analysis used LLNA results for 32 sensitizers and 12 nonsensitizers. For 14 substances with multiple LLNA: DA test results, the most prevalent outcome was used.

ICCVAM Recommendations: Future Studies

- Efforts should be made to identify additional human data and experience for test substances to further assess the usefulness and limitations of this and other versions of the LLNA for identifying human skin sensitizers.
  - Post-marketing surveillance of consumers for allergic reactions
  - Occupational surveillance of potentially exposed workers
- Additional nonsensitizing skin irritants should be tested to determine their impact on the LLNA: DA false positive rate.
- Efforts should be made to further characterize the ACD hazard potential of LLNA: DA borderline weak positive substances ($1.8 < SI < 2.5$) to determine if such results might be false positives.
- Other available information could be considered to confirm that such borderline results are potential skin sensitizers, such as:
  - Dose-response data
  - Evidence of systemic toxicity or excessive local irritation
  - Statistical significance (where appropriate) together with SI values
  - Various properties of the test substance, including whether it is structurally similar to known skin sensitizers
- Decision criteria should be reassessed as additional discriminators and data become available.

ICCVAM Recommendations: Performance Standards



- The ICCVAM-recommended performance standards (ICCVAM 2009b) for the traditional LLNA can be used to evaluate future modifications of the LLNA: DA because it is functionally and mechanistically similar to the traditional LLNA.

ICCVAM

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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 3**).

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: DA
- Comment on whether the draft BRD supports ICCVAM's draft test method recommendations

Peer Review Panel Conclusions

- Agreed that available data and test method performance supported the use of the LLNA: DA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations.
- Noted that the analysis supported using two SI decision criteria (i.e., one to identify skin sensitizers and one to identify nonsensitizers); however, the Panel questioned how indeterminate results between two criteria would be useful for regulatory purposes and emphasized that additional guidance would be needed on how to classify substances with such results.
- Concurred with ICCVAM that validation studies indicated that the standardized protocol was sufficiently transferable and reproducible.
- 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

International Acceptance of the LLNA: DA

- After the Panel review, ICCVAM agreed with the OECD Expert Consultation Group (see **Figure 3**) that a single SI ≥ 1.8 to classify substances as skin sensitizers would avoid false negative and indeterminate results, which are not useful for regulatory purposes.
- OECD Test Guideline 442A Skin Sensitization: Local Lymph Node Assay: DA, which includes the SI ≥ 1.8 to classify substances as skin sensitizers, was adopted on July 22, 2010 (OECD 2010).
- OECD Test Guideline 442A can be accessed at:
  - http://www.oecd-ilibrary.org/environment/test-no-442a-skin-sensitization_9789264090996-en
- Expected to result in broader use of LLNA tests, which will further reduce and refine animal use for ACD hazard assessments on a global basis, while ensuring human safety.

Figure 3 Timeline for Evaluation of the LLNA: DA

January 10, 2007	ICCVAM receives nomination from CPSC for several LLNA review activities,[1] including evaluation of the LLNA: DA.

March 4-–6, 2008	Independent Peer Review Panel Meeting on LLNA review activities; public meeting with opportunity for oral public comments.[2]

- Agreed with the draft ICCVAM recommendations that the LLNA: DA may be useful for identifying substances as potential skin sensitizers and nonsensitizers.
- Indicated that more information and data were needed before definitive conclusions could be made on the usefulness and limitations of the LLNA: DA.
  - Detailed test method protocol
  - Individual animal data for the validation database
  - Evaluation of interlaboratory reproducibility

April 28–29, 2009	Independent Peer Review Panel Meeting on LLNA review activities; public meeting with opportunity for oral public comments.[3]

October 20–22, 2009	OECD Expert Consultation Group meets to consider proposed updates to TG 429 and two new TG proposals for nonradioactive LLNA test methods (includes the LLNA: DA).

March 23–25, 2010	Working Group of National Co-ordinators of the Test Guidelines Programme approves the proposed updates to TG 429 and two new TG proposals for nonradioactive LLNA test methods, which includes the LLNA: DA.

June 29, 2010	*Federal Register* (75 FR 37443) notice: Announces availability of ICCVAM TMER for the LLNA: DA.

July 22, 2010	OECD Council adopts TG 442A Skin Sensitization: Local Lymph Node Assay: DA and the updated TG 429.

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods; LLNA = murine local lymph node assay; LLNA: DA = murine local lymph node assay based on ATP measurement by luciferin-luciferase assay; NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods; OECD = Organisation for Economic Co-operation and Development; TG = test guideline; TMER=Test method evaluation report.

[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

[3] The report of the 2009 Peer Review Panel meeting is available at:
http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf

References

ESAC. 2007. ESAC statement on the reduced local lymph node assay. Ispra, Italy: European Commission Directorate General, Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods.

ICCVAM. 2009a. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. NIH Publication 09-6439. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

ICCVAM. 2009b. Recommended Performance Standards: Murine Local Lymph Node Assay. NIH Publication 09-7357. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

Idehara K, et al. 2008. Characterization and evaluation of a modified local lymph node assay using ATP content as a non-radio isotopic endpoint. J Pharmacol Toxicol Meth 58:1-10.

Kimber I, et al. 2006. The local lymph node assay and skin sensitization: a cut-down screen to reduce animal requirements? Contact Dermatitis 54:181-185.

OECD. 2010.Test No. 442A: Skin Sensitization: Local Lymph Node Assay: DA. In:, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, OECD Publishing.Available at http://www.oecd-ilibrary.org/environment/test-no-442a-skin-sensitization_9789264090972-en.

Omori T, et al. 2008. Interlaboratory validation of the modified murine local lymph node assay based on adenosine triphosphate measurement. J Pharmacol Toxicol Meth 58:11-26.

Yamashita K, et al. 2005. Development of a modified local lymph node assay using ATP measurements as an endpoint. AATEX 11:136-144.

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