Evaluation of the Murine Local Lymph Node Assay (LLNA) for Assessing the Allergic Contact Dermatitis Hazard Potential of Pesticide Formulations

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Introduction

- The murine local lymph node assay (LLNA) is a test method for assessing the potential of substances to cause allergic contact dermatitis (ACD). ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeated contact with a sensitizing substance.
- In its original evaluation of the LLNA, ICCVAM recommended the LLNA as a valid alternative to traditionally accepted guinea pig test methods for assessing ACD hazard potential for most testing situations (Dean et al. 2001; Haneke et al. 2001; ICCVAM 1999; Sailstad et al. 2001).
- In response to a nomination by the U.S. Consumer Product Safety Commission in 2007, NICEATM re-evaluated the applicability domain of the LLNA.

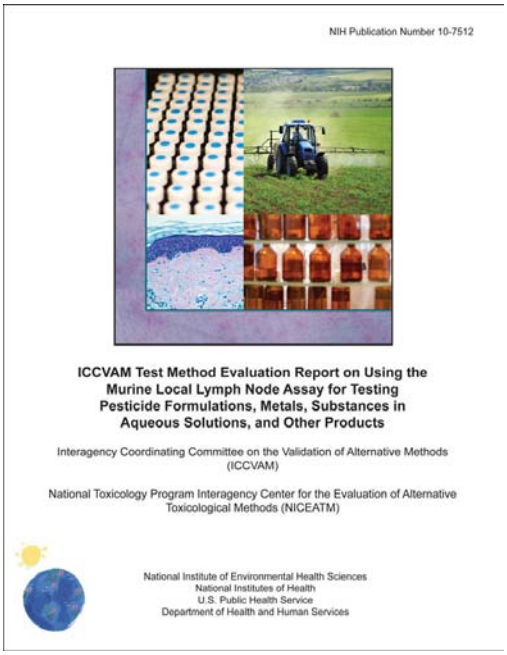


Table 1: LLNA Performance for Testing Pesticide Formulations

Comparison	n[1]	Accuracy		Sensitivity		LLNA False Negative Rate		Specificity		LLNA False Positive Rate	
		%	No.[2]	%	No.[2]	%	No.[2]	%	No.[2]	%	No.[2]
LLNA vs. GP[3]	23	57	13/23	100	3/3	0	0/3	50	10/20	50	10/20

Abbreviations: GP = guinea pig skin sensitization outcomes; No. = number.
[1]n = Number of substances included in this analysis.
[2] The data on which the percentage calculation is based.
[3] GP refers to outcomes obtained by studies conducted using either the guinea pig maximization test or the Buehler test.

Current Validation Status of the LLNA for Testing Pesticide Formulations

- NICEATM LLNA database of over 600 substances included data for 104 pesticide formulations.
  - Included both LLNA and guinea pig (GP) data on 23 formulations
  - Did not include human skin sensitization test data or postmarketing sensitization report data
- For the 23 formulations with both GP and LLNA data:
  - LLNA and the GP results agreed (accuracy) 57% (13/23) of the time (**Table 1**).
  - LLNA classified 57% (13/23) of formulations as sensitizers while GP tests classified only 13% (3/23) as sensitizers.
    - All 3 GP sensitizers were also LLNA sensitizers (i.e., no pesticide formulations were underpredicted by the LLNA compared to GP results).
  - The LLNA identified 10 formulations as sensitizers that were classified as nonsensitizers in GP tests (**Table 1**).

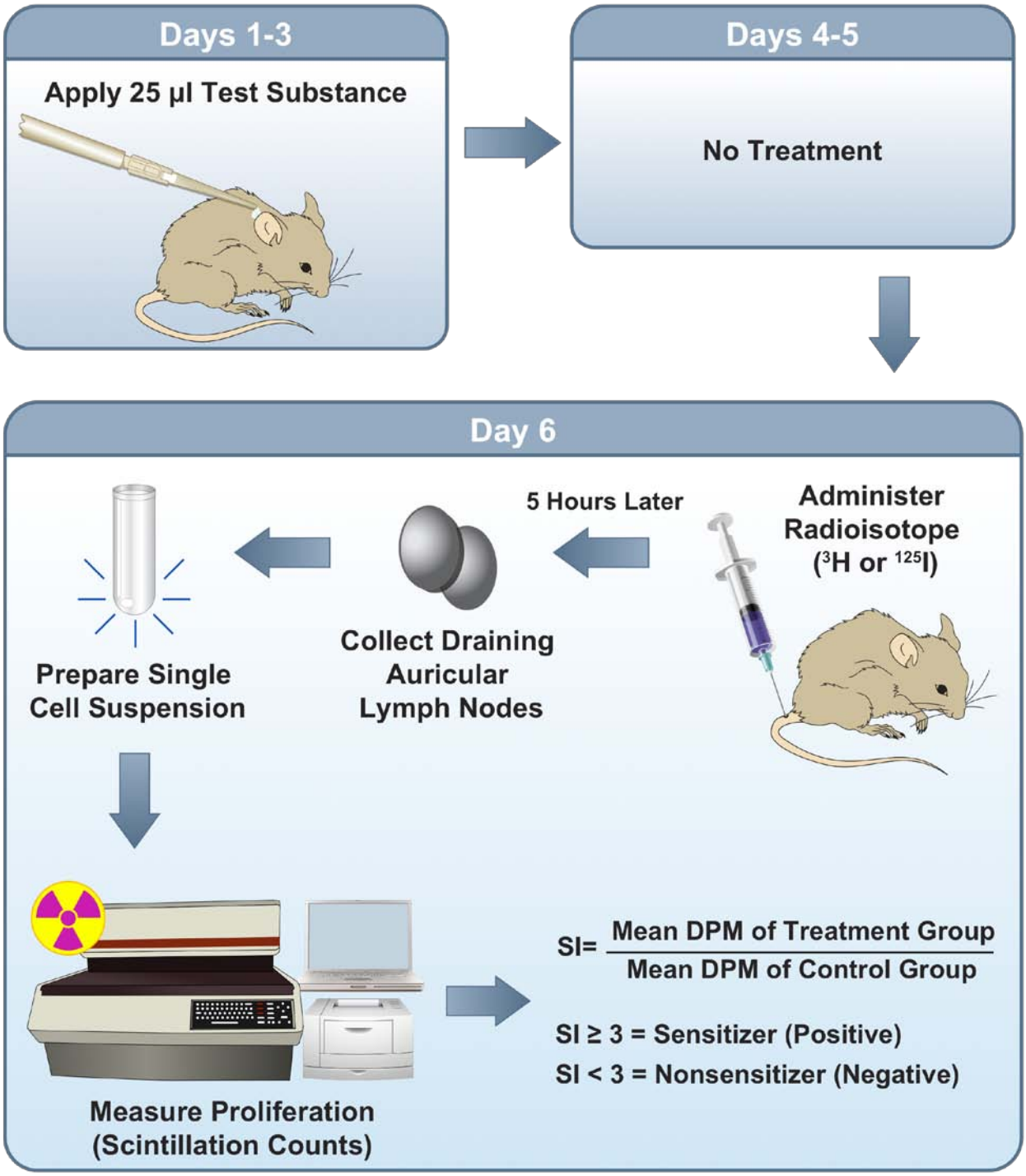
Test Method Usefulness and Limitations for Pesticide Formulations

- ICCVAM concludes that these data support the usefulness of the LLNA for testing pesticide formulations.
  - For adequate dermal exposure during the testing of aqueous formulations, an appropriate vehicle should be used to facilitate adherence of the test material to the skin (e.g., 1% Pluronic L92 [Boverhoff et al. 2008]).
  - If an LLNA variant (e.g., a nonradioactive LLNA version) is validated for use to test novel substance classes, then the findings should be relevant to the family of validated and accepted LLNA tests.
  - As indicated in **Table 1**, there is a greater likelihood of obtaining a positive result in the LLNA than in a GP test. Therefore, the potential for possible overclassification may be a limitation of the LLNA.

Test Method Protocol for Pesticide Formulations

- The updated ICCVAM-recommended LLNA test method protocol (**Figure 1**; Appendix A, ICCVAM 2009a) reduces animal use by 20% compared to the 1999 ICCVAM-recommended protocol (ICCVAM 1999).
- If dose-response information is not required or there is no basis to believe that the test article may be a sensitizer, a reduced LLNA test method protocol should be considered. By testing only the high dose, the reduced LLNA can further reduce animal use by up to 40% (ICCVAM 2009b).

Figure 1: LLNA Test Method Protocol



Abbreviations: DPM = disintegrations per minute; SI = stimulation index

Transmittal to Federal Agencies and Agency Responses

- The data supporting the ICCVAM recommendations is contained in an Addendum to the 1999 evaluation (Appendix C of the ICCVAM Test Method Evaluation Report [ICCVAM 2010]).
- In June 2010, ICCVAM forwarded final test method recommendations on the expanded uses of the LLNA for pesticide formulations and other products to U.S. Federal agencies for consideration.
- Federal agency responses include acceptance decisions and agreement with the test method recommendations for the expanded uses of the LLNA.
- Several agencies also indicated that they would communicate the ICCVAM recommendations to stakeholders and encourage their appropriate use. For example, EPA has issued a policy on the use of LLNA for pesticide formulations (see http://www.epa.gov/pesticides/science/llna-policyfinal.pdf).
- Agency responses are available on the NICEATM–ICCVAM Web site at *http:// iccvam.niehs.nih.gov/methods/ immunotox/llna.htm.*

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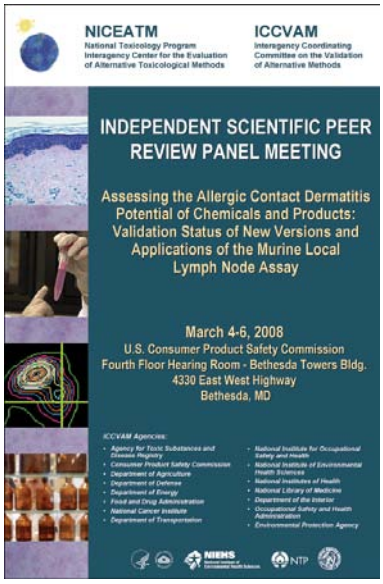
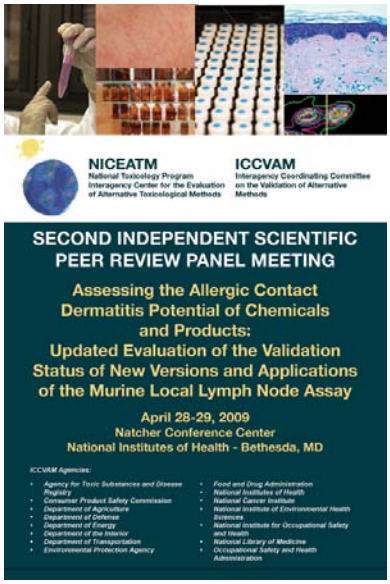
LLNA Peer Review Panel Meetings





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

Charge to the Peer Review Panel

- Review the addendum for errors and omissions
- Provide conclusions and recommendations on the current validation status of the LLNA applicability domain
- Does the information contained in the draft Addendum support ICCVAM's draft test method recommendations?

Peer Review Panel Conclusions

- Concurred that the data supported the ICCVAM draft test method recommendations for LLNA usefulness and limitations
- Considered all of the test materials as candidates for testing in the LLNA, subject to the limitations outlined in the ICCVAM draft test method recommendations
- Emphasized that before animal testing is conducted, consideration should be given to the necessity for the substance to be tested for skin sensitization potential
- Recommended including a representative positive control from the same category of materials to be tested (e.g., for testing pesticides, select one representative positive control pesticide)
- 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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International Acceptance of Expanded LLNA Applicability Domain

- The LLNA's expanded applicability domain was included in the updated OECD Test Guideline 429 (OECD 2010) based on ICCVAM's evaluation.
- The revised Test Guideline 429 was adopted by OECD in July 2010 and can be accessed at http://www.oecd-ilibrary.org/.
- Adoption of the revised test guideline is expected to result in broader use of the LLNA, which will further reduce and refine animal use for ACD assessments while ensuring human safety.

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