Updated NICEATM Evaluation and International Acceptance of the Reduced Murine Local Lymph Node Assay

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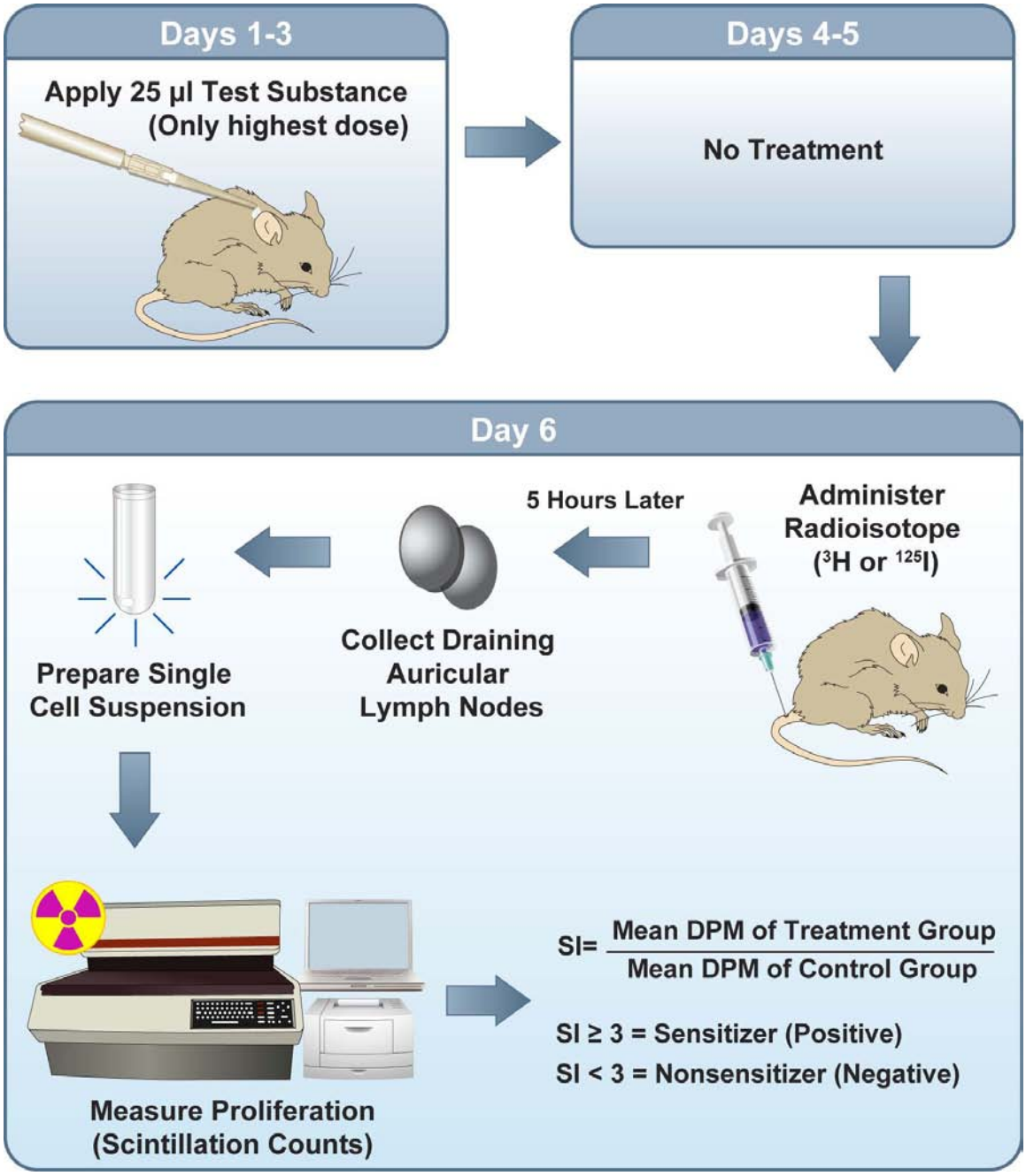
Introduction

- 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 response to a nomination by the U.S. Consumer Product Safety Commission in 2007, NICEATM evaluated the validation status of the reduced murine local lymph node assay (rLLNA) (**Figure 1**) as an alternative to the multidose LLNA for identifying the potential of substances to cause ACD.
- The only difference between the test method protocols for the multidose LLNA and the rLLNA is the number of dose levels tested.
  - In the multidose LLNA, at least three dose levels are tested for each substance.
  - Only the highest dose of a substance is tested in the rLLNA.
  - The highest dose should be based on maximum solubility and the avoidance of excessive local irritation and/or systemic toxicity.
  - The rLLNA can reduce by 40% the number of animals used for each test compared to the multidose LLNA.
- ICCVAM published a test method evaluation report (TMER) (ICCVAM 2009),

which reviewed the validation status of the rLLNA and included recommendations on:

- Usefulness and limitations of the rLLNA
- A test method protocol for the rLLNA
- Future studies to expand the applicability of the rLLNA

Figure 1 rLLNA Test Method Protocol



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

rLLNA Accuracy

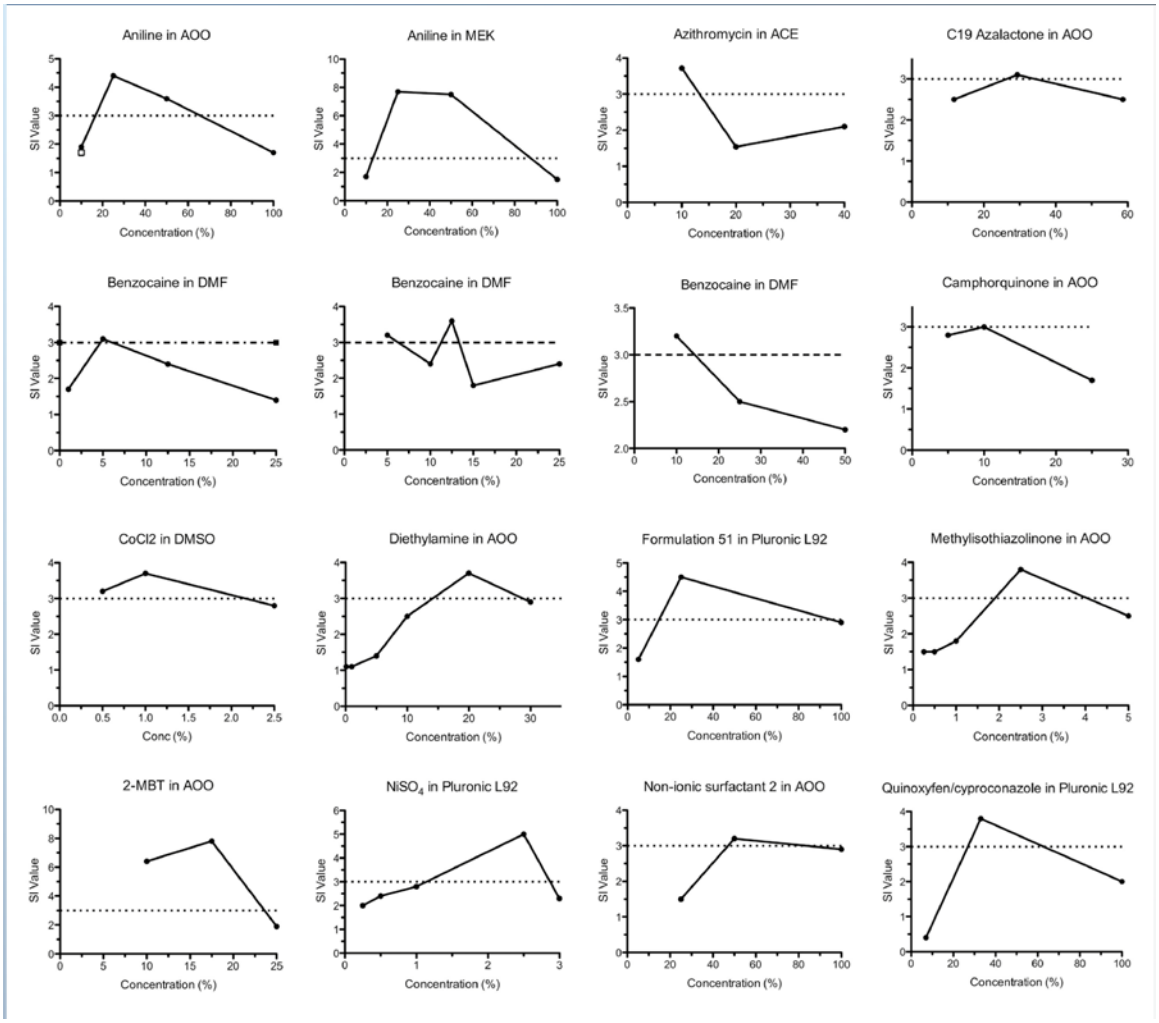
- Evaluation of rLLNA accuracy considered data from 1071 multidose LLNA studies on 668 unique substances.
- Performance characteristics for the rLLNA, as compared to the multidose LLNA, are shown in **Table 1.**
  - Results from the evaluation conducted by Kimber et al. (2006) and the ICCVAM TMER (2009) are shown for comparison.
- Sixteen tests of 13 substances were positive (SI ≥ 3) in the multidose LLNA based only on responses at doses other than the highest dose (**Figure 2**).
  - Since the rLLNA only evaluates the highest dose tested, all 16 tests incorrectly classified the substances as nonsensitizers when compared to the multidose LLNA.

Table 1. rLLNA Accuracy in Predicting Skin Sensitizers Compared to the Multidose LLNA

Data Source	N	Accuracy % (No.)	Sensitivity % (No.)	Specificity % (No.)	False Positive Rate % (No.)	False Negative Rate % (No.)
Kimber et al. (2006)	211	98.6 (208/211)	98.2 (166/169)	100 (42/42)	0 (0/42)	1.8 (3/169)
rLLNA TMER (ICCVAM 2009)	471	98.7 (465/471)	98.1 (312/318)	100 (153/153)	0 (0/153)	1.9 (6/318)
rLLNA (updated database 2011)	1071	98.5 (1055/1071)	97.9 (736/752)	100 (319/319)	0 (0/319)	2.1 (16/752)

Abbreviations: No. = number

Figure 2 Dose-Response Curves for Substances Identified as Sensitizers by the Multidose LLNA but as Nonsensitizers by the rLLNA



Note: The dotted line in each figure indicates a stimulation index of 3, which is the threshold for a positive response in the multidose LLNA and the rLLNA. Points on or above this line indicate a sensitizer (positive) response, while points below this line indicate a nonsensitizer (negative) response.

Abbreviations: ACE = acetone; AOO = acetone: olive oil (4:1 by volume); DMF = dimethylformamide; DMSO = dimethylsulfoxide; 2-MBT = mercaptobenzothiazole; MEK = methyl ethyl ketone; $NiSO_4$ = nickel sulfate; SI = stimulation index.

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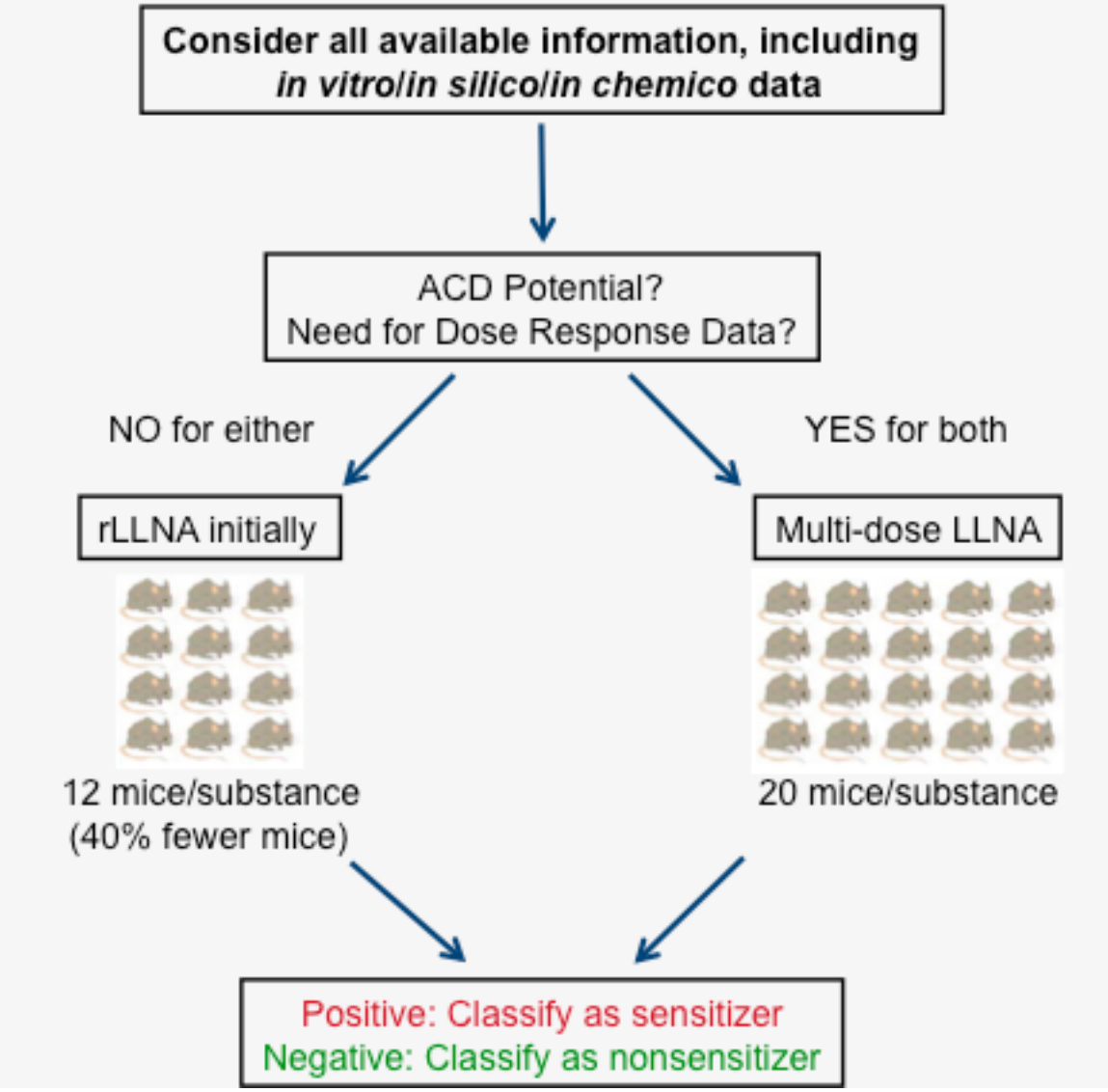
International Acceptance of the rLLNA

- Based on ICCVAM's evaluation, the rLLNA was included in the updated OECD Test Guideline (TG) 429: Skin Sensitisation: Local Lymph Node Assay (OECD 2010).
- The updated Test Guideline 429 was adopted by the OECD in July 2010. It can be accessed at http://www.oecd-ilibrary.org/.
- The availability of this international test guideline will allow for global use of the rLLNA for regulatory testing, which is expected to significantly reduce animal use for ACD hazard testing while supporting the protection of human health.

Conclusions

- The results presented here from data on 1071 LLNA studies reinforce ICCVAM's 2009 evaluation, which was based on 471 LLNA studies.
- Use the rLLNA routinely to determine the ACD hazard potential of chemicals and products unless there is a likelihood that it is a sensitizer *and* dose response information is needed
- Available information and data about the chemical/product to consider when determining whether to use the rLLNA include:
  - Physicochemical properties
  - Structural relationship to known skin sensitizers
  - Structural alerts/QSAR
  - *In vitro*/*in silico*/*in chemico* data
  - Human data
  - Test results for similar substances
  - Toxicogenomic data
- Compared to the LLNA, the rLLNA will reduce animal use by 40%.

- **Figure 3 Decision Strategy for Using rLLNA**



- This decision strategy for using the rLLNA was presented at the *ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Allergic Contact Dermatitis*, held on January 20, 2011, at the William H. Natcher Conference Center, National Institutes of Health, Bethesda, MD.

- Information about the workshop is available on the NICEATM–ICCVAM web

site at

http://iccvam.niehs.nih.gov/meetings/Implement-2011/ImplmtnWksp.htm

References

ICCVAM. 2009. 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. Available at: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/TMER.htm

Kimber I, et al. 2006. Contact Dermatitis 54:181-5.

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