1.0 Introduction

The murine local lymph node assay (traditional LLNA[9]) is an alternative skin sensitization test method that requires fewer animals and less time than currently accepted guinea pig tests (e.g., the Guinea Pig Maximization Test and the Buehler Test). It can also eliminate animal pain and distress. The LLNA measures cell proliferation in the draining auricular lymph nodes of the mouse by analyzing incorporation of a radioactive marker into newly synthesized DNA. The LLNA was the first alternative test method evaluated and recommended by the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). International regulatory authorities have now recognized the traditional LLNA as an acceptable alternative to guinea pig tests for most testing situations.

The reduced murine local lymph node assay (rLLNA), also referred to as the "cut-down" or "limit dose" LLNA, was one of several modified versions of the LLNA nominated by the U.S. Consumer Product Safety Commission (CPSC) for evaluation by ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).[10] (The term "reduced LLNA" has been adopted in this document to be consistent with the terminology used for this test method in Europe.) The proposed rLLNA could reduce the number of animals used for skin sensitization testing by 40% for each test compared to the traditional LLNA.

The ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3) charged ICCVAM with coordinating the technical evaluations of new, revised, and alternative test methods with regulatory applicability. After considering comments from the public and ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), ICCVAM members unanimously agreed that the rLLNA should have a high priority for evaluation. A detailed timeline of the rLLNA test method evaluation is provided in **Appendix A**. The updated ICCVAM-recommended LLNA test method protocol, accompanying statistical evaluation, and final rLLNA background review document (BRD) are provided in **Appendices B, C, and D**, respectively.

The ICCVAM Immunotoxicity Working Group (IWG) was formed to work with NICEATM in evaluating the test methods. Dr. Silvia Casati was the European Centre for the Validation of Alternative Methods (ECVAM) liaison, and Dr. Hajime Kojima was the Japanese Center for the Validation of Alternative Methods (JaCVAM) liaison to the IWG.

To facilitate peer review of the validation status of the rLLNA, the IWG and NICEATM, which administers ICCVAM and provides scientific support for ICCVAM activities, prepared a comprehensive BRD that provided information and data from validation studies and scientific literature. A May 17, 2007, *Federal Register* (*FR*) notice (72 FR 27815[11]) requested data and information on these test methods and nominations of individuals to serve on an international independent scientific peer review panel (Panel). The request was also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. Eight

[9] The "traditional LLNA" refers to the validated ICCVAM-recommended LLNA protocol (ICCVAM 1999; Dean et al. 2001), which measures lymphocyte proliferation based on incorporation of tritiated thymidine into the cells of the draining auricular lymph nodes.

[10] Available at http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

[11] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

individuals submitted data and three individuals or organizations nominated members to the Panel.

ICCVAM examined data from 471 traditional LLNA studies (318 sensitizers and 153 non-sensitizers) representing 457 unique substances. ICCVAM built on a recent assessment of this procedure by the ECVAM Scientific Advisory Committee (ESAC; ESAC 2007), which used data from 211 traditional LLNA studies (211 unique substances) (Kimber et al. 2006). In an April 2007 statement, ESAC concluded "that the peer reviewed and published information is of a quality and nature to support the use of the rLLNA within tiered-testing strategies to reliably distinguish between chemicals that are skin sensitisers and non-sensitisers…" (**Appendix E**)

On January 8, 2008, ICCVAM announced the availability of the ICCVAM draft BRD and a public Panel meeting to review the validation status of the rLLNA (and other modifications to the traditional LLNA) (73 FR 1360[12]). The ICCVAM draft BRD and draft test method recommendations were posted on the NICEATM–ICCVAM website.[13] All of the information provided to the Panel and all public comments received prior to the Panel meeting were made available on the NICEATM–ICCVAM website.

The Panel met in public session on March 4–6, 2008, to review the rLLNA's validation status and the completeness and accuracy of the ICCVAM draft BRD. The Panel evaluated (1) the extent to which the draft BRD addressed established validation and acceptance criteria and (2) the extent to which the BRD supported ICCVAM's draft proposed test method uses, recommended protocols, draft test method performance standards, and proposed future studies. Interested stakeholders from the public were provided opportunities to comment at the Panel meeting. The Panel considered these comments as well as those submitted prior to the meeting before concluding their deliberations. On May 20, 2008, ICCVAM posted a report of the Panel's recommendations[14] (see **Appendix F**) on the NICEATM–ICCVAM website for public review and comment (announced in 73 FR 29136[15]).

ICCVAM provided SACATM with the draft BRD and draft test method recommendations, the Panel report, and all public comments for discussion at their meeting on June 18–19, 2008, where public stakeholders were given another opportunity to comment.

ICCVAM and the IWG considered the SACATM comments, the Panel report, and all public comments when finalizing the test method recommendations provided in this report. As required by the ICCVAM Authorization Act, ICCVAM will make this Test Method Evaluation Report and the accompanying final BRD available to the public and to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses will be made available to the public on the NICEATM–ICCVAM website as they are received.

[12] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_25553.pdf
[13] http://iccvam.niehs.nih.gov/methods/immunotox/llna-panelDocs.htm
[14] Available at http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
[15] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-11195.pdf