Executive Summary

In 1999, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended the murine local lymph node assay (traditional LLNA) as a valid substitute for currently accepted guinea pig test methods to assess allergic contact dermatitis (ACD) potential of substances in most ACD testing situations. The recommendation was based on a comprehensive evaluation that included an independent scientific peer review panel (Panel) assessment of the validation status of the LLNA. The Panel report and the ICCVAM recommendations (ICCVAM 1999) are available at the NICEATM–ICCVAM website.[31]

ICCVAM forwarded to U.S. Federal agencies its recommendation that the traditional LLNA should be considered for regulatory acceptance or other non-regulatory applications for assessing the ACD potential of substances, while recognizing that some testing situations would still require the use of traditional guinea pig test methods (ICCVAM 1999). The LLNA was subsequently incorporated into national and international test guidelines for the assessment of skin sensitization (International Organization for Standardization [ISO] 10993-10: Tests for Irritation and Sensitization [ISO 2002]; Organisation for Economic Co-operation and Development Test Guideline [TG] 429 [OECD 2002]; U.S. Environmental Protection Agency Health Effects Test Guideline OPPTS 870.2600: Skin Sensitization [EPA 2003]).

In 2007, the U.S. Consumer Product Safety Commission (CPSC) nominated the rLLNA (also referred to as the "cut-down" or "limit dose" LLNA) as one of several modified versions of the LLNA for evaluation by ICCVAM. The proposed rLLNA could reduce the number of animals for skin sensitization testing by 40% per test compared with the traditional LLNA. The term "reduced LLNA" has been adopted in this document to be consistent with the terminology used for this test method in Europe.

ICCVAM assigned this activity a high priority; and the National Toxicology Program Interagency Committee on the Evaluation of Alternative Methods (NICEATM), along with the ICCVAM Immunotoxicity Working Group (IWG), collaborated closely with liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods to facilitate the evaluations requested by the CPSC. NICEATM and the ICCVAM IWG prepared this background review document (BRD), which summarizes the current validation status of the rLLNA for assessing the skin sensitization potential of substances. It includes detailed information about the reliability and relevance of the rLLNA, and the scope of the substances that were evaluated. It provides a comprehensive review of available data and information on the use of the rLLNA for hazard classification.

This information summarized in this BRD is from a retrospective review of traditional LLNA data. The database considered was obtained from 12 different sources and included 457 unique substances[32] tested in a total of 471 traditional LLNA studies. ICCVAM had considered 211 of the substances during its 1998 evaluation of the traditional LLNA (ICCVAM 1999). An additional 246 substances were obtained from the peer-reviewed literature published after that evaluation and from data submitted to NICEATM in response to a 2007 *Federal Register* (*FR*)

[31] Available at http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/llnarep.pdf

[32] Some substances were tested in more than one vehicle. In such instances, each substance-vehicle combination was considered separately, and thus there were a total of 465 unique substance-vehicle combinations that were used in the performance evaluation.

notice (72 FR 27815, May 17, 2007[33]). Specifically, three sources were published journal articles and eight were responses to the May 2007 *FR* notice. Due to the small number of repeated studies (5% of total studies), all studies were treated independently for the purpose of this accuracy evaluation.

The 1999 ICCVAM-recommended LLNA protocol accepted by U.S. regulatory agencies is consistent with procedures described in OECD TG 429 and was used as the basis for development of the OECD test guideline. Still, TG 429 allows for more procedural variation than the 1999 ICCVAM-recommended protocol (ICCVAM 1999). The protocol for the rLLNA is identical to that for the traditional LLNA (ICCVAM 1999), except that the traditional LLNA tests a substance at three dose levels, with the highest dose level being that which does not induce systemic toxicity and/or excessive skin irritation. In the rLLNA, a substance is tested at only a single dose level, which is the highest dose level that would have been tested in the traditional LLNA. As in the traditional LLNA, the threshold for classifying a substance as a skin sensitizer in the rLLNA is a stimulation index (SI) ≥ 3.

Information on chemical classes for each substance was retrieved from the National Library of Medicine's ChemIDplus® database or assigned for each test substance using a standard classification scheme based on the National Library of Medicine Medical Subject Headings classification system.[34] Chemical class information is included to indicate the variety of structural elements in the evaluated substances. One hundred and twenty-five complex substances were identified simply as pharmaceuticals. Ten substances were formulations. Seventy substances could not be assigned to a specific chemical class due to incomplete information (e.g., no Chemical Abstracts Service Registry Number or structure provided).

The ability of the rLLNA to correctly identify potential skin sensitizers was compared to that of the traditional LLNA. In the 471 studies, 318 detected skin sensitizers, and 153 detected non-sensitizers. When studies for substances tested more than once in the same vehicle (i.e., 465 unique substance and vehicle combinations) were considered together to yield an overall skin sensitization classification, 315 were classified as sensitizers, and 150 were classified as non-sensitizers.

Based on the data available from the 471 studies, the rLLNA has an accuracy of 98.7% (465/471), a sensitivity of 98.1% (312/318), a specificity of 100% (153/153), a false positive rate of 0% (0/153), and a false negative rate of 1.9% (6/318) when compared to the traditional LLNA. Based on the 465 unique substance and vehicle combinations, the rLLNA has an accuracy of 98.7% (459/465), a sensitivity of 98.1% (309/315), a specificity of 100% (150/150), a false positive rate of 0% (0/150), and a false negative rate of 1.9% (6/315).

Six substances yielded false negative results in the rLLNA (i.e., the substances were classified as sensitizers in the traditional LLNA but as non-sensitizers in the rLLNA). A review of the data for these six substances indicates that the traditional LLNA classification of the substances as skin sensitizers was based not on the highest dose level tested, which induced an SI < 3 but on a low- or mid-dose level that produced an SI ≥ 3. Because the rLLNA only tests substances at the highest dose level, all six substances would be incorrectly identified as non-sensitizers (i.e., false negatives). Four of the six substances that resulted in false negatives using the

[33] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf
[34] Available at http://www.nlm.nih.gov/mesh/meshhome.html

rLLNA compared to the traditional LLNA came from LLNA studies that used pooled data. There were no patterns of consistency for these substances with regard to physicochemical properties.

Interlaboratory reproducibility of the rLLNA was assessed with data for five substances tested independently in the same vehicle at multiple laboratories. Among these five substances, three (60%) were classified as sensitizers or non-sensitizers in all studies (i.e., 100% concordance). Each of the other two substances, tested independently in two laboratories, was classified as a sensitizer by one traditional LLNA study and as a non-sensitizer by the other traditional LLNA study. Review of the studies indicates that the discordant results were due to differences in the highest dose levels tested. However, because the traditional LLNA and the rLLNA use identical protocols and the data sets used to evaluate their accuracy are similar, the reliability of the two methods would be expected to be similar. That is, the intra- and interlaboratory reliability of the rLLNA would be expected to be the same as that of the traditional LLNA (see ICCVAM 1999 for these statistics).

A review of published literature on the rLLNA revealed only one published report in addition to that of Kimber et al. (2006). Ryan et al. (2008) described the impact of reducing the number of animals per group from five to two on the performance of the rLLNA and concluded that the sensitivity is inadequate for hazard identification of skin sensitizers.

Compared to the traditional LLNA, the rLLNA will reduce the number of animals used to assess skin sensitization. Because the rLLNA tests only the highest dose level of the test substance in addition to the concurrent control groups, the number of animals tested would decrease by at least 40% for each test.

The database included in this BRD will be updated as additional information becomes available during future use of the traditional LLNA and the rLLNA.