Preface

Allergic contact dermatitis (ACD) is an adverse health effect that frequently develops in workers and consumers exposed to skin-sensitizing chemicals and products. ACD results in lost workdays[1] and can significantly diminish quality of life (Hutchings et al. 2001; Skoet et al. 2003). To minimize the occurrence of ACD, regulatory authorities require testing to identify substances that may cause ACD. Sensitizing substances must be labeled with a description of the potential hazard and the precautions necessary to avoid development of ACD.

Skin sensitization testing has typically required the use of guinea pigs (Buehler 1965; Magnusson and Kligman 1970). However, in 1999, the U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended the murine (mouse) local lymph node assay (LLNA) as a valid test method to assess the skin sensitization potential of most types of substances (ICCVAM 1999). ICCVAM concluded that the LLNA (referred to herein as the "traditional LLNA") provided several advantages compared to the guinea pig method, including elimination of potential pain and distress, use of fewer animals, less time required to perform, and availability of dose-response information. United States and international regulatory authorities subsequently accepted the traditional LLNA as an alternative test method for ACD testing. It is now commonly used around the world.

While the traditional LLNA has many advantages, it does require the use of a radioactive marker to measure cell proliferation in lymph nodes. To avoid the use of radioactive markers, scientists have recently developed several non-radioactive versions of the LLNA. In 2007, the U.S. Consumer Product Safety Commission (CPSC) asked ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) to evaluate the scientific validity of these non-radioactive versions.[2]

When ICCVAM evaluated the LLNA in 1999, the concept of performance standards had not been developed. ICCVAM subsequently defined performance standards and described a process for their development (ICCVAM 2003). Performance standards provide criteria on which to evaluate the validity of functionally and mechanistically similar test methods. They are based on adequately validated and accepted test methods (e.g., the LLNA). Performance standards also specify test method components that must be included in modified versions in order to use the performance standards for their validation.

Following the CPSC nomination, ICCVAM decided to develop performance standards for the traditional LLNA that could be used to more rapidly and efficiently determine the validity of non-radioactive and other modified versions. NICEATM provided scientific and operational support for the ICCVAM Immunotoxicity Working Group (IWG), and scientists from the Japanese Center for Validation of Alternative Methods (JaCVAM) and the European Centre for the Validation of Alternative Methods (ECVAM) served as IWG liaisons. A detailed timeline of the development process for the LLNA performance standards is provided in the final Recommended Performance Standards document. Public

[1] http://www.bls.gov/IIF

[2] http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf

comments were solicited and considered by the IWG and ICCVAM throughout the development process.

ICCVAM released initial draft LLNA Performance Standards to the public for comment on September 12, 2007 (72 FR 52130).[3] ICCVAM considered comments from the public and from an ECVAM workshop on non-radioactive LLNA methods and published revised draft LLNA Performance Standards in January 2008. In March 2008, ICCVAM and NICEATM convened an international independent scientific peer review panel (hereafter "Panel") in public session to evaluate the revised draft ICCVAM LLNA Performance Standards. The Panel's report was made available to the public and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) for comment.

Our colleagues at ECVAM and JaCVAM agreed to work with us to develop internationally harmonized LLNA performance standards. The IWG, along with the ECVAM and JaCVAM liaisons, met several times during the summer of 2008 to further revise the performance standards and to consider the comments from the Panel, the public, and SACATM. On September 23–24, 2008, ECVAM hosted a harmonization meeting to analyze and reach consensus on the remaining issues.

An important issue addressed was the number of mice used per dose group. Organisation for Economic Co-operation and Development Test Guideline 429, which describes the LLNA procedure, specifies that at least four animals per dose group must be used when lymph nodes from all animals in the dose group are pooled into one sample but requires that at least five animals per dose group be used when individual animal data are collected and analyzed. Due to animal-use regulations that require the minimum number of animals be used in studies, the Guideline has led to only pooled data being collected in many countries, a practice considered inadequate by some regulatory authorities and discouraged by ICCVAM. NICEATM analyzed LLNA data from 83 LLNA studies (275 dose groups) from six different laboratories, which showed that the use of four animals rather than five per dose group was not likely to change the hazard-classification outcome. Based on these data, ICCVAM concluded that the number of animals per dose group could be reduced from five to four when collecting individual animal data.

ICCVAM will now forward these harmonized Recommended Performance Standards for the LLNA to U.S. regulatory authorities for their approval. At its November 4-5, 2008, meeting, the ECVAM Scientific Advisory Committee endorsed their corresponding ECVAM LLNA performance standards, on which ECVAM collaborated extensively with ICCVAM to harmonize. Approved performance standards can be used by validation organizations (e.g., ICCVAM, ECVAM, and JaCVAM) and others to assess the validity of non-radioactive and other new versions and applications of the LLNA proposed for regulatory safety testing. We anticipate that these performance standards will help promote development and validation of non-radioactive LLNA methods and other innovative approaches. These new versions are expected to lead to broader use of the LLNA, which will further reduce and refine animal use for ACD testing.

We gratefully acknowledge the many organizations and scientists who provided data and information necessary to develop these LLNA performance standards. We thank the

[3] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_18011.pdf

members of the ICCVAM IWG and ICCVAM representatives who reviewed and provided comments throughout the process. We also acknowledge the technical assistance of several individuals, including Dr. David Allen, Dr. Eleni Salicru, Ms. Catherine Sprankle, Dr. Judy Strickland, and Ms. Linda Wilson of Integrated Laboratory Systems, Inc., the NICEATM support contractor; Dr. Joseph Haseman, consulting statistician; and Dr. Raymond Tice, Deputy Director of NICEATM. We recognize the leadership of and thank the IWG co-chairs, Dr. Joanna Matheson of the CPSC and Dr. Abigail Jacobs of the U.S. Food and Drug Administration. Finally, we acknowledge and express our appreciation to our international liaisons, Dr. Hajime Kojima from JaCVAM and Dr. Silvia Casati from ECVAM, for their cooperation and contributions.

William S. Stokes, D.V.M., D.A.C.L.A.M.
Rear Admiral/Assistant Surgeon General, U.S. Public Heath Service
Director, NICEATM
Executive Director, ICCVAM

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM