Update on the April 28-29, 2009 NICEATM-ICCVAM Peer Review Panel on New Versions and Applications of the Murine Local Lymph Node Assay

NICEATM and ICCVAM convened an international independent scientific peer review panel on April 28-29, 2009, to evaluate three non-radioactive modified versions and new applications for the murine local lymph node assay (LLNA). The Panel, chaired by Dr. Michael Luster included 15 expert scientists from six countries. The public Panel meeting was held at the William H. Natcher Center on the Bethesda, MD campus of the National Institutes of Health.

The new versions and applications considered include:

- Application of the LLNA for evaluating pesticide formulations and other products
- Three modified versions of the LLNA not requiring the use of radioactive markers:
  - LLNA: DA (Local Lymph Node Assay: Daicel Adenosine Triphosphate)
  - LLNA: BrdU-FC (Local Lymph Node Assay: Bromodeoxyuridine Detected by Flow Cytometry)
  - LLNA: BrdU-ELISA (Local Lymph Node Assay: Bromodeoxyuridine Detected by ELISA)

The Panel was charged with determining (1) the extent that the validation status of each of the above proposed modifications or uses of the LLNA had been adequately characterized for its intended purpose according to established ICCVAM validation criteria,[1] and (2) whether proposed modifications or uses of the LLNA were sufficiently accurate and reliable to be used for the identification of sensitizing substances and nonsensitizing substances in place of the traditional LLNA procedure.

The Panel concluded that the available data and test method performance support the use of the LLNA: DA and the LLNA: BrdU-ELISA to identify substances as potential skin sensitizers and nonsensitizers, with certain limitations. They agreed with ICCVAM's proposal that, based on the current validation database, multiple stimulation index (SI) decision criteria should be used to identify sensitizers and nonsensitizers for each of the two methods. The Panel also noted that the limitation of these test methods when using the proposed multiple decision criteria is the indeterminate classification of substances that fall in the range of SI values for which a classification is uncertain. The Panel recommended that when such results are obtained, users should carefully interpret the results in an integrated decision strategy in conjunction with all other available information (e.g., dose response and QSAR information, statistical analyses, peptide-binding activity, molecular weight, results from related chemicals, other testing data) to determine if there is adequate information for an accurate sensitization hazard classification or if additional testing is necessary.

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[1] ICCVAM validation criteria are detailed in the document, *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods,* available at http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf.

For the LLNA: BrdU-FC, the Panel concluded that the database of more than 45 representative test substances yielded adequate accuracy based on results from one laboratory, and that intralaboratory reproducibility also had been adequately demonstrated. However, the Panel agreed with the ICCVAM proposal to defer a formal recommendation on the validity of the LLNA: BrdU-FC until an independent audit of all data supporting the analysis has been conducted and until transferability has been demonstrated in an interlaboratory validation study. The Panel recommended that ICCVAM should work with NICEATM to support and facilitate the independent audit and interlaboratory validation study. The Panel recommended that upon completion of these tasks and determination of satisfactory data quality and interlaboratory reproducibility, that the LLNA: BrdU-FC could be considered to have adequate validation and performance to support its consideration for regulatory use.

The Panel concluded that all three of the non-radioactive LLNA protocols are mechanistically and functionally similar to the traditional LLNA, and therefore do not require separate test method performance standards.

With regard to the applicability of the LLNA for testing pesticide formulations and other products (e.g., natural complex substances, dyes, aqueous solutions), the Panel concluded that any material should be suitable for testing in the LLNA unless there is a biologically-based rationale for exclusion, such as unique physicochemical properties that might affect their ability to interact with immune processes. The Panel therefore concluded that the LLNA should be considered appropriate for testing pesticide formulations and other products, unless there is a biologically-based rationale for exclusion.

The Panel's full report, including all of its conclusions and recommendations, will be published and available on the NICEATM-ICCVAM website[2] in early June 2009. The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) will meet June 25-26, 2009 and will provide comments on the Panel report, draft ICCVAM recommendations and draft background review documents. ICCVAM will consider the Panel's report along with all public and SACATM comments received and prepare final test method recommendations that will be forwarded to Federal agencies for their consideration later this year.

[2] http://iccvam.niehs.nih.gov/