U.S. CONSUMER PRODUCT SAFETY COMMISSION
4330 EAST WEST HIGHWAY
BETHESDA, MD 20814

Marilyn L. Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences

Tel: 301-504-7246
Fax: 301-504-0079
Email: mwind@cpsc.gov

January 10, 2007

Dr. William Stokes
Director, NICEATM
Executive Director, ICCVAM
NIEHS
P.O. Box 12233, EC-17
Research Triangle Park, NC 27709

Dear Dr. Stokes:

Over the past several years, U.S. Consumer Product Safety Commission (CPSC) staff has been reviewing CPSC's regulation on strong sensitizers as well as participating in the Organization for Economic and Cooperative Development (OECD) effort to review the Globally Harmonized System (GHS) for classification and labeling of sensitizers.* CPSC staff is concerned that the Local Lymph Node Assay (LLNA) is being proposed internationally for use in potency determinations for the purpose of classification. While the LLNA has been validated for hazard identification, the use of the LLNA for potency determinations for the purpose of classification, the use of non-radioactive protocols and the use of the LLNA limit test have not undergone formal evaluations of validation. There are also other issues with the use of the LLNA which are detailed below. CPSC staff requests that the Interagency Coordinating Committee for the Validation of Alternative Methods/National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (ICCVAM/NICEATM) assess the validation status of LLNA as a stand-alone assay for potency determinations (including severity) for classification purposes, the validation status of non-radioactive LLNA protocols; the LLNA limit test; the use of the LLNA to test mixtures, aqueous solutions and metals; and the applicability domain for which the LLNA has been validated.

ICCVAM evaluated the murine LLNA as a stand-alone alternative method to the Guinea Pig Maximization Test (GPMT) and the Buehler Assay (BA) (NIH publication No. 99-4494). This report focused upon the assay's performance and some of the critical assumptions. The consensus of the peer review panel was that the LLNA performed as well as the GPMT and BA

* These comments are those of CPSC staff, have not been reviewed or approved by, and may not necessarily represent the views of the Commission.

for hazard identification of strong to moderate chemical sensitizing [dermal] agents, but lacked strength in accurately predicting some weak sensitizers and some strong irritants. The report included a revised test method protocol. The potency of standard allergens was minimally evaluated. Furthermore, the report recommended that in the future, data on the testing of mixtures and pharmaceuticals be evaluated.

The LLNA was adopted as a test guideline by the OECD after ICCVAM validation of the assay as an alternative to guinea pig test methods for hazard identification. The Environmental Protection Agency (EPA) has accepted the LLNA along with the GPMT and BA, with the LLNA as a preferred alternative method, where applicable, to the traditional guinea pig test.[1] The EPA guidelines state that the LLNA may not be appropriate for all types of test materials such as certain metallic compounds, high molecular proteins and strong dermal irritants. The Food and Drug Administration (FDA) in its Guidance for Industry indicates that the sensitizing potential of a drug should be screened using an appropriate test such as the GPMT, BA, murine LLNA, the guinea pig inhalation induction and challenge assay, or other appropriate alternative assays.[2]

Extensive debate persists regarding the LLNA as a stand-alone assay. Concerns regarding the LLNA include: (1) whether it is solely appropriate for a subset class of sensitizers, type IV sensitizers; (2) that insufficient numbers of chemical classes have been validated; (3) the assay exclusively assesses the induction stage; (4) that the assay is an exaggeration of exposure compared to human exposure and lacks reflection of human exposure and response; and (5) that the assay has been validated for hazard identification but not for determination of potency or severity.

In 1992, during the United Nations' Conference on Environment and Development (UNCED) a mandate was established for the development of a Globally Harmonized System (GHS) to classify and label hazardous chemicals. Classification categories for sensitizing strength (potency) which are based solely upon LLNA EC_3 values[3] have been proposed.

In light of the extensive data collected since the 1999 ICCVAM report, global efforts for harmonization of chemical classification and labeling as well as regulatory efforts to reduce the utilization of animals in testing, we are requesting that ICCVAM evaluate the murine LLNA for current concerns, particularly for its ability to classify contact sensitizing chemicals according to potency. Questions that will need to be answered include whether the LLNA can serve as a stand-alone method and, thus, is able to provide information on the issues of severity and prevalence or whether it is useful in a weight of evidence approach for making determinations about classifying sensitizers.

Since there are some countries that do not allow the easy use of radioactive materials, non-radioactive LLNA protocols have been developed. These also need validation. Further, the use

---

[1] Skin Sensitization Health Effects Guidelines (OPPTS 870.2600), March 2003, EPA 712-C-03-197.
[2] Guidance for Industry. Immunotoxicology Evaluation of Investigational New Drugs, October 2002, Center for Drug Evaluation and Research.
[3] EC_3 value is an estimated concentration of chemical necessary to elicit a 3-fold increase in lymph node cell proliferative activity.

of an LLNA limit test has been recommended. The validation status of the limit test needs to be evaluated. As noted above there are issues with the current LLNA as well. Issues that will need to be included in any validation are the applicability domain (looking at chemical classes that now have data that didn't when previously evaluated) and the applicability of LLNA to mixtures, aqueous solutions and metals.

As indicated above, there are a number of issues that remain open since the last validation study that ICCVAM conducted as well as new ones. Also, since there is a European effort to change the GHS classification and labeling for sensitizers and some scientists in Europe are promoting the use of the LLNA in lieu of other test methods for sensitization, CPSC staff requests that ICCVAM/NICEATM assess the validation status of the LLNA as a stand-alone assay for potency determinations (including severity) for classification purposes, the validation status of non-radioactive LLNA protocols, the LLNA limit test, the use of the LLNA to test mixtures, aqueous solutions and metals, and the applicability domain for which the LLNA has been validated.

Sincerely,

for Marilyn L. Wind, Ph.D.