June 6, 2007

Dr Mary S. Wolfe
National Institute of Environmental Health Sciences
PO Box 12233, MD A3-01
Research Triangle Park, NC 27709

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT ST.
NORFOLK, VA 23510
757-622-PETA
757-622-0457 (FAX)

Re: **72 FR 23831; May 1, 2007; National Toxicology Program Liaison and Scientific Review Office; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM): Local Lymph Node Assay (LLNA) Nomination to ICCVAM by the U.S. Consumer Product Safety Commission (CPSC)**

Dear Dr Wolfe:

People for the Ethical Treatment of Animals (PETA) is the world's largest animal rights organization, with 1.7 million members and supporters. We appreciate the opportunity to provide oral comments regarding the nomination of further consideration of the Local Lymph Node Assay (LLNA) Nomination to ICCVAM by the U.S. Consumer Product Safety Commission (CPSC).

The LLNA is a widely used and accepted method for assessing skin sensitization, and several recent studies and reviews document that it is a robust assay for potency and hazard classification; therefore the execution of yet another detailed review of this method is not a high priority. Instead, ICCVAM would better fulfill it's 3Rs mandate by promoting one or more of several *in vitro* methods for regulatory classification of skin sensitizing chemicals.

In January, 2007, (ICCVAM) received a nomination from the U.S. Consumer Product Safety Commission to evaluate the validation status of: (1) The murine local lymph node assay as a stand-alone assay for determining potency (including severity) for the purpose of hazard classification; (2) the ''cut-down'' or ''limit dose'' LLNA approach; (3) non-radiolabeled LLNA methods; (4) the use of the LLNA for testing mixtures, aqueous solutions, and metals; and (5) the current applicability domain (i.e., the types of chemicals and substances for which the LLNA has been validated).

ICCVAM reviewed the nomination, assigned it a high priority, and proposed that NICEATM and ICCVAM carry out the following activities in its evaluation: (1) Initiate a review of the current literature and available data, including the preparation of a comprehensive background review document, and (2) convene a peer review panel to review the various proposed LLNA uses and procedures for which sufficient data and information are available to adequately assess their validation status. ICCVAM also recommends development of performance standards for the LLNA. At this time, NICEATM requests: (1) Public comments on the appropriateness and relative priority of these activities, (2) nominations of expert scientists to consider as members of a possible peer review panel, and (3) submission of data for the LLNA and/or modified versions of the LLNA.

In March, 1999, ICCVAM published a final peer review report concluding that the LLNA is a valid alternative to currently accepted guinea pig test methods.[1] The U.S. EPA, FDA, and OSHA announced their acceptance of the LLNA as an alternative to the guinea pig maximization test for assessing allergic contact dermatitis in October 1999. That same year, ESAC, the Scientific Advisory Committed of the European Centre for the Validation of alternative Methods (ECVAM), also endorsed the LLNA for regulatory use.

In September of 2000, the European Centre for Ecotoxicology and Toxicity of Chemicals (ECETOC) published a comprehensive review of sensitization test methods with respect to hazard identification and labeling, and to determine whether the various methods are appropriate for determining relative potency and risk assessment.[2] The conclusions from this review included that "1) the LLNA is a viable and complete alternative to traditional guinea pig test methods for the purposes of skin sensitization hazard identification, and 2) the LLNA is suitable for the determination of relative skin sensitizing potency and the adaptation of this method for derivation of comparative criteria such as EC3 values provides an effective and quantitative basis for such measurements. This report further recommends that "the LLNA is the recommended method for new assessments of relative potency and/or for the investigation of the influence of vehicle or formulation on skin sensitizing potency."

More recent work has further verified the use of the LLNA as a stand-alone method for estimating potency for regulatory purposes, including a 2005 study that concludes that there is a 'clear linear relationship' between LLNA-derived EC3 values and historical human skin patch data.[3] A 2007 review concludes that "The LLNA, when conducted according to published guidelines, provides a roust method for skin sensitization testing that not only provides reliable hazard identification in formation but also data necessary for effective risk assessment and risk management." In addition, a retrospective analysis of the regulatory use of the LLNA in the EU was published in 2006 and concluded that "the LLNA is satisfactory for routine regulatory use."[4]

The ''cut-down'' or ''limit dose'' LLNA approach (reduced, or rLLNA) has recently been reviewed by an ECVAM peer review panel. In April, 2007, ESAC issued a statement supporting the use of the rLLNA "within tiered-testing strategies to reliably distinguish between chemicals that are skin sensitisers and non-sensitisers "thereby reducing animal use by as much as 50%.[5] The statement also notes the following limitations: that "the test results provided by the rLLNA do not allow the determination of the potency of a sensitising chemical", and that "negative test results associated with testing using concentrations of less than 10% should undergo further evaluation" (and recommended further evaluation of the 10% threshold).

In light of the fact that the LLNA has been used by regulatory agencies for classifying skin sensitizers for years and both research data and regulatory use of the LLNA have been extensively reviewed in the literature, yet another review of this widely accepted method is unwarranted. Instead, we urge ICCVAM to consider spending its time and resources promoting the development

---

[1] http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm
[2] ECETOC. 2000. Skin Sensitization Testing for the Purpose of Hazard Identification and Risk Assessment.
[3] Basketter et al. Predictive identification of human skin sensitization thresholds. Contact Dermatitis. 2005; 53 (5): 260-267.
[4] Cockshott et al., The local lymph node assay in practice: a current regulatory perspective. Hum Exp Toxicol 2006; 25 (7): 387-394.
[5] http://ecvam.jrc.it/publication/ESAC26_statement_rLLNA_20070525-1.pdf

and regulatory use on non-animal methods, by engaging in an integrated approach to *in vitro* Immunotoxicity, such as the European Sens-it-iv project.[6]

Several non-animal methods for estimating sensitivity are under development, including quantitative structure activity relationship (QSAR) modeling that shows a high concordance with both guinea pig and LLNA data[7], quantification of peptide reactivity (which also shows a high concordance with LLNA data),[8] and human cell culture.[9] We urge ICCVAM to apply its resources to the regulatory validation of one or more of these non-animal methods.

In conclusion, it is a waste of ICCVAM's time and resources to carry out yet another extensive review of the widely used LLNA and/or its variants. ICCVAM needs instead to prioritize for validation and regulatory acceptance one or more existing non-animal methods for evaluating skin sensitivity.

Sincerely,

Catherine Willett, PhD
Science Policy Advisor
Regulatory Testing Division
Research and Investigations Department
People for the Ethical Treatment of Animals
501 Front Street, Norfolk, VA 23510
Tel/FAX: 617-522-3487

[6] http://www.sens-it-iv.eu/
[7] Fedorowicz et al., Structure-activity models for contact sensitization. Chem Res Toxicol. 2005; 18(6): 954-969.
[8] Gerberick et al. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. 2007; 97(2): 417-427.
[9] Schoeters et al. Microarray analyses in dendritic cells reveal potential biomarkers for chemical-induced shin sensitization. 2007; 44(12): 3222-3233.