



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

January 6, 2011

RADM William S. Stokes
Director
NICEATM
National Toxicology Program
PO Box 12233, EC-17
Research Triangle Park, NC 27709

Dear Dr. Stokes:

The US Food and Drug Administration (FDA) has reviewed the ICCVAM test method recommendations for the LLNA: BrdU-ELISA, and the LLNA: DA, two non-radioactive versions of the LLNA, and an expanded LLNA applicability domain. Our comments are listed below.

A1. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: BrdU-ELISA A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products (NIH Publication No. 10-7552)

FDA agrees that a nonradioactive method is preferable to a radioactive method. FDA agrees with ICCVAM's conclusion that the accuracy and reliability of the LLNA: BrdU-ELISA supports the use of the test method to identify substances as potential skin sensitizers and nonsensitizers. FDA agrees with ICCVAM's recommendation that a stimulation index (SI) ≥ 1.6 be used as the decision criterion to identify substances as potential sensitizers. A limitation of the LLNA: BrdU-ELISA is the potential for false positive results when borderline positive responses between an SI of 1.6 and 1.9 are obtained.

FDA does not agree with the ICCVAM recommendation that the LLNA: BrdU-ELISA can be used for testing nickel compounds based on its ability to correctly identify them as potential sensitizers.

FDA notes that, based on its experience with dermal pharmaceutical formulations in the traditional LLNA, that many dermal formulations and vehicles alone give positive results that are not seen in guinea pigs or humans. This assay would not be appropriate when the pharmacodynamic activity of the drug/ biologic was to release cytokines. Furthermore, known human sensitizers have failed in some dermal formulations. Thus, FDA is eagerly anticipating a battery of in vitro tests to assess dermal sensitivity as a screen for human dermal sensitivity.

B1. ICCVAM Test Method Evaluation Report on the Murine Local Lymph Node Assay: DA A Nonradioactive Alternative Test Method to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products (NIH Publication No. 10-7551)

FDA agrees that a nonradioactive method is preferable to a radioactive method. FDA agrees that ICCVAM's conclusion that the accuracy and reliability of the LLNA: DA support use of the test method to identify substances as potential skin sensitizers and nonsensitizers. FDA agrees that ICCVAM's recommendation that a stimulation index (SI) ≥ 1.8 be used as the decision criterion to identify substances as potential sensitizers.

A limitation of the LLNA: DA is the potential for false positive results when borderline positive responses between an SI of 1.8 and 2.5 are obtained. The use of the LLNA: DA might not be appropriate for testing substances that affect ATP levels (e.g., substances that function as ATP inhibitors) or those that affect the accurate measurement of intracellular ATP (e.g., presence of ATP degrading enzymes, presence of extracellular ATP in the lymph node). The LLNA: DA can be used for testing metal compounds, with the exception of nickel.

FDA notes that, based on its experience with dermal pharmaceutical formulations in the traditional LLNA, that many dermal formulations and vehicles alone give positive results that are not seen in guinea pigs or humans. This assay would not be appropriate when the pharmacodynamic activity of the drug/ biologic was to release cytokines. Furthermore, known human sensitizers have failed in some dermal formulations. Thus, FDA is eagerly anticipating a battery of in vitro tests to assess dermal sensitivity as a screen for human dermal sensitivity.

C1. ICCVAM Test Method Evaluation Report on Using the Murine Local Lymph Node Assay for Testing Pesticide Formulations, Metals, Substances in Aqueous Solutions, and Other Products (NIH Publication No. 10-7512)

Mixtures/Formulation

The data for pharmaceutical dermatologic formulations do not support the use of the LLNA for pharmaceutical dermatologic formulations. The pesticide formulation data submitted for review indicates there is a greater likelihood of obtaining a positive result in the LLNA (13/23; 57%) than in a GP test (3/23; 13%). There is no comparative human data. FDA's data for pharmaceutical dermatologic formulations do not support the use of the LLNA for pharmaceutical dermatologic formulations. The pivotal studies on assessment of dermal sensitivity of pharmaceuticals are conducted in humans. Based on these results, FDA will evaluate medical device mixture/formulation studies on a case-by-case basis.

Metals

FDA agrees that the LLNA can be used for testing metal compounds, with the exception of nickel, unless there are unique physicochemical properties associated with these materials that may interfere with the ability of the LLNA to detect sensitizing substances.

Aqueous Solutions

FDA agrees that the LLNA can be used for testing substances in aqueous solutions unless there are unique physicochemical properties associated with these materials that may interfere with the ability of the LLNA to detect sensitizing substances. When testing substances in aqueous solutions, it is also essential to use an appropriate vehicle, to maintain the test substance in contact with the skin (e.g. 1% Pluronic L92 [Boverhoff et al. 2008]) so an adequate exposure is achieved, as demonstrated by positive control results.

It should be recognized that the potential for possible over classification of aqueous substances may be a limitation of the LLNA (50% false positive rate). Furthermore, the aqueous solution database was heavily weighted with pesticides that the high false positive conclusions and the usefulness of 1% Pluronic L92 vehicle should be reevaluated as information about other classes of test material tested in aqueous solution become available.

Overall, FDA concurs with the ICCVAM recommendation that nonradioactive methods are preferable to radioactive methods, when they are equivalent. If you need further information, please contact Dr. Suzanne Fitzpatrick at 301-796-8527.

/s/

Jesse L. Goodman, MD, MPH
Chief Scientist and Deputy Commissioner for
Science and Public Health