DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Federal Agency Responses to Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Recommendations on Two Nonradioactive Versions of the Murine Local Lymph Node Assay (LLNA) for Assessing Allergic Contact Dermatitis (ACD) Hazard Potential of Chemicals and Products, and Expanded Uses of the LLNA for Pesticide Formulations and Other Products; Notice of Availability

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), HHS.

ACTION: Notice of Availability.

SUMMARY: U.S. Federal agency responses to ICCVAM test method recommendations on two nonradioactive versions of the LLNA for assessing the ACD hazard potential of chemicals and products and for expanded uses of the LLNA for pesticide formulations and other products are now available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm.* ICCVAM recommended the nonradioactive LLNA: 5-bromo-2-deoxyuridine-enzyme-linked immunosorbent assay

(BrdU-ELISA) and LLNA: Daicel Adenosine Triphosphate (DA), and expanded uses for the LLNA. In accordance with the ICCVAM Authorization Act (42 U.S.C. 285l–3(e)(4)), ICCVAM forwarded recommendations to Federal agencies and made these recommendations available to the public (75 FR 37443). Agencies have now notified ICCVAM in writing of their findings and ICCVAM is making these responses available to the public.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, Mail Stop: K2–16, Research Triangle Park, NC 27709, (telephone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, NIEHS, Room 2034, 530 Davis Drive, Morrisville, NC 27560.

SUPPLEMENTARY INFORMATION:

Background

In 1999, ICCVAM recommended the LLNA as a valid safety test for assessing the ACD hazard potential of many chemicals and products (NIH Publication No. 99–4494; available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel98.htm*). ICCVAM also concluded that the LLNA, when used as an alternative method to the guinea pig maximization test (GPMT) or the Buehler test (BT), could also significantly reduce animal use and improve animal welfare. Based on this evaluation, the U.S. Environmental Protection Agency (EPA 2003), the U.S. Food and Drug Administration, and the U.S. Consumer Product Safety Commission (CPSC) subsequently accepted the method as a valid substitute for the GPMT and BT (*http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel98.htm*). The Organisation for Economic Co-operation and Development (OECD) subsequently adopted the LLNA in 2002 as international OECD Test Guideline 429 (OECD, 2002). The International Organization for Standardization (ISO) adopted the LLNA as ISO standard 10993–10 in 2002 (ISO, 2002).

ICCVAM recommended an updated LLNA test method protocol in 2009 that further reduced animal use for each safety test by 20–40% (ICCVAM, 2009). Federal agencies endorsed this updated protocol (75 FR 25866). OECD Test Guideline 429 was subsequently updated in 2010 to incorporate the updated revisions (OECD, 2010a). The ISO standard was also updated in 2010 (ISO, 2010).

Compared to the LLNA, the LLNA: BrdU-ELISA and LLNA: DA do not use radioactive reagents and therefore provide additional advantages in terms of reduced hazardous waste disposal and broader availability for use by laboratories that cannot use radioactive reagents. ICCVAM concludes that the accuracy and reliability of the LLNA: BrdU-ELISA and LLNA: DA support their use to determine whether substances have the potential to cause ACD. The protocols also include reduced LLNA: BrdU-ELISA and LLNA: DA procedures that should always be considered and used where determined appropriate because they can further reduce animal use by 40% compared to multi-dose procedures. The ICCVAM evaluation and complete recommendations for the LLNA: BrdU-ELISA and LLNA: DA are provided in the *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, available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm*) and the *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, available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna-DA/TMER.htm*). The OECD subsequently adopted the LLNA: BrdU-ELISA and LLNA: DA as international test guidelines (OECD, 2010b, 2010c).

ICCVAM also concluded that available data support the use of the LLNA for safety testing of a broader range of chemicals and products, including pesticide formulations, metals with the exception of nickel, substances in aqueous solutions, and other chemicals and products, unless there are unique physicochemical properties associated with these materials that may interfere with the accuracy of the LLNA. Aqueous solutions should be tested in an appropriate vehicle that maintains sufficient contact of the test article with the skin. The ICCVAM evaluation and complete recommendations for expanded uses of the LLNA are provided in *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, available at *http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-app/TMER.htm*).

ICCVAM evaluated the new versions and applications of the LLNA in response to a 2007 nomination from CPSC (*http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/CPSC_LLNA_nom.pdf*). The nomination requested that ICCVAM assess (1) the validation status of the LLNA limit dose procedure (*i.e.,* the reduced LLNA); (2) modified LLNA test method protocols that do not require the use of radioactive materials; (3) the use of the LLNA to test mixtures, aqueous solutions, and metals; and (4) the use of the LLNA as a stand-alone assay to determine ACD potency categories for hazard classification. ICCVAM recommendations on an updated LLNA test method protocol that included the reduced LLNA were communicated to Federal agencies and each of the 15 ICCVAM agencies concurred with the ICCVAM recommendations for the reduced LLNA. ICCVAM has completed the evaluation of the LLNA for its validity for potency categorization of chemicals causing ACD in humans. Final ICCVAM recommendations will be forwarded to Federal agencies in 2011.

Agency Responses to ICCVAM Recommendations

In June 2010, ICCVAM forwarded final test method recommendations for the LLNA BrdU-ELISA, LLNA: DA and the expanded uses of the LLNA to U.S. Federal agencies for consideration (74 FR 50212), in accordance with the ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3(e)(4)). The Act requires agencies to review ICCVAM test method recommendations and notify ICCVAM in writing of their findings no later than 180 days after receipt of recommendations. The Act also requires ICCVAM to make ICCVAM recommendations and agency responses available to the public. Agency responses are to include identification of relevant test methods for which the ICCVAM test method recommendations may be added or substituted, and indicate any revisions or planned revisions to existing guidelines, guidances, or regulations to be made in response to these recommendations.

Federal agency responses include acceptance decisions and agreement with the test method recommendations for the LLNA: BrdU-ELISA, LLNA: DA and the expanded uses of the LLNA. Several agencies also indicated that they would communicate the ICCVAM recommendations to stakeholders and encourage their appropriate use. Agency responses are available at *http://iccvam.niehs.nih.gov/methods/immunotox/llna.htm.*

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological and safety-testing methods that more accurately assess the safety and hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), or replace animal use. The ICCVAM Authorization Act of 2000 established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM (42 U.S.C. 285l–3(a)). NICEATM administers ICCVAM, provides scientific and operational support for ICCVAM-related activities, and conducts independent validation studies to assess the usefulness and limitation of new, revised, and alternative test methods. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. NICEATM and ICCVAM welcome the public nomination of new, revised, and alternative test methods for validation studies and technical evaluations. Additional information about ICCVAM and NICEATM can be found on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov).*

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