Draft ICCVAM Test Method Recommendations:

Non-Radioactive LLNA: BrdU-ELISA

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1.0 Draft Recommendations: Test Method Uses and Limitations

Background: ICCVAM is currently evaluating the validation status of the LLNA: BrdU-ELISA as a non-radioactive alternative to the traditional LLNA (i.e., ICCVAM 1999, Dean et al. 2001, EPA 2003) to identify substances that may cause allergic contact dermatitis (ACD). The LLNA: BrdU-ELISA differs from the traditional LLNA only in that it assesses cell proliferation by measuring the incorporation of bromodeoxyuridine (BrdU), instead of radiolabeled thymidine or iodine, into the DNA of dividing lymphocytes. The incorporation of BrdU is measured using an enzyme-linked immunosorbent assay. A comprehensive evaluation of this test method, including its accuracy and reliability compared to the traditional LLNA, is provided in the draft ICCVAM LLNA: BrdU-ELISA Background Review Document (BRD).

Draft Recommendations:

Based on the available database of 23 substances (16 sensitizers and 7 nonsensitizers as determined by the traditional LLNA) and its performance (accuracy of 83% [19/23]) to 91% [21/23] depending on whether the traditional LLNA stimulation index decision criteria of 3.0 or a revised one of 1.3 was used) compared to the traditional LLNA, the LLNA: BrdU ELISA may be useful for identifying substances as potential skin sensitizers and nonsensitizers. However, at this time, more information and data are needed before a recommended use of the LLNA: BrdU-ELISA can be made. Specifically:

- A sufficiently detailed protocol of this test method, including a defined and adequately justified decision criteria for distinguishing between sensitizers and non-sensitizers, is required.
- Quantitative results are needed for all of the studies included in this evaluation. This is critical because there appear to be inconsistencies in test results among multiple reports that need to be reconciled.
- A formal evaluation of interlaboratory reproducibility needs to be conducted. Two interlaboratory validation studies have reportedly been completed for

the LLNA: BrdU-ELISA, but information about the study designs, the protocol used, and the results are not yet available.

- Eight of the 18 required substances in the draft ICCVAM performance standards (seven sensitizers and one nonsensitizer) have been tested in the LLNA: BrdU-ELISA. EC3 values are available for only four of the seven sensitizers tested. While all eight substances were correctly identified based on a "yes/no" decision, all four of the reported EC3 values were outside of the proposed acceptability range of 0.5x to 2.0x the historical EC3 values obtained in the traditional LLNA, as prescribed in the draft ICCVAM performance standards.

2.0 Draft Recommendations: Test Method Protocol for the LLNA: BRDU-ELISA

All aspects of the recommended ICCVAM LLNA test method protocol (ICCVAM 1999, Dean et al. 2001, EPA 2003) should be followed with the exception of the method used to assess lymphocyte proliferation. Measurement of the amount of BrdU incorporated into cells of the auricular lymph nodes using an ELISA is described in **Appendix A** of the ICCVAM BRD. However, a detailed protocol for this test method is not yet available.

3.0 Draft Recommendations: Future Studies

- To allow for a more comprehensive evaluation of the performance of the LLNA: BrdU-ELISA compared to the traditional LLNA, more nonsensitizers should be evaluated within and across laboratories.
- The ICCVAM recommended reference substances (HCA and DCNB) in the ICCVAM draft performance standards for intra- and inter-laboratory reliability assessments should be tested.
- The applicability of the LLNA: BrdU-ELISA to testing metals, mixtures, and aqueous solutions (current limitations of the traditional LLNA) should be evaluated to determine if this method can be used to assess the ACD potential of these types of substances.

- Additional studies should be designed to determine the most appropriate threshold value for the decision criteria used to identify sensitizers.

4.0 Draft Performance Standards

Performance standards for the LLNA: BrdU-ELISA are not proposed at this time although ICCVAM is currently developing performance standards for the traditional LLNA (http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm). These draft test method performance standards are proposed to evaluate the performance of LLNA test methods that incorporate specific protocol modifications to measure lymphocyte proliferation compared to the traditional LLNA. ICCVAM does not anticipate the need to develop separate performance standards for the LLNA: BrdU-ELISA.