Draft ICCVAM Test Method Recommendations
Non-Radioactive LLNA: BrdU-FC

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This document provides draft ICCVAM recommendations on the non-radioactive LLNA: BrdU-FC, a test method for assessing the allergic contact dermatitis potential of chemicals and products for regulatory testing. These draft recommendations are based on information and data provided in a draft background review document available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm, and will be considered by an independent scientific peer review panel that will meet in public session on April 28–29, 2009. Public comments are welcome. More information is available in the *Federal Register* notice of the meeting (74 FR 8974) available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E9-4280.pdf. ICCVAM will finalize these recommendations after consideration of comments from the peer review panel, the public, and its scientific advisory committee.

These draft recommendations do not represent the official position of any Federal agency.

1.0 Draft Recommendations: Test Method Uses and Limitations

Background

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is currently evaluating the validation status of the LLNA: BrdU-FC as a non-radioactive modification of the traditional LLNA (i.e., ICCVAM 1999; Dean et al. 2001) to identify substances that may cause allergic contact dermatitis (ACD). While the traditional LLNA assesses cellular proliferation by measuring the incorporation of radioactive tritiated thymidine into the deoxyribonucleic acid (DNA) of dividing lymph node cells, the LLNA: BrdU-FC assesses the same endpoint by measuring incorporation of the thymidine analog bromodeoxyuridine (BrdU) using flow cytometry. The LLNA: BrdU-FC also includes a routine assessment of ear swelling as a measure of excessive irritation. Finally, the LLNA: BrdU-FC includes enhancements (referred to hereafter as the eLLNA: BrdU-FC) for substances with a stimulation index (SI) ≥ 3 and ear swelling > 25%. The eLLNA: BrdU-FC includes assessment of immunophenotypic markers to distinguish sensitizers from irritants (see **Section 2.0** of the draft LLNA: BrdU-FC Background Review Document). The updated draft ICCVAM LLNA: BrdU-FC Background Review Document (ICCVAM 2009b) includes additional information and discussion of the evaluation of this test method.

ICCVAM has proposed test method performance standards for the LLNA (ICCVAM 2009a)[1] to evaluate the performance of modified LLNA test methods that are mechanistically and functionally similar to the traditional LLNA. However, because the validation studies for the LLNA: BrdU-FC test method were completed before development of LLNA performance standards and because data for all of the performance standards reference substances were not available, the ICCVAM LLNA performance standards were not used to evaluate the LLNA: BrdU-FC.

Draft Recommendations

Based on the available database of 45 substances (26 sensitizers and 19 nonsensitizers tested in the traditional LLNA) and the LLNA: BrdU-FC's demonstrated intralaboratory reproducibility and demonstrated accuracy compared to the traditional LLNA (accuracy of 93% [42/45], false positive rate of 13% [2/16], and a false negative rate of 3% [1/29][2]), the LLNA: BrdU-FC may be useful for identifying substances as potential skin sensitizers or

[1] Available at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm.

[2] The one false negative substance is aniline, which did not generate a strongly positive result in the traditional LLNA (EC3 = 48%, maximum SI = 3.6 at 50% in AOO).

nonsensitizers. However, although NICEATM has requested original records for all of the studies included in this evaluation, they have not been submitted yet. As a result, the reported data has not been independently audited to confirm that it is the same as that originally recorded. Validation criteria adopted by ICCVAM and its member agencies state that "all data supporting the assessment of the validity of a test method must be available for review" (ICCVAM 1997). In addition, interlaboratory reproducibility of the LLNA: BrdU-FC has not been assessed. For these reasons, ICCVAM is deferring formal recommendation of this method pending receipt of this information.

2.0 Draft Recommendations: Test Method Protocol for the LLNA: BrdU-FC

The draft ICCVAM-recommended LLNA: BrdU-FC protocol, which is based on the protocol developed by MB Research Labs (2001, see **Appendix A** of the draft ICCVAM LLNA: BrdU-FC Background Review Document), incorporates all aspects of the recently updated ICCVAM-recommended LLNA test method protocol (ICCVAM 2009a), except for those procedures unique to the conduct of the LLNA: BrdU-FC (see **Appendix A** of the draft background review document). Following are key aspects included in the ICCVAM-recommended protocol:

- The high dose should be the maximum soluble concentration that does not produce systemic toxicity and/or excessive local irritation.
- A minimum of four animals per dose group is recommended.
- Collection of individual animal data is recommended.
- Inclusion of a concurrent vehicle control and positive control in each study is recommended.

Additionally, ICCVAM recommends a measure of variability of the positive control response over time. Laboratories should maintain a historical database of positive control SI values to allow comparison of results to the mean historical SI. There could be cause for concern if a negative result for a test substance is accompanied by a positive control SI value significantly lower than the mean historical SI.

3.0 Draft Recommendations: Future Studies

- Before this test method is used in other laboratories, interlaboratory reproducibility should be evaluated to determine if acceptable results can be obtained. This can be accomplished by following the recommendations in the ICCVAM LLNA Performance Standards (ICCVAM 2009a).
- Consistent with recommendations for the traditional LLNA, additional data from LLNA: BrdU-FC studies of metal compounds with comparative human and/or guinea pig data are needed in order to more comprehensively evaluate the method's ability to test metal compounds.
- Efforts should be made to identify additional human data and human experience for test substances. This data may be used to further assess the usefulness and limitations of this and other versions of the LLNA for identifying human-sensitizing substances (e.g., formulations).
- Records from all future studies should be retained in a manner such that they can be readily accessed for review.
- The "sequential strategy" used in the enhanced LLNA: BrdU-FC protocol, which incorporates immunophenotypic endpoints via flow cytometry to identify potential false positives that are actually nonsensitizing skin irritants, should be further investigated to more fully characterize its usefulness and limitations for this purpose.

4.0 Draft Performance Standards

Unique performance standards for the LLNA: BrdU-FC are not proposed at this time. However, ICCVAM has developed draft performance standards for the traditional LLNA (http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm). Because the LLNA: BrdU-FC is mechanistically and functionally similar to the traditional LLNA, ICCVAM proposes that the ICCVAM LLNA performance standards (ICCVAM 2009a) can be used to evaluate future modifications of the LLNA: BrdU-FC.

5.0 References

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