Draft ICCVAM Test Method Recommendations:

Non-Radioactive LLNA: DA

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1.0 Draft Recommendations: Test Method Uses and Limitations

Background: ICCVAM is currently evaluating the validation status of the LLNA: DA 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: DA differs from the traditional LLNA in that it assesses cell proliferation by measuring the level of adenosine triphosphate (ATP) in the auricular lymph nodes, instead of the amount of radiolabeled thymidine or iodine incorporated into the DNA of dividing lymphocytes. The LLNA: DA also differs from the traditional LLNA in the test substance treatment and sampling schedule and the addition of pretreating the application site with sodium lauryl sulfate (SLS) (see **Section 2.0** of the draft LLNA: DA BRD).

Additional information and discussion of the evaluation of this test method are provided in the draft ICCVAM LLNA: DA BRD (ICCVAM 2007).

Draft Recommendations:

- Based on the available database of 29 substances (19 sensitizers and 10 nonsensitizers, when tested in the traditional LLNA) and demonstrated performance (accuracy of 93% [27/29], sensitivity of 95% [18/19], specificity of 90% [9/10]) compared to the traditional LLNA, the LLNA: DA may be useful for identifying substances as potential skin sensitizers and non-sensitizers. However, this recommendation is contingent upon receipt of additional data and information. Otherwise, the LLNA: DA cannot be appropriately evaluated.

- Based on the current false negative and positive rates (5% [1/19] and 10% [1/10], respectively) compared to the traditional LLNA, negative and positive results should be considered in a weight-of-evidence decision along with other relevant information. If false results are suggested based on a weight-of-evidence evaluation, confirmatory testing in the traditional LLNA or another accepted skin sensitization test method should be considered.

- In testing situations where dose-response information is not required, consideration should be given to using the LLNA: DA as a Limit Dose Procedure, which will further reduce animal use by 40% (15 vs. 25).

However, the deficiencies in the current database for the LLNA: DA include:

- A commonly used positive control in the traditional LLNA, 2-mercaptobenzothiazole, was clearly negative in the LLNA: DA. In four tests, the highest SI obtained was 2.0 at a concentration of 10%. The mean EC3 reported for this substance in the draft ICCVAM performance standards is 2.5%. A discussion regarding the potential reason for this discordance has not been provided.
- All of the studies included in the performance evaluation are based on data obtained from poster or platform presentations. Manuscripts detailing these results are reported to be currently undergoing peer review for publication. For this reason, none of the original records have been provided. As a result, an independent audit could not be conducted to confirm that the reported data is the same as the data originally recorded.
- A detailed protocol from Daicel Chemical Industries, Ltd. has not been provided.

Draft Recommendations: Test Method Protocol for the LLNA: DA

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 treatment protocol and the assessment of lymphocyte proliferation. This includes using a minimum of five treated animals per dose group, instead of four, to comply with the recommended ICCVAM LLNA test method protocol. The modifications allowed should follow the procedures described by Idehara et al. (see **Appendices A and D** of the draft LLNA: DA BRD) with respect to:

- Pretreatment with 1% SLS prior to each dose application
- The schedule for dosing (i.e., dosing on days 1, 2, 3, and 7) and subsequent removal of the lymph nodes (day 8)
- Measurement of ATP content in auricular lymph nodes

3.0 Draft Recommendations: Future Studies

- To allow for a more comprehensive evaluation of the performance of the LLNA: DA compared to the traditional LLNA, more nonsensitizers should be evaluated within and across laboratories.
- Prior to the use of this test method in other laboratories, the reference substances (HCA and DNCB) recommended for intra- and inter-laboratory reproducibility assessments in the ICCVAM draft performance standards should be tested to determine if acceptable results can be obtained.
- Additional efforts should be made to understand the potential for substances to be falsely identified compared to guinea pig and human data.
- The applicability of the LLNA: DA 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.

4.0 Draft Performance Standards

Performance standards for the LLNA: DA 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. Since the LLNA: DA has made major modifications to the traditional LLNA (i.e. test substance treatment and sampling schedule), the current LLNA draft test method performance standards do not apply. However, ICCVAM does not anticipate the need at this time to develop separate performance standards for the LLNA: DA.