DRAFT ICCVAM TEST METHOD RECOMMENDATIONS

LLNA Limit Dose Procedure

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1.0 Draft Recommendations: Test Method Uses and Limitations

Background: ICCVAM is currently evaluating the validation status of the LLNA limit dose procedure proposed as a reduction alternative to the traditional LLNA. Unlike the traditional LLNA, which requires the use of three dose groups, the LLNA limit dose procedure uses a single dose group and therefore does not generate dose response information. This evaluation examined 471 traditional LLNA studies (466 substances, 153 non-sensitizers and 313 sensitizers) and builds on a recent assessment of this procedure by the ECVAM Scientific Advisory Committee (ESAC 2007), which reviewed an evaluation of 211 traditional LLNA studies (211 substances) by Kimber et al. (2006).

Based on this analysis, the LLNA limit dose procedure correctly identified all 153 non-sensitizers (specificity of 100%) and correctly identified 308 of 313 sensitizers (sensitivity of 98.4%). Of the five incorrectly classified sensitizers, four were considered "weak" sensitizers and one was considered a "moderate" sensitizer based on a proposed potency categorization scheme using EC3 values (Gerberick et al. 2004). No "extreme" or "strong" sensitizers were underpredicted as non-sensitizers. The overall accuracy (concordance) with the traditional LLNA is 98.9% (461/466). Additional information and discussion of these results are provided in the draft Background Review Document (ICCVAM 2007).

Draft Recommendation: The LLNA limit dose procedure should be used for the hazard identification of skin sensitizing substances, if dose response information is not needed, provided there is adherence to all other LLNA protocol specifications, as described in ICCVAM (1999), Dean et al. (2001), and EPA (2003). This recommendation is based on its performance compared to the traditional LLNA and because the limit dose procedure reduces animal use by 40% compared to the traditional LLNA, In addition, users of this test method should specifically be aware that:

- The limit dose should be the highest soluble concentration that does not induce overt systemic toxicity and/or excessive local irritation (ICCVAM 1999, Dean et al. 2001); any other approach, such as one based on using a pre-established threshold dose level is inappropriate.

  - For example, Kimber et al. (2006) have proposed a 10% threshold concentration at which all negative results would be considered valid. However, 51 substances (16% [51/313]) within the NICEATM database of LLNA studies were non-sensitizers at concentrations of ≤ 10%, but sensitizers at concentrations >10%.

- Vehicle selection should be based on the recommendations provided in the ICCVAM recommended LLNA protocol (ICCVAM 1999, Dean et al. 2001).

- As noted, there is a small possibility of a false negative result (1.6% [5/313]) when compared to the traditional LLNA. This information should be considered when evaluating results from the limit dose procedure, and negative results should always be subjected to a weight-of-evidence evaluation of supplemental information (e.g., possibility of downturn in response at the limit dose, test results with similar substances, peptide binding activity, other testing data). If false negative results are suggested, confirmatory testing in the traditional LLNA or another accepted skin sensitization test method should be considered.

- All of the testing limitations that apply to the traditional LLNA apply also to the LLNA limit dose procedure. For example, consistent with the traditional LLNA, the LLNA limit dose procedure may not be suitable for use with certain types of test substances, such as metallic compounds, mixtures, high molecular weight compounds that cannot penetrate the stratum corneum, strong dermal irritants, chemicals whose pharmacodynamic activity is to release dermal cytokines that cause local lymph node proliferation (e.g., certain pharmaceuticals such as imiquimod [Gaspari 2007]), and materials that do not adhere to the ear for an acceptable time during the experiment.

2.0 Draft Recommendations: Test Method Protocol for the LLNA Limit Dose Procedure

The test method protocol used to conduct the LLNA limit dose procedure should be identical to the traditional LLNA test method protocol except for the number of dose groups tested. In the LLNA limit dose procedure, in addition to the concurrent vehicle and positive control groups, each test substance is tested at one dose level only (the high dose), whereas in the traditional LLNA, each test substance is tested at a minimum of three dose levels. In both cases, the high dose is the maximum concentration consistent with solubility and the need to avoid local and other systemic adverse effects. In both procedures, a Stimulation Index (SI) is calculated as the ratio of ^{3}H-thymidine incorporation in the auricular lymph nodes of the treated animals in the group with the highest response to that in the vehicle control animals. In the LLNA limit dose procedure, as in the traditional LLNA, the threshold for classifying a substance as a skin sensitizer is an SI $\geq$ 3.

The ICCVAM recommended LLNA protocol, which is based on an Independent Expert Peer Review Panel Evaluation of the LLNA (ICCVAM 1999), can be found on the ICCVAM-NICEATM website at http://iccvam.niehs.nih.gov/methods/immunotox/llnadocs/LLNAProt.pdf (ICCVAM 2001). The LLNA procedure is also described in the EPA Health Effects Test Guidelines (EPA 2003) and a modified version is described in OECD TG 429 (OECD 2002).

3.0 Draft Recommendations: Future Studies

To further improve the predictive performance of the LLNA limit dose procedure compared to the traditional LLNA, additional efforts should be made to understand and reduce the few substances falsely identified as non-sensitizers. Efforts should be made to identify guinea pig or human data and human experience for these and other substances that exhibit abnormal dose responses in the LLNA, including collection and assessment of post-marketing and/or occupational exposure information.

4.0 Draft Performance Standards

ICCVAM is currently developing performance standards for the traditional LLNA, which may in turn be applied to the LLNA limit dose procedure (http://iccvam.niehs.nih.gov/docs/immunotox_docs/llna/LLNAPerfStd12Sep07FD.pdf). These draft test method performance standards are proposed to evaluate the performance of LLNA test methods that incorporate specific modifications to measure lymphocyte proliferation compared to the traditional LLNA. Limit dose procedures based on modified test method protocols that adhere to the LLNA performance standards would therefore likely be considered acceptable for hazard identification purposes.