DRAFT ICCVAM TEST METHOD RECOMMENDATIONS

Updated Assessment of the Validity of the LLNA for Mixtures, Metals, and Aqueous Solutions

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1.0 Draft Recommendations: Test Method Uses and Limitations

Background: ICCVAM is currently updating the original validation report of the LLNA (ICCVAM 1999) based on a comprehensive review of available data and information regarding the current validity of the LLNA for assessing the skin sensitizing potential of mixtures, metal compounds, and substances in aqueous solutions. The information is based on a retrospective review of LLNA data derived from a database of over 500 substances (including mixtures) tested in the LLNA and builds on the previous ICCVAM evaluation of the LLNA, which was based on 209 substances (ICCVAM 1999). In the original ICCVAM report, the performance of the LLNA was compared to 1) the results from guinea pig tests and 2) information about sensitizers in humans (e.g., human maximization test [HMT] results, substances used in human repeat insult patch test [HRIPT], clinical data), where available. This addendum updates the LLNA performance analyses for mixtures, metal compounds, and substances in aqueous solutions when compared to human and guinea pig results.

Draft Recommendations - Use of the LLNA to Test Mixtures:

The updated NICEATM LLNA database contains test results on 18 mixtures, 15 of which have comparative guinea pig data while none have comparative human data. In the guinea pig, six were classified as sensitizers and nine as non-sensitizers. Ten of the 15 mixtures are pesticides (i.e., herbicides, fungicides, insecticides) and four are dyes. Information on the product class for the remaining mixture was not identified. Information on the ingredients in the various mixtures is known for only one of the 15 mixtures. Information on physical form was available for five of the 15 mixtures; four are solids and one is a liquid. In the LLNA, 11 were tested in an aqueous vehicle and four were tested in a non-aqueous vehicle. Compared to guinea pig, the LLNA has an accuracy of 53% (8/15), a sensitivity of 50% (3/6), a specificity of 56% (5/9), a false positive rate of 44% (4/9), and a false negative rate of 50% (3/6).

Due to the limitations associated with the available database for mixtures (i.e., unknown formulae, lack of human data), more data are needed before a recommendation on the usefulness and limitations of the LLNA for testing mixtures can be made at this time.

Draft Recommendations - Use of the LLNA to Test Metal Compounds:

The updated NICEATM LLNA database contains test results on 48 studies involving 17 metal compounds representing 13 different metals (mixtures containing metals are excluded from this analysis). All 17 metal compounds had comparative human data and eight had comparative guinea pig data. Among the 13 metals tested multiple times, nickel was tested four times in the LLNA as nickel sulfate, three times as nickel chloride, and once as a nickel (II) salt. Because nickel was classified as a sensitizer in four of these studies and as a non-sensitizer in the other four, a decision was made to exclude nickel compounds from the LLNA metals performance analysis.

For these remaining 14 metal compounds (13 metals), the LLNA had an accuracy of 86% (12/14), a sensitivity of 100% (9/9), a specificity of 60% (3/5), a false positive rate of 40% (2/5) and a false negative rate of 0% (0/9), when compared to human results. The two false positive compounds were copper chloride and zinc sulfate. All six of the metal compounds (six different metals with nickel compounds excluded) with comparative guinea pig test results were predicted as sensitizers by the LLNA. For these metal compounds, the LLNA had an accuracy of 83% (5/6), a sensitivity of 100% (5/5), a specificity of 0% (0/1), a false positive rate of 100% (1/1) and a false negative rate of 0% (0/5), when compared to guinea pig test results. When comparing the performance of the LLNA and the guinea pig tests, for the six metal compounds tested in all three species, to human results, the LLNA had an accuracy of 88% (7/8), a sensitivity of 100% (7/7), a specificity of 0% (0/1), a false positive rate of 100% (1/1) and a false negative rate of 0% (0/7); the accuracy of the guinea pig against the human remained the same as previously calculated.

Based on these data, the LLNA appears useful for the testing of metal compounds, with the exception of nickel. Currently, nickel compounds should not be tested in the LLNA. However, the false positive rate of 40% (2/5) should be considered when evaluating positive results for metal compounds tested in the LLNA. In this situation, p LLNA results should always be subjected to a weight-of-evidence evaluation of supplemental information (e.g., peptide binding activity, other testing data). If false positive results are

suggested, confirmatory testing in the traditional LLNA or another accepted skin sensitization test method should be considered.

Draft Recommendations - Use of the LLNA to Test Substances in Aqueous Solutions:

The updated NICEATM LLNA database contains test data on 47 studies that involved testing 21 substances in an aqueous solution (i.e., vehicle). Aqueous solutions are defined as single substances tested in a vehicle containing at least 20% water and were not evaluated separately during the original ICCVAM validation of the LLNA (ICCVAM 1999). Among these 21 substances tested in aqueous solutions, six were pesticides (i.e. herbicide, fungicides, insecticides); this is the only product class represented by more than one substance in an aqueous solution.

Human data were available for only four (3 sensitizers/1 non-sensitizer in humans) of the 21 substances tested in aqueous solutions. None of the four were pesticides. In comparison to the human data, the LLNA has an accuracy of 50% (2/4), a sensitivity of 33% (1/3), a specificity of 100% (1/1), a false positive rate of 0% (0/1) and a false negative rate of 67% (2/3). Of the 21 substances tested in aqueous solutions, guinea pig data were available for six (2 sensitizers/4 non-sensitizers in the guinea pig). Four of these six substances were pesticides (one sensitizer/3 non-sensitizers). Based on the guinea pig test data, the LLNA has an accuracy of 50% (3/6), a sensitivity of 50% (1/2), a specificity of 50% (2/4), a false positive rate of 50% (2/4), and a false negative rate of 50% (1/2). There were only two substances tested in aqueous solutions in the LLNA and for which there was comparative guinea pig and human data.

Due to the very limited number of substances tested in aqueous solutions, more data are needed before a recommendation on the usefulness and limitations of the LLNA for testing substances in aqueous solutions can be made at this time.

2.0 Draft Recommendations: Test Method Protocol for the LLNA

The ICCVAM recommended LLNA protocol, which is based on recommendations from 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. The LLNA

procedure is also described in the EPA Health Effects Test Guidelines (EPA 2003) and OECD TG 429 (OECD 2002).

3.0 Draft Recommendations: Future Studies

To more comprehensively evaluate the ability of the LLNA to be used for testing metal compounds, additional data from LLNA studies on such compounds with comparative human and/or guinea pig data are needed. In addition, efforts should be made to identify additional human data and human experience for mixtures and substances tested in an aqueous solution, in order to adequately evaluate the use of the LLNA for these testing situations relative to humans, the species of interest. It is critical that any results from studies using mixtures be linked to the individual components of the mixture to allow for an adequate assessment of potential mixture effects in the LLNA.

4.0 Draft Performance Standards

ICCVAM is currently developing performance standards for the traditional LLNA (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.

5.0 References

EPA. 2003. Health Effects Test Guideline, OPPTS 870.2600. Skin Sensitization EPA 712–C–03–197. Washington, DC: U.S. Environmental Protection Agency. Available: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf [accessed 20 September 2007].

ICCVAM 1999. The murine local lymph node assay: A test method for assessing the allergic contact dermatitis potential of chemical/compounds. NIH Publication No. 99-4494. Research Triangle Park, NC: National Toxicology Program.

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OECD. 2002. Test guideline 429. Skin Sensitisation: Local Lymph Node Assay, adopted April 24, 2002. In: OECD Guidelines for Testing of Chemicals. Paris:OECD.