

1 **Draft ICCVAM Test Method Recommendations**

2 Updated Assessment of the Validity of the LLNA for Testing Pesticide
3 Formulations and Other Products, Metals, and Substances in
4 Aqueous Solutions
5

6 **March 2009**
7

8 **This document provides draft ICCVAM recommendations on the applicability domain**
9 **of the LLNA, a test method for assessing the allergic contact dermatitis potential of**
10 **chemicals and products for regulatory testing. These draft recommendations are based**
11 **on information and data provided in draft Addendum No. 1 to the ICCVAM Report:**
12 ***The Murine Local Lymph Node Assay (LLNA): A Test Method for Assessing the Allergic***
13 ***Contact Dermatitis Potential of Chemicals/Compounds* (NIH Pub. No. 99-4494), available**
14 **at http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm, and will be**
15 **considered by an independent scientific peer review panel that will meet in public**
16 **session on April 28-29, 2009. Public comments are welcome. More information is**
17 **available in the *Federal Register* notice of the meeting (74 FR 8974) available at**
18 **<http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E9-4280.pdf>. ICCVAM will**
19 **finalize these recommendations after consideration of comments from the peer review**
20 **panel, the public, and its scientific advisory committee.**

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

23

23 **1.0 Draft Recommendations: Test Method Uses and Limitations**

24 **Background**

25 The Interagency Coordinating Committee on the Validation of Alternative Methods
26 (ICCVAM) is currently updating the original validation report of the LLNA (ICCVAM
27 1999) based on a comprehensive review of available data and information regarding the
28 current validity of the LLNA for assessing the skin-sensitizing potential of pesticide
29 formulations and other products, metal compounds, and substances in aqueous solutions. The
30 information is based on a retrospective review of data derived from over 600 substances,
31 including 104 pesticide formulations, tested in the LLNA. The current evaluation builds on
32 the previous ICCVAM evaluation of the LLNA, which was based on 209 substances
33 (ICCVAM 1999). This addendum updates the LLNA performance analyses for pesticide
34 formulations and other products, metal compounds, and substances in aqueous solutions
35 when compared to human and/or guinea pig test results.

36 **Draft Recommendations – Use of the LLNA to Test Pesticide Formulations and Other** 37 **Products**

38 **Pesticide Formulations:** The updated NICEATM LLNA database contains test results on
39 104 pesticide formulations, 22 of which have comparative guinea pig data. None have
40 comparative human data. Nine out of the approximately 450 active ingredients registered
41 with EPA were represented among these 22 formulations. Furthermore, approximately 40
42 different classes of pesticides are registered with EPA, of which these nine active ingredients
43 represent a small proportion (i.e., one insecticide, six herbicides and two fungicides). Based
44 on these 22 pesticide formulations, the concordance (accuracy) of the LLNA results
45 compared to guinea pig data is 54% (12/22), with an overprediction (“false positive”) rate of
46 53% (10/19) and underprediction (“false negative”) rate of 0% (0/3). Thus, there is a greater
47 likelihood of obtaining a positive result in LLNA (13/22; 59%) than in a guinea pig test
48 (3/22; 14%). All three formulations that were identified as positive in the guinea pig tests
49 were also identified as positive in the LLNA. Although human data are not available for
50 these pesticide formulations to confirm their human sensitization potential, these data
51 indicate that the LLNA is more likely to classify a pesticide formulation as a sensitizer than
52 the guinea pig tests. Although only a relatively small subset of registered pesticide active
53 ingredients and classes were available for analysis, these data indicate that the LLNA has
54 utility for hazard classification of pesticide formulations, provided that the potential for
55 possible overclassification is not a limitation.

56 **Essential Oils:** The current LLNA database also contains data for 12 essential oils for which
57 there are comparative LLNA and human data. Based on LLNA results for these essential oils,
58 75% (9/12) were sensitizers and 25% (3/12) were nonsensitizers. However, based on human
59 clinical studies, only 33% (4/12) of these substances tested as sensitizers. Based on this
60 limited database, the concordance (accuracy) of the LLNA results compared to human
61 sensitization data is 42% (5/12), with an overprediction (“false positive”) rate of 75% (6/8)
62 and underprediction (“false negative”) rate of 25% (1/4). There are no comparative data from
63 guinea pig tests with these essential oils. Therefore, a comparison of the performance of the
64 LLNA and the guinea pig tests relative to the human outcome is not possible. Until a larger
65 number of known human sensitizers that are essential oils have been tested in the LLNA, a
66 definitive recommendation on the usefulness of the LLNA for this applicability domain
67 cannot be made.

68 **Dyes:** The current LLNA database contains data for 6 dyes, for which there is LLNA and GP
69 data. Compared to GPMT outcomes, the LLNA concordance (accuracy) is 33% (2/6), the
70 overprediction (“false positive”) rate is 100% (1/1) and the underprediction (“false negative”) rate
71 is 60% (3/5). Due to the very limited number of dyes for which comparative human or
72 guinea pig reference data are available, more data are needed before a recommendation on
73 the usefulness and limitations of the LLNA for testing these types of substances can be made.

74 **Draft Recommendations – Use of the LLNA to Test Metal Compounds**

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

83 For these remaining 14 metal compounds (13 metals), the LLNA concordance (accuracy) is
84 86% (12/14), the overprediction (“false positive”) rate is 40% (2/5) and the underprediction
85 (“false negative”) rate is 0% (0/9), when compared to human results. The two false positive
86 compounds were copper chloride and zinc sulfate. All six of the metal compounds (six
87 different metals with nickel compounds excluded) with comparative guinea pig test results

88 were predicted as sensitizers by the LLNA. For these metal compounds, the LLNA
89 concordance (accuracy) is 83% (5/6), the overprediction (“false positive”) rate is 100% (1/1),
90 and the underprediction (“false negative”) rate is 0% (0/5), when compared to guinea pig test
91 results. When comparing the performance of the LLNA and the guinea pig tests for the six
92 metal compounds tested in all three species (i.e., mice, guinea pigs, and humans) to human
93 results, the LLNA concordance (accuracy) is 83% (5/6), the overprediction (“false positive”) rate
94 is 100% (1/1) and the underprediction (“false negative”) rate is 0% (0/5). By
95 comparison, the guinea pig test concordance (accuracy) is 100% (6/6), the overprediction
96 (“false positive”) rate is 0% (0/1) and the underprediction (“false negative”) rate is 0% (0/5)
97 against the human.

98 Based on these data, the LLNA appears useful for the testing of metal compounds (not
99 including metal formulations), with the exception of nickel. Currently, nickel compounds
100 should not be tested in the LLNA.

101 **Draft Recommendations – Use of the LLNA to Test Substances in Aqueous Solutions**

102 The updated NICEATM LLNA database contains test data on 43 studies that involved testing
103 24 substances in an aqueous solution. Pesticide formulations that were considered in the
104 analysis discussed previously were also included in this evaluation, so this database has the
105 same limitations as discussed previously. The substances included in this evaluation contain
106 at least 20% water. Most (22/24) of these substances were tested in the vehicle 1% Pluronic
107 L92. Based on LLNA results for these substances 50% (12/24) were sensitizers and 50%
108 (12/24) were nonsensitizers. However, based on guinea pig results, only 17% (4/24) tested as
109 sensitizers. Based on this limited database, the concordance (accuracy) of the LLNA
110 compared to guinea pig sensitization data is 58% (14/24), the overprediction (“false
111 positive”) rate is 45% (9/20) and the underprediction (“false negative”) rate is 25% (1/4).
112 Among the 10 substances for which LLNA and GP results were discordant, only one is
113 negative in the LLNA and positive in the guinea pig. There are no comparative data from
114 guinea pig tests with these substances. These data suggest that the LLNA is more likely than
115 the GP to classify a substance tested in an aqueous solution as a sensitizer.

116 Although the database analyzed was limited, the data evaluation indicates that the LLNA has
117 utility for regulatory hazard classification of aqueous solutions, provided that the potential
118 for possible overclassification is not a limitation.

119 **2.0 Test Method Protocol for the LLNA**

120 An updated version of the validated ICCVAM-recommended LLNA test method protocol
121 (ICCVAM 1999; Dean et al. 2001; EPA 2003; OECD 2002), which reflects the conclusions
122 and recommendations of an ICCVAM Independent Scientific Peer Review Panel convened
123 in March 2008 (see http://iccvam.niehs.nih.gov/methods/immunotox/llna_PeerPanel.htm),
124 has recently been developed (Appendix A of ICCVAM 2008). This revised protocol is
125 recommended for all future LLNA studies.

126 **3.0 Draft Recommendations: Future Studies**

127 To more comprehensively evaluate the ability of the LLNA to be used for testing metal
128 compounds, additional data from LLNA studies on such compounds with comparative
129 human and/or guinea pig data are needed. In addition, efforts should be made to identify
130 additional human data and human experience for pesticide formulations and other products,
131 as well as substances tested in an aqueous solution, in order to adequately evaluate the use of
132 the LLNA for these testing situations relative to humans, the species of interest.

133 **4.0 Performance Standards**

134 ICCVAM has developed performance standards for the traditional LLNA
135 (http://iccvam.niehs.nih.gov/methods/immunotox/llna_PerfStds.htm). These test method
136 performance standards are proposed to evaluate the performance of LLNA test methods that
137 incorporate specific modifications to measure lymphocyte proliferation compared to the
138 traditional LLNA.

139 **5.0 References**

140 Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. ICCVAM
141 evaluation of the murine local lymph node assay. Conclusions and recommendations of an
142 independent scientific peer review panel. Regul Toxicol Pharmacol 34:258-273.

143 EPA. 2003. Health Effects Test Guideline, OPPTS 870.2600. Skin Sensitization EPA 712–
144 C–03–197. Washington, DC: U.S. Environmental Protection Agency. Available:
145 [http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_G](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf)
146 [uidelines/Revised/870r-2600.pdf](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-2600.pdf) [accessed 13 January 2009].

- 147 ICCVAM. 1999. The murine local lymph node assay: A test method for assessing the allergic
148 contact dermatitis potential of chemical/compounds. NIH Publication No. 99-4494. Research
149 Triangle Park, NC: National Institute of Environmental Health Sciences.
- 150 ICCVAM. 2008. Recommended Performance Standards: Murine Local Lymph Node Assay.
151 NIH Publication Number 09-7357. Research Triangle Park, NC: National Institute of
152 Environmental Health Sciences.
- 153 OECD. 2002. Test guideline 429. Skin Sensitisation: Local Lymph Node Assay, adopted
154 April 24, 2002. In: OECD Guidelines for Testing of Chemicals. Paris: OECD.