The Regulatory Acceptance Board Report on an alternative skin sensitization test method, Reduced Local Lymph Node Assay (rLLNA)

JaCVAM Regulatory Acceptance Board

July 4th, 2012

Having received the Peer Review Panel's report[1] on an alternative skin sensitization test method, Reduced Local Lymph Node Assay (rLLNA), we discussed the following nine items. Items 2–8 are per OECD Guidance Document No. 34. It is our opinion that the use of this test as an alternative to animal testing requires careful consideration of the scope of application.

The Item Discussed

1. The submitted test method should relate to regulations or guidelines in Japan.

The rLLNA is a test method for evaluating potential for skin sensitization of chemical substances.
The principle behind the rLLNA, its procedures, and judgment criteria are identical to those in the LLNA already contained in OECD guidelines, and the only difference between the two is that[, whereas the conventional LLNA evaluates three dose levels,] the rLLNA evaluates a single dose level.
rLLNA relates to skin sensitization irritation as stipulated in regulations or guidelines governing products, raw materials, or other chemical substances used in drugs, quasi drugs, medical devices, or cosmetics.

2. The submitted test method and supporting validation data should have been subjected to a transparent and independent peer review process.

ICCVAM organized a Peer Review Panel to evaluate the test method and validity of the rLLNA based on LLNA test results obtained at 11 laboratories. The organization and the evaluation results are available from the ICCVAM website.
Also, a rLLNA Peer Review Panel was established in Japan to evaluate the conclusions of the ICCVAM review, and its findings were submitted to the Regulatory Acceptance Board. These results, as well, were published upon conclusion of the Regulatory Acceptance Board discussions.
We therefore consider the rLLNA to have been subjected to a transparent and independent peer review process.

3. The data generated by the test method should adequately measure or predict the endpoint of interest. For replacement test methods, the data should show a linkage between the proposed test method and an existing test method, and/or the proposed test method and effects in the target or model species.

Skin sensitizers stimulate lymph glands near the area of application. The conventional LLNA is based on this principle and involves applying a test substance to mice in a variety of concentrations and obtaining sensitization-induced lymphocyte proliferation by measuring the

uptake of DNA with 3H-methyl-thymidine (3H-TdR), which is used as an index of the potential for skin sensitization of the test substance. rLLNA is based on this same principle.
Provided that a suitable dosage range was used for the LLNA, a correlation between the dosage and the strength of the reaction is assumed. For the rLLNA, data is recorded only for the single dosage considered most likely to produce the strongest reaction, but as long as this dosage is established properly, the rLLNA is considered to be equivalent to the conventional LLNA in ability to predict potential for human skin sensitization of the test substance.

4. The test method should generate data useful for hazard/risk assessment purposes.

The rLLNA is used only to predict potential for skin sensitization of the test substance and not to investigate the relationship between dosage and strength of skin sensitization.

5. The submitted test method and supporting validation data should adequately cover a spectrum of chemicals and products representative of those administered by the regulatory program or agency for which the test method is proposed, and the applicability and limitations of the test method should be clearly described.

The rLLNA and supporting validation data were compiled by ICCVAM per LLNA test results from 11 laboratories covering a diverse spectrum of 465 test substances, the consistency of which was later evaluated by a rLLNA Peer Review Panel in Japan. This data demonstrated the validity of the rLLNA as a test method for skin sensitization. We consider the above-mentioned data adequately covers a spectrum of products and raw materials.
The dosage used for the rLLNA is the maximal one that does not produce excessive local irritation or obvious systemic toxicity, but compared with the LLNA, 1.9% (6/315) false negative results were seen. One reason for this could be a lower reactivity in response to the specified maximum dosage.
In the event that a false negative is suspected, supplementary information on toxicity and physical properties of the test substance are to be reviewed and a LLNA performed if dose-response information is necessary.

6. The test method should be sufficiently robust (relatively insensitive to minor changes in protocol) and transferable among properly-equipped laboratories with adequately-trained staff.

Insofar as both tests are based on the same principles, we consider the accuracy, intra- and interlaboratory reproducibility, and robustness of the rLLNA to be identical to those of the LLNA. We also recognize, however, the potential for inconsistent evaluation results due to the use of a single dose only and the effects of the selection of the dosage, solubility of the test substance, and other factors. Thus, it is necessary to conform the latest protocol recommended by ICCVAM.

With this exception of the above single precaution, we otherwise see no difficulties when comparing the rLLNA to the LLNA and consider the rLLNA to be easily transferable to a properly equipped laboratory with adequately trained staff.

7. The test method should be both time and cost effective as well as likely to be used in a regulatory context.

Both rLLNA and LLNA are performed using the same procedure, which means that there is little difference between the two in terms of cost effectiveness, but given that rLLNA uses 40% fewer animals, it is more cost effective.
In order to distinguish between chemical substances that are skin sensitizers and those that are not, it is considered necessary first to perform an initial screening per rLLNA followed by a review of supplementary information on toxicity and physical properties of the test substance as well as performance of LLNA to obtain dose-response information if a false negative is suspected.
Additional tests are also necessary when positive reactions require further data on dosage dependency in a regulatory context.

8. Justification should be provided (scientific, ethical, economical) for the new or updated test method in light of existing test methods.

The rLLNA represents an improvement over the conventional LLNA in that it uses only a single dose and therefore requires fewer animals to obtain results with a similar accuracy. Peer Review Panels in Japan and by ICCVAM have validated the rLLNA.

9. The test method should be suitable for use as regulatory documentation in the assessment of safety.

The rLLNA is used to test products, chemical substances, and raw materials used in drugs, quasi drugs, medical devices, or cosmetics to distinguish between those that are skin sensitizers and those that are not.
Insofar as the rLLNA is not suitable for obtaining dose-response information, it is not sufficient as reference material for use in evaluating risk assessment. It is possible to obviate further testing for substances that yield negative results in the rLLNA except when there is other scientific data that indicates the substance is likely to be a skin sensitizer.
Based on the above, the JaCVAM Regulatory Acceptance Board has determined the following for the rLLNA as an alternative for skin sensitization test methods.
The rLLNA represents an improvement over the conventional LLNA in that it uses only a single dose and therefore requires fewer animals to obtain results with a similar accuracy.
When performed properly in conformance with the most recent ICCVAM-recommended protocol and with an understanding of the potential for false negatives (reduced response at maximum dose, etc.), the rLLNA is a scientific means for evaluating potential skin sensitization

across a wide range of products and chemical substances as part of a regulatory program or by related government agencies.

Bibliography

1. Peer Review Panel report on an alternative skin sensitization test method: Reduced Local Lymph Node Assay
2. OECD (2005) OECD Series on testing and assessment Number 34, Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment, ENJ/JM/MONO(2005) 14
3. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), National oxicology Program (NTP), et al. Background Review Document: “in vitro Cytotoxicity Test Methods for Estimating Acute Oral systemic Toxicity”, NIH Publication No: 07-4518