Evaluation Report on an Alternative Test Method for Skin Sensitization: human Cell Line Activation Test（h-CLAT）

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Abstract

Skin sensitization is an important consideration in assessing the safety of chemicals, and the assessment of skin sensitization has conventionally involved the use of mice, guinea pigs, or other animals. Recent moves in the regulation of chemicals in the EU have promoted the use of alternative methods for safety assessment, including computer-generated quantitative structure-activity relationship models (QSAR models) and *in vitro* test methods. With the March 2013 prohibition on import or sales of cosmetics using ingredients that were tested on animals, there is a clear need for *in vitro* test methods as alternatives to animal testing. The human Cell Line Activation Test (h-CLAT) is a test method which utilizes the fact that many skin sensitisers activate dendritic cells to distinguish skin sensitizers from non-sensitisers by quantifying changes in the expression of cell surface markers associated with the process of activation of CD86 and CD54 in the human leukemia cell line THP-1. This report presents a summary of the test procedure and an evaluation of the test's utility and limitations, based on review of a validation report issued by the EURL ECVAM, a peer review report, and scientific papers from the test developers.

The h-CLAT is an *in vitro* test method that addresses the expression of cell surface markers associated with activation of dendritic cells during the third key event of the skin sensitization AOP, and provides important data points necessary for assessing the sensitization potential of chemical substances.

Simulations have shown that the h-CLAT can be used to assess the sensitization potential of chemical substances at a mere fifth of the cost associated with the murine Local Lymph Node Assay (LLNA), and insofar as the time required for testing is also shorter than that for the LLNA, it is considered more economical in terms of both time and expense. When compared with the Direct Peptide Reactivity Assay (DPRA) or ARE-Nrf2 Luciferase test method, however, h-CLAT is both more difficult to perform and subject to greater restrictions. Also, h-CLAT requires continuous measurements of the positive, negative, and vehicle controls for the creation of a historical database.

During the EURL ECVAM validation study of the h-CLAT, within-laboratory reproducibility was found to be between 73.3% and 86.7%, which overall is not a high level. An OECD panel of experts, however, did recommend stricter control of both pre-culture parameters for the THP-1 cell line and the time of exposure to test chemicals as a means of improving within-laboratory reproducibility. And since this has been reflected in the protocol included in the OECD test guidelines, we expect to see improvements in within-laboratory reproducibility. On the other hand, between-laboratory reproducibility for the 24 test chemicals tested at four participating laboratories was 79.2%. The OECD test guideline indicates an accuracy of 85%, a sensitivity of 93%, and a specificity of 66% for 142 test chemicals, excluding those with a log Kow of 3.5 or higher that tested negatively. Insofar as test substances with a log Kow of 3.5 or higher are known to test negatively and require re-testing by a different test method to confirm negative results, it is not possible to identify non-sensitizers using the h-CLAT test alone. Also, a specificity of 66% means that the potential for false positives must also be borne in mind. Furthermore, it is possible that the h-CLAT is not capable of correctly detecting the sensitization potential of chemical substances that require metabolic activation due to limitations in the metabolic capacity of the cell line used.

In consideration of the above-described limitations, the Committee does not consider the h-CLAT to be suitable for use as a standalone test for distinguishing skin sensitizers from non-sensitizers and recommends that it be used in combination with other test methods or as part of a weight-of-evidence approach.