Evaluation report on the Direct Peptide Reactivity Assay (DPRA) for alternative to skin sensitisation test

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Summary

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, and with the March 2013 prohibition on import or sales of cosmetics containing ingredients that were tested on animals, there is a clear need for *in vitro* test methods as an alternative to animal testing. The Direct Peptide Reactivity Assay (DPRA) is an assessment method that involves instrumental analysis of the reactivity of chemicals with proteins, which is often seen during initiating events of skin sensitization. The content of this report is based on a European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)-lead validation study and subsequent independent peer review by the EURL ECVAM Scientific Advisory Committee (ESAC) to determine procedures for as well as the utility and limitations of DPRA.

The DPRA entails incubating a test chemical with either a cysteine-including peptide (Ac-RFAACAA-COOH) or lysine-including peptide (Ac-RFAAKAA-COOH) solution for 24 hours, after which the test chemical is classified by measuring cysteine and lysine depletion. This test method is particularly well-suited to laboratories that are proficient in high performance liquid chromatography (HPLC). Intra-laboratory reproducibility during pre-validation testing of DPRA was between 73% and 100%, with one of the three laboratories unable to satisfy the 85% criteria because of low reproducibility of substances classified as subcategory 1B (weak sensitizers) under the United Nations Globally Harmonized Systems of Classification and Labeling (UN GHS). Thus, there is concern that results for these substances may be inconsistent. On the other hand, although the inter-laboratory reproducibility (75%) failed to satisfy the 80% criteria, when excluding results for metal compounds(which are considered outside the applicability domain), it satisfied the 80% criteria.

Pre-validation testing of the DPRA yielded an accuracy of 78%, sensitivity of 71%, and specificity of 92% when referenced against results from the Local Lymph Node Assay (LLNA), a well-known test that uses mice for predicting the risk of contact dermatitis. Even when metal compounds (which are considered outside the applicability domain) are excluded, sensitivity during validation testing was roughly 75%, and given this possibility for obtaining false-negative results, it is necessary to confirm results obtained with the DPRA using other test methods.

The DPRA does provide valuable information needed to judge the sensitization potential of chemicals by detecting reactivity with proteins, which is an early key event underlying the mechanism of sensitization. It can be performed at roughly one-tenth the cost of the LLNA and is considered to have high utility due to that fact that it is an *in chemico* test method that does not involve the use of animals. As DPRA is an *in chemico* method lacking a metabolic system, it is not capable of correctly detecting the skin sensitization potential of sensitizers requiring enzymatic bioactivation and abiotic transformation as well as weak sensitizers, metallic salts and highly-hydrophobic substances. Based on the above facts, JaCVAM (Japanese Center for the Validation of Alternative Methods) Editorial Committee recommends that the DPRA be used within a weight of evidence approach or in combination with the other test methods such as LLNA and Guinea Pig prediction tests.