Evaluation Report on an Alternative Test Method for Skin Sensitization: ARE-Nrf2 Luciferase Test

Naohiha Tsutsui[1], Reiko Adachi[2], Yukiko Kanazawa[3], Koichi Kojima[3], Kazuhiro Sato[4], Masahiro Takeyoshi [5], and Takashi Morimoto[6]

[1]Mitsubishi Tanabe Pharma Corporation, [2]National Institute of Health Sciences, [3]Hatano Research Institute, Food and Drug Safety Center, [4]University of Fukui Faculty of Medical Sciences, [5]Chemicals Evaluation and Research Institute, [6]Sumitomo Chemical Co., Ltd.

Abstract

Skin sensitization potential is an important consideration in assessing the safety of chemicals, and its assessment 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. Since the March 2013 prohibition on import or sales in the EU of cosmetics using ingredients that were tested on animals, there is a clear need for *in vitro* test methods as an alternative to animal testing.

Many skin sensitizers have been reported to induce gene expression regulated by the antioxidant response element. The ARE-Nrf2[3] Luciferase Test Method is a means of assessing skin sensitization potential by measuring the level of this induction activity in cultured cells. This assessment report provides commentary on the test protocol, the utility, and the limitations of the ARE-Nrf2 Luciferase Test Method based on information from a variety of sources, including the results of a pre-validation study on *KeratinoSens™* performed by the test's developer, Givaudan, and an independent peer review conducted by the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) Scientific committee.

The ARE-Nrf2 Luciferase Test Method is a useful means of obtaining data that is important in predicting the skin sensitization potential of chemical substances by assessing the inflammatory responses and the gene expression associated with the Nrf2-Keap1[4]-ARE pathway that take place in keratinocytes as the second key event in a skin sensitization Adverse Outcome Pathway.

The high utility of the ARE-Nrf2 Luciferase Test Method is evidenced by the fact that it is an *in vitro* test method, available at only one seventh the cost of the Murine Local Lymph Node Assay. Nevertheless, since this test is only compatible with the KeratinoSens™ cell line, it cannot be easily introduced without obtaining a license from Givaudan, which is the company that first established the cell line.

Another issue involves the fact that one of the five laboratories that participated in the pre-validation study failed to demonstrate suitable within laboratory reproducibility for both weak sensitizers (UN GHS Category 1B) and non-sensitizers. As a result, the pre-validation study failed to meet the 85% success criteria, which means that there is some concern over accuracy in predicting test chemicals that are not strong sensitizers. In contrast to this, however, the 80% success criteria for between laboratory reproducibility were met.

The ARE-Nrf2 Luciferase Test Method demonstrated a sensitivity of roughly 80%, and in cases where a test chemical is predicted to be a non-sensitizer, it is necessary to give due consideration to the

possibility of a false-negative result. Thus, it is necessary to review the results of other suitable test methods when making final decisions, and test substances cannot be classified as non-sensitizers on the basis of results from ARE-Nrf2 Luciferase Test Method alone. Also, specificity was roughly 80%, and in cases where a test chemical is predicted to be a sensitizer, it is necessary to give due consideration to the possibility of a false-positive result.

The ARE-Nrf2 Luciferase Test Method may not correctly detect the skin sensitization potential of chemical substances that require metabolic activation. Also, because of this cell lineage, it could prove problematic to assay highly-hydrophobic substances at the specified maximum concentration of 2000 µM, which could preclude negative predictions.

Taking the above into consideration, we consider the inexpensive procurement of the *KeratinoSens™,* cell line to be a prerequisite to the general utilization of the ARE-Nrf2 Luciferase Test Method in the evaluation of skin sensitization potential. Furthermore, given its limitations and the fact that it cannot be used alone to predict skin sensitization potential, we recommend that the ARE-Nrf2 Luciferase Test Method be used within a weight of evidence approach or in combination with LLNA, GPMT, or other test method.

***Key Words**: KeratinoSens™, ARE-Nrf2, skin sensitization, non-animal test methods, allergic contact dermatitis*