Evaluation report on the *in-vitro* tests for skin irritation

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Summary

We evaluated a Statement on the Scientific Validity of *in-Vitro* Tests for Skin Irritation Testing issued by the ECVAM Scientific Advisory Committee (ESAC) regarding the 3D skin model EPISKIN at the 26th meeting of the European Center for the Validation of Alternative Methods (ECVAM) held on April 26, 2007.

The ESAC statement was based on the official validation report issued by the ECVAM Skin Irritation Task Force in its efforts to find a test method capable of predicting results of primary skin irritation tests in rabbits, which began in Europe in 1998.

The objective of this validation was to determine if *in vitro* test methods were capable of predicting chemical classifications according to the EU classification system currently in effect, which labels skin irritants as R38 but does not label non-irritants.

An *in vitro* skin irritation test using the 3D skin model EPISKIN identifies irritants, using a 50% cell viability as the threshold to judge the results of a procedure in which test substances are applied to a skin surface for 15 minutes, allowed to incubate for 42 hours, and then measured for tissue viability using an MTT assay. Based on favorable results from the validation, ESAC determined that this test method using the 3D human skin model EPISKIN demonstrated reliability and was a suitable standalone test method as an alternative to the Draize skin irritation test (OECD TG404 or test B.4 in Annex V of the EU Dangerous Substances 67/548/EEC) used to distinguish the need for R38 labels or in predicting potential skin irritation in rabbits. Also, it was determined that measurement of IL-1α release was a useful adjunct determination for confirming negative results from the MTT assay, due to potential improvement of sensitivity without loss of specificity.

As a result of this committee's review and validation of documentation evaluated by the ESAC, we concluded the following:

1. We found that, due to the use of a 3D model that comromises human cells, this test method was a scientifically valid alternative to evaluating damage to human skin using a primary skin irritation test in rabbits.
2. It has been explicitly stated that this method is an alternative to OECD TG 404 as well as that the purpose of using this test method is to distinguish the skin irritation potential of chemicals according to EU labeling system using risk of R38 (2 classifications) and GHS (3 classifications).
3. The test protocol includes a method for exposing the skin model to the test substance as well as a detailed protocol for evaluation of subsequent cytotoxicity. Also, we found that the test protocol was sufficient to enable accurate implementation.
4. It was stated in the ESAC evaluation that, although the IL-1α release measurement was added to the protocol, the MTT assay is still considered the principle means of evaluation, and IL-1α release measurement data should be considered reference data. This is due to a minimal prospect for improvement as well as fact that an IL-1α valuation has yet to be completed, which relegates it to

an adjunct position. Regardless of this, the replicability, predictive ability, and reliability of the test method are valid.

5. The validation was performed in accordance with OECD Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (No. 34).
6. With regard to the manufacture of the human skin model, production facilities were inspected and quality confirmed.
7. A validation management team was organized and included an expert biostatistician.
8. Based on the fact that the validation had undergone peer review by experts, we concluded that the data satisfied international standards for reliability, quality, and accuracy.
9. Data analysis was used to evaluate both intra- and inter-laboratory variability, and found no problem with either intra- or inter-laboratory reproducibility.
10. A set of 58 test substances was chosen per valid selection criteria from three databases containing reliable *in vivo* data. PBS was used as the negative control and 0.5% SDS was used as a positive control.

From the above and as a result of having reviewed the ESAC evaluation concerning approval of the *in vitro* skin irritation test method using the 3D human skin model EPISKIN, we concluded that although the IL-1α release measurement was added to the protocol during the ESAC evaluation, the MTT assay is still considered the principle means of evaluation, and IL-1α release measurement data should be considered reference data. This is due to a minimal prospect for improvement as well as fact that an IL-1α valuation has yet to be completed, which relegates it to an adjunct position. Even when evaluation is performed using only an MTT assay, results with suitable replicability, predictive ability, and reliability are obtained. Thus thisshould be careful *in vitro* test method to predict classification of chemicals according to EU labeling system using risk of R38 and aloso can be used in place of OECD TG404.