An update on the development of *in vitro* skin sensitization testing in Japan

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For some time, the Guinea Pig Maximization Test (GPMT) and the Buehler guinea pig test were commonly used for regulatory screening of human allergic contact dermatitis in Japan. Moreover, the murine Local Lymph Node Assay (LLNA) as described in OECD Test Guideline No.429 has come into extensive use since 2002. The LLNA is problematic, however, in its use of radioisotopes (RI), which has led Japanese researchers to develop modified methods that work without RI. Two such methods, the LLNA-DA and the LLNA:BrdU-ELISA, have now been validated in Japan. LLNA:DA is based on quantification of adenosine triphosphate (ATP) content and was developed by Daicel Chemical Industries. LLNA:BrdU-ELISA uses 5-bromo-2-deoxyuridine (BrdU) as an indicator of lymphocyte proliferation and was developed by Chemicals Evaluation and Research Institute (CERI). Peer review of these two test methods by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) resulted in the publication of OECD test guidelines 442A and 442B in 2009.

In addition, there are other *in silico* and alternative test methods for screening human allergic contact dermatitis that have been developed and evaluated in Japan. The human cell line activation test (h-CLAT) is an *in vitro* skin sensitization test method based on the enhancement by sensitizers of CD86 and/or CD54 expression on THP-1 cells and was developed jointly by Shiseido Co. Ltd and Kao Corporation. Having concluded preliminary ring studies involving multiple cosmetic companies, a pre-validation study is now underway at the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM). Also, a novel in vitro test to screen skin sensitizers using THP-G8, a stable THP-1-derived IL-8 reporter cell line, has been developed by Dr. S. Aiba. et al at the Tohoku University Graduate School of Medicine with the support of the New Energy and Industrial Technology Development Organization (NEDO) of Japan. A validation study is now underway with the support of the Ministry of Economy, Trade and Industry, Japan.

We look forward to further progress in the development and use of these methods within the context of international research into AOP (Adverse Outcome Pathway) skin sensitization.