

set



**Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen**

Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

Mainzer Landstraße 55, 60329 Frankfurt am Main
www.stiftung-set.de

Project

Improved genotoxicity tests with the Phenion® Full Thickness Skin Model

Dr. Kerstin Reisinger
Phenion GmbH & Co. KG

September 2007 – August 2008

The logo for 'set' consists of the lowercase letters 'set' in a bold, sans-serif font. Below the letters are two vertical columns of horizontal bars. The left column has 10 blue bars, and the right column has 10 pink bars. The bars in each column are of varying lengths, creating a stepped effect.

Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen

Improved genotoxicity tests with the Phenion® Full Thickness Skin Model

In vitro genotoxicity tests were the first regulatory accepted animal free alternative methods and are in use for a long time. However, the currently used tests exhibit an insufficient predictability. DNA damaging substances are identified as mutagens to a very high extent (nearly 89%), but nearly 75% of the harmless substances are classified as mutagens as well and result in a high rate of false positive results. Consequently, additional *in vivo* experiments have to be performed in order to verify the obtained *in vitro* data.

However, the use of animal tests for assessing the genotoxic potential of cosmetic ingredients is banned by the legislator starting 2009. In addition, due to amendments of the chemical legislation a risk assessment of nearly 30,000 already existing substances is mandatory. Hence, the genotoxic potential of a variety of these substances which were brought to the market before 1981 has to be determined. Both circumstances require the development of additional *in vitro* genotoxicity tests which show an improved predictability to avoid animal tests.

The low predictability of the currently used tests might be caused by the use of bacteria, hamster, mouse or rat cell cultures mainly instead of human cells. Furthermore, the cells are cultivated in simple test systems, so called monolayer cultures which do not allow the adequate formation of specific cell or tissue characteristics. The current project intend to address these circumstances with the development of two genotoxicity tests based on a full thickness skin model which is commercially available since January 2006. The Phenion® Full Thickness Skin Model allows a repeated topical application of test substances, mirrors the barrier function of native skin which is absent in monolayer cultures and exhibit a human skin-specific xenobiotic metabolism.

Based on this test system which is close to the *in vivo* situation a micronucleus test will be developed. The test allows the detection of permanent DNA damages like DNA double strand breaks or the disintegration of whole chromosomes during mitosis. An OECD draft guideline for an *in vitro* micronucleus test based on cell lines is already prepared. As a second test a protocol for the Comet-Assay, a sensitive indicator test, will be implemented. The Comet Assay is a widely used and scientifically well accepted method detecting different kinds of DNA damages of transient and permanent nature. Recommendations for methodological standards of international expert groups are available. Since both tests detect different kinds of DNA damages and allow the collection of data of higher biological relevance these tests might supplement the existing *in vitro* test batteries. If the project proceeds successfully it might help to reduce or even to replace animal tests.

set



Stiftung zur Förderung
der Erforschung von
Ersatz- und
Ergänzungsmethoden
zur Einschränkung von
Tierversuchen

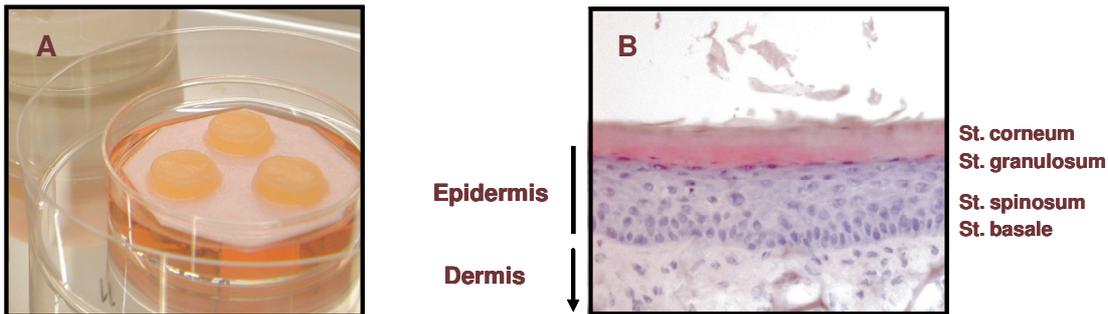


Figure: **(A)** Culture of Phenion® Full Thickness Skin Models at the air-liquid-interface which allows cornification of the uppermost layer of the skin model. **(B)** Cross section of a cryo preserved skin model which mirrors the characteristic layers of human native skin perfectly: Epidermis and dermis (the latter only displayed partly). The epidermis of the skin model can be subdivided the four typical layers of human native skin: Stratum corneum (development only at air-liquid-interface), St. granulosum, St. Spinosum, and St. Basale.

Operating institution:

Phenion GmbH & Co. KG
Merowinger Platz 1a
40225 Düsseldorf

Project director:

Dr. Kerstin Reisinger

December 2008