Human Skin Model Test: *in vitro* Skin Corrosion

(EpiDerm™ and EPISKIN-SM™)

The prediction and classification of skin corrositivity potential of substances can be performed by the assessment of the effect on EpiDerm™ and EPISKIN-SM™ models.

The Human Skin Model Test is validated by the EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) and is performed in accordance with the OECD guidance (OECD 431) at Eurofins BioPharma Product Testing Munich GmbH [1] with chemicals, cosmetics or personal care products and pharmaceuticals.

Assessment of skin corrosion potential

- Corrosion represents irreversible tissue damage of the skin (visible necrosis) after application of a substance. The underlying mechanisms are either the destruction of the skin penetration barrier or the rapid penetration of highly cytotoxic chemicals through the skin without its destruction.

- The EpiDerm™ and EPISKIN-SM™ represent a reconstituted three-dimensional human epidermis (RhE) model which consists of human epidermal keratinocytes. This *in vitro* model mimics biochemical and physiological properties of the upper human skin.

- To determine skin corrosion potential chemicals are applied directly on the skin tissue surface and the cell viability is measured by MTT assay.

- The EpiDerm™ and EPISKIN-SM™ human skin model test can be used as a reliable *in vitro* method to distinguish between corrosive and non-corrosive chemicals. Additionally, sub-categorization in "Category 1A" or a combination of sub-categories "1B" and "1C" is possible with this test [2].

- The skin corrosion test can be performed subsequently to a positive skin irritation test for a final classification. If the skin corrosion test is performed at first, a negative result can be followed by an irritation test to conclude if the chemical has irritant potential or not.

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Procedure

Principles of the Human Skin Model Test

Protocol		
Model	EpiDerm	EpiSkin
Supplier	MatTek	Skin Ethic
Analysis	Corrosivity potential: cytotoxicity measurement with MTT (mean tissue viability compared to negative control)	
Test chemical concentrations	Liquids: 50 µL (undiluted) Solids: 25 mg + 25 µL H_2O	Liquids: 50 µL (undiluted) Solids: 20 mg + 100 µL NaCl
Exposure time	3 min and 60 min incubation with dose groups	3 min, 60 min and 240 min incubation with dose groups
Quality controls	Positive control: 8 N (KOH) Negative control: H_2O	Positive control: glacial acetic acid Negative control: NaCl
Pre-tests	**To determine if additional controls are needed:** **NSMTT***: mixing of test item with MTT medium to determine if test item alone can reduce MTT → blue colouring: in main experiment two killed tissues treated with test item and two untreated killed tissues were added as controls **NSC**_{living}[#]: mixing of test item with H_2O or isopropanol to determine if strong own colour of test item can discolour at contact with this liquids → optical discolouring (measuring of spectrum): in main experiment two living tissues without incubation with MTT medium were added as controls **NSC**_{killed}[§]: if the two other controls were determined → in main experiment two killed tissues without incubation with MTT medium were added as controls	
Application	Direct topical application of chemicals on skin tissue Two tissue replicates per dose group	
Data delivery	Optical density (OD) value with microplate spectrophotometer at 570 nm	

*NSMTT: non-specific MTT reduction
[#]NSC_{living}: non-specific colouring of living tissues
[$]NSC_{killed}: non-specific colouring of killed tissues

Procedure

Prediction Model of the Human Skin Model Test

Prediction EpiDerm[TM]		Prediction EPISKIN-SM[TM]	
Mean tissue viability (% negative control)	**Prediction optional sub-categorisation**	**Mean tissue viability (% negative control)**	**Prediction optional sub-categorisation**
Step 1		--	
< 50% after 3 min exp.	**Corrosive**	< 35% after 3 min exp.	**Corrosive** Optional sub-category 1A (risk of over-prediction)
≥ 50% after 3 min exp. **and** < 15% after 60 min exp.	**Corrosive** A combination of optional sub-categories 1B and 1C	≥ 35% after 3 min **and** < 35% after 60 min exp. **or** ≥ 35% after 60 min **and** < 35% after 4 h exp.	**Corrosive** A combination of optional sub-categories 1B and 1C
≥ 50% after 3 min exp. **and** ≥ 15% after 60 min exp.	**Non-Corrosive**	≥ 35% after 4 h exp.	**Non-Corrosive**
Step 2			
< 25% after 3 min exp.	Optional sub-category 1A		
≥ 25% after 3 min exp.	A combination of optional sub-categories 1B and 1C		

Exp. = exposure

Table 1: Prediction model of the corrosivity potential of the EpiDerm[TM] and the EPISKIN-SM[TM] tissues dependent on the mean tissue viability. [1]

Based on the mean tissue viability, chemicals can be classified with the skin corrosion test into corrosive and non-corrosive and can be furthermore sub-categorized.

Data

Eurofins Data for demonstration technical proficiency of the Human Skin Model Test (EpiDerm™ and EPISKIN-SM™)

Chemical	UN GHS category	EF category for EpiDerm™	EF category for EPISKIN-SM™
Corrosive chemicals (sub-category 1A)			
Bromoacetic acid	1A	1A	1A
Boron trifluoride dihydrate	1A	1A	1A
Phenol	1A	1A	1A
Dichloroacetyl chloride	1A	1A	1A
Corrosive chemicals (combination of sub-categories 1B and 1C)			
Glyoxylic acid monohydrate	1B and 1C	1B and 1C	1B and 1C
Lactic acid	1B and 1C	1B and 1C	1B and 1C
Ethanolamine	1B and 1C	1B and 1C	1B and 1C
Hydrochloric acid (14.4%)	1B and 1C	1B and 1C	1B and 1C
Non-corrosive chemicals			
Phenethyl bromide	NC	NC	NC
4-Amino-1,2,4-triazole	NC	NC	NC
4-(Methylthio)-benzaldehyde	NC	NC	NC
Lauric acid	NC	NC	NC

EF = Eurofins Munich GmbH   NC = Non-Corrosive

Table 2: Eurofins data of *in vitro* skin corrosion with EpiDerm™ and EPISKIN-SM™ of twelve tested proficiency chemicals compared to the data of the OECD guideline 431.[1]

In Table 2 the obtained data from the *in vitro* skin corrosion with EpiDerm™ and EPISKIN-SM™ of eight corrosive and four non-corrosive chemicals are shown. The prediction of all tested chemicals was correct in comparison to the classification of the OECD guideline 431.

References

1) OECD (2016). OECD Guideline for the Testing of Chemicals. No. 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method, 29 July, 2016

2) UN (2015), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Sixth revised edition, UN New York and Geneva