

DB-ALM Protocol n° 118 : EpiSkin™ Skin Corrosivity Test

Skin Irritation and Corrosivity

The corrosivity potential of a chemical may be predicted by measurement of its cytotoxic effect, as reflected in the MTT assay, on the EPISKIN™ reconstituted human epidermis. (*Validation study protocol*)

Objective & Application

TYPE OF TESTING	: replacement
LEVEL OF ASSESSMENT	: toxic potential, toxic potency, hazard identification
PURPOSE OF TESTING	: classification and labelling

The test method was granted regulatory approval as a replacement for the *in vivo* skin corrosivity test (Method B.40 bis, EU 2000, 2008; OECD Test Guideline 431, OECD 2004) and it is used for hazard identification and classification of corrosive potential in order to fulfil the regulatory requirements concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (EU, 2008). Furthermore, the test method is recommended for its use within the context of the sequential skin corrosivity testing strategy (OECD Test Guideline 404, OECD 2002 and Method B.4, EU 2004 and 2008).

The test allows the identification of corrosive chemical substances and mixtures and enables the identification of non-corrosive substances and mixtures when supported by a weight-of-the-evidence determination using other existing information (OECD, 2004 and EU, 2008).

Résumé

Most international regulatory classification schemes define chemically induced dermal corrosion as full thickness destruction (necrosis) of the skin tissue, while some extend the definition of corrosion to include any irreversible alterations caused to the skin.

The potential to induce skin corrosion is an important consideration in establishing procedures for the safe handling, packing and transport of chemicals.

The determination of the skin corrosion potential is therefore included in international regulatory requirements for the testing of chemicals, such as the U.S. Code of Federal Regulations (US DOT, 1991), the updated OECD Test Guideline No 404 (OECD, 2002) and the Method B.4 of the Annex to Commission Regulation 440/2008/EC (EU, 2008). Corrosivity was usually determined *in vivo* using the Draize rabbit skin test (Draize *et al.*, 1944).

The present test is based on the experience that corrosive chemicals show cytotoxic effects following short-term exposure of the stratum corneum of the epidermis.

The test is designed to predict and classify the skin corrosivity potential of a chemical by assessment of its effect on a reconstituted human epidermis.

EPISKIN Standard Model™ is a three-dimensional human skin model comprising a reconstructed epidermis with a functional stratum corneum. Its use for skin corrosivity testing involves topical application of test materials to the surface of the skin, and the subsequent assessment of their effects on cell viability. Cytotoxicity is expressed as the reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Fentem *et al.*, 1998 and Fentem, 1999).

Experimental Description

Endpoint and Endpoint Measurement:

CELL VIABILITY: Cell viability as determined by reduction of mitochondrial dehydrogenase activity measured by formazan production from MTT

Endpoint Value:

Experimental System(s):

RHE EpiSkin™: Small Model (EpiSkin™-SM, EPISKIN SNC Lyon, France) is a three-dimensional human *epidermis* model. Human-derived epidermal keratinocytes are seeded on a dermal substitute

consisting of a collagen type I matrix coated with type IV collagen. A highly differentiated and stratified *epidermis* model is obtained after 13-day culture period comprising the main basal, supra basal, spinous and granular layers and a functional stratum corneum (Tinois *et al.*, 1994).

Basic Procedure

Test materials are applied to the stratum corneum of the epidermal model (one epidermis unit per test material) for three different exposure periods: 3 minutes, 1 hour, and 4 hours. Exposure to the test chemical was terminated by rinsing with phosphate buffered saline (PBS). EPISKIN™ cultures exposed to the control compounds for 240 min serve as the controls for all three exposure periods. For each test material, three independent tests with three different batches of EPISKIN™ are to be undertaken.

The viability of the epidermis is assessed by measuring the mitochondrial activity. The tissues are incubated for 3 hours with MTT solution (0.3 mg/l; 2.2 ml per well). MTT, a yellow-coloured tetrazolium salt, is reduced by succinate dehydrogenase into a blue formazan precipitate in the mitochondria of living cells. The precipitated formazan is extracted overnight by using acidified isopropanol (0.85 ml), and is then quantified spectrophotometrically at a wavelength between 545nm and 595nm. All experimental procedures have to be conducted at room temperature (18-28°C); if the temperature is below 20°C, the 3 hours MTT incubation should be carried out in a warmer environment of 20-28°C. NaCl (50 µl) and glacial acetic acid (50 µl) are used as negative and positive controls, respectively.

Some highly reactive chemicals can produce fumes which may affect adjacent units in the same plate. It is recommended that if there is any suspicion that a material could cause fumes, it should be tested alone in a single plate. It is particularly important that the negative control units are not exposed to fumes from other units, hence it is recommended to routinely incubate positive and negative controls in a separate plate.

Data Analysis/Prediction Model

The test results are interpreted on the basis of the exposure time needed to cause cell viability to decrease below 35%.

The determination of the UN packing groups and EU classifications is summarized in the table reported in the section 4.1. "**Interpretation of test results**" of the present standard operating procedure.

Test Compounds and Results Summary

A total of 60 test compounds, consisting of 11 organic acids, 10 organic bases, 9 neutral organics, 5 phenols, 7 inorganic acids, 4 inorganic bases, 3 inorganic salts, 8 electrophiles, 3 soaps/surfactants (Barratt *et al.*, 1998) have been tested in the ECVAM validation study.

Status

Participation in Evaluation Studies:

An in-house evaluation and prevalidation study of the test method protocol was carried out during 1994-1996 (ECVAM, 1996).

Participation in Validation Studies:

During 1996 and 1997, this method has been evaluated in the **ECVAM International Validation Study on *In Vitro* Tests for Skin Corrosivity** (Barratt *et al.*, 1998 and Fentem *et al.*, 1998). Based on the outcome of the study, the ECVAM Scientific Advisory Committee concluded that the test was able to distinguish between corrosive and non-corrosive chemicals for all of the chemical types studied; it was also able to distinguish between known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals.

ESAC unanimously endorsed the statement that the EPISKIN™ test was scientifically validated for use as a replacement for the animal test and that this test was ready to be considered for regulatory acceptance (ESAC, 1998).

Regulatory Acceptance:

In 2000, the human skin model assays, which meet certain criteria (such as EPISKIN™ and EPIDERM™), have been included into "Annex V. Part B.40 on Skin Corrosion" of the "Directive 67/548/EEC on the Classification, Packaging and Labelling of Dangerous Substances" (EU, 2000).

The test method was further recommended to be used as one of the *in vitro* methods for skin corrosivity

testing in the OECD Test Guideline 404 (OECD, 2002) and in the Method B.4 of Annex V of the Directive 67/548/EEC (EU, 2004) laying down the step-wise testing strategy for classifying skin corrosives by the sequential application of three alternative methods: structure-activity relationships, pH measurements and a single *in vitro* method (Worth *et al.*, 1998).

In 2004, *In Vitro* Skin Corrosion: Human Skin Model Test was adopted as the OECD Test Guideline No 431 which is applicable to the assays for skin corrosion employing reconstituted human skin (EPISKIN™ and EPIDERM™) models (OECD, 2004).

In May 2008, all the test methods of Annex V of the Directive 67/548/EEC have been incorporated into the Annex to the Commission Regulation 440/2008/EC, laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EU, 2008).

Last update: November 2008

PROCEDURE DETAILS, March 1997*

EpiSkin™ Skin Corrosivity Test DB-ALM Protocol n° 118

this protocol presents the standard operating procedure used in the ECVAM International Validation Study on In Vitro Tests for Skin Corrosivity (Fentem et al., 1998).

* The accuracy of the SOP has been confirmed in October 2000. During the preparation of the regulatory test guidelines some refinements have been introduced into the test method. Therefore, the proposed update of the SOP has been sent to the person responsible for the method for review and can be provided on request. As soon as new information will become available this version of the protocol will be updated.

Contact Details

Dr. David J. Esdaile
Scientific Director
LAB Research - Hungary
Veszprem
Szabadsagpuszta H-8200
email: david.esdaile@labresearch.hu
telephone: +36 88 545-242

1. Introduction

Product Description

The EPISKIN-SM™ (Standard Model) kit contains 12 reconstructed epidermis units. Each reconstructed epidermis unit consists of a human collagen (Types III and I) matrix, representing the dermis, covered with a film of Type IV human collagen, upon which stratified differentiated epidermis derived from human keratinocytes has been laid. Test materials can be applied directly to the stratum corneum.

Precautions

The epidermal cells are taken from healthy volunteer donors negative to anti-HIV 1 and 2, and to hepatitis C, antibodies, and to hepatitis B antigens. Nevertheless, normal handling procedures for biological materials should be followed:

(a) it is recommended that gloves are worn during handling;

and

(b) after use, the epidermis, the material in contact with it, and the culture medium, should be decontaminated (for example, by using a 10% solution of bleach or a 1% solution of pyosynthene), prior to disposal.

Quality Control

EPISKIN-SM kits are manufactured according to defined quality assurance procedures (certified ISO 9001). All biological components of the epidermis and the kit culture medium have been tested for the presence of viruses, bacteria and mycoplasma. The quality of the final product is assessed by undertaking an MTT cell viability test and a

cytotoxicity test with sodium dodecylsulphate (SDS).

For reasons connected with the nature of the product, it is shipped before all of the necessary checks have been completed. A release form certifying the conformity (or otherwise) of the batch is sent to the user, by fax, on the day of delivery of the kit.

2. Materials

2.1. KIT CONTENTS

DESCRIPTION	USE
1 EPISKIN-SM plate containing 12 reconstructed epidermis units (area: 0.38cm ²)	each reconstructed epidermis is attached to the base of a tissue culture vessel with an O-ring set and maintained on nutritive agar for transport
1 12-well assay plate	for assays
1 flask of sterile assay medium	basic medium for use in assays
1 EPISKIN-SM biopsy punch	for easy sampling of epidermis
<i>1 lot of "MTT reagents" :</i>	
1 flask MTT reagent	to reconstitute
1 flask PBS 10x wash solution	to dilute
1 flask 4N NaOH	to adjust pH of wash solution
1 flask extraction solution of isopropanol acid (ready to use)	
1 flask negative control (NaCl, 9g/l)	specific controls for the corrosivity test
1 flask positive control (glacial acetic acid)	

2.2 MATERIALS NOT PROVIDED WITH THE KIT

- 500ml wash bottle
- 5ml glass tubes with corks
- 200µl micropipette
- Multidispenser micropipette (2.2ml)
- 50µl or 100µl positive displacement micropipette (for applying thick or viscous samples)
- Vacuum source and Pasteur pipettes
- Small forceps
- Timers
- Microplate reader with filter of 545-595nm and 96-well microplates; or spectrophotometer and 1ml microcells
- Vortex mixer

- Non-sterile ventilated cabinet

3. Experimental Procedures and Timing

Details of the kit and assay procedures should be registered on the reporting form (Annex 1).

3.1 RECEIPT OF TEST KIT

Check the date of dispatch written on the package. Before opening the EPISKIN-SM kit:

a) inspect the colour of the agar medium used for transport and check that its pH is acceptable: orange colour = good; yellow or violet colour = not acceptable;

and

b) inspect the colour of the temperature indicator to verify that the kit has not been exposed to a temperature above 40°C: the indicator changes from white to grey at 40°C.

In the event of any anomaly, immediately contact the Sales Administration Department at SADUC (Tel: +33 78 56 72 72; Fax: +33 78 56 00 48).

Place the assay medium supplied with the kits at 2-8°C. Leave the EPISKIN-SM kits in their packaging at room temperature until the assays are to be undertaken.

3.2 APPLICATION AND RINSING

Safety precautions: MTT and corrosive materials are dangerous. Work in a non-sterile, ventilated, cabinet, wear protective gloves, and a mask and safety glasses, as necessary.

Pre-warm the assay medium to 37°C. An approximate timing for conducting the test procedure is given below as a guide.

9.30: proceed with the application of test material for the **4 hours** samples

(a) Fill the appropriate number of wells of an assay plate with pre-warmed culture medium (2.2ml per well). Mark the plate lids with the application time (4 hours) and the code numbers of the chemicals to be tested (1 well per chemical), or negative control (3 wells) or positive control (3 wells).

(b) Open the EPISKIN-SM kits and place an epidermis unit into each prepared well. Mark each epidermis unit with the appropriate code number.

9.45: application of the products during **4 hours**:

(c) Add 50µl of test material to each well by using the positive displacement pipette.

(d) In the case of solids, the material should be crushed to a powder, if necessary, and 20mg applied evenly to the epidermal surface (with difficult materials, use sufficient to cover the epidermal surface); add 100µl NaCl (9g/l saline) to ensure good contact with the epidermis.

(e) Add 50µl NaCl (9g/l saline) to each of the three negative control wells.

(f) Add 50µl glacial acetic acid to each of the three positive control wells.

(g) Replace the lid on the plate and incubate for 4 hours (\pm 5 minutes) in a ventilated cabinet at room temperature (18-28°C).

Note: The negative and positive controls incubated for 4 hours will act as controls for all of the incubation times.

10.00: proceed with the application of test material for the **1 hour** samples

(a) Fill the appropriate number of wells of an assay plate with pre-warmed culture medium (2.2ml per well). Mark the plate lids with the application time (1 hour) and the code numbers of the chemicals to be tested (1 well per chemical).

(b) Open the EPISKIN-SM kits and place an epidermis unit into each prepared well. Mark each epidermis unit with the appropriate code number.

10.15: application of the products during **1 hour**

(c) Add 50µl of test material to each well by using the positive displacement pipette.

(d) In the case of solids, apply 20mg and add 100µl of NaCl (9g/l), as described previously for the 4 hours samples.

(e) Replace the lid on the plate and incubate for 1 hour (\pm 5 minutes) in a ventilated cabinet at room temperature (18-28°C).

10.30: proceed with the application of test material for the **3 minute** samples

(a) Prepare the MTT solution (0.3mg/ml; enough for 2.2ml per well for the entire assay) and the PBS 1x wash solution, as indicated in the "MTT reagents" leaflet accompanying the test kit.

(b) Fill the appropriate number of wells of an assay plate with pre-warmed culture medium (2.2ml per well). Mark the plate lids with the application time (3 minutes) and the code numbers of the chemicals to be tested (1 well per chemical).

(c) Open the EPISKIN-SM kits and place an epidermis unit into each prepared well. Mark each epidermis unit with the appropriate code number.

10.45: application of the products during **3 minutes**

(d) Add 50µl of test material to each well by using the positive displacement pipette. Proceed well-by-well at 20-second intervals, with the aid of multiple timers (test a maximum of 5 or 6 materials at a time). Ensure that the exposure period is exactly 3 minutes for each well

(e) In the case of solids, apply 20mg and add 100µl of NaCl (9g/l), as described previously for the 4 hours samples.

(f) Remove the EPISKIN-SM unit and rinse thoroughly with PBS 1x solution, to remove all of the test material from the epidermal surface.

(g) Replace the EPISKIN-SM unit in the culture medium.

(h) When all of the units have been rinsed:

- remove the culture medium
- place the units on absorbant paper, or remove the rest of the PBS from the epidermal surface with a Pasteur pipette linked to a vacuum source (be careful not to touch the epidermis)
- add 2.2ml of the MTT solution (0.3mg/ml) to each well
- replace the lid on the plate. If the ambient temperature is 20-28°C, leave to incubate for 3 hours (\pm 5 minutes) in a ventilated cabinet at room temperature, **protected from light**. If the ambient temperature is below 20°C, then leave to incubate for 3 hours (\pm 5 minutes) at temperature of 20-28°C, **protected from light**. An incubator (with or without CO₂), or a warm location within the laboratory, may be used. It is important that all the samples from each exposure time are treated identically.

11.15: rinse the 1 hour samples and replace the culture medium with 2.2ml of MTT solution (0.3mg/ml), as described above.

11.45: place 0.85ml of acidified isopropanol into labelled glass tubes (one tube corresponding to one well of the tissue culture plate). Label each tube with the name of the test material and the incubation time.

13.45: rinse the 4 hours samples and replace the culture medium with 2.2ml of MTT solution (0.3mg/ml), as described above.

3.3 FORMAZAN EXTRACTION

At the end of each incubation with MTT (14.15, 14.45 and 17.00), the formazan extraction should be undertaken:

- (a) place the units on absorbant paper
- (b) remove the MTT solution from each well
- (c) take a biopsy of the epidermis by using the biopsy punch, by placing the epidermis unit on the plate lid
- (d) separate the epidermis from the collagen matrix with the

aid of forceps, and place both parts (epidermis and collagen matrix) into the acidified isopropanol

(e) cork each tube and mix thoroughly by using a vortex mixer

(f) ensure that the acidified isopropanol is in good contact with all of the material

(g) store at room temperature overnight, protected from light.

3.4 ABSORBANCE/OPTICAL DENSITY MEASUREMENTS

Following the formazan extraction (left overnight):

(a) mix each tube by using a vortex mixer

(b) let the solution settle for 1-2 minutes, so that any cell fragments do not interfere with the absorbance readings

(c) place a 200µl sample from each tube into the wells of a 96-well plate (labelled appropriately)

(d) read the optical densities (OD) of the samples at a wavelength between 545nm and 595nm using acidified isopropanol solution as the blank.

(e) record the results on the template given in Annex 2.

NB. if a spectrophotometer is used rather than a plate reader, place a 500µl sample from each tube and 500µl isopropanol (not acidified) in a 1ml microcell and read the OD at 545-595nm using the acidified isopropanol solution as the blank.

4. Calculations of Viability Percentages and Acceptability Criteria

Record all calculations on the Data Report Form (Annex 3).

Viability (%) = $100 \times (\text{OD test material} / \text{mean OD negative control at 4 hours})$

(a) calculate the mean OD of the 3 negative control values: this corresponds to 100% viability. Based on historical data the minimum acceptable mean OD for negative controls is 0.115 (mean \pm 2SD). The maximum acceptable mean OD for the negative control is 0.4 (to allow for incubations at 28° C).

(b) calculate the mean OD of the 3 positive control values: the % viability of the positive control is calculated relative to the mean negative control. Based on historical data (mean \pm 2SD), the acceptable mean percentage viability range for positive controls is 0-20%.

(c) calculate the % viability following exposure to the test material at each incubation time as the OD expressed as a percentage of the mean negative control value.

(d) *assay acceptability criteria*: for an assay to be acceptable, the mean positive and negative control values should fall within the ranges given above. In those cases where the mean values fall outside the range, the assay should be repeated, except in cases where the same chemical has been tested on at least two other occasions (with acceptable control values) and the results of all of the tests give the same corrosivity

classification.

4.1 INTERPRETATION OF TEST RESULTS

The test results are interpreted on the basis of the exposure time needed to cause cell viability to decrease below 35%. The determination of the packing group is summarized in the following table:

Classification	Packing group	Criteria for <i>In Vitro</i> interpretation
UN	Corrosive class I	If viability < 35% after 3 min exposure
	Corrosive class II	If viability \geq 35% after 3 min exposure and < 35% after 1 hour exposure
	Corrosive class III	If viability \geq 35% after 1 hour exposure and < 35% after 4 hours exposure
	Non corrosive	If viability \geq 35% after 4 hours exposure
EU	Corrosive class R35	If viability < 35% after 3 min exposure
	Corrosive class R34	If viability \geq 35% after 3 min exposure and < 35% after 4 hours exposure
	Non corrosive	If viability \geq 35% after 4 hours exposure

In cases where the viability values from individual skin units are highly variable, causing different corrosivity classifications, the chemical should normally be retested. If one or more sets of data are considered to be incorrect (or inconsistent with data from other runs), the results should be replaced by those generated in a repeat run.

In cases where the viability values fall below 35% but longer exposure times give values of $>35\%$ (or values higher than the earlier time point), the results should be considered to be doubtful. The run should normally be repeated.

After having undertaken three independent tests with three different batches of EPISKIN-SM™ for each test material, the Data Compilation Form (Annex 4) should be completed.

ANNEX 1

<p>ECVAM SKIN CORROSIVITY VALIDATION STUDY</p> <p>EPISKIN™</p> <p>Assay report form</p>

Experimental center: _____

Kit Reception

<p>Lot number:</p> <p>Date of Receipt:</p> <p>Observations:</p>

Assay

<p>Date of Assay:</p> <p>Code of tested product:</p> <p>Observations:</p>

ANNEX 2

FEUILLE DE DEPOTS

Date: (jj-mm-aa):

N. de plaque:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

ANNEX 3

ECVAM SKIN CORROSIVITY VALIDATION STUDY EPISKIN™

Data report form

EXPERIMENTAL CENTER:

Lot n.:

Date of assay:

Wavelength (nm) used for lecture:

Negative control	
OD 1	
OD2	
OD3	
Mean	#D/V/O!
% Viability	#D/V/O!

Positive control	
OD 1	
OD2	
OD3	
Mean	#D/V/O!
% Viability	#D/V/O!

Product Code	Physical Appearance	3 min exposure		1 hour exposure		4 hours exposure	
		OD	% viability	OD	% viability	OD	% viability
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!
			#D/V/O!		#D/V/O!		#D/V/O!

Alternatives to Laboratory Animals (ATLA) 24, 653-655

- ESAC (1998)
Statement on the scientific validity of the EPISKIN™ test (an *in vitro* test for skin corrosivity), 10th meeting of ECVAM Scientific Advisory Committee, 31 March 1998, European Commission-Joint Research Centre, Institute for Health and Consumer Protection, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy. ECVAM News and Views.
Alternatives to Laboratory Animals (ATLA) 26, 278-280
- EU (2008)
Council Regulation (EC) No 440/2008 of 30 May 2008, laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Annex, part B. Methods for the determination of toxicity and other health effects.
Official Journal of the European Communities L 142, 141-443
- EU (2004)
Commission Directive 2004/73/EC of 29 April 2004, adapting to technical progress for the 29th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal of the European Communities L 152, 1
- EU (2000)
Commission Directive 2000/33/EC of 25 April 2000, adapting to technical progress for the 27th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal of the European Communities L 136, 90
- Fentem J.H. (1999)
Validation of *in vitro* tests for skin corrosivity.
Alternatives to Animal Experimentation (ALTEX) 16, 150-153
- Fentem J.H., Archer G.E.B., Balls M., Botham P.A., Curren R.D., Earl L.K., Esdaile D.J., Holzhütter H.G. and Liebsch M. (1998)
The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the Management Team.
Toxicology In Vitro 12, 483-524
- OECD (2015)
OECD Test Guideline No. 404: Acute Dermal Irritation/Corrosion. First adopted in 1981. Latest update adopted on the 28th of July 2015. [Link to document](#) (last access: 16.06.2016)
OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects
- OECD (2004)
OECD Test Guideline No. 431: *In Vitro* Skin Corrosion: Human Skin Model Test, adopted on the 13th of April 2004. [Link to document](#) (last access 16.08.2012)
OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects
- Tinois, E., Gaetini, Q., Gayraud, B., Dupont, D., Rougier, A., and Pouradier Duteil, X. (1994)
The Episkin model: successful reconstruction of human epidermis *in vitro*.
Alternative Methods in Toxicology 10, 133-140
- US DOT (1991)
U.S. Department of Transportation. Code of Federal Regulations. Method of Testing Corrosion to the Skin.
Transportation Title 49, Part 173.136, Appendix A
- Worth A.P., Fentem J.H., Balls M., Botham P.A., Curren R.D., Earl L.K., Esdaile D.J. and Liebsch M. (1998)
An evaluation of the proposed OECD testing strategy for skin corrosion.
Alternatives to Laboratory Animals (ATLA) 26, 709-720