In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method

Commonly used acronym: *RHE irritation test method, OECD TG 439*

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Organisation

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Department Health

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	Reconstructed human Epidermis (RhE) obtained from human derived non-transformed keratinocytes

DESCRIPTION

Method keywords

3D Reconstructed Human Tissue model

Human epidermis

cytotoxicity

Cell viability

MTT

Skin irritation

Scientific area keywords

Hazard identification

Irritating chemicals

GHS

Local toxicity of substances and mixtures

Classification

Labelling

Method description

The method is described in detail in OECD TG 439 as SkinEthicTM RHE and provides an *in vitro* procedure that may be used for the hazard identification of irritant chemicals (substances and mixtures) in accordance with the United Nations Globally Harmonized System of Classification and Labelling (UN GHS) Category 2. This Test Guideline (TG) can also be used to identify non-classified chemicals. Therefore, depending on the regulatory framework and the classification system in use, this procedure may be used to determine the skin irritancy of test substances as a stand-alone replacement test for *in vivo* skin irritation testing, or as a partial replacement test, within a tiered testing strategy. It is based on reconstructed human epidermis (RhE), which in its overall design closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e. the epidermis. The test chemical is applied topically to a three-dimensional RhE model. The method measures the initiating events in the cascade, e.g. cell/tissue damage, using cell viability as readout. Cell viability is measured by enzymatic conversion of the vital dye MTT into a blue formazan salt that is quantitatively measured after extraction from tissues. Irritant chemicals are identified by their ability

to decrease cell viability below defined threshold levels (i.e. ? 50%, for UN GHS Category 2). Test chemicals that produce cell viabilities above the defined threshold level, may be considered non-irritants (i.e. > 50%, No Category).

Lab equipment

- Standard equipment for working with cell cultures;
- Microplate reader (OD) or HPLC/UPLC-spectrophotometer.

Method status

Validated by an external party (e.g. OECD, EURL ECVAM,…)

PROS, CONS & FUTURE POTENTIAL

Advantages

- It is based on an *in vitro* test system of reconstructed human epidermis (RhE), which closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e. the epidermis.
- The RhE test system uses human-derived non-transformed keratinocytes as cell source to reconstruct an epidermal model with representative histology and cytoarchitecture.
- Performance Standards (PS) are available to facilitate the validation and assessment of similar and modified RhE-based test methods.
- The method is applicable to solids, liquids, semi-solids and waxes. The liquids may be aqueous or non-aqueous; solids may be soluble or insoluble in water. Whenever possible, solids should be ground to a fine powder before application; no other pre-treatment of the sample is required.

For more information: see OECD TG 439

Challenges

- It does not allow the classification of chemicals to the optional UN GHS Category 3 (mild irritants).

- Gases and aerosols have not been assessed yet in a validation study.

- A lack of applicability of the RhE based *in vitro* skin irritation test for agrochemical formulations.

- Test chemicals absorbing light in the same range as MTT formazan and test chemicals able to directly reduce the vital dye MTT (to MTT formazan) may interfere with the cell viability measurements and need the use of adapted controls for corrections.

- This method does not provide adequate information on skin corrosion. It should be noted that OECD TG 431 on skin corrosion is based on the same RhE test system, though using another protocol.

For more information: see OECD TG 439

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

OECD TG 439.pdf

Links

OECD Test Guideline No.439: In Vitro Skin Irritation: Reconstructed Human Epide…