

# In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method

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

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## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Regulatory use - Routine production
<b>Type of method</b>	In vitro - Ex vivo
<b>This method makes use of</b>	Human derived cells / tissues / organs
<b>Specify the type of cells/tissues/organs</b>	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 SkinEthic™ 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.  $\leq$  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...](#)

## PARTNERS AND COLLABORATIONS

### Organisation

**Name of the organisation** VITO

**Department** Health

**Country** Belgium

**Geographical Area** Flemish Region

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