

## In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method

**Commonly used acronym:** RhE Corrosion Test Method, OECD TG 431

Created on: 23-02-2021 - Last modified on: 04-03-2021

### Contact person

Sandra Verstraelen

### Organisation

**Name of the organisation** Vlaamse Instelling voor Technologisch Onderzoek (VITO)

**Department** Health

**Country** Belgium

**Geographical Area** Flemish Region

## 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>Specify the type of cells/tissues/organs</b> | Reconstructed human epidermis (RhE) obtained from human derived non-transformed epidermal keratinocytes |

## DESCRIPTION

### Method keywords

Reconstructed human epidermis  
Three-dimensional human skin model  
cytotoxicity  
Cell viability  
MTT  
Skin corrosion  
Reconstructed human epidermis  
Skin corrosion

### Scientific area keywords

Hazard identification  
Corrosive chemical substances and mixtures  
Classification  
Labelling  
GHS  
in vitro toxicology

### Method description

This method has been described in detail in OECD TG 431 and allows the identification of non-corrosive and corrosive substances and mixtures in accordance with the UN GHS. This method further supports the sub-categorisation of corrosive substances and mixtures into optional Subcategory 1A, in accordance with the UN GHS, as well as a combination of Subcategories 1B and 1C. A limitation of this method is that it does not allow discriminating between skin corrosive Sub-category 1B and Sub-category 1C in accordance with the UN GHS due to the limited set of well-known *in vivo* corrosive Sub-category 1C chemicals. There are five validated test methods described under test guideline 431 which are able to discriminate sub-categories 1A versus 1B-and-1C versus NC. The test chemical is applied topically to a three-dimensional RhE model, comprised of non-transformed, human-derived epidermal keratinocytes, which have been cultured to form a multi-layered, highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multi-layered stratum corneum containing intercellular lamellar lipid layers representing main lipid classes analogous to those found *in vivo*. The RhE test method is based on the premise that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion, and are cytotoxic to the cells in the underlying layers. 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. Corrosive chemicals are identified by their ability to decrease cell viability below defined threshold levels. The RhE-based skin corrosion test methods have shown to be predictive of *in vivo* skin corrosion effects assessed in rabbits according to the OECD guideline 404.

### 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

- Commercially available test method.
- This method allows the identification of non-corrosive and corrosive substances and mixtures in accordance with the UN GHS. It supports the sub-categorisation of corrosive substances and mixtures into optional Subcategory 1A, in accordance with the UN GHS, as well as a combination of Subcategories 1B and 1C.
- This test can also be used for regulatory purposes for distinguishing corrosive from non-corrosive substances.
- This method is applicable to a wide range of chemical classes and physical states including liquids, semi-solids, 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 prior treatment of the sample is required.

For more information: see OECD TG 431

### Challenges

- A limitation of this method is that it does not allow discriminating between skin corrosive Sub-category 1B and Sub-category 1C in accordance with the UN GHS due to the limited set of well-known *in vivo* corrosive Sub-category 1C chemicals.
- This method does not provide adequate information on skin irritation.

- This method is assumed to be applicable to mixtures as an extension of its applicability to substances. However, due to the fact that mixtures cover a wide spectrum of categories and composition, and that only limited information is currently available on the testing of mixtures, it might not be used for specific categories of mixtures.
- 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 tissue viability measurements and need the use of adapted controls for corrections.
- Gases and aerosols cannot be tested with this method.

For more information: see OECD TG 431

## REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

### Associated documents

[OECD TG 431.pdf](#)

### Links

[Test Guideline No. 431 In Vitro Skin Corrosion: Reconstructed Human Epidermis \(...\)](#)

Coordinated by



Financed by



Vlaanderen  
verbeelding werkt

