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

Commonly used acronym: *RhE Corrosion Test Method, OECD TG 431*
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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
This method makes use of	Human derived cells / tissues / organs
Specify the type of cells/tissues/organs	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 corrosionReconstructed 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 (...

PARTNERS AND COLLABORATIONS

Organisation
Name of the organisation VITO
Department Health
Country Belgium
Geographical Area Flemish Region

Coordinated by

Financed by