An Initial Evaluation of the CellSystems EST-1000 Reconstructed Human Skin Model for distinguishing R34 and R35 corrosives *in vitro*

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Introduction

Skin corrosion refers to the production of irreversible tissue damage in the skin following the application of a test material (as defined by the Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS)[1]). Assessment of the potential of chemicals to induce skin corrosion is important in establishing procedures for their safe handling, packaging and transportation. Validation studies have shown that tests employing reconstructed human skin models are able to reliably distinguish between known skin corrosives and non-corrosives[2,3,4]. The reconstructed human epidermis model EST-1000 can be used in the assessment of skin corrosivity potential as indicated in the OECD Guideline for the testing of chemicals No 431[5].

The purpose of this study was to assess whether the EST-1000 model can be used to correctly identify R35 (UN packing group I) and R34 (UN packing group II & III) chemicals by performing corrosivity testing in accordance with INVITTOX Protocol No 118. Two R35 corrosive chemicals (1,2 diaminopropane and acrylic acid), two R 34 corrosive chemicals (2-tert.butylphenol and octanoic acid) and two non-corrosive chemicals (phenethyl bromide and 4-(methylthio)-benzaldehyde) were applied to EST-1000 tissues for periods of 3, 60 or 240 minutes and tissue viability was determined by measurement of mitochondrial dehydrogenase activity using the MTT assay.

Materials and Methods

Test materials (coded and used as supplied)
Material R-4180: Phenethyl bromide
Material R-4181: 4-(Methylthio)-benzaldehyde
Material R-4182: 2-tert. Butylphenol
Material R-4183: 1, 2-Diaminopropane
Material R-4184: Acrylic acid
Material R-4189: Octanoic acid

Positive control material: Glacial acetic acid.
Negative control material: 0.9% (w/v) Sodium chloride solution

Human skin model cultures: EST-1000 reconstructed human epidermis cultures (0.63cm^2) supplied by CellSystems® Biotechnologie Vertrieb GmbH, St Katharinen, Germany

Briefly, duplicate EST-1000 tissue cultures were topically exposed to the test or control substances (25mg of solids or 50µL of liquids) for periods of 3, 60, and 240 minutes at room temperature. At the end of the exposure period the tissues were subjected to a washing procedure. The rinsed tissues were treated with 1.0mg/ml MTT (300µL for 3 hours). The relative viability of the MTT treated tissues was assessed by extraction of formazan and assay of optical density at 540nm.

Prediction Model for Corrosivity

Classification of corrosivity potential was based on relative viabilities for each exposure time according to the following table:

Treatment Time (minutes)	Relative mean tissue viability (% of negative control)	Prediction	EU Classification
3	<35	Corrosive	R35
3/60	≥35 / <35	Corrosive	R34
60/240	≥35 / <35	Corrosive	R34
240	≥35	Non-Corrosive	No label

Results

Relative mean tissue viability (% of negative control) for the Test Material treatment groups following exposure

Test Material	Exposure Period (minutes)			In Vitro Classification	EU Classification
	3	60	240		
R-4180	88.89	62.93	39.38	Non-Corrosive	Non-Corrosive
R-4181	104.31	82.13	48.05	Non-Corrosive	Non-Corrosive
R-4182	3.22	4.24	4.49	R35	R34
R-4183	2.25	3.84	1.90	R35	R35
R-4184	4.02	3.68	3.17	R35	R35
R-4189	47.07	4.26	3.00	R34	R34

Conclusions

•Concordance between the *in vitro* predictions of skin corrosivity potential obtained with the CellSystems EST-1000 human skin model and EU Classification was generally very good.

•The results of the study demonstrated that using the EST-1000 model in accordance with the INVITTOX protocol, it was possible to correctly distinguish and identify the corrosive and non corrosive chemicals. Both R35 corrosive chemicals were correctly identified and one of the R34 corrosive chemicals was also correctly identified. The other R34 chemical (2-tert.Butylphenol) was overpredicted as an R35 corrosive chemical.

• It is concluded that although the study used only a limited number of chemicals, the results indicate that the EST-1000 human skin model is predictive of corrosivity potential and is a suitable candidate for distinguishing R35 (UN PG I) and R34 (UN PG II & III) chemicals when used in accordance with INVITTOX Protocol NO 118.

References

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