The Open Source Reconstructed Epidermis – Results of a catch-up validation study for skin irritation testing

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Introduction

Until today four epidermal equivalents, provided by 3 different companies, have been validated as stand-alone replacements of animal tests for *in vivo* skin irritation testing of chemicals and thus are subject of the OECD Test Guideline 439 [1]. In order to facilitate woldwide access to epidermal equivalents and skin irritation testings, a novel human epidermal equivalent, based on a published protocol [2], was developed: the Open Source Reconstructed Epidermis (OS-REp). As a prerequisite for official regulatory acceptance of the test method a catch-up validation study according to the Performance Standards (PS) of the OECD TG 439 was conducted. The study was performed completely under open source conditions, meaning that each of the three test laboratories produced its own OS-REp equivalents for subsequent irritation tests according to standard operation procedures that were shared beforehand.

Materials & Methods

OS-REp models were produced from human primary keratinocytes. Final differentiation was achieved after 19 days at the air-liquid interface (figure 1).

Skin irritation tests according to OECD TG 439 were performed with OS-REp models which had passed the quality checks. Twenty chemicals – 10 irritants and 10 non-irritants – were tested in each laboratory in three valid runs under double-blinded conditions. Briefly, 25 µl of liquids or an equivalent of 25 µl of solids, respectively, were topically applied to the OS-REp models. After 35 minutes incubation time, the models were washed with PBS and incubated for 42 hours at 37°C and 5% CO_2. After an MTT viability assay, formazan was extracted from the tissues with isopropanol. The optical density of the formazan solution was measured in a photospectrometer at λ = 540 – 600 nm. From the sample OD the relative tissue viability with regard to the OD of the negative control was calculated.

Prediction model

The chemicals are classified with respect to their skin-irritating properties according to the GHS system:

Tissue viability ≤ 50%	GHS category 2 (skin irritant)
Tissue viability > 50%	no category (no-skin irritant)

Results & Discussion

All acceptance criteria, as defined in the OECD TG 439, were met or even exceeded by each of the 3 laboratories. In table 1 the results for every laboratory and every test substance is depicted. Table 2 summarizes the predicitive parameters for each study partner.

Three out of 10 non-classified chemicals were predicted false positive in every laboratory similar to the results of the validated reference method (VRM) at EURL-ECVAM, which thus had defined 70% specificity as acceptance criterion. Three out of 10 irritants were predicted falsely negative in single laboratories only. In the cases of di-n-propyl disulphide and 1-bromohexane only about 20% and 50% of humans developed irritation reactions in a 4h patch test study, respectively [3, 4]. Because the keratinocytes for the OS-REp models in the partner labs originated from different donors, variable classifications due to donor-specific cellular properties can be expected *in vitro*, too. Misclassification of 1-methyl-3-phenyl-1-piperazine can be presumably attributed to its special physical properties.

The catch-up validation study data are currently under review at EURL-ECVAM for official regulatory acceptance.

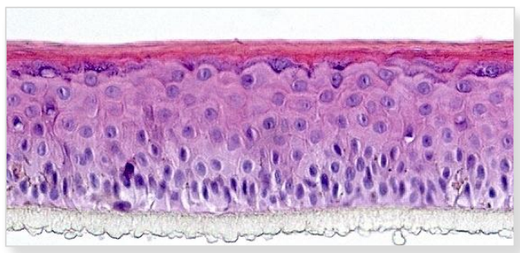

Figure 1: OS-REp models at day 19 of air-liquid interface culture. All 4 human layers, including a cornified layer, are developed. Underneath the basal layer the membrane of the inserts, in which the models are grown, is visible. H&E staining of paraffin sections.

Table 1: Test results for 10 non-classified and 10 skin-irritating chemicals. Every classification comprises the mean value of 3 valid runs with 3 OS-REp models each. Results highlighted in red: falsely classified chemicals. Reference result: *in vivo* GHS classification; VRM *in vitro*: validated reference method based on epidermal models

Test substance	Reference result	Lab A	Lab B	Lab C	VRM in vitro
1-bromo-4-chlorobutane	No Cat.	Cat. 2	Cat. 2	Cat. 2	Cat. 2
4-methyl-thio-benzaldehyde	No Cat.	Cat. 2	Cat. 2	Cat. 2	Cat. 2
allyl phenoxy-acetate	No Cat.	NI	NI	NI	NI
cinnamaldehyde	No Cat.	Cat. 2	Cat. 2	Cat. 2	Cat. 2
diethyl phthalate	No Cat.	NI	NI	NI	NI
heptyl butyrate	No Cat.	NI	NI	NI	NI
hexyl salicylate	No Cat.	NI	NI	NI	NI
Isopropanol	No Cat.	NI	NI	NI	NI
methyl stearate	No Cat.	NI	NI	NI	NI
naphtalene acetic acid	No Cat.	NI	NI	NI	NI
1-bromohexane	Cat. 2	Cat. 2	NI	Cat. 2	Cat. 2
1-decanol	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
1-methyl-3-phenyl-1-piperazine	Cat. 2	Cat. 2	Cat. 2	NI	Cat. 2
2-chloromethyl-3,5-dimethyl-4-methoxypyridine HCl	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
benzenethiol-5-(1,1-dimethylethyl)-2-methyl	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
cyclamen aldehyde	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
di-n-propyl disulphide	Cat. 2	Cat. 2	NI	Cat. 2	NI
heptanal	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
potassium hydroxide (5% aq)	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2
tetrachloroethylene	Cat. 2	Cat. 2	Cat. 2	Cat. 2	Cat. 2

Table 2: Main predictive parameters calculated for every participating laboratory with a 2x2 contingency table. Overall values: mean values from all 3 labs.
Sensitivity: % of correctly predicted irritants; Specificity: % of correctly predicted non-irritants; Accuracy: % of all correctly predicted chemicals; BLR (between-laboratory reproducibility): % of concordantly predicted chemicals

Predictive parameters	Lab A	Lab B	Lab C	overall	Acceptance criteria
Sensitivity [%]	100	90	80	90	80
Specificity [%]	70	70	70	70	70
Accuracy [%]	85	80	75	80	75
BLR [%]	-	-	-	85	80

Literature

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