In vitro skin corrosion testing according to UN-GHS sub-categorization using **EST**1000

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Introduction

In addition to OECD TG431 which differentiates between corrosive or non-corrosive chemicals the United Nations Globally Harmonized System (UN-GHS) requires an advanced classification into three sub-categories: 1A, 1B or 1C. Within these sub-categories especially 1A labeling has major consequences e.g. complex transport restrictions. This underlines the importance for a reliable discrimination between 1A and 1B/C sub-categories.
EST1000 (CellSystems, Germany) is a reconstructed human epidermis which is validated and accepted for skin corrosion testing and can be used for predicting the corrosive potential of substances according to OECD TG431. As described above this guideline does not satisfy the international packing standards for the labeling of dangerous chemicals according to UN-GHS.
In this study **EST**1000 was used to classify 42 chemicals (part of the the ECVAM validation study) according to UN-GHS. This set of liquid and solid chemicals included similar numbers of non corrosive, 1A and 1B/C chemicals.

Material and Methods

Test System

Technology:

- Organotypic culture
- Human keratinocytes forming a multilayered epidermis
- Intact barrier function
- Epidermis surface: 0.6 cm^2

Applications:

- Skin corrosion
- Skin irritation
- Sensitisation
- Wound healing
- Phototoxicity
- Genotoxicity

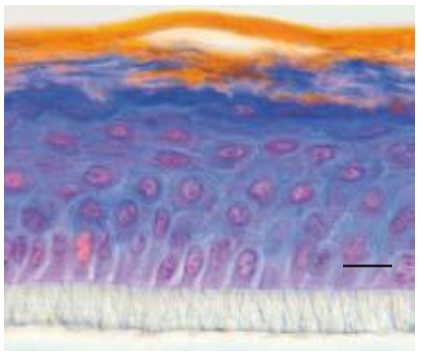

Figure 1: EST1000 morphology
Histological analysis demonstrates strong similarity to human skin. (Azan staining, bar 10 µm)

Test Protocol

- Tissues (n=2) are exposed to 50 µl of liquid or 25 mg of solid chemical of a chemical for 3 and 60 minutes.
- One freeze-killed (FK) **EST**1000 is additionally used for each tested chemical and time point 3 and 60 minutes).
- Tissues are rinsed extensively with PBS and transferred to a 24-well plate containing 1mg/ml MTT in assay medium.
- Incubation 3 hrs at 37° C
- Extraction of formazan with 2 ml isopropanol
- The optical density (OD) of the solution is measured by using a spectro-photometer at 550 nm.
- The viability is determined as follows:

$$\text{Viability} = \frac{\text{OD } \mathbf{EST}1000 \text{ viable} - \text{OD } \mathbf{EST}1000 \text{ FK}}{\text{OD } \mathbf{EST}1000 \text{ negative control}}$$

Results

Prediction Model

A

Old: no sub-categories	
Corrosive:	viability < 50 % at 3 min **or**
	viability ≥ 50 % at 3 min **and**
	viability < 15 % at 60 min
Non-Corrosive:	viability ≥ 50 % at 3 min **and**
	viability ≥ 15 % at 60 min

B

New: with sub-categories	
Category 1A	viability < 50 % at 3 min
Category 1B+1C	viability ≥ 50 % at 3 min **and**
	viability < 15 % at 60 min
Non-Corrosive	viability ≥ 50 % at 3 min **and**
	viability ≥ 15 % at 60 min

Table 1: Comparison of different prediction models.
The validated test protocol for TG431 remained unchanged. An adaptation of the old prediction model (A) was necessary to satisfy the needs of the UN-GHS (B).

Analysis

Sensitivity (C, NC)	**95,20%**	**20 of 21**
1A	88,90%	8 of 9
1A classified as 1B+1C	11,10%	1 of 9
1A classified NC	0%	0 of 9
1B+1C	50%	6 of 12
1B+1C classified as 1A	41,70%	5 of 12
1B+1C classified as NC	8,30%	1 of 12
Specificity (C, NC)	**76,20%**	**16 of 21**
NC classified as 1B+1C	23,80%	5 of 21
NC classified as 1A	0%	0 of 21
Accuracy (C, NC)	**85,70%**	**36 of 42**
Accuracy (1A, 1B+1C, NC)	**71,40%**	**30 of 42**

Table 2: Statistical analysis shows the results for the different sub-categories.

List of Chemicals

Chemical	Physical State liquid (l)/solid (s)	Viability 3 min [%]	Viability 60 min [%]	CAS#	MTT reducer	Classification *in vivo*	Classification **EST**1000
2.4-Xylidin	l	96,94	29,60	95-68-1		NC	NC
20 % SDS	l	84,49	62,56	151-21-3		NC	NC
Eugenol	l	92,65	28,63	97-53-0	yes	NC	NC
Potassium hydroxide (5 %)	l	91,23	-1,23	1310-58-3	yes	NC	1B/1C
Lauric acid	s	104,29	93,05	143-07-7		NC	NC
Potassium hydroxide (5 %)	l	91,23	-1,23	1310-58-3	yes	NC	1B/1C
4-Methy-Thio-Benzaldehyde	l	91,90	65,10	3446-89-7		NC	NC
Tetrachloroethylen	l	87,22	27,50	127-18-4		NC	NC
L-Glutamic acid hydrochloride	s	110,90	106,10	138-18-8		NC	NC
Phenethyl bromide	l	93,97	55,26	103-63-9		NC	NC
Alpha-Ketoglutaric acid	s	80,30	2,80	3280-50-7		NC	1B/1C
4-Amino-1,2,4-triazole	s	98,00	81,00	584-13-4		NC	NC
Sulfamic acid	s	88,40	2,40	5329-14-6		NC	1B/1C
Butyl carbamate	s	96,70	54,20	592-35-8		NC	NC
Sodium bicarbonate	s	83,20	86,40	144-55-8		NC	NC
Oxalic acid dihydrate	s	86,10	6,90	6153-56-6		NC	1B/1C
Isostearic acid	l	97,00	79,00	30399-84-9		NC	NC
Methyl 2,2-dimethylpropanoate	l	93,00	22,69	598-98-1		NC	NC
Sodium carbonate (50%)	l	89,40	98,60	497-19-8		NC	NC
Cuaiacol	l	82,10	15,24	90-05-1		NC	NC
1,9-Decadiene	l	87,03	16,57	1647-16-1		NC	NC
n-Heptylamine	l	14,38	19,09	111-68-2	yes	1B/1C	1A
Octanoic acid	l	27,32	3,03	124-07-2		1B/1C	1A
2-tert. Butylphenol	l	10,26	4,95	88-18-6	yes	1B/1C	1A
Sulphuric acid (10 %)	l	97,93	61,96	7664-93-9		1B/1C	NC
1-(2-Aminoethyl)piperazine	l	102,09	13,47	140-31-8		1B/1C	1B/1C
Sodium bisulphate	s	-0,90	16,40	7681-38-1	yes	1B/1C	1A
Hexanoic Acid	l	82,49	2,74	142-62-1		1B/1C	1B/1C
Cyclohexylamine	l	17,98	0,10	108-91-8		1B/1C	1A
Lactic Acid	l	74,81	0,01	598-82-3		1B/1C	1B/1C
Sodium bisulphate monohydrate	s	117,13	14,30	10034-88-5		1B/1C	1B/1C
Methacrolein	l	52,42	6,45	78-85-3	yes	1B/1C	1B/1C
Dimethylisoproylamine	l	64,67	1,45	996-35-0		1B/1C	1B/1C
Dimethyldipropylenetriamine	l	74,40	3,23	10563-29-8		1A	1B/1C
Phosphrus tribromide	l	14,91	1,02	7789-60-8		1A	1A
Boron trifluoride dihydrate	l	6,07	2,58	13319-75-0		1A	1A
1, 2-Diaminopropane	l	12,40	10,22	78-90-0	yes	1A	1A
Acrylic acid	l	4,93	3,74	79-10-7		1A	1A
Phenol	s	9,7	5,3	108-95-2		1A	1A
Silver nitrate	s	17,5	20,8	7761-88-8		1A	1A
Bezalkonium cholride	s	39,6	22,7	8001-54-5		1A	1A
Brom acetic acid	s	4,4	3,7	79-08-3		1A	1A

Table 3: Overview of all tested chemicals.
The table shows the results for all recently tested chemicals including viabilities and classifications. 1A chemicals are marked in red, 1B/1C chemicals in yellow and NC chemicals in green.

Discussion and Conclusion

During this study 42 chemicals were classified following the OECD TG431 using the validated EST1000 **skin corrosion test. In addition, a new prediction model to sub-categorize the corrosive chemicals into 1A or 1B/1C according to UN-GHS is introduced. Excellent results were found for the detection of 1A and non corrosive chemicals. We chose a high number of borderline chemicals which are frequently misclassified *in vitro*. This explains the relatively high rate of over prediction of 1B/1C chemicals. In further studies we are going to test the remaining 40 chemicals from the ECVAM skin corrosion validation study which include a lower number of borderline chemicals. We expect that these studies will reduce the rate of over prediction significantly.**
Conclusion: The EST1000 ***in vitro*** **skin corrosion test is suitable for sub-categorization of chemicals according to UN-GHS.**