Sub-categorization of dermal corrosives *in vitro* using the reconstructed human skin model epiCS®

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Introduction

The epiCS skin corrosion test method classifying chemicals either as corrosive (Category 1) or non corrosive (no Category) was validated (ESAC 2009) according to OECD TG 431. Recently, the EU Classification, Labeling and Packaging Regulation (EU CLP) system required the sub-categorization of corrosive chemicals into the UN GHS optional subcategories 1A and 1B/C. Protection measures for human health are not affected by sub-categorization, i.e. independent of the sub-category the protection measures for human health are the same. The purpose of the present study was to assess whether epiCS skin models can reproducibly discriminate the sub-categories of corrosive chemicals with the classifications 1A (strong corrosive) and 1B/C (weak corrosive).

Methods

MTT Test

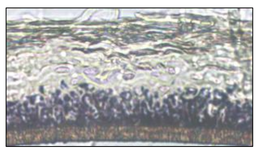

Fig. 1 epiCS cryo slide preparation. MTT reducing cells are located in the basal layers.

Duplicate epiCS tissue models were topically exposed to the test or control substances (25 mg of solids or 50 µl of liquids) for 3 minutes at room temperature and 60 minutes at 37 °C. At the end of the exposure period tissues were subjected to a washing procedure using PBS (pH 7,0). The rinsed tissues were treated with 1 mg/ml MTT (300 µl for 3 hours). The relative viability of the MTT treated tissues was assessed by extraction of formazan with isopropanol and subsequent measurement of optical density at 550 nm wavelength.

Prediction Model

Viability measured after exposure time points (t=3 and 60 minutes)	Prediction to be considered
< 50% after 3 min exposure	**Corrosive:** • Optional Sub-category 1A
≥ 50% after 3 min exposure **AND** < 15% after 60 min exposure	**Corrosive:** • A combination of optional Sub-categories 1B and 1C
≥ 50% after 3 min exposure **AND** ≥ 15% after 60 min exposure	Non-corrosive

Fig. 2 Prediction model for the sub-categorization of corrosives.

Eighty chemicals including solids, semi-solids and liquids of different chemical classes (e.g., electrophiles, organic bases and acids, neutral organics, surfactants, inorganic salts and acids, phenols), selected by the OECD expert group on skin corrosion, were tested in two independent runs. Freeze-inactivated tissues were used to correct for direct MTT reduction and interference by colouring agents.
The classification is based on the following prediction model (PM, Fig.2): A chemical is classified 1A corrosive, if the viability is < 50% after 3 min exposure. A chemical is classified as 1B/C corrosive, if the viability is ≥ 50% after 3 min and < 15% after 1 hr exposure. Non corrosive (NC) classification is achieved if the viability is ≥ 50% after 3 min and ≥ 15% after 1 hr exposure.

Results

No.	Chemical name	CAS #	*In vivo* (DSD)	*In vivo* (GHS/CLP)	MTT Reducer (Y / N)	New Data NC	1C	1B	1A
1	o-Methoxyphenol (guaiacol)	90-05-1	NC	NC	yes			2	
2	2,4-Xylidine (2,4-dimethylaniline)	95-68-1	NC	NC	yes	1		1	
3	Phenethyl bromide (2-bromoethy benzene)	103-63-9	NC	NC	yes	2			
4	Butyl carbamate	592-35-8	NC	NC		2			
5	L-Glutamic acid hydrochloride	138-15-8	NC	NC		2			
6	1-(o-Tolyl)biguanide	93-69-6	NC	NC	yes	2			
7	Butyl glycolate (polysolvan)	7397-62-8	NC	NC				2	
8	2-Hydroxyisobutyiric acid	594-61-6	NC	NC				2	
9	Oxalic acid dihydrate	6153-56-6	NC	NC				2	
10	alpha-Ketoglutaric acid	328-50-7	NC	NC				2	
11	Sulphamic acid	5329-14-6	NC	NC				2	
12	Dodecanoic acid (lauric acid)	143-07-7	NC	NC		2			
13	Sodium lauryl sulphate (20%)	151-21-3	NC	NC		2			
14	Methyl trimethylacetate	598-98-1	NC	NC		2			
15	4-Amino-4H-1,2,4-triazole	584-13-4	NC	NC		2			
16	1,9-Decadiene	1647-16-1	NC	NC		2			
17	Sodium carbonate (50%)	497-19-8	NC	NC		2			
18	Benzylacetone (4-phenyl-2-butanone)	2550-26-7	NC	NC		2			
19	Eugenol	97-53-0	NC	NC	yes	2			
20	Tetrachloroethylene	127-18-4	NC	NC		2			
21	Sodium undecylenate (33%)	3398-33-2	NC	NC				2	
22	4-Amino-5-methoxy-2-methylbenzensulphonic acid	6471-78-9	NC	NC		2			
23	Potassium hydroxide (5%)	1310-58-3	NC	NC				2	
24	3,3-Dithiopropionic acid	1119-62-6	NC	NC		2			
25	Isopropanol	67-63-0	NC	NC		2			
26	2-Phenylalcohol (2-Phenetyl etanol)	60-12-8	NC	NC	yes			2	
27	n-Butyl propionate	590-01-2	NC	NC		2			
28	Methyl palmitate	112-39-0	NC	NC		2			
29	Methyl laurate	111-82-0	NC	NC		2			
30	Sodium bicarbonate	144-55-8	NC	NC		2			
31	2-Bromobutane	78-76-2	NC	NC		2			
32	4-(Methylthio)-benzaldehyde	3446-89-7	NC	NC	yes	2			
33	2-Ethoxyethyl methacrylate	2370-63-0	NC	NC		2			
34	Cinnamaldehyde	14371-10-9	NC	NC	yes			2	
35	4,4'-Methylene-bis-(2,6-ditert-butylphenol)	118-82-1	NC	NC		2			
36	Sodium bislufite	7631-90-5	NC	NC	yes	2			
37	10-Undecenoic acid	112-38-9	NC	NC		2			
38	N,N-Dimethylbenzylamine	103-83-3	R34	1C	yes				2
39	Fluoboric acid (hydrogentetrafluoroborate) (48%)	16872-11-0	R34	1C					2
40	Maleic anhydride	108-31-6	R34	1C			1		1
41	60/40 Octanoic/decanoc acid	68937-75-7	R34	1B/1C				2	
42	55/45 Octanoic/decanoc acid	68937-75-7	R34	1B/1C				2	
43	65/35 Octanoic/decanoic acid	68937-75-7	R34	1B/1C				2	
44	N,N-Dimethylisopropylamine	996-35-0	R34	1B/1C	yes			2	
45	Hydrochloric acid (14.4%)	7647-01-0	R34	1B/1C				2	
46	n-Heptylamine	111-68-2	R34	1B/1C	yes				2
47	Octanoic acid (caprylic acid)	124-07-2	R34	1B/1C					2
48	Carvacrol	499-75-2	R34	1B/1C	yes			1	1
49	2-tert-Butylphenol	88-18-6	R34	1B/1C	yes				2
50	Methacrolein	78-85-3	R34	1B/1C	yes			2	
51	Lactic acid	598-82-3	R34	1B/1C				2	
52	Sodium bisulphate monohydrate	10034-88-5	R34	1B/1C				2	
53	Glyoxylic acid monohydrate	563-96-2	R34	1B/1C				2	
54	Sodium bisulphate	7681-38-1	R34	1B/1C		2			
55	Cyclohexylamine	108-91-8	R34	1B/1C	yes				2
*56	2-Methylbutyric acid	600-07-7	R34	1B/1C					2
57	Glycol bromoacetate (85%)	3785-34-0	R34	1B/1C				1	
58	3-Methoxypropylamine	5332-73-0	R34	1B/1C	yes				2
59	Allyl bromide	106-95-6	R34	1B/1C	yes			2	
60	1-(2-Aminoethyl)piperazine	140-31-8	R34	1B	yes			2	
61	Iron(III) chloride	7705-08-0	R34	1B		2			
62	Phosphoric acid	7664-38-2	R34	1B					2
63	Propionic acid	79-09-4	R34	1B					2
64	Butyric acid	107-92-6	R34	1B					2
65	Boron trifluoride-acetic acid complex	373-61-5	R34	1B					2
66	Ethanolamine	141-43-5	R34	1B	yes			2	
67	Hydrobromic acid (48%)	10035-10-6	R34	1B					2
68	HCl + sulphuric acid + citric acid (5, 5, 5% wt)	-	R34	1B				2	
69	1,2-Diaminopropane	78-90-0	R35	1A	yes				2
70	Phosphorus tribromide	7789-60-8	R35	1A					2
71	Boron trifluoride dihydrate	13319-75-0	R35	1A					2
72	Acrylic acid	79-10-7	R35	1A					2
73	Formic acid	64-18-6	R35	1A					2
74	Dichloroacetyl chloride	79-36-7	R35	1A					2
75	Silver nitrate	7761-88-8	R35	1A					2
76	Phenol	108-95-2	R35	1A					2
77	Acetic acid	64-19-7	R35	1A					2
78	Bromoacetic acid	79-08-3	R35	1A					2
79	N,N-Dimethyldipropylenetriamine	10563-29-8	R35	1A	yes			2	
80	Sulphuric acid (98%)	7664-93-9	R35	1A					2

*CAS 600-07-7 not available; is now CAS 116-53-0

Table 1 Chemicals tested twice for sub-categorization in this study

epiCS® *In vitro* sub-categorization results				
***In vivo* results**	**1A**	**1BC**	**NC**	
1A	**22 (91.67%)** Correct prediction for 1A	**2 (8.33%)** 1A Under-predicted as 1BC	**0 (0.00%)** 1A Under-predicted as NC	**26**
1BC	**28 (45.90%)** 1BC Over-predicted as 1A	**29 (47.54%)** Correct prediction for 1BC	**4 (6.56%)** 1BC Under-predicted as NC	**61**
NC	**0 (0.00%)** NC Over-predicted as 1A	**21 (28.38%)** NC Over-predicted as 1BC	**53 (71.62%)** Correct prediction for NC	**74**
	50	**52**	**57**	**159**
Accuracy = 65.41%				

Table 3 Summary of in vitro sub-categorization data achieved with epiCS.

STATISTICS ON ENTIRE SET OF CHEMICALS (n= 80 chemicals tested over 2 or 3 runs, i.e. 159-240 classifications)		
	Other RhE Test Methods	**epiCS®**
Overclassifications:		
Cat. 1B/C chemicals Overclassified 1A	21.5 - 46.24%	45.90%
Cat. NC chemicals Overclassified 1B/C	20.72 - 24.32%	28.38%
Cat. NC chemicals Overclassified 1A	0 - 2.70%	0.00%
Cat. NC chemicals Overclassified Corrosive	20.72 - 27.03%	28.38%
Global Overclassification rate (all categories)	17.92 - 30.42%	30.82%
Underclassifications:		
Cat. 1A Underclassified 1B/C	8.33 - 16.67%	8.33%
Cat. 1A Underclassified NC	0.00%	0.00%
Cat. 1BC Underclassified NC	0.00 - 7.53%	6.56%
Global Underclassification rate (all categories)	2.47 - 5.00%	3.77%
Correct Classifications:		
1A Correctly classified	83.33 - 91.67%	91.67%
1B/C Correctly classified	46.24 - 76.34%	47.54%
NC Correctly classified	72.97 - 79.28%	71.62%
Accuracy (Predictive capacity)	64.58 - 78.75%	65.41%

Table 2 Analysis of the results from sub-categorization of corrosives achieved with epiCS and other RhE test methods.

The results with the epiCS test method showed correctly classified corrosive and non corrosive chemicals with a high sensitivity (91,67 %) and specificity (71,62 %). The overall accuracy regarding sub-categorization into UN GHS subcategories 1A and 1B/C was 65,41 %. According to the current prediction model, predictions for Category 1B/C chemicals have quite high over-prediction rates, i.e. over-predicted as Category 1A, while predictions for Category 1A are mostly correct.

Conclusion

The results demonstrate that the epiCS skin corrosion test method is able to correctly identify corrosive and non corrosive chemicals and can distinguish between 1A and 1B/C chemical categories with the current PM. Results will be listed in the revised version of the OECD TG 431 (2014). We recommend to examine whether modifications of the current PM can lead to higher accuracy of the test method and improved prediction of 1 B/C chemicals.