Appendix 8.13 Predictive capacity of the Vitrigel-EIT for 114 chemicals considering DRD

Predictive capacity and relevance

Total 114 test chemicals were tested at the lead laboratory (Appendix 8.14 and Appendix 8.15, law data). Hence, the predictive capacity was evaluated by the 114 results comprise the data for 27of the 36 chemicals during Phase III shown in Table 21 and for 87 chemicals obtained at the lead laboratory shown in Appendix 8.14. The test chemicals were selected to ensure that a diverse range of substances were represented, and aspects such as eye-irritant level per UN GHS categories, physical state, chemical class. Also, quality and reliability of in vivo data for the chemicals were carefully considered by reference to the Draize eye test Reference Database (Adriaens et al., 2014; Barroso et al, 2017). The 114 test chemicals are composed of 89 liquids and 25 solids. Also, their contents are 27 Category 1 chemicals, 1 Category 2 or higher, 1 Category 2, 5 Category 2A or higher, 15 Category 2A, 2 Category 2B or higher and 11 Category 2B chemicals, and 52 No Category chemicals by UN GHS classification. There were 27 coded chemicals tested for Phase III and 87 non-coded chemicals were tested at the lead laboratory. These 114 test chemicals were examined by the Vitrigel-EIT method in accordance with the protocol versions described in Chapter 3.1.3.4 and Appendix 8.8. However, the temperature at which all measurements were made during the chemical exposure experiments was strictly controlled at 28±2°C (Table 19 and Appendix 8.8). Thus we consider this data sufficient for assessing the suitability of the Vitrigel-EIT method for use in a bottom-up approach for identifying ocular non-irritants and in a top-down approach for identifying UN GHS Category 1 ocular irritants. In a bottom-up approach, 75 of the test chemicals were classified as irritant and the other 39 as non-irritant, with results for 85 of the 114 test chemicals matching their UN GHS categories.

In contrast, 8 of the 62 test chemicals classified as irritants by in vivo data were identified as non-irritants, a false-negative rate of 13%. Additionally, 21 of the 52 test chemicals classified as non-irritants under UN GHS were identified as irritants, a false-positive rate of 40%. Thus, the Vitrigel-EIT method achieved a sensitivity of 87% (54/62), a specificity of 60% (31/52), and an accuracy of 75% (85/114), as shown in Table A8.13-1. Data from the lead laboratory also demonstrated that predictive capacity could be improved by expanding the sample size. For example, the specificity achieved in Phase III of this validation study was lower than that obtained from the data of 52 non-irritants resulted in a higher specificity. The list of test chemicals that were either false negative or false positives is shown in Table A8.13-3.

On the other hand, analysis per a top-down approach for identifying UN GHS Category 1 ocular irritants was also performed as a part of this validation study, as shown in Tables A8.13-2. Regarding identifying test chemicals classified as UN GHS Category 1 in a top-down approach, the Vitrigel-EIT method demonstrated a sensitivity of 89% (24/27), a specificity of 41% (36/87), and an accuracy of 53% (60/114). Specificity is an important criterion in a top-down approach, which means that Vitrigel-EIT method is not well suited for use in a top-down approach to identifying UN GHS Category 1 ocular irritants.

Table A8.13-1. Contingency table used for 114 test chemicals in a bottom-up approach

		Vitrigel-EIT		Total
		I	NI	
UN GHS	Cat.1, 2A, 2B	54	8	62
	No Category	21	31	52
Total		75	39	114

Sensitivity: 87% (54/62)

Specificity: 60% (31/52)

Accuracy: 75% (85/114)

Table A8.13-2. Contingency table used for 114 test chemicals in a top-down approach

		Vitrigel-EIT		Total
		I	NI	
UN GHS	Cat.1	24	3	27
	Cat.2A, 2B, No Category	51	36	87
Total		75	39	114

Sensitivity: 89% (24/27)

Specificity: 41% (36/87)

Accuracy: 53% (60/114)

Table A8.13-3. False test chemicals in a bottom-up approach for 114 test chemicals

No.*	Test chemicals	Rank	Applicability limitation
11	Lactic acid	False negative	pH of 2.5% solution ≤ 5.0
14	Captan		Insoluble after 5 m
22	Acetic acid (10%)		pH of 2.5% solution ≤ 5.0
41	3,3'-Dithiodipropionic acid		pH of 2.5% solution ≤ 5.0
46	2,6-Dichlorobenzoyl chloride		pH of 2.5% solution ≤ 5.0
54	Camphene		Protocol revised
55	Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate		pH of 2.5% solution ≤ 5.0
62	Glycolic acid (10%)		pH of 2.5% solution ≤ 5.0
66	Gluconolacton	False positive	pH of 2.5% solution < 5.0 after 10 m
67	Methyl amyl ketone		
68	Methyl isobutyl ketone		
69	*N*,*N*-Dimethylguanidine sulfate		
70	Glycerol		
75	2-Ethoxyethyl acetate		
76	Ethyl acetate		
77	2,4-Pentanediol		
78	Triethanolamine		
85	Cyclohexanone		
89	2,4-Pentanedione		
90	Butyl acetate		
91	Xylene		

93	EDTA,di-potassium		
94	3-Glycidoxypropyltrimethoxysilane		
96	Ethyl trimethyl acetate		
97	2,2-Dimethyl-3-pentanol		
98	Betaine monohydrate		
104	1,2,3-trichloropropane		
108	2,4-Difuronitrobenzene		
109	Potassium tetrafluoroborate		

*Each number corresponds to the number in Appendix 8.14.

Applicability domain

Analysis of the false-negative reactions shows that six of the eight false-negative chemicals were acidic, and the 2.5% solutions used for exposure had a pH level lower than 5, as shown in Table 25. The TEER values of the HCE models after exposures to each of these six acidic test chemicals that yielded false-negatives increased from their initial values. Interestingly, it was reported that isolated rabbit esophageal mucosal epithelium and normal human bronchial epithelial cell layers in culture displayed increased TEER values when exposed to weak acidic solutions (Farré et al., 2008; Oshima et al., 2012). On the other hand, one of the two non-acidic false-negative chemicals were water-insoluble solids that were easily separated from the culture medium at room temperature, as shown in Table A8.13-3. Therefore, the lead laboratory added two restrictions to the applicability domain in consideration of above scientific rationales:

- Exclude all test chemicals that have a pH level of 5 or less in solution (affected 9 tested chemicals).
- Exclude all solids that have both a logP value of 2.5 or more and a density of either less than 0.95 g/cm^3 or over 1.10 g/cm^3 (affected 3 test chemicals).

Under this applicability domain, 12 of the original 114 test chemicals were excluded, as shown in Tables A8.13-4, which improve sensitivity from 87 to 98%, specificity from 60 to 61%, and accuracy from 75 to 79%, as shown in Table A8.13-5.

Eight of the 36 test chemicals in Phase III are excluded under the new applicability domain:

No. 3-2 2-Benzyl-4-chlorophenol (insoluble)

No. 3-3 2,2-Dimethyl butanoic acid (pH $\leq$ 5)

No. 3-4 Captan (insoluble)

No. 3-5 tetra-n-Octylammonium bromide (insoluble)

No. 3-13 Myristyl alcohol (insoluble)

No. 3-14 2,6-Dichlorobenzoyl chloride (pH $\leq$ 5)

No. 3-15 Dibenzyl phosphate (pH $\leq$ 5)

No. 3-25 tetra-aminopyrimidine sulfate (pH $\leq$ 5)

After excluding these eight test chemicals, sensitivity improved from between 75 and 83% to between 88 and 94% (15 to 16/17), specificity changed from 42% to 36% (4/11), and accuracy improved from between 64 and 69% to between 68 and 71% (19 to 20/28).

Of the 17 irritants, two others that yielded false-negatives were No. 3-18, camphene, and No. 3-33, alpha-hexylcinnamaldehyde. Camphene is a waxy, water-insoluble solid, and the false-negative was due to the technique used for dissolving, as described in section 4.1.4 Phase III. Alpha-hexylcinnamaldehyde is a water-immiscible liquid and was identified as an irritant by the lead laboratory (Yamaguchi, 2016). The reason for the discordance of the judgment is currently under investigation although the classification of alpha-hexylcinnamaldehyde in several studies in vivo was reported as NC and 2A or higher (Barroso et al, 2017).

Table A8.13-4-1. Limitations on applicability (pH level 5 or less in 2.5% solution) in a bottom-up approach

No.*	Test chemical	GHS category	Vitrigel-EIT results	pH
11	Lactic acid	1	False negative	3
22	Acetic acid (10%)	1	False negative	4
41	3,3'-Dithiodipropionic acid	2A	False negative	4
46	2,6-Dichlorobenzoyl chloride	2A	False negative	3
47	Dibenzyl phosphate	2A		3
55	Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	2B	False negative	5
56	3-Chloropropionitrile	2B		5
62	Glycolic acid (10%)	2B	False negative	4
93	EDTA,di-potassium	NC	False positive	5

*Each number corresponds to the number in Appendix 8.14.

Table A8.13-4-2. Limitations on applicability (solid chemicals with a logP value of 2.5 or more and a density under 0.95 g/cm^3 or over 1.10 g/cm^3 in a bottom-up approach.

No.*	Test chemical	GHS category	Vitrigel-EIT results	LogP	Density (g/cm^3)
10	Acid red 92	2		7.13	2.16
13	2-Benzyl-4-chlorophenol	1		3.60	1.19
14	Captan	1	False negative	2.80	1.74

*Each number corresponds to the number in Appendix 8.14

Table A8.13-5. Contingency tables used for 102 test chemicals within the applicability domain in bottom-up approach.

		Vitrigel-EIT		Total
		I	NI	
UN GHS	Cat.1, 2A, 2B	50	1	51
	Not Classified	20	31	51
Total		70	32	102

Sensitivity: 98% (50/51)

Specificity: 61% (31/51)

Accuracy: 79% (81/102)