

Attachment 4a: Within laboratory reproducibility of the KeratinoSens assay for 28 chemicals

All chemicals were tested in the initial screening on the list referred to as the 'Silver list', summarized in Attachment 2.

In this initial screening 2 - 4 repetitions each in triplicate were run on all chemicals, and the individual results of these repetitions are given in the rows 'GIV_hist'

The 28 chemicals listed in Attachment 3 and below were then repeated with three repetitions in the ring study, and the results are given in the rows 'GIV_RS'

Thus for each chemical 5 to 7 independent experiments are listed below.

		lmax rep1	rep2	rep3	rep4	Avg	EC 1.5 rep1	rep2	rep3	rep4	Geomean	positive up to 1000 µM	positive up to 1000 µM all reps	positive / negative in full experiment	Comments
NON-SENSITIZERS															
GIV_hist	Methyl salicylate	1.198	1.237			1.22	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.202	1.344	1.705		1.42	n.i.	n.i.	1612.0		n.i.	0 of 3		negative	
GIV_hist	Chlorobenzene	1.068	1.262			1.16	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.126	1.140	1.496		1.25	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
GIV_hist	Sulfanilamide	1.287	1.456			1.37	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.061	1.080	1.017		1.05	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
GIV_hist	Isopropanol	1.151	1.196			1.17	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.212	1.241	1.329		1.26	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
GIV_hist	Salicylic acid	1.117	1.143			1.13	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.109	1.034	1.107		1.08	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
GIV_hist	Sodium lauryl sulfate	1.111	1.212			1.16	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	Significant Induction in all reps at cytotoxic conc. only
GIV_RS		6.344	3.748	7.736		5.94	34.3	37.9	33.8		35.3	3 at cytotox		negative	
GIV_hist	Lactic acid	1.246	1.240	1.241	1.548	1.32	n.i.	n.i.	n.i.	219.2	n.i.	1 of 4	1 of 7	negative	
GIV_RS		2.258	1.051	1.845		1.72	1329.9	n.i.	1555.3		n.i.	0 of 3		negative	
GIV_hist	Glycerol	1.216	1.220	1.120	1.127	1.17	n.i.	n.i.	n.i.	n.i.	n.i.	0 of 4	0 of 7	negative	
GIV_RS		1.238	1.193	1.634		1.36	n.i.	n.i.	1793.9		n.i.	0 of 3		negative	
GIV_hist	Diethyl phthalate	1.047	1.088			1.07	n.i.	n.i.			n.i.	0 of 2	0 of 5	negative	
GIV_RS		1.427	1.049	1.081		1.19	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
SENSITIZERS															
GIV_hist	Hexyl cinnamic aldehyde	2.090	3.265			2.68	18.3	16.2			17.2	2 of 2	3 of 5	positive	
GIV_RS		1.494	1.414	2.362		1.76	n.i.	n.i.	19.4		n.i.	1 of 3		negative	
GIV_hist	Citral	84.555	108.277			96.42	25.1	21.2			23.1	2 of 2	5 of 5	positive	
GIV_RS		50.835	35.860	93.894		60.20	15.3	19.2	17.3		17.2	3 of 3		positive	
GIV_hist	Ethylene glycol dimethacrylate	178.723	198.067			188.39	47.0	67.8			56.5	2 of 2	5 of 5	positive	
GIV_RS		203.537	117.700	209.331		176.86	70.3	98.4	78.2		81.5	3 of 3		positive	
GIV_hist	2,4-Dinitro- chlorobenzene	10.928	18.770			14.85	2.8	2.1			2.5	2 of 2	5 of 5	positive	
GIV_RS		12.576	10.435	15.611		12.87	3.7	2.5	3.8		3.3	3 of 3		positive	
GIV_hist	4-Methylamino- phenol sulphate	16.889	16.807			16.85	3.1	2.1			2.6	2 of 2	5 of 5	positive	
GIV_RS		5.807	6.033	5.884		5.91	6.0	7.2	15.0		8.7	3 of 3		positive	

GIV_hist	(5-chloro)-Methyl- isothiazolinone	9.062	5.433			7.25	0.4	0.6			0.5	2 of 2	5 of 5	positive	
GIV_RS		6.837	9.316	6.732		7.63	9.6	3.5	12.9		7.6	3 of 3		positive	
GIV_hist	Phenyl benzoate	1.041	1.757	1.148	1.181	1.28	n.i.	203.1	n.i.	n.i.	n.i.	1 of 4	1 of 7	negative	
GIV_RS		1.095	0.972	0.992		1.02	n.i.	n.i.	n.i.		n.i.	0 of 3		negative	
GIV_hist	Imidazolidinyl urea	1.507	1.299	2.425	6.337	2.89	61.9	n.i.	41.6	32.7	43.9	3 of 4	5 of 7	positive	Rep 1 and 2 EC1.5 manual extrapolation
GIV_RS		2.146	1.942	1.460		1.85	50.0	50.0	n.i.		50.0	2 of 3		positive	
GIV_hist	Oxazolone	2.257	2.453	2.553	58.483	2.42	168.2	113.9	244.4	43.8	119.7	4 of 4	7 of 7	positive	Averages EC1.5 and I _{max} calculated without Rep 4, which appears to be an outlier
GIV_RS		9.593	7.333	55.940		24.29	196.8	192.0	184.8		191.2	3 of 3		positive	
GIV_hist	4-Phenylene-diamine	25.060	28.547			26.80	6.4	3.6			4.8	2 of 2	5 of 5	positive	
GIV_RS		22.210	37.277	76.003		45.16	8.8	9.9	11.3		9.9	3 of 3		positive	
GIV_hist	Cinnamic aldehyde	17.866	18.447	11.938	18.321	16.24	18.2	14.5	15.1	13.5	15.2	4 of 4	7 of 7	positive	
GIV_RS		17.510	33.161	16.488		22.39	15.9	13.1	14.0		14.3	3 of 3		positive	
GIV_hist	Isoeugenol	5.517	7.509	62.675	6.208	6.41	15.2	20.4	14.4	14.3	15.9	4 of 4	7 of 7	positive	
GIV_RS		19.279	8.076	12.235		13.20	12.1	29.4	17.4		18.4	3 of 3		positive	
GIV_hist	tetramethyl- thiuramdisulfide	7.321	6.180			6.75	0.9	0.8			0.8	2 of 2	5 of 5	positive	
GIV_RS		37.316	13.119	16.364		22.27	<0.97	<0.97	<0.97		<0.97	3 of 3		positive	
GIV_hist	2-Mercapto- benzothiazole	50.151	5.394	7.218	13.888	8.83	66.6	36.1	37.6	52.2	46.6	4 of 4	7 of 7	positive	
GIV_RS		25.078	6.823	41.747		24.55	34.4	51.3	49.2		44.3	3 of 3		positive	
GIV_hist	Eugenol	1.192	1.215	1.264	1.329	1.25	n.i.	n.i.	n.i.	n.i.	n.i.	0 of 4	1 of 7	negative	
GIV_RS		1.614	1.077	1.384		1.36	505.5	n.i.	n.i.		n.i.	1 of 3		negative	
GIV_hist	Cinnamyl alcohol	1.705	1.993	1.648	1.635	1.75	83.7	189.1	160.2	61.3	111.6	4 of 4	7 of 7	positive	
GIV_RS		18.028	6.727	7.784		10.85	110.7	117.3	87.0		104.1	3 of 3		positive	
GIV_hist	Glyoxal	4.270	3.521	23.632	81.323	28.19	88.6	89.1	87.0	91.7	89.1	4 of 4	7 of 7	positive	
GIV_RS		78.651	21.802	100.886		67.11	130.5	131.2	140.8		134.1	3 of 3		positive	
GIV_hist	4-nitro- benzylbromide	7.250	6.451			6.85	1.0	1.6			1.3	2 of 2	5 of 5	positive	
GIV_RS		6.605	4.906	9.594		7.03	1.2	1.1	2.0		1.4	3 of 3		positive	
GIV_hist	Methyldibromo glutaronitrile	5.044	3.019			4.03	10.0	5.5			7.5	2 of 2	5 of 5	positive	
GIV_RS		5.705	3.862	6.032		5.20	15.7	8.8	13.9		12.4	3 of 3		positive	