Attachment 2 – Chemicals used for the test development

Table 1. Chemicals used to select sensitive clone and to define the SOP

Name	Rationale for inclusion	CAS Number	Formula	MW	LLNA EC 3
2,4-Dinitrochlorobenzene	Extreme sensitizer	97-00-7	$C_6H_3ClN_2O_4$	202.55	0.05
Benzyl salicylate	Moderate sensitizer, not directly reactive	118-58-1	$C_{14}H_{12}O_3$	228.24	2.9
5,6,7-Trimethyl-(2E)- 2,5-Octadien-4-one	Moderate sensitizer, reactive with peptides	357650-26-1	$C_{11}H_{18}O_1$	166	1.6
Lyral HMPCC	Weak sensitizer	31906-04-4	$C_{13}H_{22}O_2$	210.31	17.1
Tert-butyl-hydroquinone	Reference Nrf2 inducer	1948-33-0	$C_{10}H_{14}O_2$	166.22	n.a.
Methyl salicylate	Non-sensitizer	119-36-8	$C_8H_8O_3$	152.15	var. /negative

Table 2. Full data set for the chemicals in the silver list tested according to the SOP and used to for the evaluation of the prediction model and predictivity of the KeratinoSens assay

Name	CAS Number	Formula	MW	cLogP	LLNA EC 3	KeratinoSens results						
						I_{max}	EC1.5	Reps. Positive [7]	Overall result	IC50	EC2	EC3
Sensitizers												
Oxazolone	15646-46-5	$C_{12}H_{11}NO_3$	217.22	1.9	0.003	2.4	**175.5**	4/4	Positive	1370.9	335.3	490.4
Benzoquinone	106-51-4	$C_6H_4O_2$	108.09	0.3	0.01	15.2	**6.5**	4/4	Positive	104.5	25.0	32.8
(5-chloro)-Methylisothiazolinone	26172-55-4	C_4H_4ClNOS	149.6	1.2	0.01	7.2	**0.5**	2/2	Positive	7.1	0.9	1.7
2,4-Dinitrochlorobenzene	97-00-7	$C_6H_3ClN_2O_4$	202.55	2.2	0.05	14.8	**2.5**	2/2	Positive	8.2	3.3	3.9
4-nitrobenzylbromide	100-11-8	$C_7H_6BrNO_2$	216.03	2.8	0.05	6.9	**1.3**	2/2	Positive	9.1	1.7	2.1
4-Phenylenediamine	106-50-3	$C_6H_8N_2$	108.14	0.9	0.11	26.8	**5.0**	2/2	Positive	438.9	13.3	46.7
Glutaraldehyde	111-30-8	$C_5H_8O_2$	100.12	0.6	0.12	80.7	**24.3**	2/2	Positive	242.6	57.2	69.4
Benzoyl peroxide	94-36-0	$C_{14}H_{10}O_4$	242.23	2.6	0.22	1.4	**n.i.**	0/2	Negative	567.6	n.i.	n.i.
Glyoxal	107-22-2	$C_2H_2O_2$	58.04	-0.6	0.75	28.2	**89.1**	4/4	Positive	677.9	192.4	307.8
4-Methylaminophenol sulphate	55-55-0	$C_7H_{11}NO_5S$	221.23	1.4	0.80	9.5	**1.5**	2/2	Positive	11.7	2.1	2.7
Formaldehyde	50-00-0	CH_2O	30.03	-0.2	0.84	16.9	**63.2**	2/2	Positive	201.6	66.4	72.2
Methyldibromo glutaronitrile	35691-65-7	$C_6H_6Br_2N_2$	265.93	2.3	0.90	4.0	**7.8**	2/2	Positive	25.6	12.4	18.1
Cinnamic aldehyde	104-55-2	C_9H_8O	132.16	1.9	1.3	16.2	**16.1**	4/4	Positive	194.4	36.6	63.9
2-Hydroxyethyl acrylate	818-61-1	$C_5H_8O_3$	116.12	-0.3	1.4	54.9	**32.3**	2/2	Positive	207.2	59.5	92.9
Isoeugenol	97-54-1	$C_{10}H_{12}O_2$	164.2	2.4	1.5	6.4	**16.1**	4/4	Positive	731.4	72.6	259.4
Ethylenediamine	107-15-3	$C_2H_8N_2$	60.1	-1.1	2.2	13.2	**99.9**	4/4	Positive	>2000	188.2	453.4
Benzylidene Acetone	122-57-6	$C_{10}H_{10}O$	146.19	2.3	2.2	503.9	**9.7**	2/2	Positive	174.5	19.7	31.5
Methyl-2-nonynoate	111-80-8	$C_{10}H_{16}O_2$	168.23	2.1	2.5	33.1	**1.8**	2/2	Positive	121.9	8.9	26.7
2-Mercaptobenzothiazole	149-30-4	$C_7H_5NS_2$	167.25	2.4	2.5	8.8	**48.1**	4/4	Positive	1003.1	108.0	340.1
Benzyl salicylate	118-58-1	$C_{14}H_{12}O_3$	228.24	3	2.9	5.5	**8.4**	2/2	Positive	111.0	18.7	40.9
Tetramethylthiuramdisulfide	137-26-8	$C_{10}H_{20}N_2S_4$	296.54	2.7	3.1	6.8	**0.8**	2/2	Positive	39.1	5.3	10.7
Diethylenetriamine	111-40-0	$C_4H_{13}N_3$	103.17	-1.5	3.3	1.7	**1259.4**	2/4	Negative	>2000	n.i.	n.i.

Thioglycerol	96-27-5	$C_3H_8O_2S$	108.16	-0.53	3.5	1.5	**n.i.**	1 /4	Negative	>2000	n.i.	n.i.
Phenylacetaldehyde	127-78-1	C_8H_8O	120.15	1.4	4.5	11.3	**28.5**	2/2	Positive	116.2	50.4	69.0
Resorcinol	108-46-3	$C_6H_6O_2$	110.11	1.1	5.9	1.0	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Dihydroeugenol	2785-87-7	$C_{10}H_{14}O_2$	166.22	2.4	6.8	1.5	**462.0**	2/2	Positive	759.2	n.i.	n.i.
Benzoisothiazolione	2634-33-5	C_7H_5NOS	151.19	1.2	7.8	24.0	**3.2**	2/2	Positive	50.9	6.3	14.8
Citral	5392-40-5	$C_{10}H_{16}O$	152.23	2.9	9.8	96.4	**23.2**	2/2	Positive	182.8	53.9	67.4
Hexyl cinnamic aldehyde	101-86-0	$C_{15}H_{20}O$	216.32	4.2	9.9	2.7	**17.3**	2/2	Positive	26.3	24.9	n.i.
Eugenol	97-53-0	$C_{10}H_{12}O_2$	164.2	2.1	10.1	1.3	**n.i.**	0/4	Negative	1505.7	n.i.	n.i.
Abietic acid	514-10-3	$C_{20}H_{30}O_2$	302.45	5.2	11.6	11.4	**16.6**	2/2	Positive	104.6	30.8	34.7
Phenyl benzoate	93-99-2	$C_{13}H_{10}O_2$	198.22	2.9	13.6	1.3	**n.i.**	1 /4	Negative	191.6	n.i.	n.i.
Lyral HMPCC	31906-04-4	$C_{13}H_{22}O_2$	210.31	2.9	17.1	16.1	**79.6**	2/2	Positive	355.4	117.3	197.1
Benzocaine	94-09-7	$C_9H_{11}NO_2$	165.19	1.4	17.1	3.0	**18.2**	2/2	Positive	>2000	101.6	n.i.
Benzyl cinnamate	103-41-3	$C_{16}H_{14}O_2$	238.28	3.7	18.4	8.7	**11.0**	2/2	Positive	>2000	26.4	38.3
2,4-Dichloronitrobenzene	611-06-3	$C_6H_3Cl_2NO_2$	192	3.06	20.0	2.9	**68.3**	4/4	Positive	816.0	197.3	456.0
Cinnamyl alcohol	104-54-1	$C_9H_{10}O$	134.18	1.7	21.0	1.7	**123.6**	4/4	Positive	774.6	n.i.	n.i.
Hydroxycitronellal	107-75-5	$C_{10}H_{20}O_2$	172.26	2.2	23.0	137.1	**79.4**	2/2	Positive	>2000	110.1	142.9
Imidazolidinyl urea	39236-46-9	$C_{11}H_{16}N_8O_8$	388.29	-4.8	24.0	2.9	**45.4**	3 /4	Positive	90.4	44.4	41.9
Butyl glycidyl ether	2426-08-6	$C_7H_{14}O_2$	130.18	1.2	30.9	340.7	**218.5**	2/2	Positive	>2000	289.8	381.6
Ethylene glycol dimethacrylate	97-90-5	$C_{10}H_{14}O_4$	198.22	1.2	32.9	188.4	**57.4**	2/2	Positive	1655.8	135.9	253.4
Cobalt chloride	7646-79-9	$CoCl2$		n.a.	Pos.	23.3	**298.6**	2/2	Positive	1330.2	450.0	661.4
Nickel sulfate	7786-81-4	$NiSO4$		n.a.	var.	4.2	**329.0**	4/8 (4)	Positive	998.7	235.2	291.5
Benzaldehyde	100-52-7	C_7H_6O	106.12	1.5	>25	2.3	**443.1**	2/2 (6)	Positive	>2000	n.i.	n.i.
Non-sensitizers												
Sodium lauryl sulfate	151-21-3	$C_{12}H_{25}NaO_4S$	288.38	3.4	var.	1.2	**n.i.**	0/2	Negative	44.7	n.i.	n.i.
Salicylic acid	69-72-7	$C_7H_6O_3$	138.12	1.1	var.	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Methyl salicylate	119-36-8	$C_8H_8O_3$	152.15	1.2	var.	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Sulfanilamide	63-74-1	$C_6H_8N_2O_2S$	172.2	-0.1	NC (1)	1.4	**n.i.**	0/2	Negative	>2000	n.i.	n.i.

Diethyl phthalate	84-66-2	$C_{12}H_{14}O_4$	222.24	2	> 100%	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Glycerol	56-81-5	$C_3H_8O_3$	92.09	-1.7	> 100%	1.2	**n.i.**	0/4	Negative	>2000	n.i.	n.i.
Propylene glycol	57-55-6	$C_3H_8O_2$	76.09	-0.6	> 100%	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Benzoic acid	65-85-0	$C_7H_6O_2$	122.12	1.4	> 20%	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
1-Butanol	71-36-3	$C_4H_{10}O$	74.12	0.8	> 20%	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
4-Hydroxybenzoic acid	99-96-7	$C_7H_6O_3$	138.12	1.1	> 25%	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Sulfanilic acid	121-57-3	$C_6H_7NO_3S$	173.19	-0.1	> 25%	1.3	**n.i.**	0/2	Negative	>1000	n.i.	n.i.
Tartaric acid	87-69-4	$C_4H_6O_6$	150.09	-2.1	> 25%	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Propylparaben	94-13-3				> 25%	9.7	**14.5**	2/2 (6)	Positive	813.1	41.9	n.i.
Ethyl vanillin	121-32-4	$C_9H_{10}O_3$	166.17	1.6	> 50%	5.4	**161.7**	2/2 (7)	Positive	>2000	534.5	700.6
Isopropanol	67-63-0	C_3H_8O	60.1	0.4	> 50%	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Benzyl alcohol	100-51-6	C_7H_8O	108.14	1.4	> 50%	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Dimethylisophtalate	1459-93-4	$C_{10}H_{10}O_4$	194.18	1.83	NC (1)	2.1	**694.9**	3 /4	Positive	>2000	1253.5	n.i.
Dextran	9004-54-0	n.a.	n.a.	n.a.	NC (1)	1.5	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Tween 80	9005-65-6	n.a.	n.a.	n.a.	NC (1)	2.7	**19.3**	2/2	Positive	399.8	53.2	n.i.
Chlorobenzene	108-90-7	C_6H_5Cl	112.56	2.4	Neg. (2)	1.2	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Lactic acid	50-21-5	$C_3H_6O_3$	90.08	-0.5	Neg. (2)	1.3	**n.i.**	1 /4	Negative	>2000	n.i.	n.i.
Phenol	108-95-2	C_6H_6O	94.11	1.4	Neg. (2)	1.3	**n.i.**	0/2	Negative	>2000	n.i.	n.i.
Octanoic acid	124-07-2	$C_8H_{16}O_2$	144.21	2.4	>50	1.1	**n.i.**	0/2	Negative	>2000	n.i.	n.i.

n.i. = no significant induction above threshold

(1) Level not specified

(2) Negative reference according D. Basketter, 1999, Food Chem. Toxicol. 37, 1167-1174.

(3) diethylentriamine, 2 negative repetitions with I_{max} 1.46 / 1.43, but statistically significant induction and clear dose response in all 4 repetitions but EC1.5 > 1000, considered negative with the corrected prediction model

(4) Nickel sulfate, only four repetitions positive out of eight, but very strong induction, considered positive

(5) Propylparaben, known to be a weak human sensitizer, nevertheless for Cooper statistics the negative prediction from animal tests was considered

(6) Benzaldehyde is negative in the LLNA and was long considered a non-sensitizer. It is now considered a human sensitizer by the fragrance association due to recent human HRIPT test

(7) Indicates number of experiments (each conducted in triplicate) with statistically significant induction > 1.5-fold / number of experiments conducted.