Forreryd et al.:

Exploration of the GARD™skin Applicability Domain: Indirectly Acting Haptens, Hydrophobic Substances and UVCBs

Supplementary Data

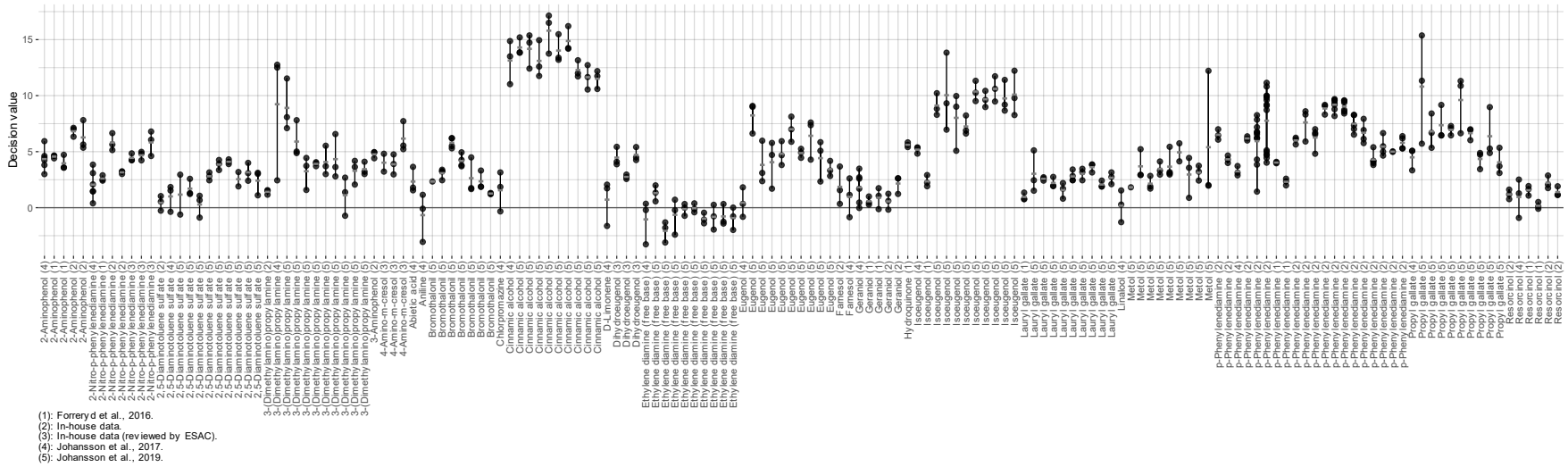

Fig. S1: GARDskin results of indirectly acting haptens
The study origin of each assessment is referenced 1-5. Each datapoint represents a unique replicate sample within each GARDskin study. The mean decision value used for classification is indicated with a vertical line.

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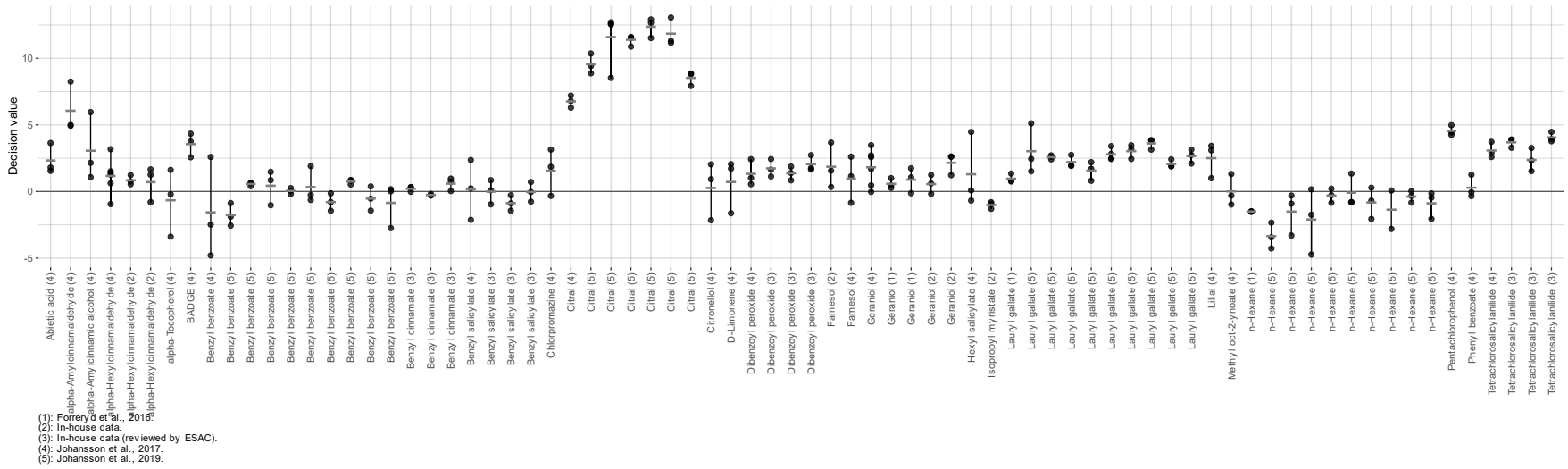

Fig. S2: GARDskin results of hydrophobic substances

The study origin of each assessment is referenced 1-5. Each datapoint represents a unique replicate sample within each GARDskin study. The mean decision value used for classification is indicated with a vertical line.

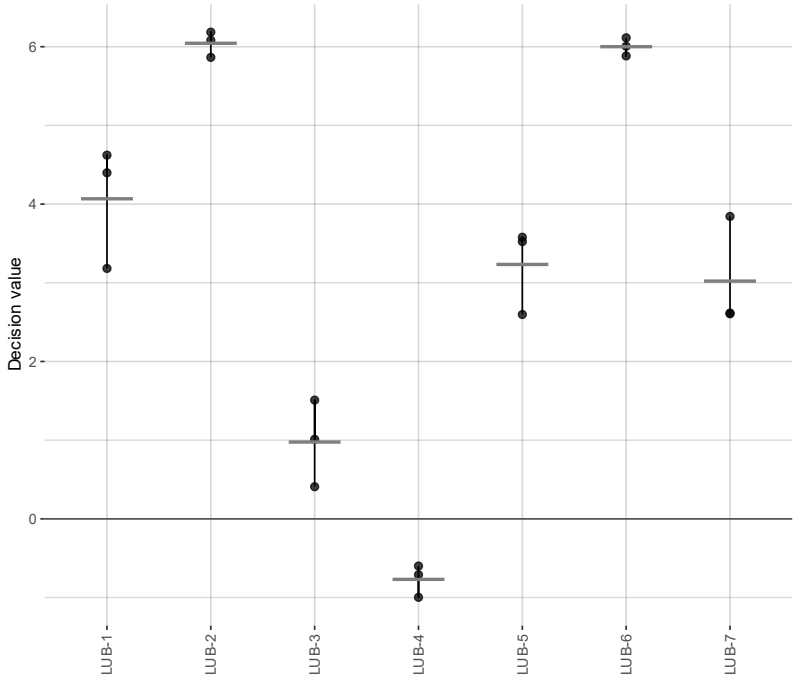

Fig. S3: GARDskin results of UVCBs
Each datapoint represents a unique replicate sample within each GARDskin study. The mean decision value used for classification is indicated with a vertical line.

Tab. S1: GARD^{TM}skin assay details: Indirectly acting haptens

Test Chemical Descriptors		GARD^{TM}skin assay details			
Name	**CASRN**	**Maximum Solubility**	**GARD^{TM} Input Concentration**	**Cytotoxic?**	**Solvent**
2-Aminophenol	95-55-6	500	80-100	Yes	DMSO
2-Nitro-p-phenylenediamine	5307-14-2	500	200-400	Yes	DMSO
2,5-Diaminotoluene sulfate	615-50-9	14-500	14-100	Yes & No	Water & DMSO
3-(Dimethylamino)propylamine	109-55-7	500	500	No	DMSO
3-Aminophenol	591-27-5	500	5	Yes	DMSO
4-Amino-m-cresol	2835-99-6	500	30-40	Yes	DMSO
Abietic acid	514-10-3	200	200	No	DMSO
Aniline	62-53-3	500	500	No	DMSO
Bromothalonil	35691-65-7	300-500	16-35	Yes	DMSO
Chlorpromazine	50-53-3	100	10	Yes	DMSO
Cinnamic alcohol	104-54-1	500	500	No	DMSO
D-Limonene	5989-27-5	500	500	No	DMSO
Dihydroeugenol	2785-87-7	500	500	Yes	DMSO
Ethylene diamine (free base)	107-15-3	500	500	No	DMSO
Eugenol	97-53-0	500	300-500	Yes	DMSO
Farnesol	4602-84-0	500	500	No	DMSO
Geraniol	106-24-1	500	500	No	DMSO
Hydroquinone	123-31-9	500	45	Yes	DMSO
Isoeugenol	97-54-1	500	300-500	Yes	DMSO
Lauryl gallate	1166-52-5	10-500	3-5	Yes	DMSO
Linalool	78-70-6	500	500	No	DMSO
Metol	55-55-0	209-359	10-23	Yes	Water & DMSO
p-Phenylenediamine	106-50-3	500	70-75	Yes	DMSO
Propyl gallate	121-79-9	500	100-200	Yes	DMSO
Resorcinol	108-46-3	500	500	No	Water

Tab. S2: GARD^{TM}skin assay details: Lipophilic/hydrophobic compounds

Test Chemical Descriptors		GARD^{TM}skin assay details			
Name	**CASRN**	**Maximum Solubility**	**GARD^{TM} Input Concentration**	**Cytotoxic?**	**Solvent**
Abietic acid	514-10-3	200	200	No	DMSO
α-Amylcinnamaldehyde	122-40-7	500	110	Yes	DMSO
α-Amylcinnamic alcohol	101-85-9	500	260	Yes	DMSO
α-Hexylcinnamaldehyde	101-86-0	500	32-100	Yes & No	DMSO
α-Tocopherol	59-02-9	100	100	No	DMSO
BADGE	1675-54-3	200	50	Yes	DMSO
Benzyl benzoate	120-51-4	100-500	100-500	Yes & No	DMSO
Benzyl cinnamate	103-41-3	100-120	100-120	No	DMSO
Benzyl salicylate	118-58-1	50-500	50-200	Yes & No	DMSO
Chlorpromazine	50-53-3	100	10	Yes	DMSO
Citral	5392-40-5	400-500	40-80	Yes	DMSO
Citronellol	106-22-9	500	500	No	DMSO
D-Limonene	5989-27-5	500	500	No	DMSO
Dibenzoyl peroxide	94-36-0	67-500	67-90	Yes & No	DMSO
Farnesol	4602-84-0	500	500	No	DMSO
Geraniol	106-24-1	500	500	No	DMSO
Hexyl salicylate	6259-76-3	500	120	Yes	DMSO
Isopropyl myristate	110-27-0	50	50	No	DMF
Lauryl gallate	1166-52-5	10-500	3-5	Yes	DMSO
Lilial	80-54-6	500	160	Yes	DMSO
Methyl oct-2-ynoate	111-12-6	500	50	Yes	DMSO
n-Hexane	110-54-3	300-500	300-500	No	Water & DMSO
Pentachlorophenol	87-86-5	200	150	Yes	DMSO
Phenyl benzoate	93-99-2	200	200	No	DMSO
Tetrachlorosalicylanilide	1154-59-2	40-500	20	Yes	DMSO

Tab. S3: GARD^{TM}skin assay details: UVCBs

[a] Unless otherwise indicated, molecular weights are approximated with a weighted mean, based on relative concentrations of multiconstituent components. [b] The molecular weight of LUB-4 was conservatively approximated, based on known information of corresponding test chemicals with similar chemistry, i.e., LUB1-3, 5-7.

Test Chemical Descriptors				GARD^{TM}skin assay details		
NAME	**CASRN**	**Molecular weight[a] (g/mol)**	**Maximum solubility**	**GARD^{TM} Input Concentration**	**Cytotoxic?**	**Solvent**
LUB-1	-	1420	100 µM	58 µM	Yes	DMSO
LUB-2	-	640	500 µM	500 µM	Yes	DMF/glycerol
LUB-3	-	291	100 µM	60 µM	Yes	DMSO
LUB-4	-	2000[b]	500 µM	500 µM	No	Xylene
LUB-5	-	550	250 µM	200 µM	Yes	DMF/glycerol
LUB-6	-	420	500 µM	130 µM	Yes	DMF/glycerol
LUB-7	-	264.4	200 µM	200 µM	Yes	DMSO

Tab. S4: Classifications by alternative non-animal assays on indirectly acting haptens
(Data extracted from Annex 2, supporting document to OECD TG 497)

Test chemical descriptors		Alternative assays		
Name	**CASRN**	**DPRA**	**KeratinoSens™**	**hCLAT**
2-Aminophenol	95-55-6	1	1	1
2-Nitro-p-phenylenediamine	5307-14-2	1	1	1
2,5-Diaminotoluene sulfate	615-50-9	1	1	1
3-(Dimethylamino)propylamine	109-55-7	0	1	1
3-Aminophenol	591-27-5	0	0	1
4-Amino-m-cresol	2835-99-6	1	1	1
Abietic acid	514-10-3	1	1	0
Aniline	62-53-3	0	0	1
Bromothalonil	35691-65-7	1	1	1
Chlorpromazine	50-53-3	0	0	1
Cinnamic alcohol	104-54-1	1	1	1
D-Limonene	5989-27-5	0	0	1
Dihydroeugenol	2785-87-7	0	1	0
Ethylene diamine (free base)	107-15-3	0	1	1
Eugenol	97-53-0	1	0	1
Farnesol	4602-84-0	0	1	1
Geraniol	106-24-1	0	1	1
Hydroquinone	123-31-9	1	1	1
Isoeugenol	97-54-1	1	1	0
Lauryl gallate	1166-52-5	1	1	1
Linalool	78-70-6	0	0	1
Metol	55-55-0	1	1	1
p-Phenylenediamine	106-50-3	1	1	1
Propyl gallate	121-79-9	1	1	1
Resorcinol	108-46-3	0	0	1

Tab. S5: Classifications by alternative non-animal assays on lipophilic compounds
(Data extracted from Annex 2, supporting document to OECD TG 497).

Test chemical descriptors		Alternative assays		
Name	**CASRN**	**DPRA**	**KeratinoSens™**	**hCLAT**
Abietic acid	514-10-3	1	1	0
α-Amylcinnamaldehyde	122-40-7	0	1	1
α-Amylcinnamic alcohol	101-85-9	0	0	1
α-Hexylcinnamaldehyde	101-86-0	0	1	0
α-Tocopherol	59-02-9	0	1	0
BADGE	1675-54-3	1	1	1
Benzyl benzoate	120-51-4	0	1	0
Benzyl cinnamate	103-41-3	0	1	0
Benzyl salicylate	118-58-1	0	1	0
Chlorpromazine	50-53-3	0	0	1
Citral	5392-40-5	1	1	1
Citronellol	106-22-9	1	0	1
D-Limonene	5989-27-5	0	0	1
Dibenzoyl peroxide	94-36-0	1	0	0
Farnesol	4602-84-0	0	1	1
Geraniol	106-24-1	0	1	1
Hexyl salicylate	6259-76-3	0	0	1
Isopropyl myristate	110-27-0	0	0	1
Lauryl gallate	1166-52-5	1	1	1
Lilial	80-54-6	1	0	1
Methyl oct-2-ynoate	111-12-6	1	1	1
n-Hexane	110-54-3	0	0	0
Pentachlorophenol	87-86-5	1	0	1
Phenyl benzoate	93-99-2	1	0	1
Tetrachlorosalicylanilide	1154-59-2	1	1	1

Tab. S6: Confusion matrices and performance metrics for DPRA

Dataset		Indirectly acting haptens				Hydrophobic substances			
Reference		**LLNA**		**Human**		**LLNA**		**Human**	
Reference classification		NS	S	NS	S	NS	S	NS	S
		(0)	(25)	(0)	(8)	(1)	(24)	(4)	(9)
DPRA classifications	NS	0	11	0	4	1	13	3	3
	S	0	14	0	4	0	11	1	6
Accuracy		56.0%		50.0%		48.0%		69.2%	
Sensitivity		56.0%		50.0%		45.8%		66.7%	
Specificity		-		-		100%		75.0%	
Balanced accuracy		-		-		72.9%		70.8%	

Tab. S7: Confusion matrices and performance metrics for KeratinoSens™

Dataset		Indirectly acting haptens				Hydrophobic substances			
Reference		**LLNA**		**Human**		**LLNA**		**Human**	
Reference classification		NS	S	NS	S	NS	S	NS	S
		(0)	(25)	(0)	(8)	(1)	(24)	(4)	(9)
KeratinoSens™ classifications	NS	0	7	0	3	1	10	3	4
	S	0	18	0	5	0	14	1	5
Accuracy		72.0%		62.5%		60.0%		61.5%	
Sensitivity		72.0%		62.5%		58.3%		55.6%	
Specificity		-		-		100%		75.0%	
Balanced accuracy		-		-		79.2%		65.3%	

Tab. S8: Confusion matrices and performance metrics for hCLAT

Dataset		Indirectly acting haptens				Hydrophobic substances			
Reference		**LLNA**		**Human**		**LLNA**		**Human**	
Reference classification		NS	S	NS	S	NS	S	NS	S
		(0)	(25)	(0)	(8)	(1)	(24)	(4)	(9)
hCLAT classifications	NS	0	3	0	1	1	7	2	1
	S	0	22	0	7	0	17	2	8
Accuracy		88.0%		87.5%		72.0%		76.9%	
Sensitivity		88.0%		87.5%		70.8%		88.9%	
Specificity		-		-		100%		50.0%	
Balanced accuracy		-		-		85.4%		69.4%	