Natsch and Gerberick:

Integrated Skin Sensitization Assessment Based on OECD Methods (II): Hazard and Potency by Combining Kinetic Peptide Reactivity and the "2 out of 3" Defined Approach

Supplementary Data: ESM3

Tab. ESM3-1: Chemicals ≥ 5-fold under-predicted by the PoD as compared to LLNA data when applying scheme in Figure 1

Name	CAS-No.	LLNA.MLLP EC3 OECD	LLNA EC3 literature	Predicted PoD with 2o3	Equation	Discussion
Chemicals with clear underpredictions						
3,4-Dihydrocoumarin	119-84-6	5.6	5.6	55.6	4	Low certainty for PoD; outside of kDPRA AD due to predominant Lys-reactivity; EQ6 cannot be applied
Ethylenediamine	107-15-3	NC, 2 of 3 LLNA studies negative	2.2	14.8	6	Inconcl. 2o3 (Borderline positive, Bp); Outside kDPRA AD – primary amine/pro-hapten; EQ6 incl. Bp result used (for WoE only); OECD data review also indicates LLNA is equivocal
3-Dimethyl-amino-1-propylamine	109-55-7	3.5	2.2	11.1	6	Outside kDPRA AD – primary amine/pro-hapten; EQ6 used
1-Naphtol	90-15-3	1.3	1.3	14.5	1	Potential prohapten, but not *a priori* obvious from structure. **EQ6 based on cellular assays would predict EC3 of 2.6%**
Glyoxal	107-22-2	1.4	1.4	9.2	1	High vapor pressure by TIMES for glyoxal. It actually is tested as aqueous solution (*in vitro* and LLNA) where it is mainly present as the **hydrate** (Michailoudi et al., 2021). The hydrate has a normalized vapor pressure of 0, and based on this value, the predicted **EC3 with EQ 1 is 1.6%**; thus high EC3 predicted due to high predicted volatility for non-hydrated form
Diethyl sulfate	64-67-5	3.3	3.3	25.3	1	
Chemicals with high sensitization potential in LLNA, low PoD predicted *in vitro,* but still significant under-prediction						
2,5-Diaminotoluene sulphate	615-50-9	0.4	0.4	2.4	1	Aromatic amine, but inside kDPRA AD due to Log k_{max} > -2
Propyl gallate	121-79-9	POS	0.32	5.8	1	*In vivo* value is far extrapolation (lowest concentration tested in LLNA is 5%); Hydroquinone, but inside kDPRA AD due to Log k_{max} > -2
Maleic anhydride	108-31-6	0.16	0.16	1.1	4	
Glutaraldehyde	111-30-8	0.0795	0.1	0.65	6	Predominant Lys reactivity; Outside kDPRA AD; EQ6 used
1,4-Hydrochinone	123-31-9	0.19	0.1	0.85	1	Hydroquinone, but inside kDPRA AD due to Log k_{max} > -2
Tetrachlorsalicylanilide	1154-59-2	0.0265	0.04	3.0	1	Sensitizer, but predominantly photo-sensitizer
Bandrowski's Base	20048-27-5	0.03	0.02	0.37	1	Aromatic amine, but inside kDPRA AD due to Log k_{max} > -2
p-Benzoquinone	106-51-4	1A	0.01	0.10	1	*In vivo* value is far extrapolation (lowest concentration tested in LLNA is 0.5%)

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Diphenylcyclopropenone	886-38-4	1A	0.003	0.18	1	*In vivo* value is far extrapolation (lowest concentration tested in LLNA is 0.3%)
Oxazolone	15646-46-5	0.002	0.003	1.5	1	Sensitization potential explained by unique amine reactivity kinetics, amine reaction kinetics not assessed in scheme for PoD (Natsch et al., 2010)
Chlorothalonil	1897-45-6	0.004	0.004	0.11	1	
Kathon CG	26172-55-4 & 2682-20-4	0.0076	0.0076	0.05	1	

Tab. ESM3-2: Chemicals ≥ 5-fold over-predicted by the PoD as compared to LLNA data when applying scheme in Figure 1

Name	CAS-No.	LLNA.MLLP EC3 OECD	LLNA EC3 literature	Predicted PoD with 2o3	Equation	Human sensitization potential and discussion
False positives 2o3 vs LLNA						
Methyl 3-bromopropionate	3395-91-3	NC	> 50	5.8	1	Highly reactive, alkylating molecule, probably FN in LLNA
Ethyl 4-amino-benzoate (Benzocaine)	94-09-7		> 50 / variable	6.9	6	Var. in GPMT and LLNA (Basketter et al., 1995); moderate frequency of pos. patch tests (Uter et al., 2021; Warshaw et al., 2008), weak human potency probably over-predicted
4-Methyl-2-nitroanisole	119-10-8	NC	> 50	12.8	6	No *in vivo* data except single LLNA study
Cinnamyl nitrile	1885-38-7		> 10	11.9	6	Known human 1B sensitizer (OECD, 2021)
Majantol	103694-68-4		> 30	6.1	6	Frequent positive cases in the clinic (Heisterberg and Johansen, 2009; Schnuch et al., 2007)
Propylparaben	94-13-3	NA	> 50	3.4	6	Parabens are only weak human sensitizers (Schnuch et al., 2011), clearly overpred. by PoD
Highly reactive molecules with very high volatility, VP correction cannot fully predict the weak sensitization in LLNA due to volatility						
2-Ethylbutyraldehyde	97-96-1	76	68.2	2.5	1	VP correction cannot fully predict the weak sensitization in LLNA probably due to volatility. These highly reactive chemicals may be significantly more potent under occlusion, and the predicted PoD may be more relevant in such a situation.
Ethyl acrylate	140-88-5	32.75	28	3.6	1	
Methyl acrylate	96-33-3	20	20	3.5	1	
Butyl acrylate	141-32-2	11.2	20	1.7	1	
2,4-Heptadienal	5910-85-0	4	4	0.8	1	
Chemicals with significant human sensitization potency, LLNA may be an underestimation and *in vitro* PoD more relevant						
Tetramethylthiuram disulfide	137-26-8	5.2	5.2	0.1	1	Important glove allergen; tested as thiuram mix leading to a very high frequency of reactions in the past (Warshaw et al., 2013) indicating high human sensitization potential
1,2-Benzisothiazolin-3-one	2634-33-5	4.8	2.3	0.2	1	Human sensitization in an HRIPT study; ICCVAM (ICCVAM, 2011) derived a DA05 of 50 µg/cm^2 (~ EC3 of 0.2%), which is in exact alignment with the predicted PoD, although OECD review found a confounding factor in the co-formulation in the human study.
α-Damascone	24720-09-0		3.3	0.7	1	Different damascone derivatives are positive in HRIPT (at 500 µg/cm^2 ~ EC3 of 2%); NESIL IFRA 100 µg/cm^2 ~ 0.4%)
Imidazolidinyl urea	39236-46-9	24	24	2.1	1	Known human sensitizer, formaldehyde releaser, significant risk relative to exposure SEQ 1.5 (Schnuch et al., 2011), probably underestimated by the weak LLNA outcome
Iodopropynyl butylcarbamate	55406-53-6	0.9	0.9	0.1	1	Known human sensitizer, frequent case of preservative allergy
Chemicals with limited human evidence						
cis-6-Nonenal	2277-19-2	23	23.1	3.8	6	

α-Amylcinnamic aldehyde	122-40-7	10.6	11	1.5	6	
Farnesal	502-76-0	12	11.7	2.3	1	Frequent positive cases for farnesol – the prohapten for farnesal – in clinical studies
Abietic acid	514-10-3	15	14.7	0.9	1	Key pre-hapten in colophony (Hausen et al., 1989; Karlberg, 1988)
Trimellitic anhydride	552-30-7	9.2	9.2	1.7	4	
trans-2-Decenal	3913-71-1	2.5	2.5	0.5	1	Potent Micheal acceptor
4-Amino-m-cresol	2835-99-6	1.8	1.5	0.2	1	

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