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: ESM4

Tab. ESM4-1: Chemicals classified into the wrong GHS-class using the combination of the 2o3 DA and the kDPRA

Name	CAS-No.	OECD LLNA EC3 MLLP	Discussion and human sensitization evidence
FP (1B instead of NC) n = 3			
2-Hydroxypropyl methacrylate	923-26-2	NC	Frequent allergen in dental workers (Heratizadeh et al., 2018) and nail artists (Fisch et al., 2019), indicating **FN LLNA result**. Lower alkyl methacrylates are weak sensitizers (Kimber and Pemberton, 2014). Covalently reacts with Cor1-peptide (38% depletion, direct adduct formation (Natsch et al., 2015).
1-Iodohexane	638-45-9	NC	Covalently reacts with Cor1-peptide by addition-elimination reaction (63% depletion, direct adduct formation: [M+H]+ = 993.4 [908.4+212-126.9-1]+1)(Natsch et al., 2015). **Alkylating potential** indicates FN in LLNA.
4-Methyl-2-nitroanisole	119-10-8	NC	No *in vivo* data except 1 LLNA study
FP (1A instead of NC) n = 1			
Methyl 3-bromoproprionate	3395-91-3	NC	Covalently reacts with Cor1-peptide by addition-elimination reaction (98% depletion, direct adduct formation of [M+H]+ = 995.3 [908.4+165.9-78.9-1]+1) (Natsch et al., 2015). **Very strong alkylating potential** indicates FN in LLNA.
FN (NC instead of 1B) n = 16			
3-Aminophenol	591-27-5	3.2	No other data
α-Amylcinnamic alcohol	101-85-9	NA	Negative in LLNA, but close to threshold (Max SI 2.9-fold), hence rated positive by OECD group (OECD, 2021c)
Anisyl alcohol	105-13-5	7.1	No other data
Benzyl salicylate	118-58-1	2.9	Weak human sensitizer based on clinical data (Schnuch et al., 2007); Negative in HRIPT at 15% (Api et al., 2015)
DMSO	67-68-5	72	Human non-sensitizer (Marren, 2011; OECD, 2021d)
p-Isobutyl-α-methylhydrocinnamaldehyde	6658-48-6	9.5	Weak human sensitizer by read-across to p-*tert*-butyl-(α-methylhydrocinnamaldehyde)
α-Isomethylionone	127-51-5	21.8	Human non-sensitizer (OECD, 2021d), tested in HRIPT at 60% in 106 and 23 panelists with no positive reactions. Very low frequency of positive reactions (n = 1 of 2004 (Schnuch et al., 2007) in clinic despite high use leading to low SEQ[1] 0.23/0.08 (Schnuch et al., 2015)). Completely shielded Michael acceptor alert, leading to not even traces of peptide adducts (Natsch and Emter, 2017)
Isopropyl myristate	110-27-0	44	Negative at 20% in human HRIPT (close to OECD decision threshold of 25%), Simple alkyl esters lack reactivity and acyl-transfer potential, "extremely weak or non-sensitizer" based on extended patch-testing (16 reactions in 12,600 patients; less frequent than, e.g., propylene glycol) (Uter et al., 2004). Very wide-spread use as cosmetic excipient.

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[1] SEQ: Sensitization exposure quotient indicates frequency of positive reaction relative to use frequency/ volume and hence gives a rough sensitization risk under normal use. See Schnuch et al. (2015)

Linalool	78-70-6	35.5	SEQ 0.08/0.1 (Schnuch et al., 2015) indicates very low frequency compared to use. Negative in HRIPT (NOEL 13793 μg/cm^2 (Basketter et al., 2014; Gerberick et al., 2001) and HMT (NOEL 55176 μg /cm^2 (Greif, 1967), 20% top concentration close to OECD decision threshold of 25%. SCCNFP opinion (SCCS, 2012) lists Linalool as a well-established contact allergen, and this assessment is based on data on Linalool put under forced oxidation for several months (Brared Christensson et al., 2012), which is not relevant for the regulatory assessment of the parent compound and also appears not to represent commercial use (Kern et al., 2014). Oxidation products (hydroperoxides) from linalool are indeed positive in *in vitro* tests (Raffalli et al., 2018), but these are not assessed here.
Methyl pyruvate	600-22-6	2.4	No other data
OTNE	54464-57-2	14.2	Human non-sensitizer (OECD, 2021d). (Tested at 40% in 101 with no positive reactions.)
Pyridine	110-86-1	72	No structural alert, positive in LLNA only at the 100% dose. Considered human negative by Basketter et al. (2014) but positive by OECD (OECD, 2021d) based on a single positive individual in HMT.
Resorcinol	108-46-3	6.3	Rare contact allergen in the clinic even if specifically tested in an exposed population such as hairdressers (Darcis and Goossens, 2016)
Salicylic acid	69-72-7	12.2	SCCS concluded salicylic acid as non-sensitizer based on WoE (EC, 2020). Negative in human predictive test at 20% (OECD, 2021d) (close to OECD decision threshold). Widely used up to 2% in leave-on cosmetics as an active ingredient, but allergic reactions are not reported. Negative in Buehler test at 25% induction concentration. Negative LLNA performance standard.
Sodium lauryl sulfate	151-21-3	3.7	Human non-sensitizer (OECD, 2021d). Widely documented as false-positive in the LLNA (Loveless et al., 1996)
2,2,6,6-Tetramethylheptane-3,5-dione	1118-71-4	27	No other data
Overpredicted (1A instead of 1B) n = 14			
Abietic acid	514-10-3	15	Pre-hapten in colophony
1,2-Benzisothiazol-3(2H)-one	2634-33-5	4.8	Strong human sensitizer in an HRIPT study, ICCVAM (ICCVAM, 2011) derived a DA05 of 50 μg/cm^2, i.e., 1A, OECD review found a confounding factor in the co-formulation in the human study
Butyl acrylate	141-32-2	11.2	Highly reactive molecule. Very high volatility may lead to underprediction under LLNA conditions
trans-Dec-2-enal	3913-81-3	2.5	Close to LLNA classification threshold
Diethyl maleate	141-05-9	2.1	Human 1A (OECD, 2021b), close to LLNA classification threshold
Ethyl acrylate	140-88-5	32.75	Highly reactive molecule. Very high volatility may lead to underprediction under LLNA conditions
2-Ethylbutanal	97-96-1	76	Highly reactive molecule. Very high volatility may lead to underprediction under LLNA conditions
Hepta-2,4-dienal	5910-85-0	4	Highly reactive molecule. Very high volatility may lead to underprediction under LLNA conditions
trans-Hex-2-enal	6728-26-3	4.05	Human 1A (OECD, 2021b), Highly reactive molecule with very high volatility evaporating under LLNA conditions
Imidazolidinyl urea	39236-46-9	24	Known human sensitizer, formaldehyde releaser, significant risk relative to exposure SEQ 1.5 (Schnuch et al., 2011), probably underestimated by the weak LLNA outcome
Methyl acrylate	96-33-3	20	Highly reactive molecule. Very high volatility may lead to underprediction under LLNA conditions
Safranal	116-26-7	7.5	Human 1A (OECD, 2021b)
Thiram	137-26-8	5.2	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
Trimellitic anhydride	552-30-7	9.2	No other data
Underpredicted (1B instead 1A) n=7			
2-Aminophenol	95-55-6	0.45	Aromatic amine with Log kmax < -2; Lag time for oxidation leads to an estimation of low reactivity rate; outside of kDPRA AD according to TG 442C (OECD, 2021a). Correctly predicted as 1A when taking AD of kDPRA into account and applying EQ6 as indicated in Figure 1.
BADGE	1675-54-3	1.5	Log k_{max} and LLNA EC3 close to decision threshold for 1A classification
DNBS, sodium salt	885-62-1	2	Log k_{max} and LLNA EC3 close to decision threshold for 1A classification
Glutaraldehyde	111-30-8	0.08	Predominant amine reactivity; outside of kDPRA AD according to TG 442C; EQ6 predicts EC3 of 0.7% / 1A but only to be

			used for WoE; Correctly predicted as 1A when taking AD of kDPRA into account and applying EQ6 as indicated in Figure 1.
1-Naphthol	90-15-3	1.3	Potential prohapten, but not *a priori* obvious from structure
p-Phenylenediamine	106-50-3	0.11	Aromatic amine with Log kmax < -2; Lag time for oxidation leads to an estimation of low reactivity rate; outside of kDPRA AD according to TG 442C; Correctly predicted as 1A when taking AD of kDPRA into account and applying EQ6 as indicated in Figure 1.
Tetrachlorosalicylanilide	1154-59-2	0.0265	

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