7. Oct 2022

Proposal for application of RCPL

Evaluating the Regression based potency DA vs. the RCPL

Givaudan

Human by nature

Agenda

- Overview on the published Defined Approach for potency assessment
- Predictivity of the DA when applied to fragrance chemicals in the PV-list:
  - A) Predictivity vs. LLNA EC3 values
  - B) Predictivity vs. Potency values
- Predictivity of the DA when applied to non-fragrance chemicals in the PV-list:
  - A) Predictivity vs. LLNA EC 3 values
  - B) Predictivity vs. Potency values
- Assessing the chemical space by fitting a model to the PV list
- The big outstanding question: Models trained to human data?

Deriving Point of Departure (PoD) without animal testing

Our published approach is entirely trained vs. LLNA data – hence this is the model supposed to best fitting LLNA potency data

Our overall approach:

1. Combine KeratinoSens and/or h-CLAT with kDPRA
2. Perform multiple regression vs. LLNA data
3. Predict 'most likely' LLNA EC3 value as Point of departure (PoD)
4. Optional: Gather additional data with specific tests
5. Uncertainty assessment: Check prediction accuracy for similar molecules with *in vitro* AND *in vivo* data
6. Adjust PoD based on uncertainty assessment and use it in QRA2 for risk assessment

Cases study Givaudan: Deriving NESIL without animal testing

- All the input data are Log-transformed and normalized (set to zero if molecule is inactive)
- Multiple regression model is trained to predict pEC3
  - pEC3 = Logarithmic molar EC3 value
- This predicts a **Likely LLNA EC3** as point of departure (PoD)
- Models can work with data from 2 or 3 tests

Microsoft Excel Worksheet

$$\mathbf{pEC3} = 0.42 + 0.40 \times \mathbf{Log\ k_{max}} + 0.15 \times Log\ \mathbf{EC1.5}_{norm} + 0.36 \times Log\ \mathbf{IC50}_{norm} - 0.21 \times Log\ VP_{norm}$$

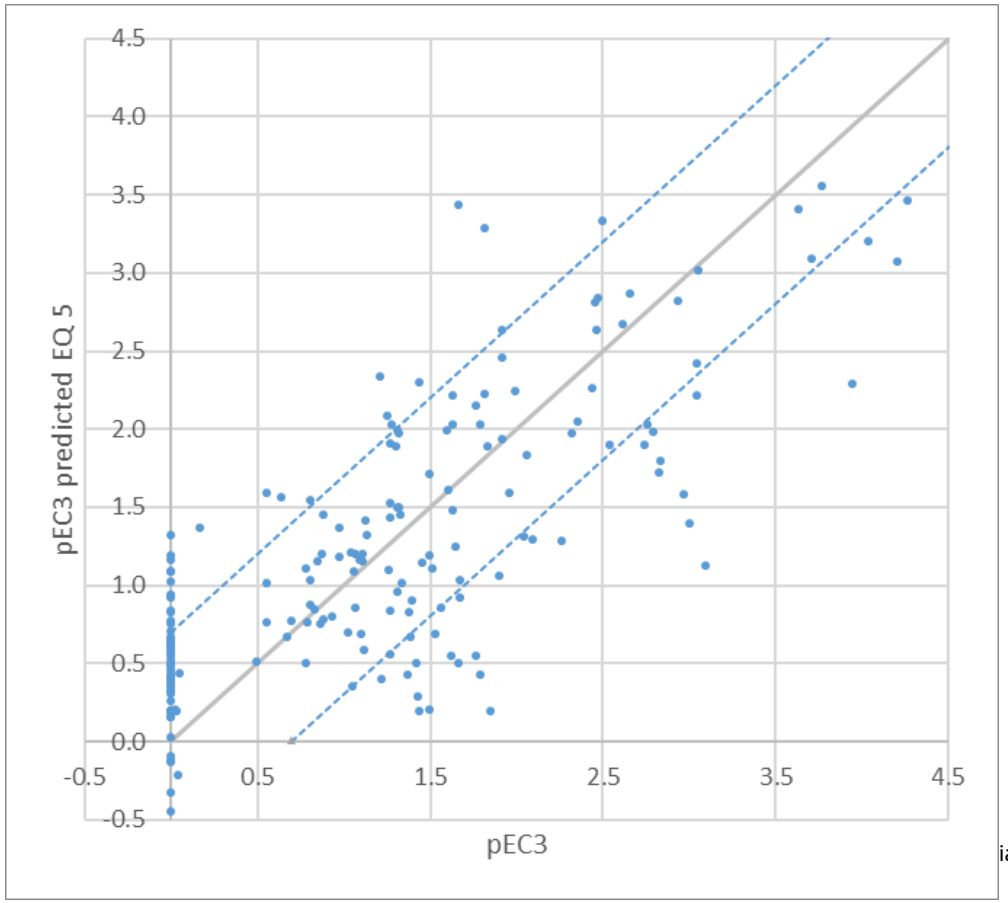
Peptide reactivity **KeratinoSens** **Volatility**

$$\mathbf{pEC3} = 0.18 + 0.36 \times \mathbf{Log\ K_{norm}} + 0.21 \times Log\ \mathbf{MIT}_{norm} + 0.35 \times Log\ \mathbf{CV75}_{norm} - 0.19 \times Log\ VP_{norm}$$

Peptide reactivity **h-CLAT** **Volatility**

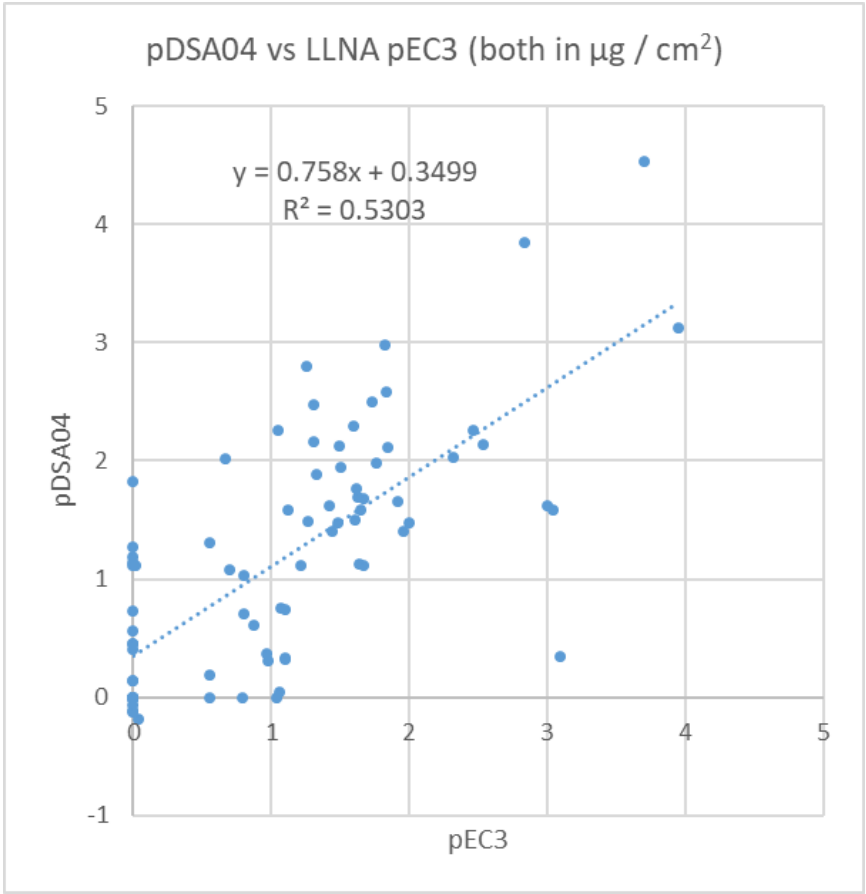
Predictivity of correlation model

- 75% of chemicals are within a 5-fold margin around the LLNA value
- 75% of chemicals are less than 3-fold underpredicted
- This uncertainty includes variability of the LLNA itself and predictive limitations of the LLNA



Prediction for 188 chemicals with kDPRA, hClat and KS data vs in vivo LLNA value

As a reference: Comparison of LLNA and Human data



Predictivity of correlation model

- Similar predictivity of the models based on
  - kDPRA and KeratinoSens
  - kDPRA and h-CLAT
  - kDPRA, KeratinoSens and h-CLAT

Model	Input parameters	Fold-misprediction[1] (Geomean)	Fold-misprediction (Median)	Chemicals > 5 – fold underpre-dicted[2] n, (%)	Chemicals > 10 – fold under-predicted n, (%)	Chemicals > 5 – fold over-predicted[2] n, (%)	Chemicals > 10 – fold over-predicted n, (%)
EQ1	kDPRA, KS	3.3	2.5	33 (18%)	20 (11%)	16 (9%)	7 (4%)
EQ4	kDPRA, h-CLAT	3.2	2.4	30 (16%)	17 (9%)	16 (9%)	7 (4%)
EQ5	kDPRA, KS, h-CLAT	3.1	2.3	35 (19%)	17 (9%)	18 (10%)	6 (3%)
EQ6	KS, h-CLAT	3.5	2.6	33 (18%)	19 (10%)	19 (11%)	8 (4%)
EQ7	KS, h-CLAT	3.4	2.7	31 (16%)	19 (10%)	18 (10%)	6 (3%)

Predictivity for case studies: Evaluation of predictivity in the Publication

				Predicted EC3		
	LLNA EC3 [1)]	LLNA studies (n)	LLNA EC3 range	EQ1	EQ4	EQ5
Aniline	**NC**	14	13.25 - (> 100)	60	52	**57**
Penicillin G	**31.3**	8	11.2 - 46.5	>100	>100	**>100**
Hydroxycitronellal	**21.1**	8	18.8 - 33	18.7	11.3	**10.9**
Geraniol	**16.1**	6	5.6 - 57	18.3	14.3	**14.2**
Eugenol	**11.6**	16	3.8 - 16.6	19.9	6.8	**10.4**
alpha-hexyl cinnamic aldehyde	**10.8**	29	1.2 - 33.8	5.9	*(25)*	**17.4**
Lilial	**8.6**	5	3 - 18.6	20.5	9.3	**12.5**
Citral	**5.8**	16	1.5 - 26.8	9.4	5.0	**4.8**
Formaldehyde	**3.8**	15	0.35 - 14.5	1.5	0.8	**1.0**
3- dimethylaminopropylamine	**3.5**	7	1.8 - (>10)	40	37	**32**
Isoeugenol	**1.3**	31	0.5 - 6.4	1.8	*(4.6)*	**4.2**
Cinnamic aldehyde	**1**	12	0.5 - 3.1	1.0	0.8	**0.8**
Hydroquinone	**0.19**	20	0.07 - 1.67	0.9	0.4	**0.4**
PPD	**0.11**	10	0.06 - 0.2	3.5	1.9	**1.7**
DNCB	**0.054**	20	0.012 - 0.096	0.18	0.19	**0.17**
Kathon CG	**0.008**	10	0.005 - 0.063	0.05	0.05	**0.05**
Oxazolone	**0.002**	7	0.001 - 0. 003	1.5	0.5	**0.7**

- Chemicals with at least 5 LLNA studies as case studies
- For these the certainty of the LLNA value is high
- **Overall accurate prediction of these chemicals with strong in vivo evidence**
- Similar predictivity with different models
- **Flexibility which model to apply**

Predictivity of the DA when applied to fragrance chemicals in the PV-list: (A) Predictivity vs. LLNA

- Our key concern is potency prediction of fragrance molecules
- As the model is trained on LLNA, we first looked at the LLNA predictions for the RPLC list
- For most molecules the LLNA is predicted within a margin of two-fold (green)
- 3 molecules are overpredicted (stronger sensitization potential (Hexenal, safranal, Coumarin), light green
- 4 molecules are 2 – 5-fold underpredicted (orange)
  - Allyl phenoxyacetate is strongly underpredicted, but in vivo value is based on a single LLNA study
  - Benzyl salicylate is underpredicted, but salicylates known to be overpredicted in LLNA

Name	EC3	EC3 PREDICTED EQ5
trans-2-Hexenal	1013	203
Methyl 2-nonynoate	<1250	541
Methyl 2-octynoate	125	482
Phenylacetaldehyde	750	586
Safranal	1875	479
Isoeugenol	325	1041
Citral	1450	1198
Allyl phenoxyacetate	775	17938
3-Propylidenephthalide	925	4061
Cinnamic aldehyde	250	199
Furaneol	450	no invitro data
Perillaldehyde	2175	1379
Benzaldehyde	>6250	10151
Lyral (HICC)	4275	3632
Hydroxycitronellal	5275	2728
Cinnamic alcohol	5775	4466
Eugenol	2900	2593
Benzyl salicylate	725	5001
Geraniol	4025	3555
Coumarin	neg	9838
Carvone	3250	2620
Hexyl cinnamic aldehyde	2700	4345
Benzyl Alcohol	neg	10880
Benzyl benzoate	4250	10813
Isomethylionone (α-)	5450	3357
Methyl salicylate	5000*	16111
Vanillin	neg	16726

Predictivity of the DA when applied to fragrance chemicals in the PV-list: (B) Predictivity vs. Potency value

- For 12 of 37 the PV is predicted within a margin of two-fold (green)
- 6 molecules are overpredicted (stronger sensitization potential predicted; cinnamic aldehyde, eugenol, BS, geraniol, Carvone, HCA), light green
  - These are mostly clinical relevant allergens, hence this overprediction is probably correctly conservative

- 7 molecules are 2 – 5-fold underpredicted (orange)
  - Signicant sensitization potency is predicted for these molecules, underprediction is mostly 3 - 4 fold
  - Hence the overall ranking is still correct

Name	Potency Value	EC3 PREDICTED EQ5	
trans-2-Hexenal	39.3	203	
Methyl 2-nonynoate	109	541	
Methyl 2-octynoate	125	482	
Phenylacetaldehyde	750	586	
Safranal	106	479	
Isoeugenol	325	1041	
Citral	1450	1198	
Allyl phenoxyacetate	775	17938	
3-Propylidenephthalide	925	4061	
Cinnamic aldehyde	885	199	
Furaneol	1181	) invitro data	no
Perillaldehyde	2175	1379	
Benzaldehyde	4094	10151	
Lyral (HICC)	4275	3632	
Hydroxycitronellal	5275	2728	
Cinnamic alcohol	5775	4466	
Eugenol	7357	2593	
Benzyl salicylate	17715	5001	
Geraniol	9197	3555	
Coumarin	11792	9838	
Carvone	17573	2620	
Hexyl cinnamic aldehyde	23620	4345	
Benzyl Alcohol	>25000	10880	(Pred.) non-sens
Benzyl benzoate	>25000	10813	(Pred.) non-sens
Isomethylionone (α-)	>25000	3357	(Pred.) non-sens
Methyl salicylate	NS	16111	(Pred.) non-sens
Vanillin	NS	16726	(Pred.) non-sens

Fragrance chemicals: Some difference between evaluation vs. LLNA EC3 and vs. PV

- In some cases, the predicted value is in between the LLNA EC3 and the PV (Safranal and trans-2-hexenal)
- In some cases, the EC3 is better predicted and both the prediction and the LLNA EC3 are more conservative

Name	Potency Value	EC3	EC3 PREDICTED EQ5	
trans-2-Hexenal	39.3	1013	203	Predicted EC3 between PV and LLNA
Safranal	106	1875	479	Predicted EC3 between PV and LLNA
Cinnamic aldehyde	885	250	199	Predicted EC3 closer to LLNA, conservative
Benzaldehyde	4094	>6250	10151	
Geraniol	9197	4025	3555	Predicted EC3 closer to LLNA, conservative
Coumarin	11792	neg	9838	Predicted EC3 closer to PV
Carvone	17573	3250	2620	Predicted EC3 closer to LLNA, conservative

Predictivity of the DA when applied to non-fragrance chemicals in the PV-list: (A) Predictivity vs. LLNA EC3

- For some of the extreme sensitizers, the LLNA EC3 value is clearly underpredicted
- Still, except for Glutaraldehyde, these chemicals are rated as strong sensitizers
  - EC3 < 500 µg/cm^2, < 2%, i.e. GHS1A
  - This is in line with our published observation that the model not completely covers to potency scale of the extreme sensitizers

Name	EC3	EC3 EQ5
5-chloro-2-methyl-4-isothiazolin-one (CMIT)	2	30
2,4-dinitrochlorobenzene (DNCB)	14	41
1,4-Phenylenediamine (PPD)	28	429
Glutaraldehyde (act. 50%)	20	995
1,4-Dihydroquinone	48	104
Benzyl bromide	50	64

Predictivity of the DA when applied to non-fragrance chemicals in the PV-list: (B) Predictivity vs. potency values

- For some strong sensitizers, the potency values derived from human DSA04 are clearly lower than the LLNA EC3, e.g. PPD* and DNCB – for these the underprediction by the model are even more pronounced than vs. LLNA
  - As for the LLNA evaluation - This is in line with our observation that the model not completely covers to potency scale of the extreme sensitizers

Name	Potency Value	EC3 EQ5
5-chloro-2-methyl-4-isothiazolin-one (CMIT)	2.25	30
2,4-dinitrochlorobenzene (DNCB)	3.4	41
1,4-Phenylenediamine (PPD)	3.9	429
Glutaraldehyde (act. 50%)	19.9	995
1,4-Dihydroquinone	47.5	104
Benzyl bromide	50	64

* Note: For PPD the initial peptide reactivity is slow, high reactivity and lower PoD is observed if the chemicals is pre-incubated for oxidation to start

Ranking the PV list with all different models

- The full PV list was ranked with Spearman rank correlation – as one of the goals of the RCLP list was to check whether models can rank potency

- The LLNA and the LLNA-trained model can similarly rank the chemicals
  - rho = 0.816; p = 0.000 for the LLNA vs. The PV
  - rho = 0.823; p = 0.000 for our published (LLNA-based) model vs PV

  - We also made an alternative model based on human data, not published and not discussed here for time reason
  - This model gives also a similar ranking
  - rho = 0.821; p = 0.000 for new human data trained model vs PV

Uncertainty assessment

- In our initial approach for potency assessment with regression models we propose to use an assessment factor for *in vitro* to *invivo* uncertainty of 2 in case we have a good predictivity for close analogues

- We generally use a factor of 3 as general factor accounting for the uncertainty of the models if we do not have close analogues (this corresponds to the 75% percentile, i.e. 75% of the chemicals are less than 3-fold underpredicted
  - We have to keep in mind that the uncertainty associated with a single LLNA values is normally not factored in and the value is used as such – (all toxicological assessments never use a 95%-percentile assumption)

- Uncertainty is also factored in by assessment factors at subsequent steps of the risk assessment

Predictivity of PV by Regression DA in published model: Conclusion

- The model (trained on LLNA) based on KeratinoSens, h-Clat and kDPRA predicts the LLNA potency of the fragrance chemicals in the RCPL quite well, with two outliers

- The model predicts the strong sensitization potential of the non-fragrance chemicals, but underestimates the exact potency in some cases

- The model is somewhat less accurate for the potency values (PV) on fragrance molecules
  - The overall ranking is correct
  - In some cases both the LLNA and the model are more conservative
  - In some cases the prediction is between PV and EC3
  - For the stronger human fragrance sensitizers based on the PV, the model tends to underestimate the potency, but this is in the range of the factor 3
  - This would be mostly corrected with an assessment factor for in vitro – to in vivo extrapolation of 3 which we usually apply in absence of close analogues

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