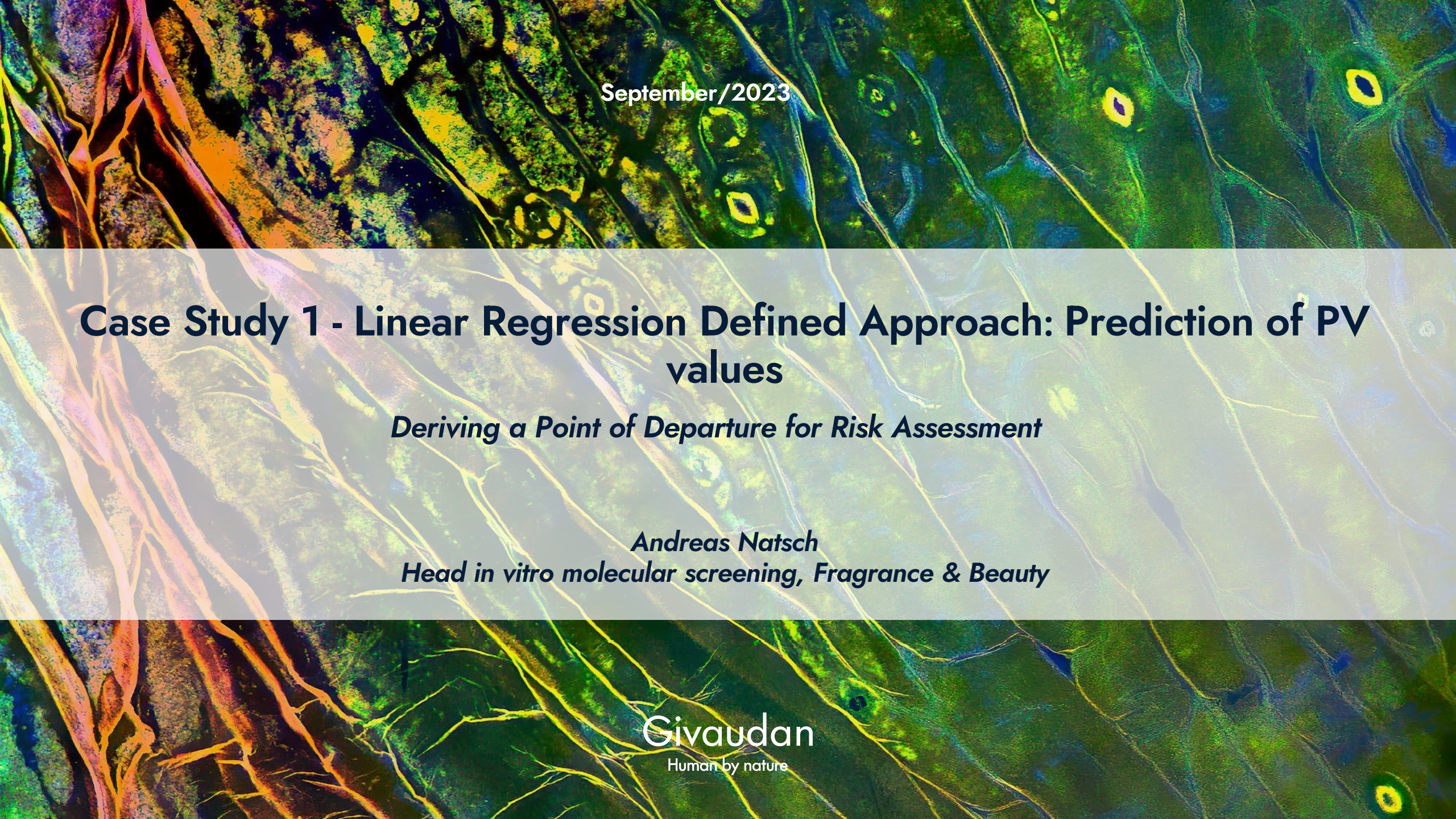

September/2023

Case Study 1 - Linear Regression Defined Approach: Prediction of PV values

Deriving a Point of Departure for Risk Assessment

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Human by nature

Agenda

- Risk assessment without animal testing: Overall approach
- Input data: KeratinoSens, h-CLAT and kDPRA
- The Databases
- Regression models
- Spreadsheet - Application in practice
- Predictivity
- Key under- and overpredictions
- Robustness and redundancy
- Predictivity of the DA when applied to fragrance chemicals in the PV-list
- Adding human data (ALTEX publication 2023)
- Uncertainty factors

Overall Approach

01

In vitro data

Using KeratinoSens and/or h-CLAT and/or Kinetic DPRA (kDPRA)

02

Regression Model

Perform multiple regression vs. LLNA data (or human data)

03

LLNA Prediction

Predict the 'most likely' LLNA EC_3 as the Point of Departure (PoD)

04

Uncertainties Factors

Adjust PoD based on uncertainty assessment and use it for Quantitative Risk Assessment (QRA2)

Input data: KeratinoSens, h-CLAT and kDPRA

01

***In vitro* data**

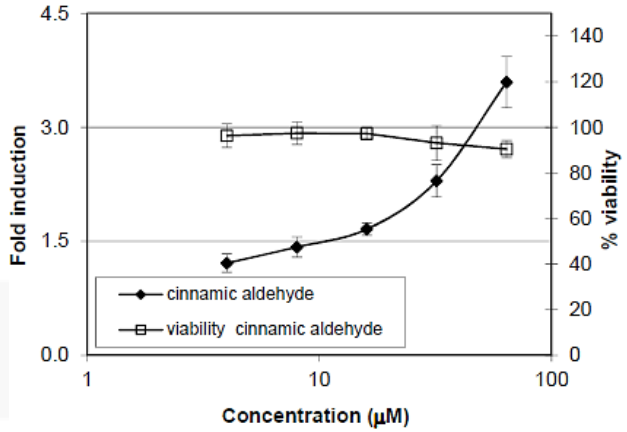
- **KeratinoSens**

EC1.5 –Dose for 1.5-fold induction

EC3 - Dose for 3-fold Luciferase induction

IC50 for 50% reduction in cell viability

KeratinoSens Example



- **hClat**

EC150 – dose for 1.5-fold induction of CD86

EC200 – dose for 2-fold induction of CD54

MIT minimum of EC150 and EC200

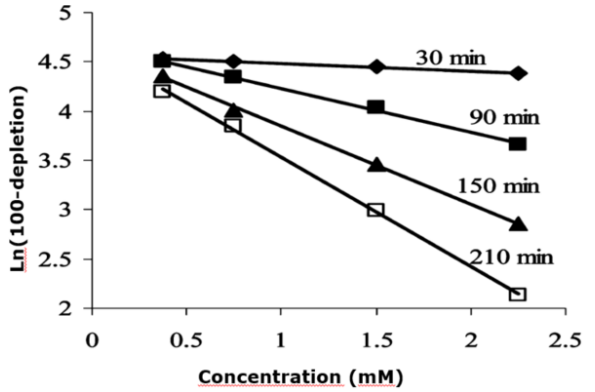
CV75 for 25% reduction in cell viability

- **Kinetic DPRA**

Kinetic rate for Cys-peptide depletion

D. Roberts, A. Natsch, *Chem Res. Toxicol.* **2009,** *22,592-603.*

kDPRA Example



The Databases

01

Regression Model

- **The Database and data preparations**

Dataset with LLNA, KeratinoSens and kDPRA: **n = 203**

Dataset with LLNA, KeratinoSens, h-CLAT and kDPRA: **n = 188**

Dataset with OECD curated LLNA data, KeratinoSens, h-CLAT and kDPRA: **n = 149**

✓ In the skin sensitization field we have a unique size of the dataset with in vitro and in vivo data

✓ All data are log transformed and normalized

Regression models

02

Regression Model

The data gives key input parameters for the equation(s) in the Regression Model :

- KS Log EC1.5norm
- KS Log IC50norm
- h-CLAT Log MITnorm
- h-CLAT Log CV75norm
- kDPRA Log kmax
- Physchem: Log VP norm

EQ 1: $pEC3 = 0.42 + [0.40 \times \textbf{Log } k_{max}]_{\text{Peptide reactivity}} + [0.15 \times \text{Log } \textbf{EC1.5}_{norm} + 0.36 \times \text{Log } \textbf{IC50}_{norm}]_{\text{KeratinoSens}} - [0.21 \times \text{Log } VP_{norm}]_{\text{Volatility}}$

EQ 4: $pEC3 = 0.18 + [0.36 \times \textbf{Log } k_{norm}]_{\text{Peptide reactivity}} + [0.21 \times \text{Log } \textbf{MIT}_{norm} + 0.35 \times \text{Log } \textbf{CV75}_{norm}]_{\text{H-CLAT}} - [0.19 \times \text{Log } VP_{norm}]_{\text{Volatility}}$

Four different models can be applied using the '2 out of 3 approach':

- EQ1: Combining KeratinoSens with kDPRA
- EQ4: Combining h-CLAT with kDPRA
- EQ6: Combining KeratinoSens and h-CLAT

} **Can be used for partial evidence**

- EQ5: All evidence: Combining KeratinoSens AND h-CLAT with kDPRA

Spreadsheet - Application in practice

03

LLNA Prediction

Prediction in Practice

- The key benefit of regression models is simplicity and transparency
- Using the equations from the previous slide the results can be calculated directly – no proprietary software or hidden algorithm
- For ease of application a public spreadsheet can be used
- Just enter the test results from the study report and voilà…

Chemical Identification

Chemical identifier	Cinnamic aldehyde
Chemical Name	
CAS Nr.	105-55-2
MW	132.16
Vapor pressure	4.6

Microsoft Excel Worksheet

Lognorm VP	0

KeratinoSens Assay Result

KeratinoSens assay data			
Rating PM	1	EC 1.5 (µM) consolidated	16.13
EC 1.5 (µM)	16.13	LOG norm EC1.5 KS	2.394425624
EC 3 (µM)	63.93	EC 3 (µM) consolidated	63.93
IC 50 (µM)	194.37	LOG norm EC3 KS	1.796355287
If data not in micromolar, enter in ppm		IC 50 (µM) consolidated	194.37
EC 1.5 (ppm)		LOG norm IC 50 KS	1.313430757
EC 3 (ppm)			
IC 50 (ppm)		If no induction above threshold, give default value = 4000	

kDPRA Assay Result

kDPRA assay data			
Log kmax (s-1M-1)	-1.35	LOG norm Kmax	2.15

h-CLAT Assay Result

h-CLAT assays data		If no induction above threshold, give default value = 5000	
Rating PM	1		
CD86 EC150 (µg/ml)	10.2	MIT (µM)	77.17917676
CD54 EC200 (µg/ml)	12.3	Log norm MIT h-CLAT	2.510439867
MIT (µg/ml)	10.2	CV 75 (µM)	211.8644068
CV 75 (µg/ml)	28	Log norm CV75 h-CLAT	2.071882007

LLNA Prediction

	pEC3	EC3
Global model KS + kDPRA Equation 1	2.11	1.02
Global model kDPRA + h-CLAT Equation 4	2.22	0.81
Global model KS + kDPRA + h-CLAT Equation 5	2.22	0.80
Global model KS + h-CLAT Equation 6	2.07	1.11
Global model KS + h-CLAT Equation 7	2.16	0.92

Predictivity

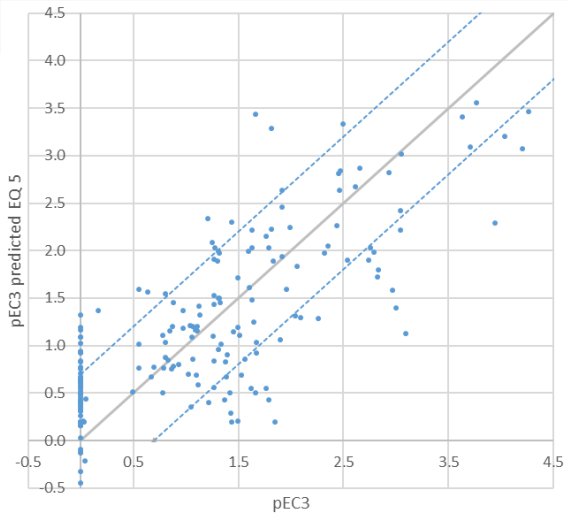
03

Prediction

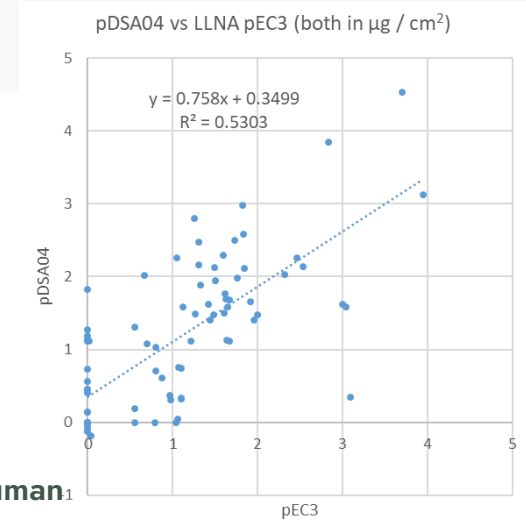
75% of chemicals are within a 5-fold margin around the LLNA value

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



Comparison of LLNA and Human data

Givaudan Approach: Database integrating i*n vitro and in vivo* data & Regression Model for PoD calculation

03

Prediction

Similar predictivity of the models based on the following:

- 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 underpredicted[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%)

[1] The ratio between the higher and the lower values of the measured and predicted EC3 value. Predicted EC3 > 100% were set to 100%.
[2] Under-predicted chemicals: those for which the measured LLNA EC3 is < than the predicted EC3; over-predicted chemicals: Those with measured LLNA EC3 > than the predicted value.

Predictivity for case studies

03

Prediction

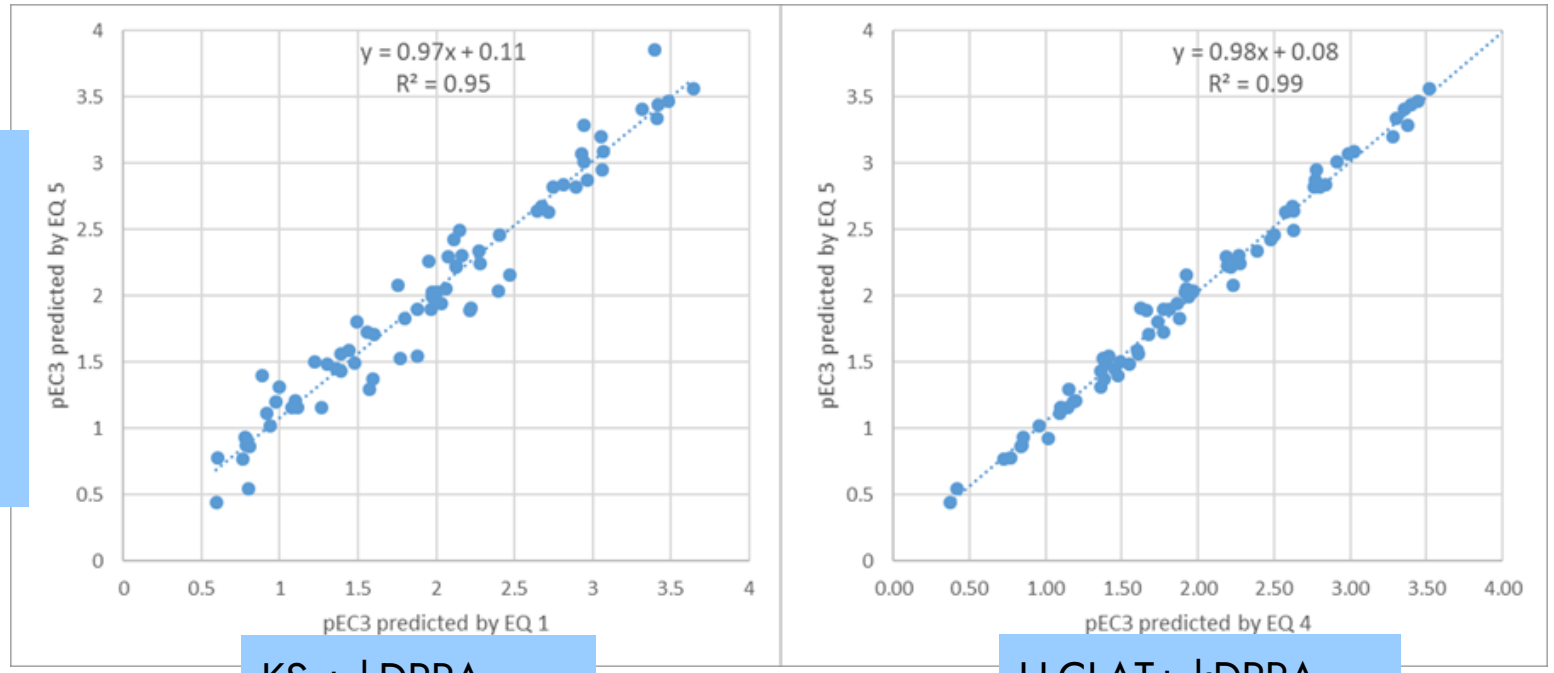
- Chemicals with at least 5 LLNA studies in OECD DB as case studies
- For these the certainty of the LLNA value is high
- Overall accurate prediction of these chemicals with strong *in vivo* evidence. Mostly within variability of the LLNA studies
- Similar predictivity with different models
- Flexibility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

				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**

Robustness and redundancy

- The data on predictivity and case studies show that
  - Similar predictions for individual chemicals with EQ1, EQ4 and EQ5
  - The overall fold-misprediction is quite similar by different models
- Further illustrated by individual predictions for chemicals positive in three tests

All three tests



KS + kDPRA

H-CLAT+ kDPRA

- This indicates data-redundancy
- Partial evidence is sufficient
- Having a third positive tests often does not change the assessment
- Will additional OECD-tests provide non-redundant information?

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	nc
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	LLNA EC3	EC3 EQ5
5-chloro-2-methyl-4-isothiazolin-one (CMIT)	2.3	11.8
2,4-dinitrochlorobenzene (DNCB)	10	41
1,4-Phenylenediamine (PPD)	40	429
Glutaraldehyde (act. 50%)	25	995
1,4-Dihydroquinone	25	104
Benzyl bromide	50	64

For CMIT Equation 1 is used due to missing h-CLAT data

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	PV	EC3 EQ5
5-chloro-2-methyl-4-isothiazolin-one (CMIT)	2.25	11.8
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

For CMIT Equation 1 is used due to missing h-CLAT data

- Less concern for fragrance materials which do not cover the extreme sensitizer scale

* 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 (see below)
  - This model gives also a similar ranking
  - rho = 0.821; *p* = 0.000 for new human data trained model vs. PV

Conclusions on predicting the chemicals in the RCPL

- For fragrance chemicals in the RCPL, we have overall a good predictivity of potency
- The PoD from regression models is not less conservative than the LLNA for fragrance chemicals, and using the PoD instead of the LLNA EC3 would not decrease safety assessments
  - Allyl phenoxyacetate is a clear exception – however for this chemical we just have a single LLNA study as all available *in vivo* evidence
- For the strong sensitizers, a significant sensitization potential is predicted, yet the models do not cover the full dynamic range for the very strong sensitizers
- Considerations of applicability domain are important – thus for example PPD needs oxidation to occur (hours) to become highly reactive, or Glutaraldehyde prediction should also take into account ist strong amine reactivity and possibility to cross-link proteins.

ALTEX 2023: Training models on the RCPL?

- Models are trained on LLNA data only – **what about training vs. Potency values (PV) in RCPL?**
- The RCPL list is **too small for representative models** – training vs. LLNA target in RCPL gives significantly different models
- Comparing model trained vs. LLNA target or PV in the RCPL shows a significant difference – **much lower weight for cytotoxicity**

Regression coefficients and statistics for predictive models trained on the **RCPL dataset**

Model	**Constant**	**k_{max}**	**EC1.5**	**IC50**	**MIT**	**CV75**	**VP_{norm}**	**R^2**	**n** [4)]
EQ5 (KS+h-CLAT+kDPRA)	0.20	0.34	0.09	0.11	0.20	0.21	-0.19	62%	188
vs. pEC3	-0.39	**0.13**	0.30	**-0.1**	**0.18**	**0.70**	-0.26	75%	31
vs. pPV	-0.43	**0.32**	0.44	**-0.4**	**0.39**	**0.35**	0.12	74%	31

Full set (n=188) for **LLNA target**

RCPL (n=31) different equation for **LLNA target: Not representative of full database**

RCPL (n=31) different equation for **PV target compared to LLNA target:**

- **Higher weight cell activation and reactivity**
- **Lower weight cytotoxicity**

ALTEX 2023: Training models on human data?

- Models are trained on LLNA data only – **what about training vs. Human DSA04 values?**
- The list with human data also not representative – training vs. LLNA target gives significantly different models
- Comparing model trained vs. LLNA target or vs. Human DSA04 shows a significant difference – **much lower weight for cytotoxicity is confirmed!**

Regression coefficients and statistics for predictive models trained on the **RCPL dataset**

Model	**Constant**	**k_{max}**	**EC1.5**	**IC50**	**MIT**	**CV75**	**$VP_{no\ rm}$**	**R^2**	**n** [4)]
EQ5 (KS+h-CLAT+kDPRA)	0.20	0.34	0.09	0.11	0.20	0.21	-0.19	62%	188
vs. pEC3	0.07	**-0.02**	**0.26**	**0.26**	**0.06**	**0.35**	-0.06	60%	62
vs. human DSA04	0.34	**0.04**	**0.44**	**-0.06**	**0.35**	**-0.15**	0.01	45%	62

Full set (n=188) for **LLNA target**

Human set (n=62) different equation for **LLNA target: Not representative of full database**

Human set (n=62) different equation for **DSA04 target compared to LLNA target:**

- **Higher weight cell activation and reactivity**
- **Lower weight cytotoxicity**

Adding human data (ALTEX 2023)

- RCPL List appears too small to train stable models
- Chemical set with human data is biased

- However – human data can be integrated along with LLNA according the RCPL workflows
  - Similar list was created for n = 139 chemicals
  - This list is heavily influence by LLNA data… but contains as much human evidence as possible

- Regression analysis
  - A) vs. LLNA data (same chemicals, n = 139)
  - B) **vs. Potency values (PV) integrating human evidence where available**

Irizar, A., et al., *Reference Chemical Potency List (RCPL): A new tool for evaluating the accuracy of skin sensitisation potency measurements by New Approach Methodologies (NAMs)*. Regul Toxicol Pharmacol, 2022. **134**: p. 105244.

ALTEX 2023: Training models on the extended RCPL

- Models trained on LLNA: Almost identical on set of 139 chemicals – this set is representative of the full database
- Comparing model trained vs. LLNA target or vs. Potency Values shows a significant difference – **much lower weight for cytotoxicity is again confirmed!**
- Indicates importance of cytotoxicity/irritancy for LLNA response?

Regression coefficients and statistics for predictive models trained on the **RCPL dataset**

Model	Constant	k_{max}	EC1.5	IC50	MIT	CV75	VP_{norm}	R^2	n [4)]
EQ5 (KS+h-CLAT+kDPRA)	0.20	0.34	0.09	0.11	0.20	0.21	-0.19	62%	188
vs. pEC3	0.15	**0.34**	**0.09**	**0.03**	**0.18**	**0.33**	-0.15	65%	139
vs. pPV	0.24	**0.38**	**0.26**	**-0.17**	**0.36**	**0.01**	-0.06	64%	139

Full set (n=188) for **LLNA target**

Extended PV list (n=139) different equation for **LLNA target: Almost identical model!**

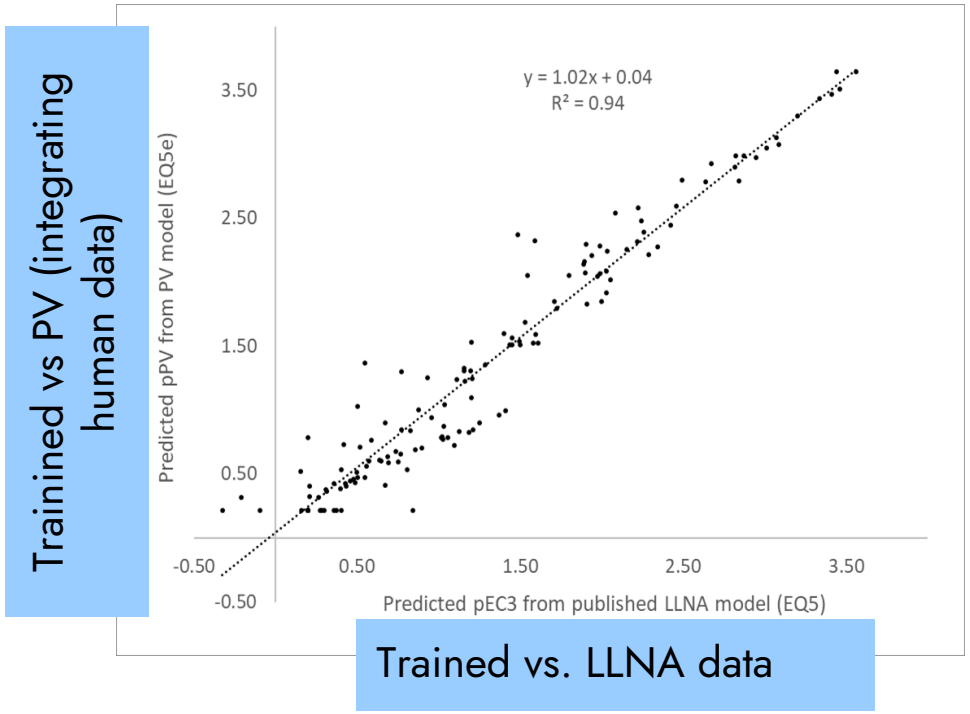
RCPL (n=139) different equation for **PV target compared to LLNA target:**

- **Higher weight cell activation and reactivity**
- **Lower weight cytotoxicity again confirmed**

Adding human data (ALTEX 2023)

- Two key learnings:
  - Adding human data indicates that **cytotoxicity has much less importance** for predicting potency
  - BUT: the resulting regression equations give very similar predictions
  - THUS: The regression approach – only trained on LLNA/animal data is quite **robust also in regards of which target data are used**

Predictions by LLNA model and PV model



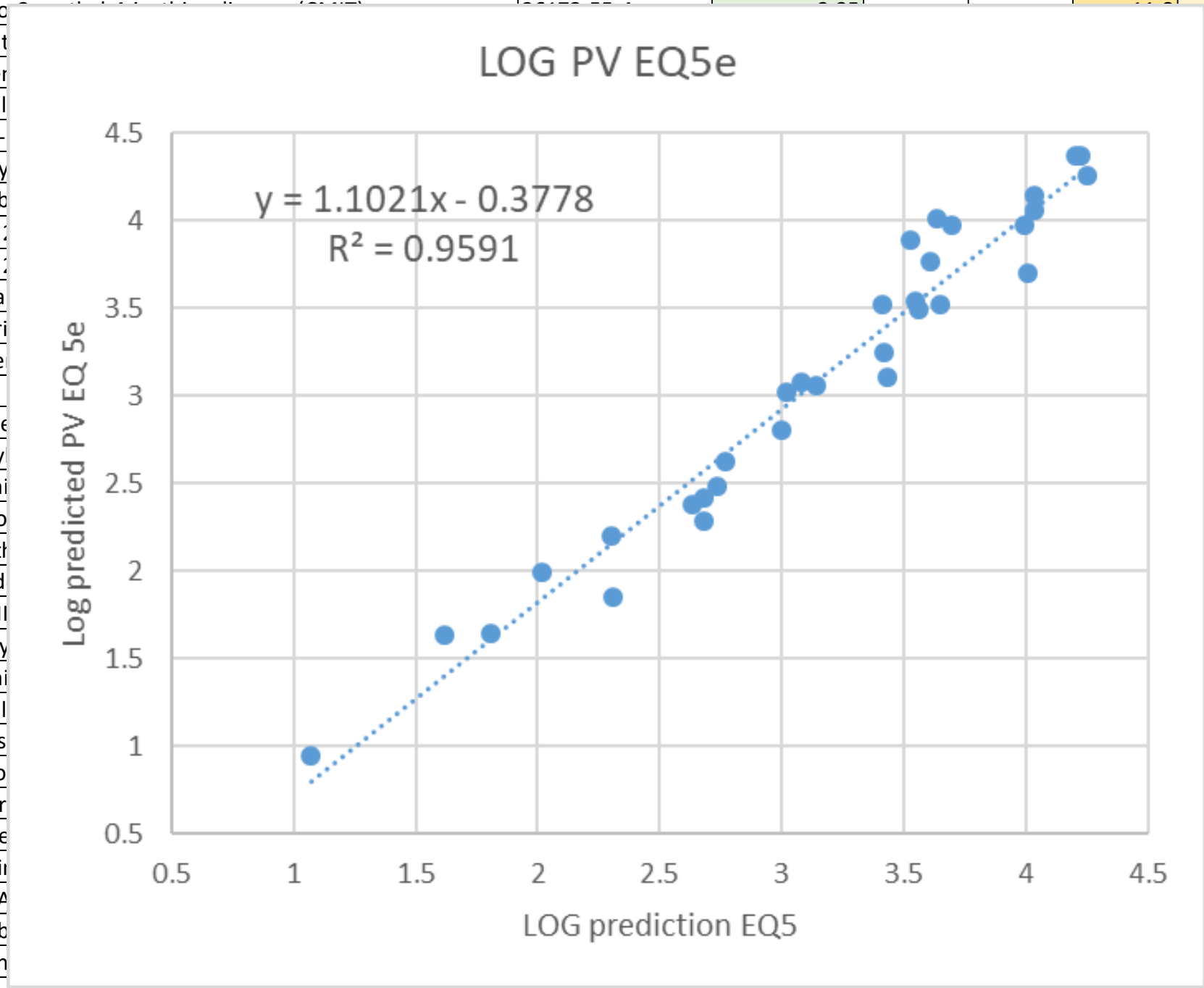
Using the PV models to predict the RCPL

- I showed you all predictions for the RCPL list with the original LLNA-trained models, i.e. the calculations I made *before* looking at predicting PV values
- I do not want to run the risk that I 'fitted the model' to the RCPL – as this is exacly what I warn people not to do…
- Still – we can look at the model trained on the 139 PV values….

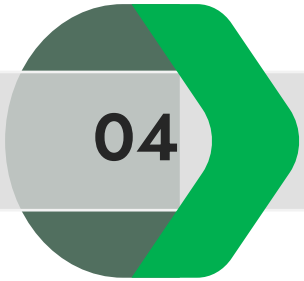
Using the PV models to predict the RCPL

- It doesn't matter!
- … indicating our models are quite stable …

Name	CAS No	Potency Value	RIFM NESI	LLNA EC3	EC3 EQ5	PV EQ5e
5-chloro						8.8
2,4-dinit						43
1,4-Pher						239
Glutaral						631
trans-2-						71
1,4-Dihy						98
Benzyl b						44
Methyl 2						303
Methyl 2						195
Phenyla						425
1,1,3-Tri						260
Isoeuge						1049
Citral						1184
Allyl phe						17966
3-Propy						5856
Cinnami						159
4-Hydro						data
p-Menth						1154
Benzald						5015
Lyral (HI						3126
Hydroxy						1265
Cinnami						3331
Eugenol						3279
Benzyl s						9307
Geranio						3493
Coumar						9327
Carvone						1775
Hexyl ci						10287
Benzyl A						11278
Benzyl b						13724
Isometh						7722
Methyl salicylate	119-36-8	No PV derived-	35000	25000	16111	23454



Uncertainty factors



Uncertainties Factors

- **Uncertainty assessment**
- In our initial approach for potency assessment with regression models we propose to use an assessment factor for in vitro to in vivo 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 application of sensitization assessment factors (SAF) at subsequent steps of the quantitative risk assessment process (i.e., QRA2)

Conclusions regression models

- All the key event based test guidelines (except classical GARD in TG) deliver – next to hazard identification – **dose-response** data which contribute to potency prediction
- Regression models are a **facile** and **transparent** way to integrate these data to derive a **Point-of-Departure** for quantitative risk assessment (QRA2)
- Already with these three tests there is **data redundancy**, and two tests often give very similar predictions to three tests
  - This may indicate we are in a 'as good as it can get' situation for the prediction model
- The **simple spreadsheet** makes application straightforward
- This is an *in vitro*-only **Defined Approach** –*in vitro* data directly leads to the PoD*
- '2 out of 3' DA (TG497) combined with kDPRA (TG442D) give
  - Hazard ID
  - GHS potency class
  - PoD from the same data! **No additional testing!**

*DA does not yet include *in silico* evaluation, structural alert and read-across. **These additional lines of evidence can then be used to refine the assessment and assess uncertainty** (they are not 'used up' in the DA)

Publications

The work presented here is the summary of three publications in 2022 and 2023

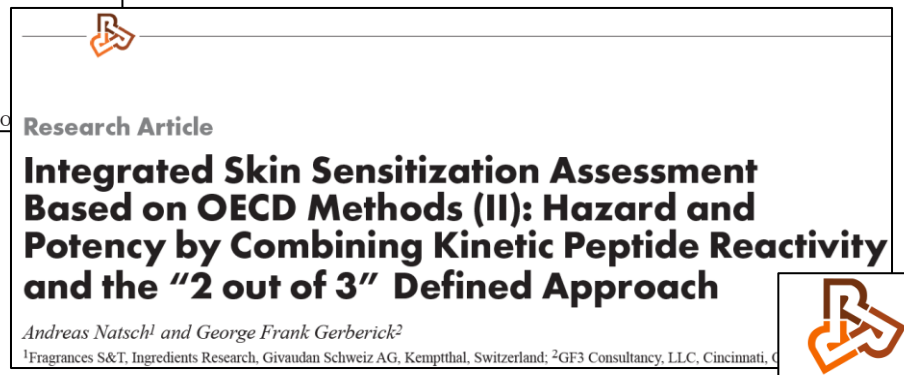
Research Article

Integrated Skin Sensitization Assessment Based on OECD Methods (I): Deriving a Point of Departure for Risk Assessment

Andreas Natsch[1] and George Frank Gerberick[2]

[1]Fragrances S&T, Ingredients Research, Givaudan Schweiz AG, Kemptthal, Switzerland; [2]GF3 Consultancy, LLC, Cincinnati, O

https://pubmed.ncbi.nlm.nih.gov/35404469/



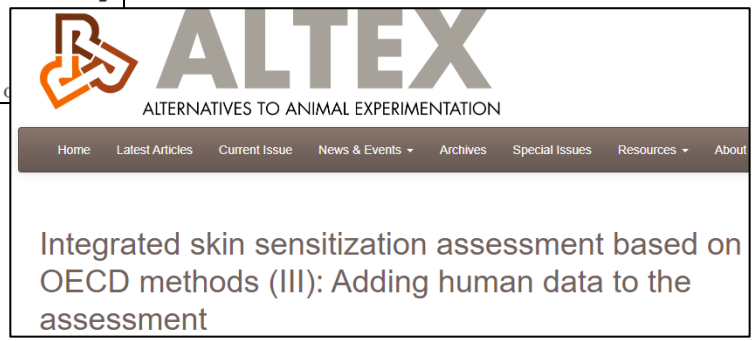
Research Article

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

Andreas Natsch[1] and George Frank Gerberick[2]

[1]Fragrances S&T, Ingredients Research, Givaudan Schweiz AG, Kemptthal, Switzerland; [2]GF3 Consultancy, LLC, Cincinnati,

https://pubmed.ncbi.nlm.nih.gov/35404468/



ALTEX

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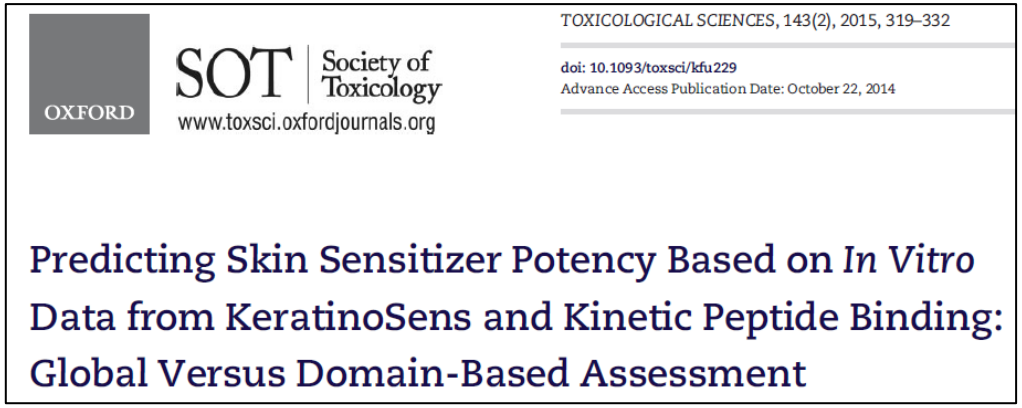
Integrated skin sensitization assessment based on OECD methods (III): Adding human data to the assessment

https://pubmed.ncbi.nlm.nih.gov/37074977/

Publications

This is following up on earlier research using a non-validated peptide reactivity assay. The 2018 paper goes into more detail on uncertainty analysis and application within an IATA.



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- Susanne Kolle (kDPRA validation and 2o3 validation)

OECD DASS expert group
- Data curation and compilation:

The IMS team

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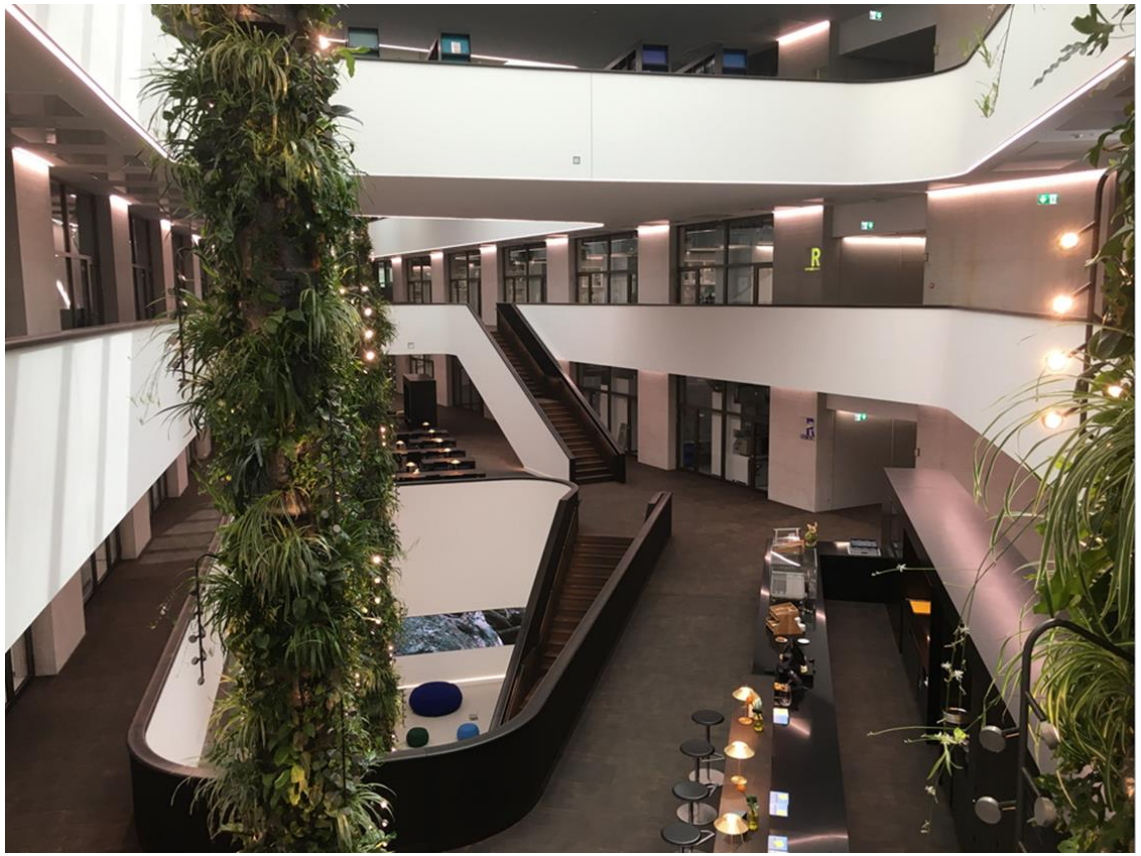


Thank you

Andreas Natsch
Andreas.natsch@givaudan.com

Givaudan
Human by nature

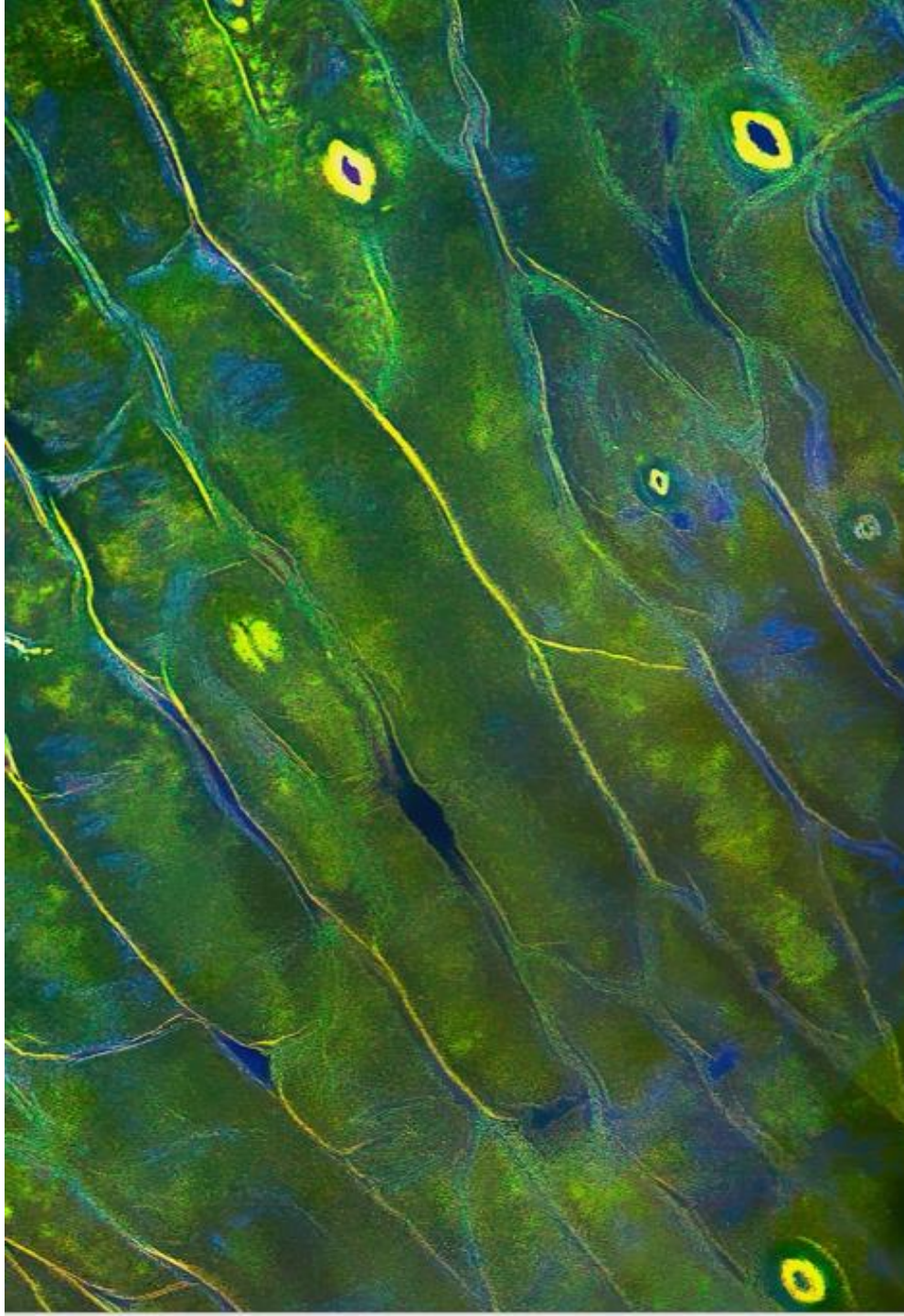

Backup slides with additional information
Givaudan
Human by nature

Do we always needs three tests?

Does the sequence of testing affect the result?

If we have all evidence, which PoD to select?

Looking For An Answer?

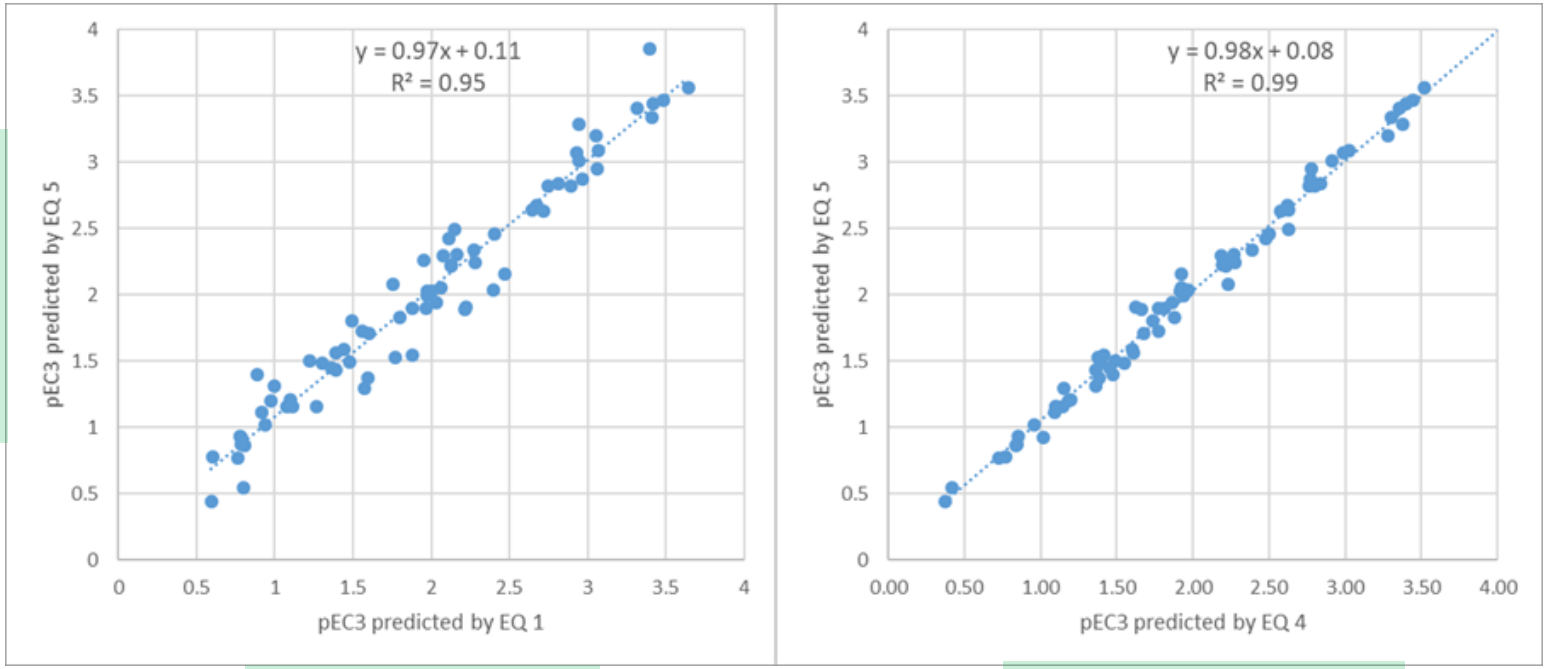


Givaudan

Model choice – Do we always need three tests?

As shown in Part I: Partial evidence gives similar predictions as using complete evidence

All three tests



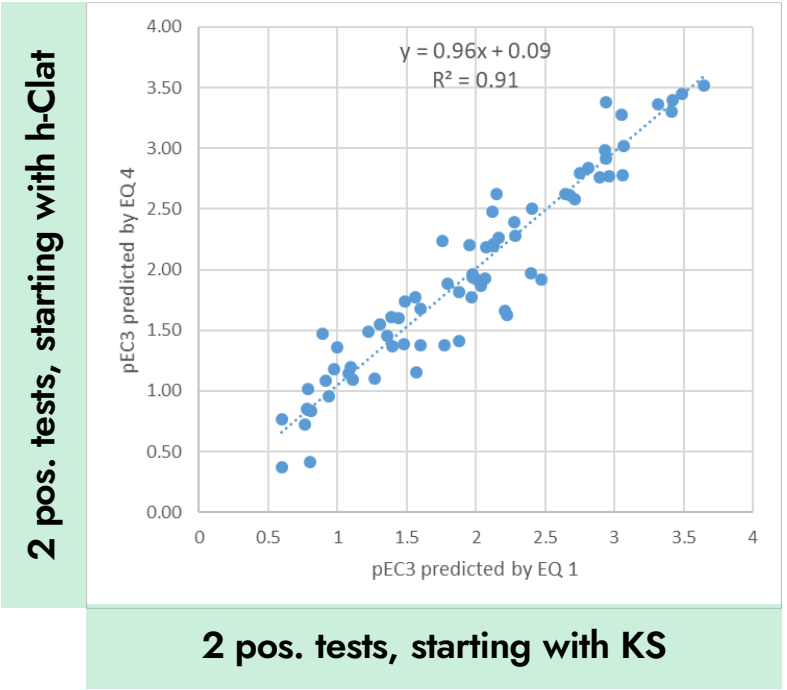
KS + kDPRA

H-CLAT+ kDPRA

This indicates that we can make an assessment with partial evidence, e.g. in a 2o3 assessment with only two tests conducted

Model choice – Does the sequence of testing affect the result?

- For '2 out of 3' hazard assessment, the sequence of testing does NOT affect the result
- For PoD with quantitative data: If we stop after two tests, because we have a positive outcome based on two tests – does it matter with which test we started?
- Due to the data redundancy, the result overall is **very similar, independent of which test we start with!**

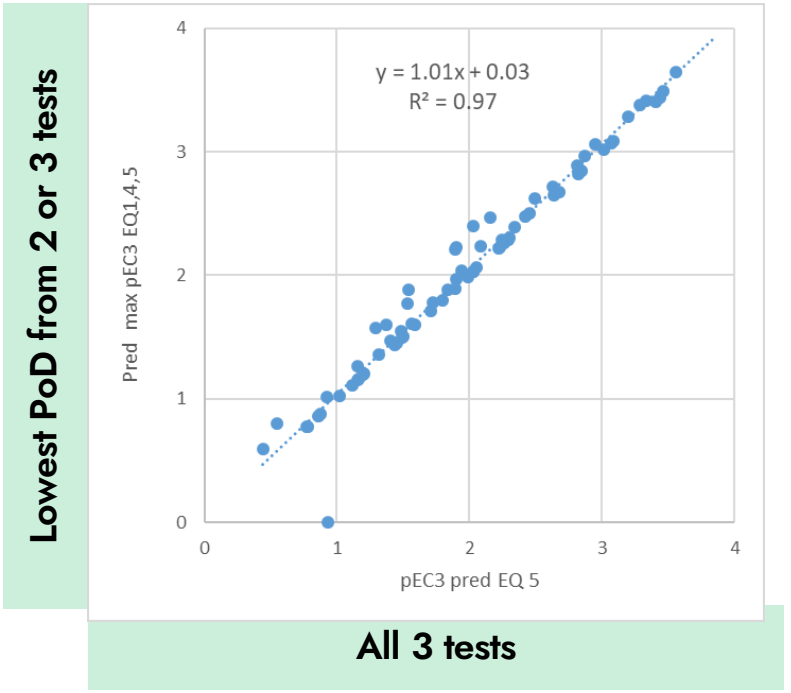


Model choice – If we have all evidence, which PoD to select?

If all three tests were done – which model should we use?

- **CASE A: All three are positive**
  - EQ5 integrating all evidence?
  - OR
  - The model with the lowest PoD (Conservative approach)?

The difference is small, and integrating all positive evidence (EQ 5) appears most appropriate



Model choice – If we have all evidence, which PoD to select?

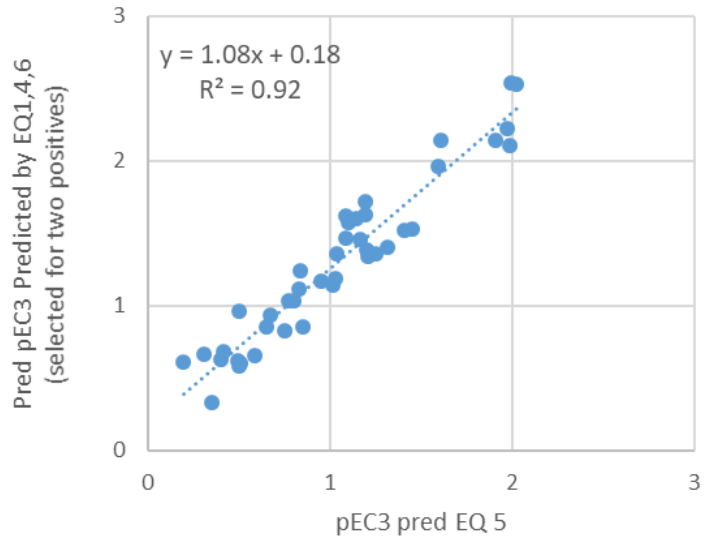
If all three tests were done – which model should we use?

- **CASE B: Only two tests are positive**
  - EQ5 also integrating negative evidence?
  - OR
  - The model for the two positive tests?

Positive Y intercept of 0.18 indicates that 1.5-fold lower PoD is predicted using evidence from the two positive tests only

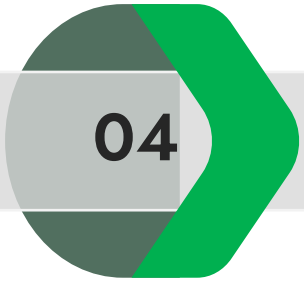
However, predictivity ignoring the negative evidence decreases and leads to some overprediction (conservative choice)

Lowest PoD from 2 or 3 tests



All 3 tests

Limitations

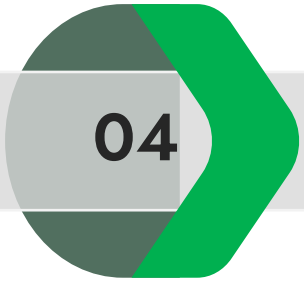


Uncertainties Factors

- **Limitations**
  - Especially chemicals which are outside of applicability domain of kDPRA may be underpredicted:
  - Aromatic amines with Lag-phase for oxidation
  - Prohaptens requiring enzymatic activation
  - Selectively amine reactive chemicals

- **Chemicals which technically interfere with the kDPRA may not be assessed**
  - Thiols
  - Fluorescent chemicals
    - See alternative fluorescent probe in SOP
  - Chemicals absorbing in exitation / emission wavelengths
    - See alternative fluorescent probe in SOP
  - However, these may be assessed with a classical DPRA with repeated injection into HPLC over time – these data then can also be converted to rate constants

PoD determinations: Under-predictions



Uncertainties Factors

- In supporting information to paper II we discuss the key under- and over-predictions for the individual chemicals
- PoD for 6 chemicals is underpredicted as weak (EC3 of 9.2% - 55%), while they are moderate sensitizers in the LLNA.
  - These include inter alia primary amines/pro-haptens and amine-reactive chemicals, which are outside of the applicability domain of the kDPRA
- PoD for 12 chemicals indicates a significant sensitization potency (EC3 0.05%-5%), but the individual values are clearly below the strong to extreme potency in LLNA.
  - This indicates that the dynamic range for some extreme sensitizers using the regression models is limited

PoD determinations: Over-predictions

04

Uncertainties Factors

6 false positives in the 2o3 vs. LLNA outcome

- For four chemicals, positive human sensitization or a strong alkylating potency indicate that **LLNA underpredicts the sensitization potential.**
- For the other two (propyl paraben and benzocaine) a weak human sensitization potential is also reported

5 very reactive and volatile chemicals are overpredicted

- The models corrects for high volatility, but do not fully predict the weak sensitization in LLNA observed for these highly reactive chemicals that evaporate rapidly under LLNA conditions
- **Conservative assessment** for use conditions under occlusion

5 chemicals are overpredicted when assessed vs. LLNA data, but either clinical data or human repeat insult patch tests indicate that these are very **relevant human sensitizers**

- PoD may be more correct for human situation than LLNA result

Combining ‘2 out of 3’ with kDPRA: GHS subclassification and potency

- GHS classification: not the key subject of these presentation
- But: As kDPRA is combined here with the tests within ‘2 out of 3’ – it is noteworthy to highlight how all assessments can be combined

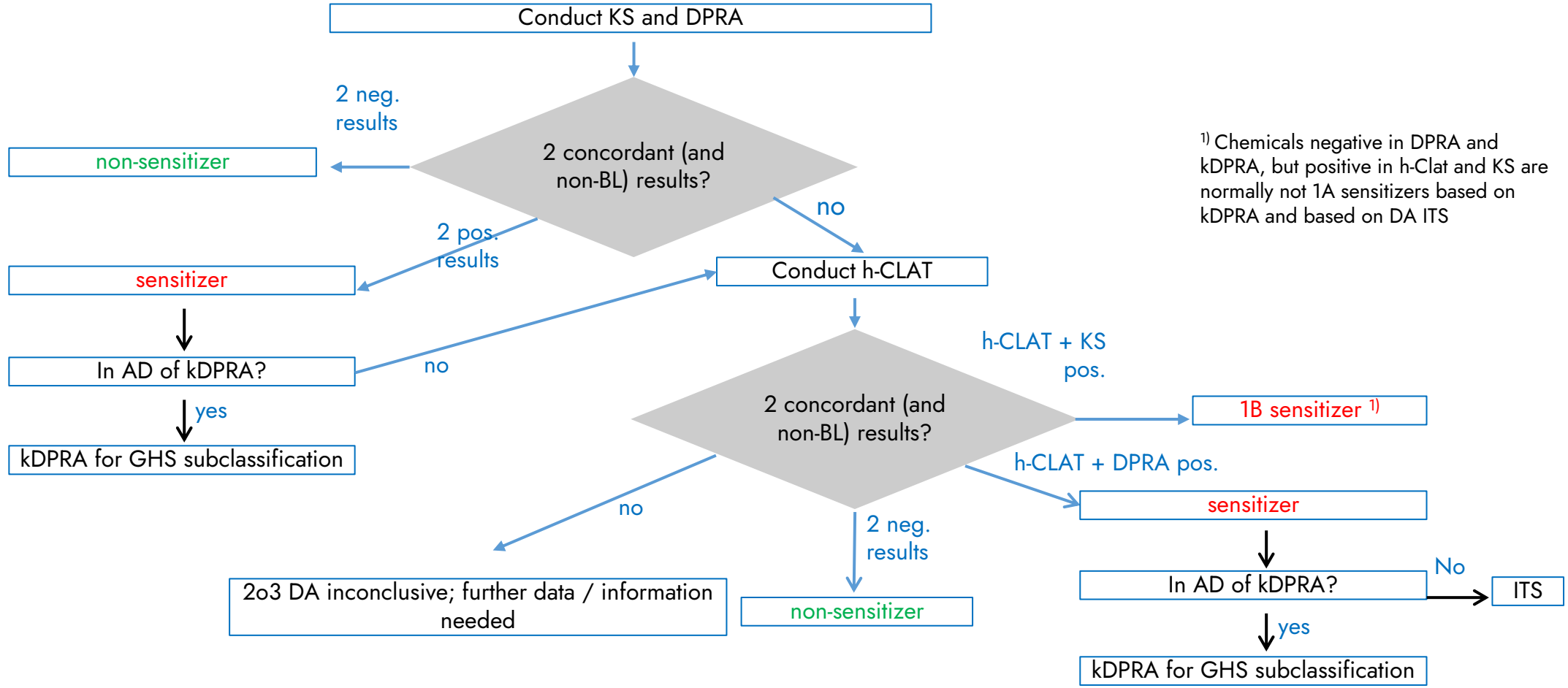
- ‘2 out of 3’ is accepted to discriminate GHS 1A/1B from GHS 2 (non-sensitizers)
- kDPRA is accepted to discriminate GHS 1A from GHS 1B/2 (if in Defined Approach)

Thus with ‘2 out of 3’ and kDPRA in combination, we have a fully validated approach to discriminate the three GHS classes! (no further validation needed)

The same in vitro data are used for the PoD, no parallel testing needed

Combining ‘2 out of 3’ with kDPRA: GHS subclassification

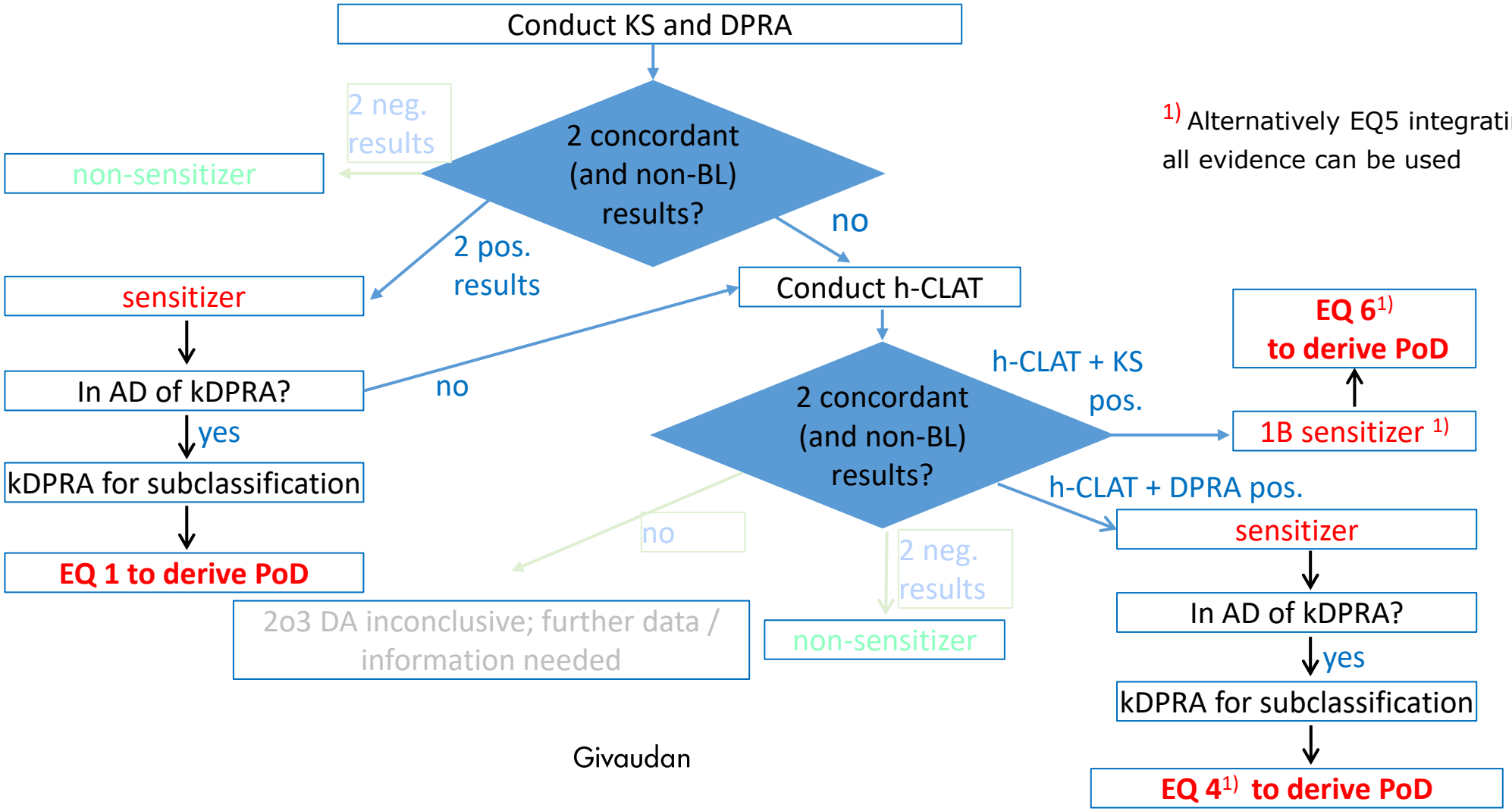
Workflow starting with KS and DPRA, equally works starting with h-CLAT



1) Chemicals negative in DPRA and kDPRA, but positive in h-Clat and KS are normally not 1A sensitizers based on kDPRA and based on DA ITS

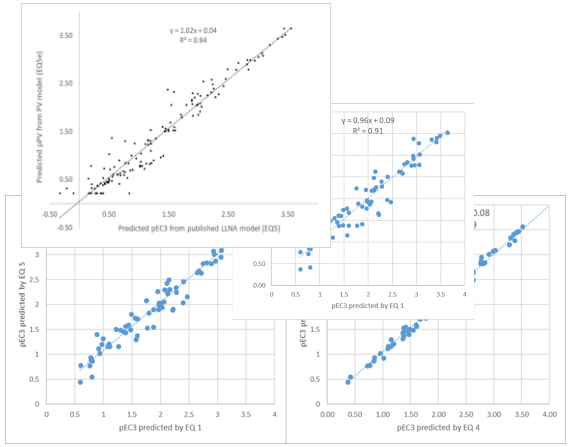
Combining ‘2 out of 3’ with kDPRA: Addition of PoD models

- No additional data generated for PoD determination – same workflow – just add the quantitative data to the Spreadsheet…



[1] Alternatively EQ5 integrating all evidence can be used

Outlook: Adding other tests



- We have shown multiple illustrations of data redundancy
- Mechanistic, additional tests certainly may improve ***local*** models
  - E.G. We use a specific amine-reactivity test for aldehydes only
  - Test with S9 fractions in KeratinoSens for phenolic compounds
- But: Will additional tests improve the ***global*** models (as the ones shown here) for all chemicals?
- The fact that often KS and h-CLAT already give very similar information for potency raises a question mark… we may be in a 'as good as it gets' situation

- BUT: as soon as a dataset is available with CAS number and data on > 120 chemicals of the database with the 188 chemicals with all information, we can test data redundancy and improved prediction very easily….