Sensitizer potency prediction based on Key event 1 + 2

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Description of the information sources and readouts used

- Peptide reactivity (Key event 1):
  - LC-MS evaluation of direct peptide modification
  - Molecular weight of adduct to interpret possible reaction mechanism
  - Peptide depletion after 24 h
  - Dose-response of peptide depletion at earlier time-points
  - **Kinetic rate constant** derived from the multiple depletion values
- KeratinoSens™ (Key event 2, Keratinocyte activation):
  - Positive/negative rating according prediction model
  - $EC1.5_{KS}$ /$EC2_{KS}$ / $EC3_{KS}$ concentration for 1.5/2/3-fold **luciferase gene induction**
  - $IC50_{KS}$ concentration for 50% **reduction in viability**
- Physicochemical parameters:
  - cLogP, **Vapor pressure**

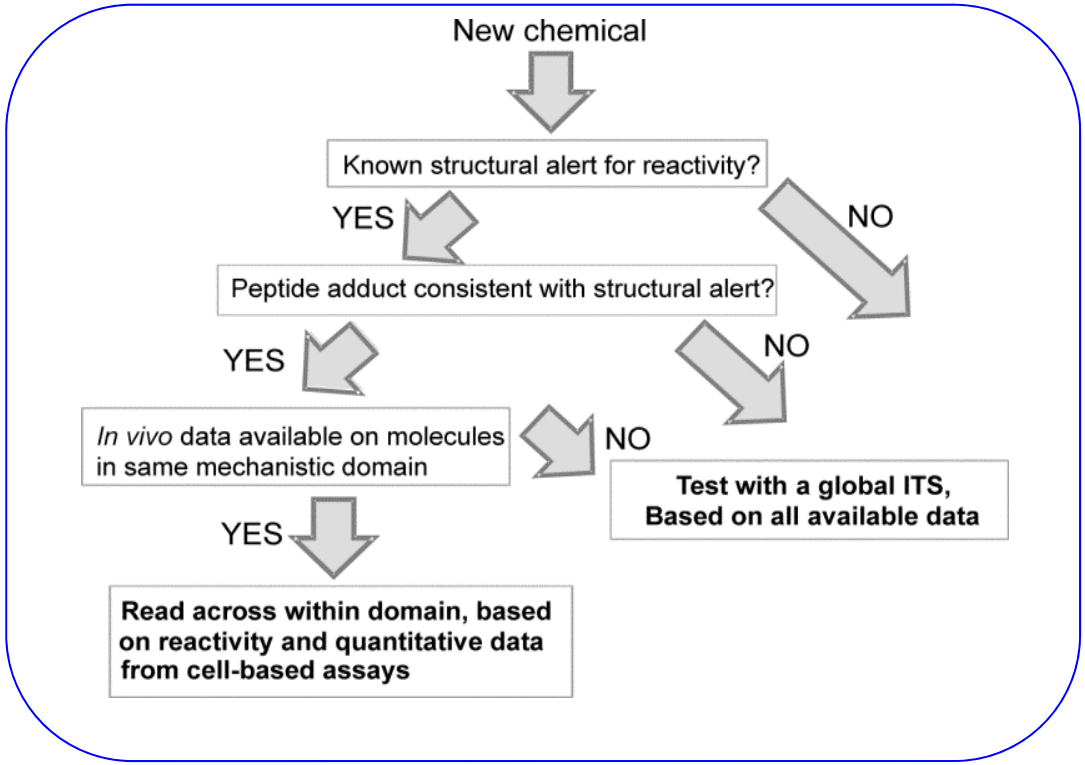
Underlying rationale: A) Global model

- **LLNA EC3 best available parameter for *in vivo* potency**
  - linearized by Log transformation = pEC3
- **Quantitative *in vitro* data partly correlate to LLNA potency**
  - dose response in KeratinoSens™
  - rate constant in peptide reactivity
    - All data can be linearized by Log transformation
- **Multiple regression uses most predictive combination of linear parameters**
  - Treats all chemicals equal
  - Fixed coefficients over whole potency range

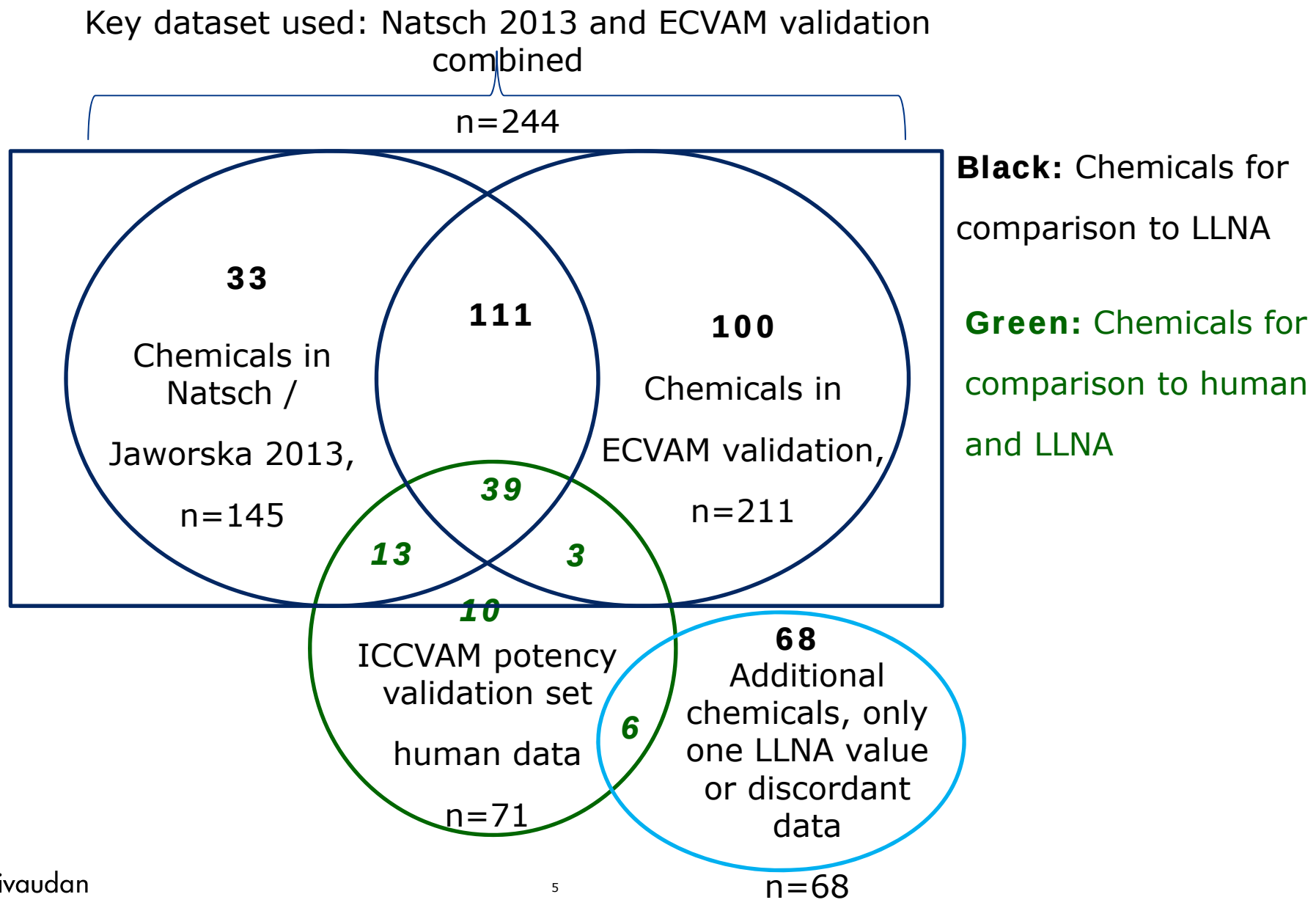
Parameter	R^2 adjusted (%)	*p* value
Peptide reactivity kinetic: K_{max}	51.7	< 0.0005
Peptide reactivity: $K_{24\ h\ depletion}$	43.6	< 0.0005
Luciferase $EC1.5_{KS}$	42.5	< 0.0005
Luciferase $EC2_{KS}$	44.8	< 0.0005
Cytotoxicity $IC50_{KS}$	33.5	< 0.0005

Underlying rationale: B) Global vs. mechanistic domain models

- The concept of grouping of chemicals is widely accepted (e.g. used in OECD toolbox)
- Chemicals should be predicted in domains if:
  - They can be grouped in domains with related chemicals
  - Related chemicals have been tested *in vitro* and *in vivo*



Chemical used to develop and test the approach



Process applied to derive the prediction/assessment

- **Global model**:
  - Regression equation can the be used to make predictions
  - Rate constant peptide reactivity highest influence
  - Followed by luciferase from KeratinoSens

Equation 1: A global regression analysis on prediction of $EC3_{LLNA}$ by *in vitro* and *in chemico* data

$$pEC3_{LLNA} = 0.04 + 0.38 \times \text{Log } K_{norm} + 0.25 \times \text{Log } EC1.5_{norm} + 0.25 \times \text{Log } IC50_{norm} - 0.19 \times \text{Log } VP_{norm}$$

Constant	T = 0.51, p = 0.612	**Log K_{norm}**	T = 9.55, ***p* < 0.0005**
Log $EC1.5_{norm}$	T = 4.06, ***p* < 0.0005**	Log $IC50_{norm}$	T = 3.05, p = 0.003
Log VP_{norm}	T = - 3.39, p = 0.001	R^2 (adj) = 62.3%	

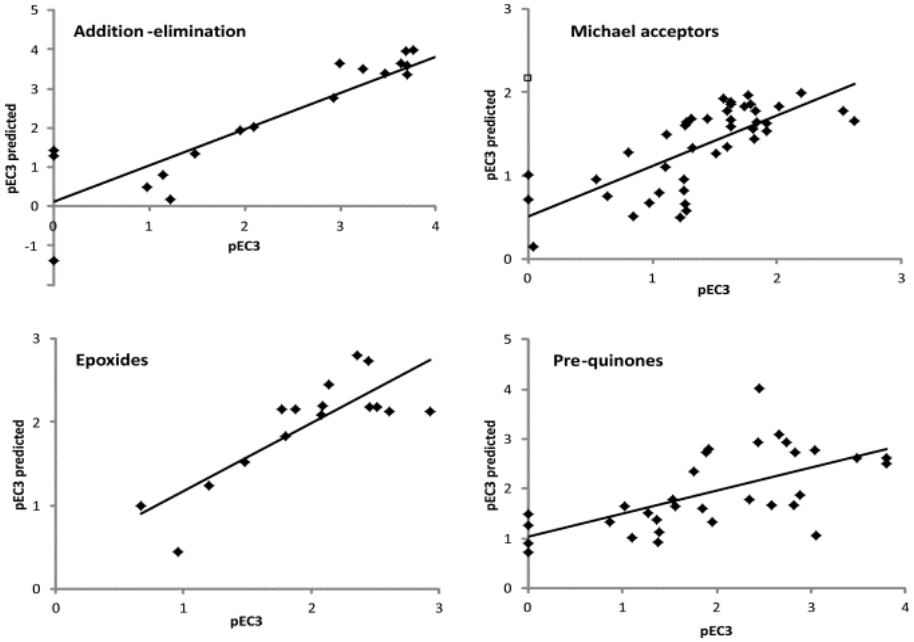
- **Local models: Multiple regression with leave-one-out analysis**
  - Each chemical is predicted with the remaining chemicals in dataset as training set
  - Avoids bias due to too small groups

Predictive capacity of the approach

Domain models – leave one-out analysis.

Domain models allow fold misprediction of 2 – 3 fold for many chemicals

This may be more useful as point of departure in risk assessment as compared to 10-fold potency classes



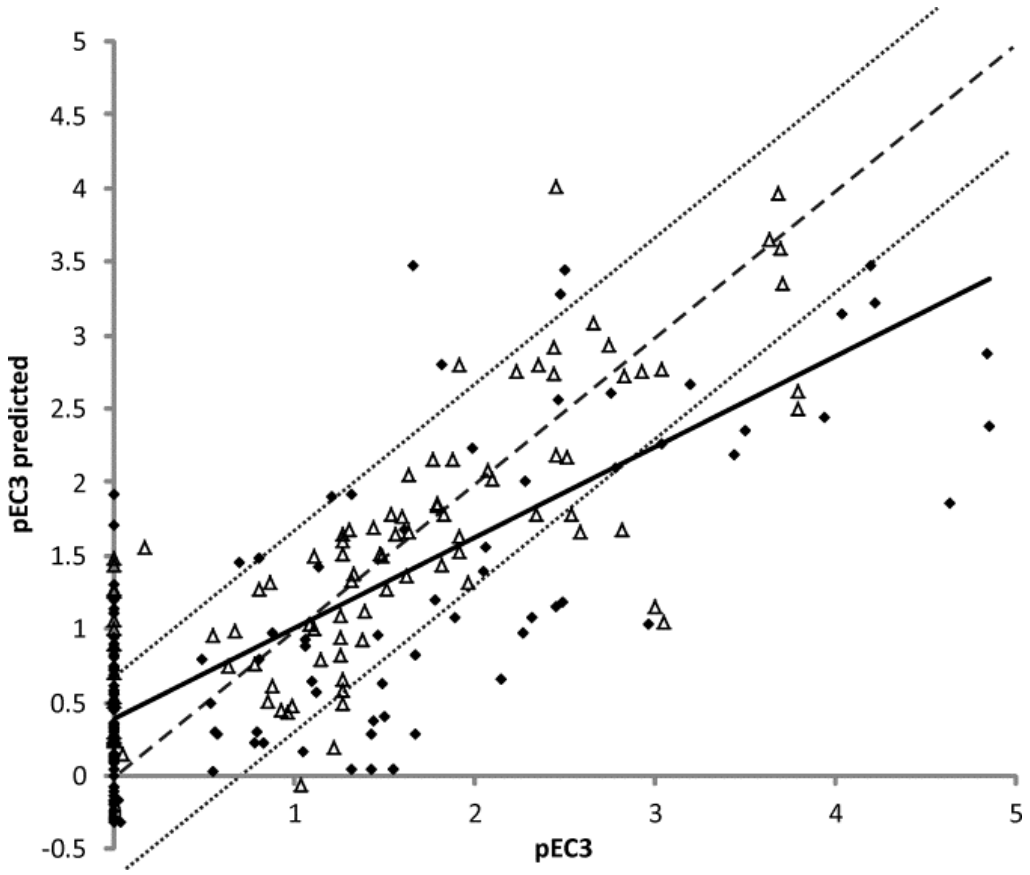
- In general prediction by global model somewhat less accurate as compared to local model

Domain [1)]	N	R^2-adj. of best model (*p*-value)	Fold-misprediction domain model	Fold- misprediction global model
Michael acceptors	44	58.4% (< 0.0005)	**2.26**	3.22
Addition-elimination	19	85.9% (< 0.0005)	**2.60**	3.43
Epoxides	16	81.2% (< 0.0005)	**1.97**	2.88
Aldehydes	28	43% (0.001)	**3.16**	3.26
pre-quinone-domain	32	48.2% (< 0.0005)	**4.54**	6.45

Predictive capacity of the approach

- Combined view of predictions with domain models (open triangels) and global predictions according (closed diamonds).
  - Chemicals attributable to domain predicted by domain model.
  - Remaining chemicals predicted by global model.

- Solid line indicates regression line
- dashed line indicates line of identity
- dotted lines indicate the area of chemicals with ≤ 5 fold misprediction.



Limitations in the application of the approach:

- Applicable for chemicals with MW < 500 and with a cLogP < 5, excluding polymers and mixtures.
- Phase I metabolic pathways are not fully represented

- Full dynamic range for very strong and extreme sensitizers not represented
- Quantitative reactivity of amine reactive chemicals not fully represented
  - No kinetic assay for amine reactive chemical implemented
- Only effects based on key events 1 + 2 measured; impact of other steps in prediction not reflected
  - But redundancy of data detected: Adding hClat AND KeratinoSens to peptide reactivity gives marginal improvement as compared to using either of the two.

Conclusions

- Quantitative readouts from Peptide reactivity and Nrf2-induction can partly explain sensitization potency
- Predictions are most accurate within domains of chemicals reacting with similar mechanism
- Within several domains, predictions with an average 2-fold misprediction are possible
  - Working on a continous scale may be more useful as point of departure in risk assessment as compared to predicting 10-fold potency classes
- There is also a correlation to human data (not shown here, see paper)
  - However, prediction of human data by in vitro data and LLNA is limited, which may be partly due to the very heterogeneous nature of the available human data.

Thank you

Contact

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