The kinetic Direct Peptide Reactivity Assay (kDPRA): An *in chemico* Method to Characterize the Skin Sensitization Potency of Chemicals

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INTRODUCTION

The reaction of electrophilic chemicals with nucleophilic residues in skin proteins is the molecular initiating event (MIE) in skin sensitization. While additional steps are involved in the acquisition of skin sensitization, the MIE is of predominant importance. To characterize the potency of a skin sensitizer, it is therefore important to characterize the reactivity of the test chemicals with skin proteins or surrogate nucleophilic residues with similar reactivity.

Reactivity in chemical terms is ideally expressed as a rate constant, which indicates how fast two chemicals react with each other, or more precisely how much reaction product is formed from a given amount of chemicals in a unit of time.

The kinetic direct peptide reactivity assay (kDPRA) is a modification of the DPRA (OECD TG 442C) assessing several test substance concentrations and incubation times. The kDPRA uses kinetic rates of cysteine- peptide depletion to distinguish between two levels of skin sensitization potency, i.e. to discriminate between CLP/GHS sub-categories 1A and 1B/ not classified. In addition, kinetic rates generated with this method have a strong quantitative correlation to sensitizing potency and can therefore be used in defined approaches (DA) with a quantitative data integration procedure (DIP) for skin sensitization potency assessment.

VALIDATION STUDY OUTLINE

Pre-phase
- Protocol refinement and standardization between the two lead labs
- 12 (non-coded) chemicals, two repetitions

Phase I
- Transfer of protocol to the 5 naïve labs
- 6 (non-coded) chemicals, two repetitions

Phase II
- Blinded testing (total of 24 blinded chemicals) in 7 labs
- 24 coded chemicals, 1 repetition in all participating labs
- 12 of these 24 coded chemicals were tested in 2 further repetitions

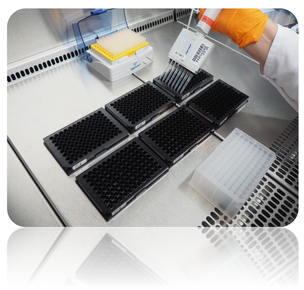
Data base

- Evaluation of the predictive capacity

METHOD

day -X

- Solubility assessment
- pH 7.5 phosphate buffer preparation



day 0

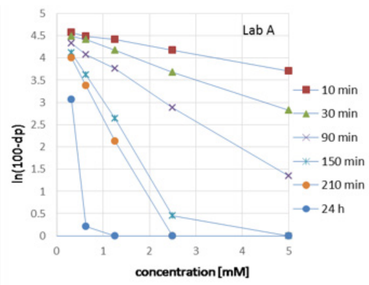
- Monobromobimane (mBrB) preparation for day 0
- Preparation of substance stock solutions (20 mM)
- Preparation of cysteine-peptide stock solution (0.667 mM, 0.501 mg/mL)
- Preparation:

  1 Application plate containing:
  - Test substance samples
  - PC
  - NC (= VC)
  - background controls (BC)
  - substance controls (SC)

  6 Assay plates (one for each time point)
- Start of incubation at 25°C (all assay plates)
- Fluorescence measurement after respective incubation time

day 1

- mBrB preparation for day 1
- Fluorescence measurement (24 h incubation)



	t [min]	log k
Calculation of k for each time point	10	-0.52 (=log k_{max})
	30	-0.61
	90	-0.77
	150	-0.69
	210	-0.85
	1440	-0.92

SUMMARY

Transfer to naïve labs

- Very similar average k_{max} and standard deviations in experienced (lead) labs and in naïve labs
- Transferability (without hands-on training) and quantitative reproducibility proven

Intra-/ interlaboratory testing

- Overall good reproducibility of log k_{max} values
- Low variability for most chemicals
- Few chemicals have higher variability – these also have higher inter-lab variability
- Intra-laboratory reproducibility (GHS Cat 1A vs. GHS Cat 1B/ not classified) of 96%
- Inter-laboratory reproducibility (GHS Cat 1A vs. GHS Cat 1B/ not classified) of 88%

k_{max}

- k_{max} is a quantitative measure of reactivity
- This is important beyond the GHS classifications – as the quantitative measure can be used in DA approaches for potency later

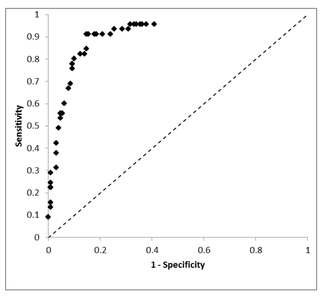
Refining the prediction cut-off

- Log k_{max} = -2 optimizes predictivity for LLNA and human data (GHS Cat 1A vs. GHS Cat 1B/ not classified)

RESULTS

Building the database and testing the prediction cut-off

Eventually the database contains log k_{max} data on a total of 182 chemicals with LLNA and 121 chemicals with additional human potency category attribution. Six chemicals which were tested were excluded from evaluation due to strong interference (quenching or autofluorescence).



Based on the receiver operating characteristic (ROC) analysis of the complete database, a refined cut-off of log k_{max} = -2.0 appears as an optimal prediction model to balance accuracy for LLNA and human data to differentiate GHS Cat 1A from GHS Cat 1B/ not classified.

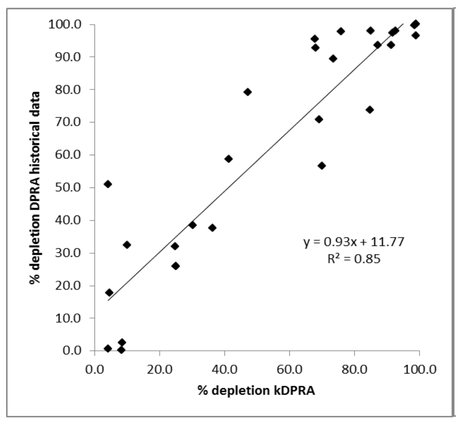
ROC analysis for different log kmax cut-off values to predict GHS Cat 1A vs. LLNA data.

Summary of the predictivity with the published and the refined cut-off.

		Sensitivity [%]	Specificity [%]	Balanced accuracy [%]	n tested
Reference Data	LLNA vs human	58	92	75	120
Refined cut-off (log k_{max} -2.0)	kDPRA vs LLNA	86	86	86	176
	kDPRA vs human	64	89	76	120

Comparision of kDPRA (5 mM, 24 h) to standard DPRA according to OECD TG 442C

A



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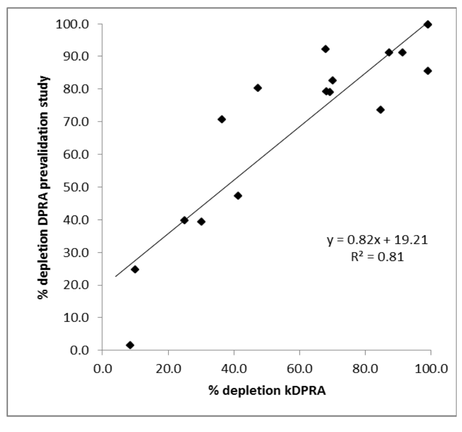
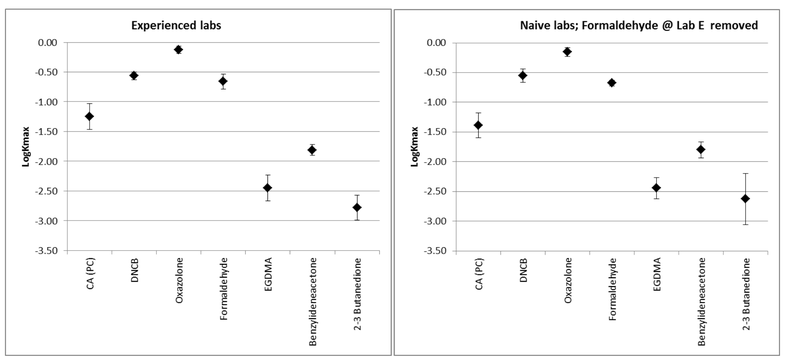


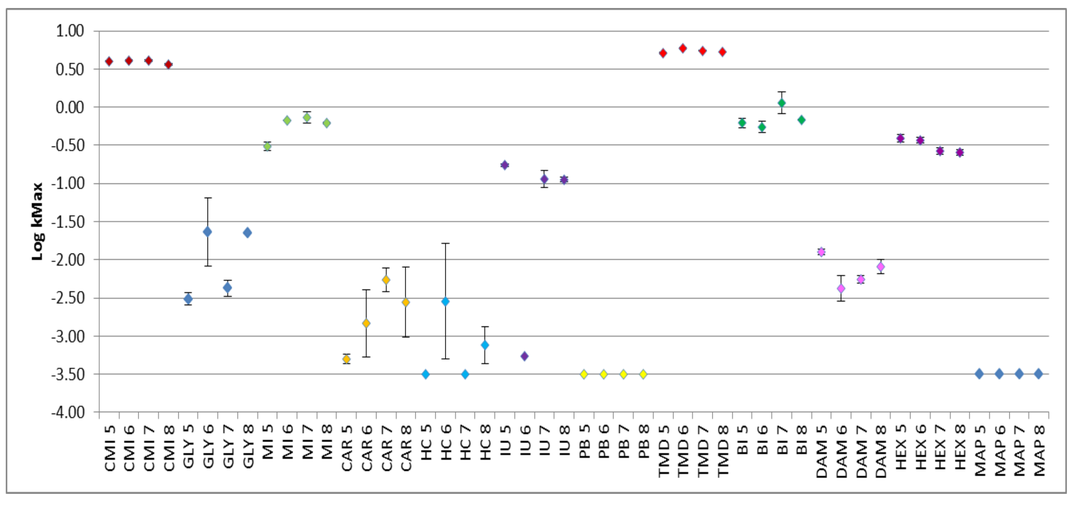
Correlation between 5 mM / 24 h values recorded in the kDPRA and in the DPRA for (A) published reference values and (B) data from the pre-validation study.

Transfer to naïve labs



Reproducibility of log k_{max} values for PC and Set A tested in the experienced (lead) and in the naive labs. Shown are averages and standard deviations of 4 runs (experienced labs) and 11 runs (naïve labs).

Intralaboratory testing



Intra-laboratory testing: Variability expressed as average values and standard deviation in repeated intra-laboratory testing (3 times each) in 4 labs. For chemicals not reactive (log k_{max} < -3.46) a default value of -3.5 was indicated to allow plotting the results. Abbreviated chemical names and default laboratory number are indicated on the x-axis.