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Skin sensitization potency assessment: Regression based DA

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Human by nature

Agenda

1. ***In vivo*** reference databases used for training
2. ***In vitro*** tests, *in vitro* input data parameters and reference databases used for training
3. **The prediction model** – different regression equations
4. **DA approach** with defined model choice
5. **Predictivity** vs. LLNA data
6. Robustness and redundancy with different partial data inputs
7. Conclusions

Databases of curated *in vivo* reference data

01

***In vivo* data**

- The OECD prepared a curated reference data set (https://one.oecd.org/document/ENV/CBC/MONO(2021)11/en/pdf) on
- A) Local lymph node assay data (n = 194)
- B) Human reference data (n = 66)

- We have an extended the set to 213 chemicals with LLNA and *in vitro* data

- The IFRA IDEA project proposed a workflow to combine human and animal data to derive "potency values" and applied it to a list of chemicals (n = 33) (https://www.sciencedirect.com/science/article/pii/S0273230022001313)

- We extended the list with PV values (n = 139 chemicals)* https://www.altex.org/index.php/altex/article/view/2617
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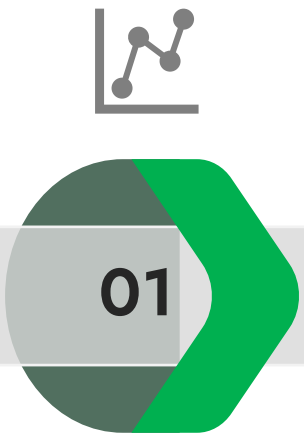
* independent of recent RCPL extension

Databases of curated *in vitro* reference data



***In vitro* data**

- Several institutions generated and collected *in vitro* data for the chemicals in the *in vivo* reference databases, mainly:
  - Kao and Shisheido (mainly h-Clat and DPRA)
  - Givaudan (KeratinoSens and kDPRA)
  - BASF (mainly kDPRA and U-Sens)
  - Procter and Gamble (Mainly DPRA)
  - Research institute for fragrance Materials (RIFM; all endpoints)

- With these large set of *in vitro* data AND *in vivo* reference data , it is possible to perform quantitative modeling

- While these data collection effort were first targeted *at qualitative hazard identification,* **most of these *in vitro* assay also provide quantitative dose-response data**

Input data: Quantitative data in KeratinoSens, h-CLAT and kDPRA

01

***In vitro* data**

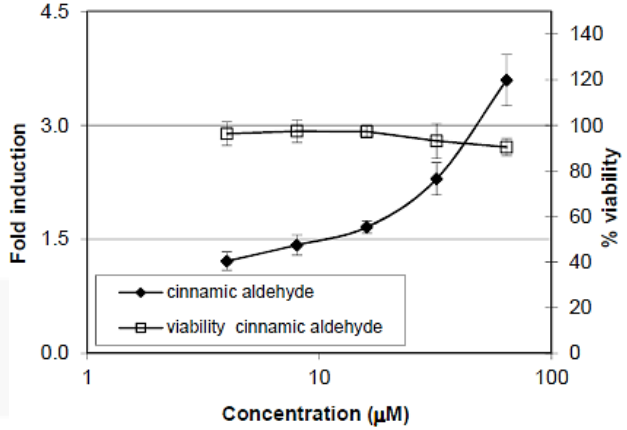
- **KeratinoSens**

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

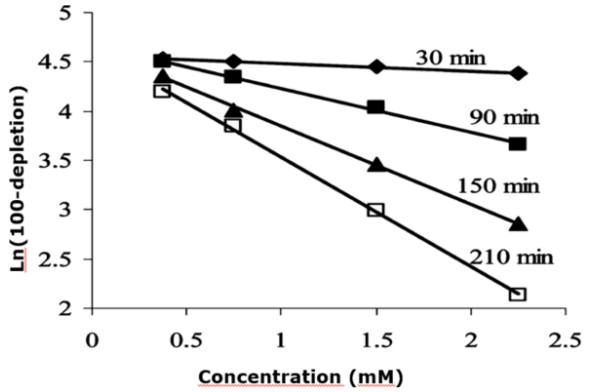
MIT minimum of EC150 and EC200

CV75 for 25% reduction in cell viability

- **Kinetic DPRA**

Kinetic rate for Cys-peptide depletion

kDPRA Example



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

The combined *in vitro / in vivo* Databases used for the Regression model

02

Regression Model

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**

Dataset with Potency values (PV) and KeratinoSens, h-CLAT and kDPRA: **n = 139**

✓ All data are log transformed and normalized

Regression models

02

Regression Model

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: $pEC3 = 0.42 + 0.40 \times \textbf{Log } k_{max} + 0.15 \times \text{Log } \textbf{EC1.5}_{norm} + 0.36 \times \text{Log } \textbf{IC50}_{norm} - 0.21 \times \text{Log VP}_{norm}$

Peptide reactivity | KeratinoSens | Volatility

EQ 4: $pEC3 = 0.18 + 0.36 \times \textbf{Log } k_{norm} + 0.21 \times \text{Log } \textbf{MIT}_{norm} + 0.35 \times \text{Log } \textbf{CV75}_{norm} - 0.19 \times \text{Log VP}_{norm}$

Peptide reactivity | H-CLAT | 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

Models were trained on:

a) LLNA EC3 values
b) Potency values

Regression model: Spreadsheet - Application in practice

https://www.altex.org/index.php/altex/article/view/2617

02

Regression model

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

KeratinoSens Assay Result

kDPRA Assay Result

h-CLAT Assay Result

PoD Prediction

Chemical identifier			Yellow fields only to be filled by user	
Chemical Name				
CAS Nr.				
MW				
Vapor pressure (Pa)			Lognorm VP	0
KeratinoSens assay data				
Rating PM (0/1)			EC 1.5 (µM) consolidate	
EC 1.5 (µM)			LOG norm EC1.5 KS	#VALUE!
EC 3 (µM)			EC 3 (µM) consolidated	
IC 50 (µM)			LOG norm EC3 KS	#VALUE!
If data not in micromolar, enter in ppm			IC 50 (µM) consolidated	
EC 1.5 (ppm)			LOG norm IC 50 KS	#VALUE!
EC 3 (ppm)				
IC 50 (ppm)			If no induction / cytotoxicity above threshold, give default value = 4000	
kDPRA assay data				
Log kmax (s-1M-1)			LOG norm Kmax	
h-CLAT assays data			If no induction/cytotoxicity above threshold, give default value = 5000	
Rating PM (0/1)				
CD86 EC150 (µg/ml)			MIT (µM)	#DIV/0!
CD54 EC200 (µg/ml)			Log norm MIT h-CLAT	#DIV/0!
MIT (µg/ml)	0		CV 75 (µM)	#DIV/0!
CV 75 (µg/ml)			Log norm CV75 h-CLAT	#DIV/0!

RESULTS for models trained on LLNA data	pEC3	EC3 (%)	EC3 (DSA in µg/cm²)
Global model KS + kDPRA Equation 1	not sufficient data	not sufficient data	not sufficient data
Global model kDPRA + h-CLAT Equation 4	not sufficient data	not sufficient data	not sufficient data
Global model KS + kDPRA + h-CLAT Equation 5	not sufficient data	not sufficient data	not sufficient data
Global model KS (EC1.5)+ h-CLAT Equation 6	not sufficient data	not sufficient data	not sufficient data

RESULTS for the models trained on the extended list of Potency values (PV)	pPV	PV (%)	PV (DSA in µg/cm²)
Global model KS + kDPRA Equation 1d	not sufficient data	not sufficient data	not sufficient data
Global model kDPRA + h-CLAT Equation 4d	not sufficient data	not sufficient data	not sufficient data
Global model KS + kDPRA + h-CLAT Equation 5d	not sufficient data	not sufficient data	not sufficient data
Global model KS + h-CLAT Equation 6d	not sufficient data	not sufficient data	not sufficient data
Global model KS + kDPRA + h-CLAT **Equation 5e**	not sufficient data	not sufficient data	not sufficient data

FINAL result for use as Defined	Model used	pPoD_DA	PoD_DA (%)	PoD_DA (DSA in µg/cm
Fixed model choice	Eq1 (EC3)	not sufficient data	not sufficient data	not sufficient data

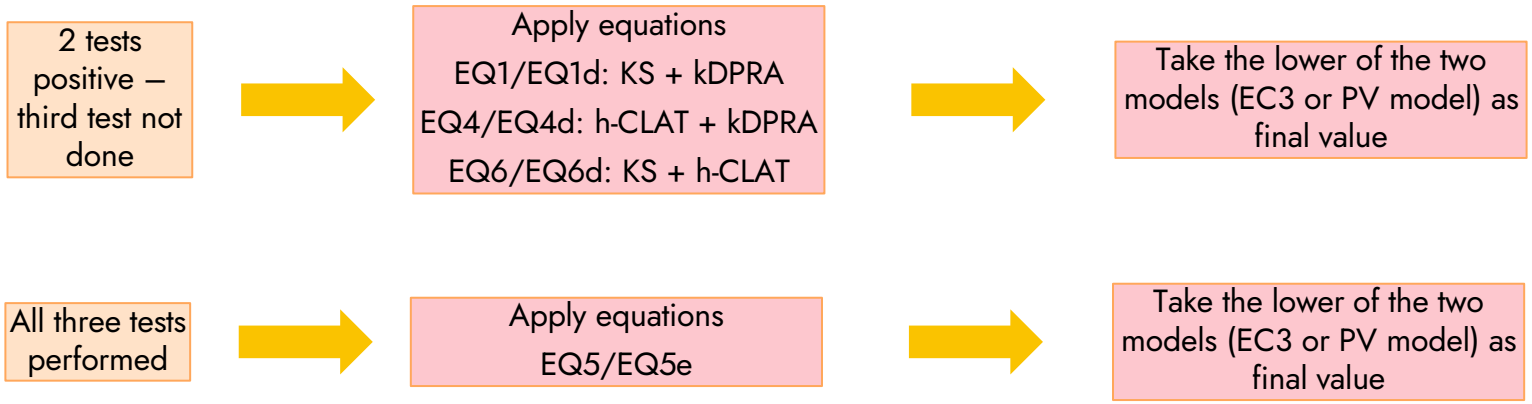
Automated model choice in a Defined Approach

The Regression approach makes multiple predictions – based on

a) the training dataset (LLNA EC3 or potency values (PV)) and

b) based on input data (all tests, partial evidence)

For OECD approval – the approach selects one outcome based on the input data:



This approach is coded into the Excel-Spreadsheet, so that, depending on the available data, one final value will automatically be generated (i.e. it is a DEFINED APPROACH (DA).

Regression model: Predictivity

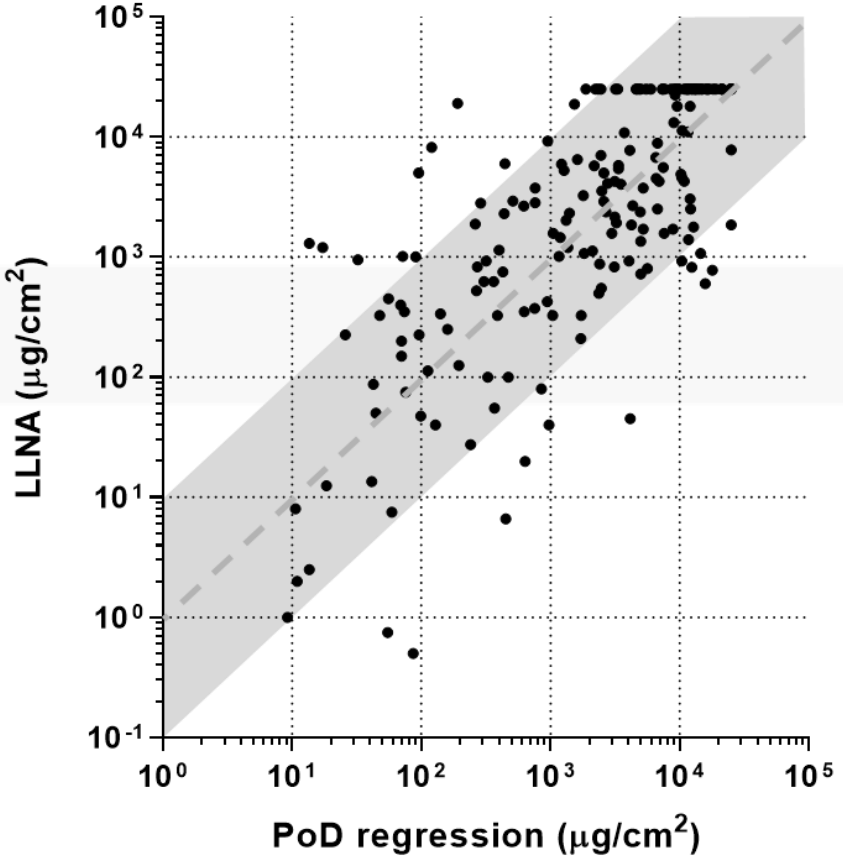
03

Prediction

Overall predictivity vs. LLNA EC3 data

- All chemicals with all three tests available
- Compared versus OECD MLLP LLNA EC3 data or other LLNA data according [1] in case no OECD MLLP EC3 is available

- Grey dashed line: line of identity
- Grey area – area of less than 10-fold misprediction



Regression model: Predictivity for case studies

03

Prediction

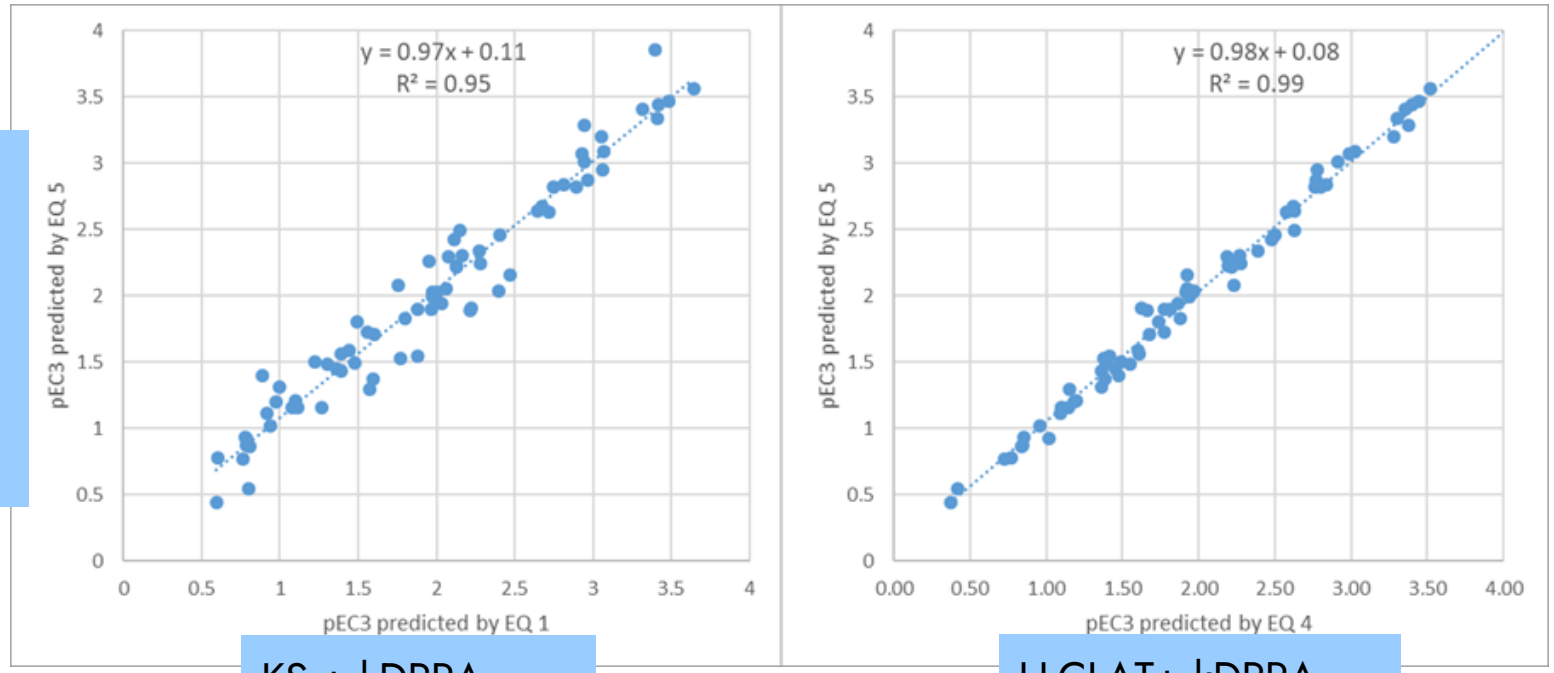
- Chemicals with at least 5 LLNA studies in OECD DB as case studies
- For these the certainty of the LLNA value is high
- Overall accurate prediction of these chemicals with strong *in vivo* evidence. Mostly within variability of the LLNA studies
- Similar predictivity with different models
- Flexibility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

Regression model: 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

Conclusions

- 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 public spreadsheet** makes application of the regression model straightforward
- These are *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

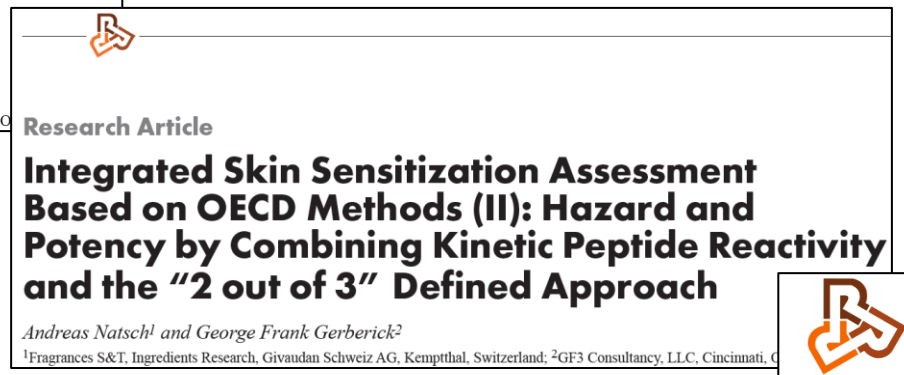
Research Article

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/



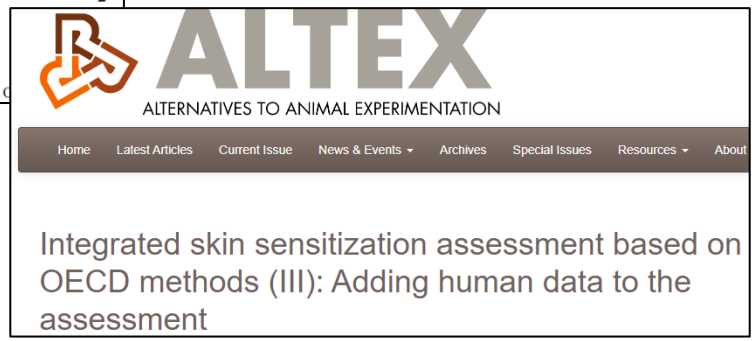
Research Article

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/



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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/

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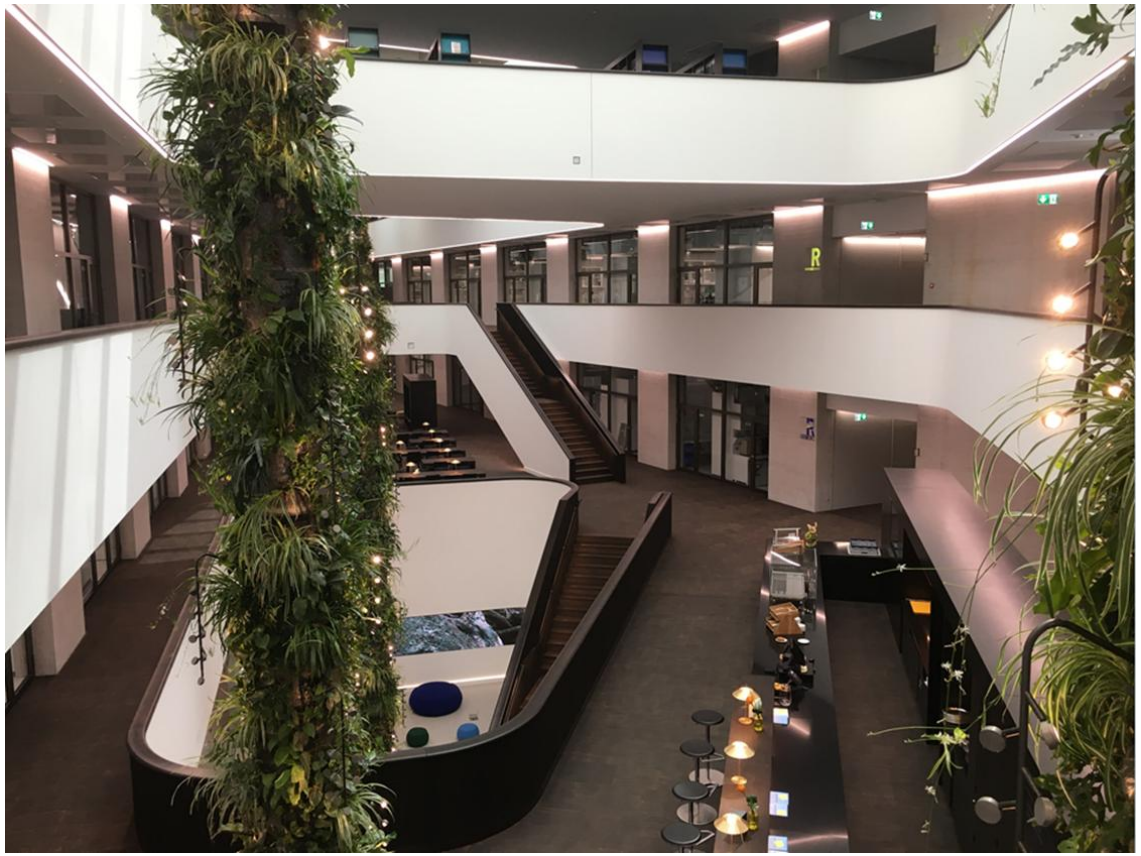
- Data curation and compilation:

The IMS team



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Thank you

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