Derivation of Skin Sensitization Potency using the Bayesian Net Integrated Testing Strategy

P. Kern, E. Deconinck, C. Ryan, G. Dameron, J. Jaworska
P&G

May 2018

IDEA Case Study Workshop

Touching lives, improving life. P&G™

Global Product Stewardship

Safety • Sustainability • Regulatory • Technical Relations

Introduction

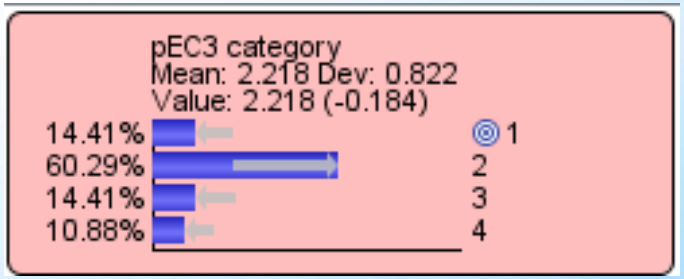
- Use of Bayesian Net approach for a sensitization potency prediction
- Generates a probabilistic hypothesis about skin sensitization hazard and potency
- Decision support system for a risk assessor, providing a quantitative weight of evidence.

- One of the OECD case studies for defined approaches

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Bayesian Net ITS3- Skin Sensitization

- Predicts a skin sensitization potency (even when data are missing)
- Expressed as probability distribution of LLNA pEC3, 4 potency classes: nonsensitizers (NS), weak (W), moderate (M), and combined strong and extreme (S) sensitizers.



P(LLNA=NS, W, M, S| evidence)

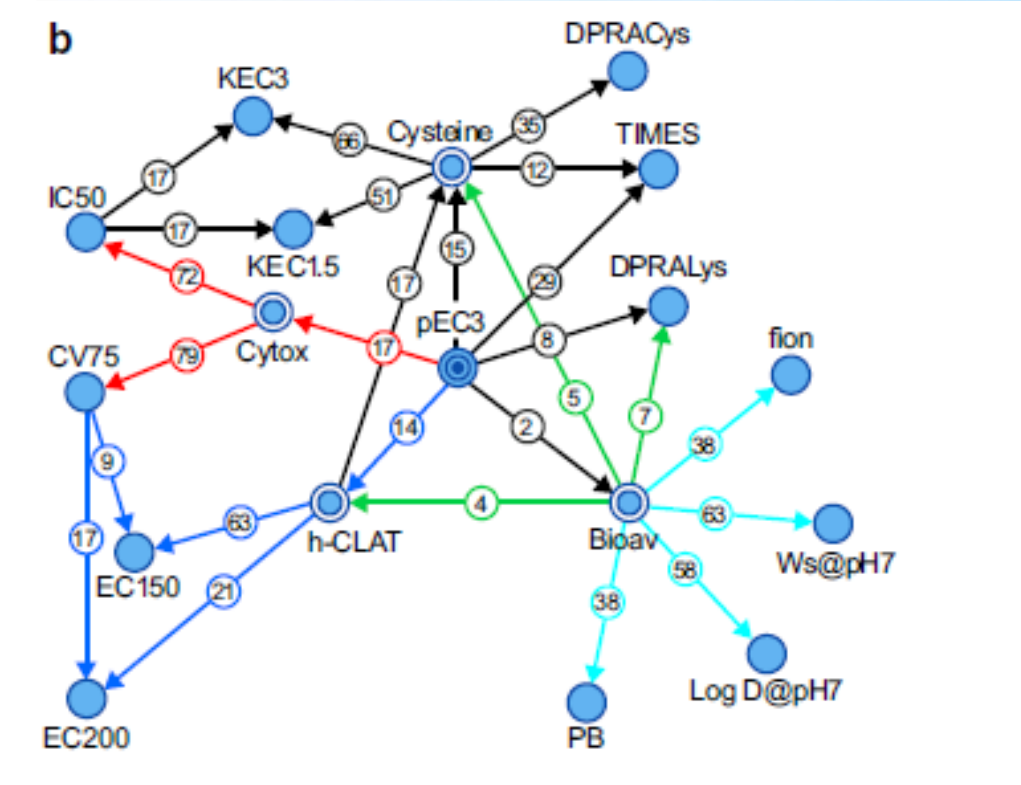
EC3% (50th or any other percentile)

- Can be used:
  - For classification and labeling under the GHS C&L scheme
  - To set NESILs for QRA
  - For the development of testing strategy if data are missing. Measures progress by uncertainty reduction.

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Bayesian Net ITS3- Structure and Inputs

- Phys Chem properties
- In silico potency prediction considering metabolism and potential for auto-oxidation (TIMES-SS)
- **KE1:** Cys and Lys binding in DPRA
- **KE2:** KEC1.5, KEC3 and IC50 in KeratinoSens™
- **KE3:** EC150, EC200 and CV75 in h-CLAT



Jaworska et al. Arch. Toxicol. 2015
Or : www.its.douglasconnect.com

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Hazard Potency Prediction Process (1)

- Collect evidence:
  - TIMES-SS prediction
    - Highest potency among parent molecule and metabolites
    - Reactivity alerts: determine direct Michael Acceptors
  - Phys chem parameters: (logD, Ws@pH7, f_ion, PB)
  - Conduct DPRA, Keratinosens, hCLAT
- Assessment of applicability domains:
  - Biological: Potential to be a pre- or prohapten?
  - Chemical:
    - Ionization: chemicals that are 100% ionized not suitable.
    - Water solubility at pH=7 cut-offs

Ws at pH=7 [M/l]	DPRA	Keratinosens	hCLAT
<2.5e-08	x	x	x
2.5e-08 - 1.7e-04	ok	x	x
1.7e-04 - 2.1e-04	ok	ok	x
> 2.1e-04	ok	ok	ok

Global Product Stewardship

Safety • Sustainability • Regulatory • Technical Relations

Hazard Potency Prediction Process (2)

- Integrate all the "in domain" evidence to obtain the pEC3 probability distribution ("run BN –ITS3")
- Post processing step: correction for direct Michael acceptors – adjust pEC3 probability distribution
- Evaluate confidence: Conversion of probability distribution to Bayes' Factors
  - Bayes factor removes biases in the predicted probability distribution introduced by distribution of a training set.

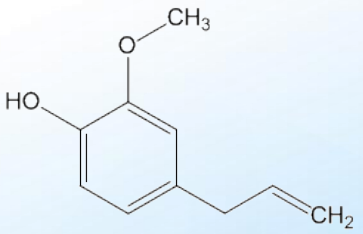
$$B = \frac{P(H = x|e)/P(H = not\_x|e)}{P(H|x)/P(H = not\_x)} = \frac{posterior\ odds}{prior\ odds}$$

Jeffreys, 1961

Bayes Factor	Strength of evidence
<1	Negative (supports alternative)
1-3	weak
3-10	Substantial
>30	Strong

- Finalise hazard or potency prediction depending on uncertainty information
  - Potency class NS, W, M, S
- Conversion from pEC3 to EC3% (if needed)

Case Study Eugenol



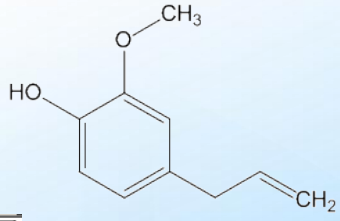
- TIMES predicted metabolites as weak sensitizers
- DPRA, h-CLAT and Keratinosens generated (raw data of assays used as input to BN-ITS3)
- Phys chem data calculated

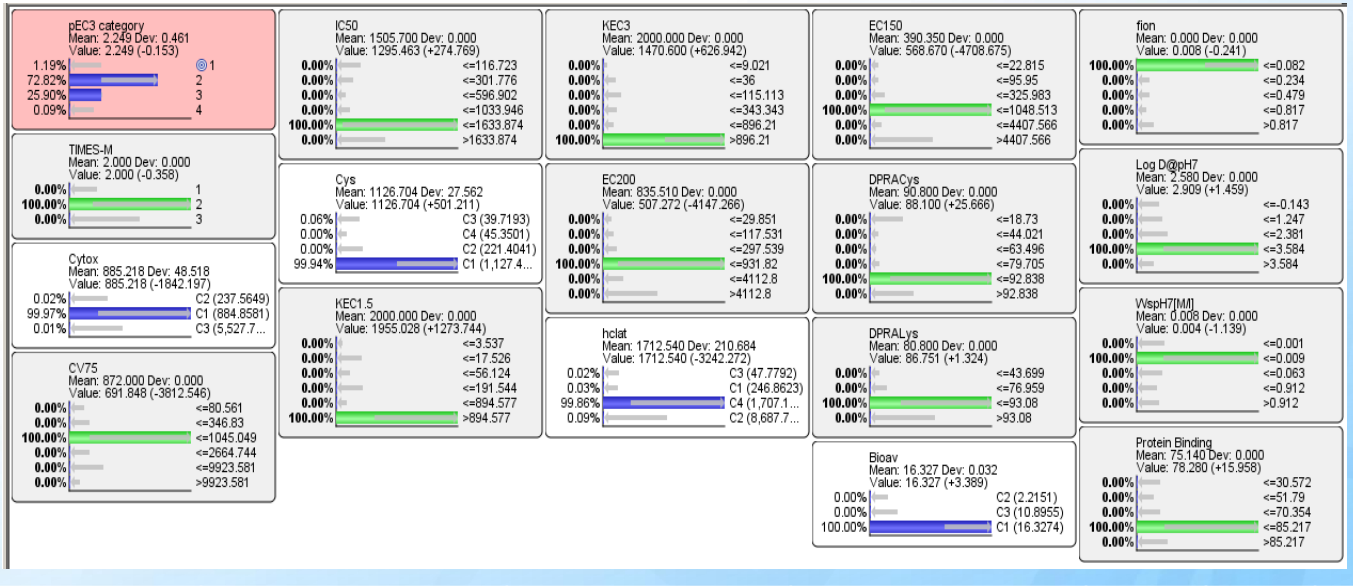
Name	DPRA		Keratinosens			h-CLAT			TIMES-M	TIMES-P
	Cys depl [%]	Lys depl [%]	EC1.5 [µM]	EC 3 [µM]	IC 50% [µM]	CD86 EC150 [µg/ml]	CD54 EC200 [µg/ml]	CV75 [µg/ml]		
Eugenol	9.2	19.2	>2000	>2000	1505.7	64.4	137.2	143.2	strong	NS
	Pos		Neg			Pos			Pos	

- Mainly concordant data (except Keratinosens)
- Within applicability domain

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Case Study Eugenol





pEC3 category.1	pEC3 category.2	pEC3 category.3	pEC3 category.4	B(NS)	B(W)	B(M)	B(S)
0.011862659	0.728209336	0.258984556	0.000943449	0.03	7.42	0.93	0.00

- High probability to be in Category 2 (weak sensitizer)- in line with LLNA
- High Bayes factor- substantial evidence

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

From pEC3 class to NESIL derivation

- NESIL is a human threshold: Conservatism needs to be factored in when NESIL is derived based on BN ITS3 prediction.
- Translate pEC3 potency class into a "potency class NESIL"
  - Conservative value for QRA?
  - LLNA- EC3 13% (3250ug/cm2; IFRA Standard WoE NESIL 5900 ug/cm2)

pEC3 Potency Class	Default NESIL (ug/cm^2)	ECETOC values (ug/cm^2)	EC3 Range (%)
Non Sensitiser NS	>10000		>100
Weak W	1000	2500	10~100
Moderate M	100	250	1~10
Strong S	10	25	0.1~1

Eugenol

- The output of BN –ITS3 is a probability distribution. Could that be transformed to a deterministic value to define a NESIL?

EC3 Conversion for Eugenol

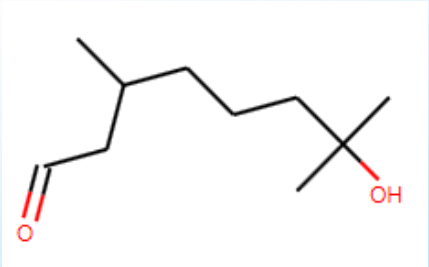
pEC3 category.1	pEC3 category.2	pEC3 category.3	pEC3 category.4	B(NS)	B(W)	B(M)	B(S)
0.011862659	0.728209336	0.258984556	0.000943449	0.03	7.42	0.93	0.00

- Convert probability value into EC3
  - the 50-percentile, i.e., the dose at which the likelihoods for a lower or higher EC3 values are balanced- *probably underestimation*
  - the 90 % percentile, i.e., the concentration at which the chance of a lower real EC3 value is only 10 %.- *very conservative*
  - 70th percentile corresponded best to 0.5*EC3 for the all weak/ moderate sensitizers investigated

	% tile	EC3%	ug/cm^2*
LLNA Eugenol		**13**	**3250**
	50	**16.5**	**4125**
	70	**9.9**	**2475**
	90	**3.0**	**750**

*1% in LLNA = 250 ug/cm^2

Case Study Hydroxycitronellal



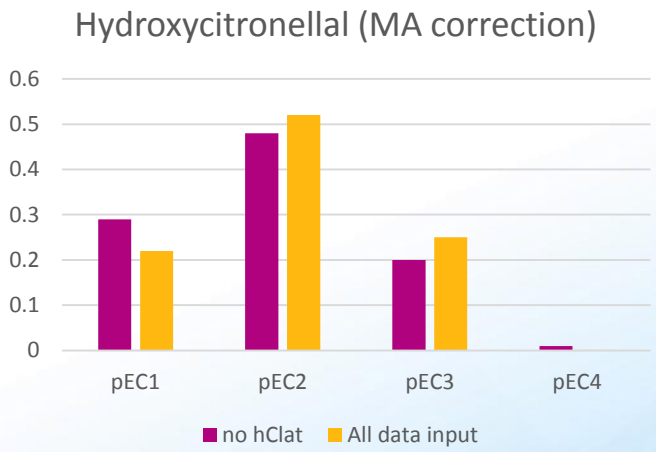
- Experimental LLNA EC3- 22% (5500 ug/cm^2)
  - IFRA Standard NESIL 5000ug/cm^2
- Collect Input Parameters

hCLAT			Ksens			DPRA			Phys Chem properties			
EC150	EC200	CV75	KEC1.5	KEC3	IC50	DPRACys depletion	DPRALys depletion	TIMES -M	Log D @pH7	Protein Binding %	Ws@ pH=7	fion
205.5	155.6	4063.5	79.4	142.9	2028	17.5	6.5	3	1.65	45.2	0.019	0
Pos			Pos			Pos		Pos				

- Concordant data, in domain
- Identified as direct Michael acceptor

- Simulate Prediction with missing data:
  - Use all data as evidence or leave h-CLAT data out

Case Study Hydroxycitronellal



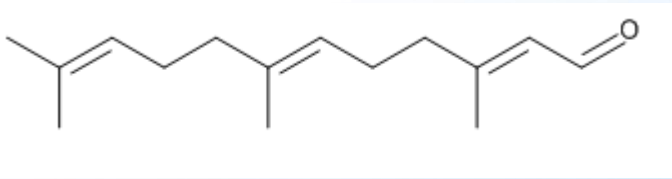
Bayes' factors

	B(NS)	B(W)	B(M)	B(S)	
No h-Clat	1.2	**2.6**	0.7	0.07	*Weak evidence*
All evidence	0.77	**3.1**	0.92	0.006	*Substantial evidence*

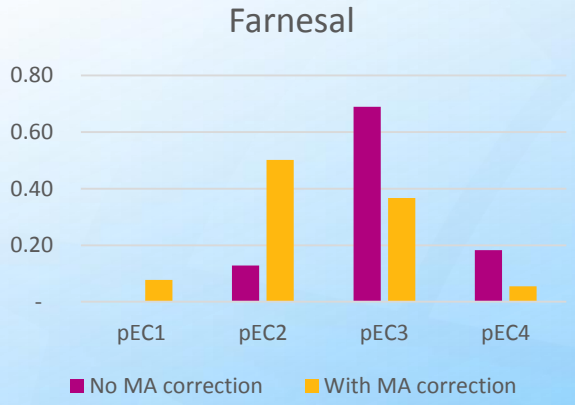
- Adding more input data changes probability distribution and increases evidence- lowers uncertainty in prediction
- Allows planning testing strategy
- Weak Sensitizer- substantial evidence with all data input
- Potency class NESIL: 1000-2500 ug/cm^2
- pEC3-EC3% conversion:
  - 22% (50th %tile= 5500ug/cm^2),
  - 11% (70%tile= 2750 ug/cm^2)

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Case Study Farnesal



- Experimental LLNA 12% (weak, 3000 ug/cm^2)
- Collecting input parameters
- All in domain, concordant data
- Determine pEC3 probability distribution, correct For Michael acceptors and calculate Bayes factors

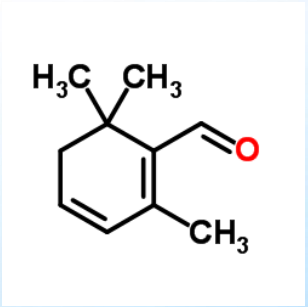


	B(NS)	B(W)	B(M)	B(S)
All evidence	0.00	0.4	**5.9**	0.91
All evidence+ MA correction	0.23	**2.8**	1.6	0.24

- Switch from Moderate sensitizer (substantial evidence) to weak
- Potency class NESIL weak (to moderate)
  - 1000 ug/cm^2 (100 ug/cm^2) ; can be converted to EC3

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Case Study Safranal



- Collecting input parameters

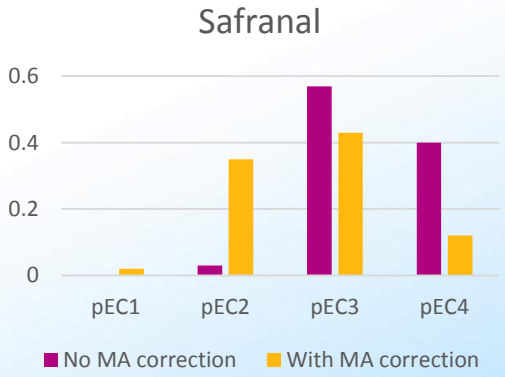
hCLAT			Ksens			DPRA			Phys Chem properties			
EC150	EC200	CV75	KEC1.5	KEC3	IC50	DPRACys depletion	DPRALys depletion	TIME S-M	Log D @pH7	Protein Binding %	Ws@ pH=7	fion
176.2	256.2	456.8	5.4	33.5	337.3	91.8	0	3	2.8	40	0.008	0
Pos			Pos			Pos		Pos				

- All in domain, use all for BN-ITS3
- Concordant data
- Determine pEC3 probability distribution and calculate Bayes factors

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Case Study Safranal

- Identified as Michael acceptor
- Adjust pEC3 values as per BN-ITS guidance and adjust BFs



Bayes' factors

	B(NS)	B(W)	B(M)	B(S)
All evidence	0.00	0.1	**3.6**	2.7
All evidence+ MAcorrection	0.01	1.7	**2.3**	0.6

- Moderate - strong sensitizer
- Potency class NESIL: 10-100 ug/cm^2
- pEC3 to EC3% conversion (70th %tile): 2.5 (625 ug/cm^2)
- Matches the experimental LLNA EC3- 7.5%, **BUT:**
- WoE NESIL IFRA Standard = 29 ug/cm^2
  - How can this be addressed?

EC3% experimental = 7.5

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

Uncertainties in BN ITS3

- As in all Tox assays or methods, don't take the ITS results in isolation- careful review of all data is always needed.

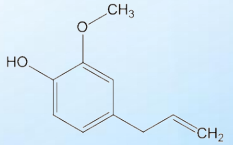
- …in the model structure due to uncertainty in knowledge around AOP
- …in experimental data due to variability of biological data

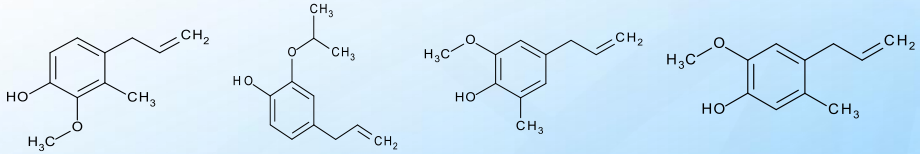
- LLNA variabilities of BN ITS3 training set considered in 4 way classification
- Probabilistic models handle data uncertainty.
  - Evidence used in the model is represented as range spanning over discretization bins.
- Conversion to Bayes factors allows for consistency when accepting uncertainty in predictions

Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations

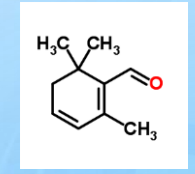
Increase Confidence using Analogs

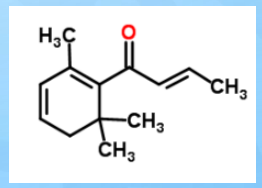
- BN allows to quantitatively weigh in analogue information about potency and quality
- Example Eugenol
  - Analogs identified (3 weak, 1 NS) (Wu et al. 2013, Blackburn et al. 2014)



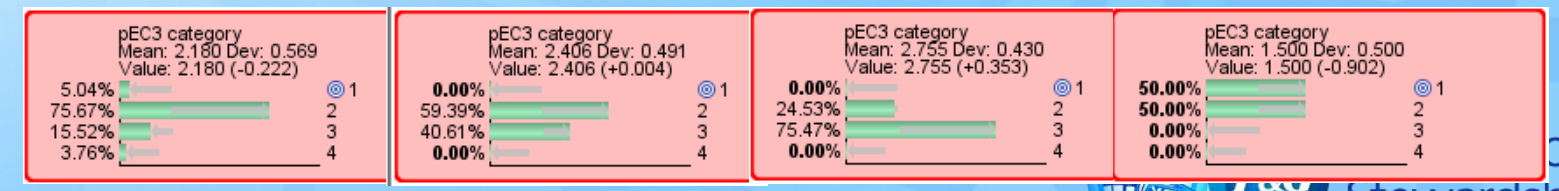


- Example Safranal
  - 1 Analog moderate- strong sensitizer





- Prior distribution x evidence=posterior distribution
  - w/o analogue data = prior evenly distributed over 4 classes
  - With prior -> expert converts analog data into a distribution
- Possible adaptation of priors, depending on potency of analogs:



BN-ITS3 Checklist for each Chemical

- ✓ Gather evidence and check for completeness:
  - ✓ TIMES-SS prediction
  - ✓ Phys chem parameters: (logD, Ws@pH7, f_ion, PB)
  - ✓ DPRA, Keratinosens, hCLAT
- ✓ Assessment of applicability domains:
  - ✓ Pre or prohapten ?
  - ✓ Direct Michael acceptor chemistry ?
  - ✓ Ionization: 100% ionized ?
  - ✓ Water solubility at pH=7 ?
- ✓ Integrate all the "in domain" evidence to obtain the pEC3 probability distribution
- ✓ Post processing step of probability distribution correction for direct Michael acceptors
- ✓ Evaluate confidence: Conversion of probability distribution to Bayes' Factors
- ✓ Finalise hazard or potency prediction depending on uncertainty information
  - ✓ Potency class NS, W, M, S
- ✓ Conversion from pEC3 to EC3% (if needed)

Conclusions and Outlook

- BN ITS3 allows hazard and potency prediction
  - Limitations in applicability domain
  - Not proprietary- but some adaptations planned to switch to non license software
- Confidence estimation via Bayes factors
  - Define acceptable Bayes factors
  - if the ITS output does not provide substantial evidence for a potency class then analog data might become important
- Setting of potency class NESILs or EC3 values possible- Input for QRA
  - Explore more chemicals to define best approach for EC3 value determination
  - Define degree of conservatism needed to reflect human thresholds
- Prediction also possible with incomplete data sets
  - Define minimum data requirements
  - Allows to define testing strategy
- Additional information e.g. from analog structures can be considered
  - Develop guidance

P&G Global Product Stewardship
Safety • Sustainability • Regulatory • Technical Relations