Evaluating a Skin Sensitization Model and Examining Common Assumptions of Skin Sensitizers

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Abstract

Skin sensitization is an adverse outcome that has been well studied over many decades. Knowledge of the mechanism of action was recently summarized using the Adverse Outcome Pathway (AOP) framework as part of the OECD work programme (OECD, 2012). Currently there is a strong focus on how AOPs can be applied for different regulatory purposes including the development and application of Integrated Approaches to Testing and Assessment (IATA). One example is an Integrated Testing Strategy developed by Jaworska et al. (2013) known as ITS-2 which was derived using a Bayesian network and relied upon outcomes from different *in vitro, in chemico* and *in silico* approaches that mapped onto the events within the AOP. Here we assessed the global performance of ITS-2 model using a cross validation approach, and the local performance using reaction domains. Re-training the network using protein binding alerts (categorized by domain) as extracted from the OECD Toolbox in place of predictions from TIssue MEtabolism Simulation for Skin sensitization (TIMES-SS), a commercial expert system resulted in a comparable predictive performance. Skin penetration is denoted as the first event within the AOP since there is a long standing belief that substances that cannot penetrate through the skin will not be sensitizers. Physicochemical parameters such as $LogK_{o/w}$ and MW which are typical inputs to estimate skin penetration (specifically the skin permeability constant, Kp) have been used to propose thresholds for skin sensitization. Namely for a substance to be a sensitizer, it should have a MW<500 and a $LogK_{o/w}$>1. A large dataset of 1482 substances that had been evaluated for their skin sensitization potential together with available measured $LogK_{o/w}$ values was compiled from the REACH dissemination database to investigate whether these thresholds were a myth or a reality. 197 compounds with a MW > 500 were identified, 33 of these were sensitizers. These findings complemented those of Roberts et al. (2012) who examined the training set within TIMES-SS and found 13 compounds above a MW of 500 of which 5 were sensitizers. Comparing the incidence of sensitizers above and below a threshold of $LogK_{o/w}$ >1 showed no significant difference. Existing reaction chemistry principles were found to be appropriate to rationalize the sensitization behavior of substances that exceeded the MW and $LogK_{o/w}$ thresholds.

Aims

Aim 1: Integrated Testing Strategies-2 (ITS-2)

- Modify ITS-2 to replace the TIMES-SS predictions with protein binding alerts (herein termed reaction domains) as taken from the freely available OECD Toolbox.
- Evaluate the global and local performance (based on reaction domain) of the ITS-2 Bayesian network using cross validation.

Aim 2: Bioavailability

- Examine the impact $LogK_{o/w}$ plays in discriminating for skin sensitization potential
- Examine the impact MW plays in determining skin sensitization potential

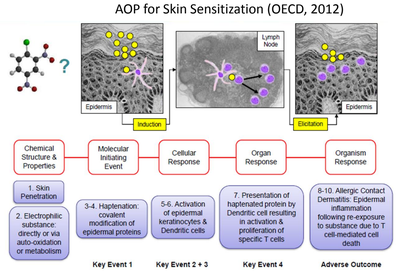
Effects of Skin Sensitization

- Chemicals classified as contact skin sensitizers have the capacity to cause allergic contact dermatitis (ACD).
- ACD is responsible for 10% to 20% of all work related health complaints and ~4 million lost work days each year.
- In many countries, occupational contact dermatitis ranks first among all occupational diseases.
- In the US, the total cost of ACD is estimated to be between $400 million and $1 billion a year.

Background

Predictive test methods to determine skin sensitization hazard and potency still rely on animals. Historically skin sensitization hazard identification was conducted using guinea pigs. The local lymph node assay (LLNA) is the recommended alternative that provides a quantitative measure of relative skin sensitizing potency. However given the legislative environment, particularly in EU, such as the Cosmetics Regulation (EU, 2009) that bans animal testing of cosmetic products, there has been a concerted effort to identify alternative approaches for assessing skin sensitization potential and potency. The adverse outcome pathway (AOP) for skin sensitization provides a convenient roadmap to integrate outcomes from computational modeling, *in vitro* and cell based assays . In the long term models could be derived to simulate the entire pathway. A recently published model which aims to take into account some of the steps in the AOP is the ITS-2 Bayesian network (Jaworska et al, 2013).

AOP for Skin Sensitization (OECD, 2012)



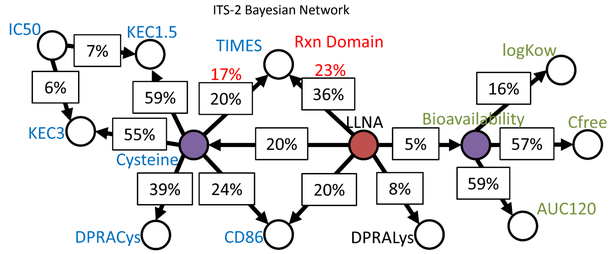
We evaluated the ITS-2 model and attempted to replace one of the key components of the model the TIMES-SS predicted score, with a reaction domain prediction generated using the OECD QSAR Toolbox. We also examined two key factors that are thought to be important to skin penetration, molecular weight and $LogK_{o/w}$.

Understanding the relative importance of the initial events leading to the induction of skin sensitization potential is critical in devising an appropriate IATA which obviates animal testing.

Integrated Testing Strategies-2

The Bayesian network ITS-2 (Jaworska et al, 2013) uses information on chemical properties, and experimental data characterizing the first 3 key events in the AOP to make a prediction of skin sensitization potential as measured in the LLNA. (See the components chart below for more details on those included.) We evaluated the performance of the original ITS-2 and two modified versions. In one network, the TIMES-SS node was removed and in the second, the TIMES-SS prediction was replaced with the reaction domain prediction generated using the OECD QSAR Toolbox. The performance of these three networks was evaluated using the same data set as the original ITS-2 model. This data set contained 42 non-sensitizers, 33 weak sensitizers, 40 moderate sensitizers, and 30 strong/extreme sensitizers. (For the purposes of this model, the two were grouped together.) Stratified 10-fold cross-validation, run 100 times was used to judge the relative performance of 3 models. The performance of the model using reaction domains in place of the TIMES-SS predictions was comparable to the original ITS-2 model.

ITS-2 Bayesian Network



Components of the ITS2 Model

LLNA	Skin sensitization result from the local lymph node assay
$logK_{o/w}$	Octanol/water partition coefficient
Cfree	Maximum free concentration in the mid-epidermis
AUC120	Amount absorbed by the epidermis over 120 hours
DPRALys, DPRACys	Percentage of peptide remaining after reaction in the DPRA assays
CD86	Amount needed for 150% cell surface activation in the U937 assay
KEC3, KEC1.5, IC50	Amount needed to see a 1.5 or 3 fold increase of luciferase activity in the KeratinoSens assay, IC50 is used to control for cell viability in the assay
TIMES	Skin sensitization potential predicted by the TIMES-SS module
Rxn Domain	Binary for if a reaction domain was found in the OECD QSAR toolbox or not.

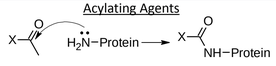
Global Accuracy based on 10-fold Stratified Cross Validation

Test Set	Sensitization Potential Accuracy	Sensitization Potency (LLNA) Accuracy
ITS-2 Network	89% ± 2%	65% ± 4%
ITS-2 Network without TIMES-SS	79% +3%/ -4%	49% +3%/ -4%
ITS-2 Network with Rxn Domain Replacing TIMES-SS	84% +2%/ -3%	54% +3%/ -4%

"Sensitization Potential Accuracy," indicates how often a given model correctly predicted that a compound was a sensitizer in the LLNA.
"Sensitization Potency (LLNA) Accuracy," indicates how often the network predicted the potency class of a skin sensitizer, based on the LLNA.

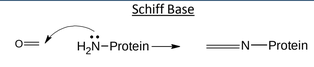
Local Accuracy: Based on Reaction Domain

Acylating Agents

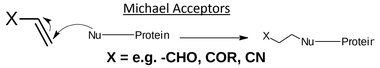


X = e.g. F, Cl, Br, I, -OC_6H_5

Schiff Base

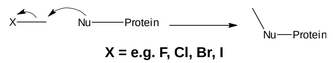


Michael Acceptors



X = e.g. -CHO, COR, CN

S_N2



X = e.g. F, Cl, Br, I

LLNA Accuracy per Reaction Domain

Reaction Domain	No. Compounds	Sensitization Potential Accuracy	Sensitization Potency (LLNA) Accuracy	Over Prediction (Potency Class)	Under Prediction (Potency Class)
Acylation	15	84% +3%/ -4%	45% +15%/ - 12%	34% +13%/ -14%	21% +6%/ -8%
Michael Addition	21	100% ± 0%	76% ± 10%	21% +8%/ -7%	3% +7%/ -0%
No Domain Found	64	85% +3%/ -2%	68% ± 5%	10% ± 4%	22% ± 3%
Schiff Base Formation	23	89% +7/ -6%	66% +8%/ -5%	15% +7%/ -6%	19% +7%/ -6%
SN2	14	100% ± 0%	57% +7%/ -14%	28% +8%/ -7%	15% +6%/ -8%

Compounds containing a Michael addition domain had the greatest accuracy in predicting the correct LLNA class, at 76% it even exceeds the overall maximum accuracy. The most difficult compounds to predict were those with acylation and SN2 domains, these compounds fell below the minimum predictions given by the network. Interestingly those with an SN2 domain always gave a correct prediction for sensitization.

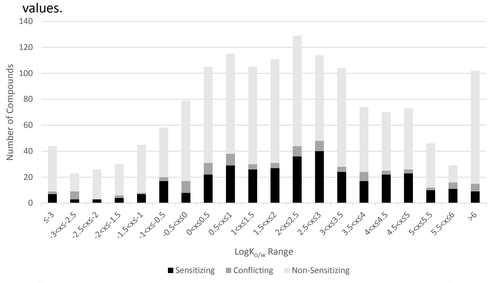
Information about which classes are predicted best could be used to assess whether a sensitization prediction for a certain compound is likely to be accurate, and what value should be placed on the results of the LLNA class.

Overall Accuracy	
Minimum	60%
1st Quintile	63%
Median	65%
Mean	65% ± 2%
3rd Quintile	67%
Maximum	71%

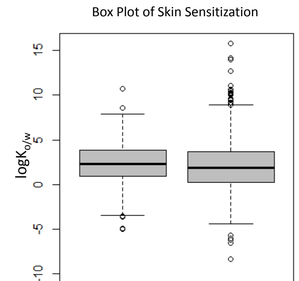
Bioavailability

It has been previously suggested that compounds must have a molecular weight under 500 and a $LogK_{o/w}$ above 1 in order to be skin sensitizers. The reasoning behind this is that compounds must pass through the stratum corneum a lipophilic region of the epidermis in order to reach the viable epidermis, where haptenation takes place. It has also been noted that very few skin sensitizers have been reported which have a molecular weight above 500 or a $LogK_{o/w}$ less than 1.

Using the OECD eChemPortal which allows the ECHA REACH dissemination database to be searched, we collected a large dataset of compounds tested for their skin sensitizing ability. We also collected data for compounds with experimentally determined $LogK_{o/w}$ values.

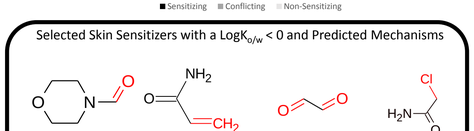


Box Plot of Skin Sensitization



Data on 1482 compounds was collected and is shown in the $LogK_{o/w}$ vs. skin sensitization chart, due to size constraints. These data clearly indicated that although there many more skin sensitizers above a $LogK_{o/w}$ of 1, even having a negative $LogK_{o/w}$ value does not rule out a compound from being a skin sensitizer.

Selected Skin Sensitizers with a $LogK_{o/w}$ < 0 and Predicted Mechanisms



CAS# 4394-85-8 — $LogK_{o/w}$ = -1.2 — Mechanism: Acylation

CAS# 79-06-1 — $LogK_{o/w}$ = -0.9 — Mechanism: Michael Addition

CAS# 107-22-2 — $LogK_{o/w}$ = -1.2 — Mechanism: Schiff Base

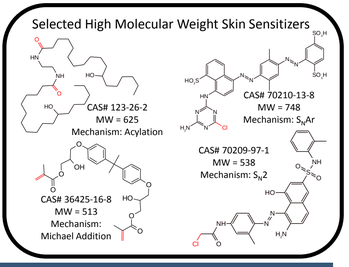
CAS# 79-07-2 — $LogK_{o/w}$ = -0.6 — Mechanism: S_N2

We gathered a large data set of skin sensitizers with a MW >500. While it appears that compounds with a MW below 500 may be more likely to be skin sensitizers, compounds above a MW of 500 should not be automatically ruled out from assessment.

Skin Sensitization Information From ECHA

	MW > 500		MW ≤ 500	
	# of Compounds	% of Compounds	# of Compounds	% of Compounds
Sensitizers	33	17%	735	27%
Non-Sensitizers	164	83%	1972	73%
Total Compounds	197		2707	

Selected High Molecular Weight Skin Sensitizers



CAS# 123-26-2 — MW = 625 — Mechanism: Acylation

CAS# 70210-13-8 — MW = 748 — Mechanism: S_NAr

CAS# 36425-16-8 — MW = 513 — Mechanism: Michael Addition

CAS# 70209-97-1 — MW = 538 — Mechanism: S_N2

Conclusions

Aim 1: ITS-2

- Adding reaction domains to the ITS-2 network restores some, but not all of the predictive value of TIMES-SS.
- A compound's reaction domain can be used to inform the likelihood of a correct prediction within the ITS-2 network.

Aim 2: Bioavailability

- There is no $LogK_{o/w}$ < 1 or MW > 500 cutoff for skin sensitizing compounds.
- Structure based reaction domains are still valid to rationalize the sensitization caused by these compounds.
- Chemical skin sensitizers may enter the viable epidermis via alternative routes to the stratum corneum, such as hair shunts or pores.

References

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Images

Images of the effects of contact dermatitis were provide by a colleague.

Images of products representative of those which caused the sensitization were taken from the following sites and were all used under the creative common license.

https://commons.wikimedia.org/wiki/File:Lancome_Tresor.jpg

https://commons.wikimedia.org/wiki/File:Afwasmiddel_Una_Aldi.JPG

http://myrnakeupblog.blogspot.com/2011/05/because-once-isnt-enough-i-wash-my-face.html

https://en.wikipedia.org/wiki/File:Stick_deodorant.jpg

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