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 which relied upon information generated from different *in vitro* and *in chemico* assays that characterized the key events within the AOP. Here we evaluated the performance of ITS-2 model using cross validation. We explored replacing TIMES-SS, a commercial expert system with the freely available OECD QSAR Toolbox Protein binding alerts. Re-deriving the model resulted in a comparable predictive performance. We also examined whether penetration, expressed as a percentage of the total amount, is a relevant predictor of skin sensitization potential and potency. General dogma supposes size and hydrophobicity as modelled by molecular weight (MW) and $LogK_{o/w}$ are important parameters for evaluating penetration, with a MW>500 and $LogK_{o/w}$ >1 often being cited as thresholds for skin sensitization. Roberts et al (2013) examined the training set within TIMES-SS and the extent to which substances with a MW > 500 were sensitizing. Their dataset was limited with 13 compounds above a MW of 500 and of those only 5 were sensitizers. Here we present preliminary findings using the ECHA REACH dissemination dataset which identified 176 compounds with a MW greater than 500 and of those 31 were sensitizers. The findings confirm those of Roberts et al. (2013) and provide greater confidence that penetration is not a relevant predictor for skin sensitization. In addition we also found that 24 % of compounds with a $LogK_{o/w}$ below 1 were skin sensitizers where as 31 % of compounds with a $LogK_{o/w}$ 1 or above were sensitizers, using the same ECHA REACH dataset.

Aims

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

- Evaluate the performance of the ITS-2 Bayesian network using cross validation.
- Modify ITS-2 to replace the TIMES-SS predictions with protein binding alerts (herein termed reaction alerts) as taken from the OECD QSAR Toolbox.

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

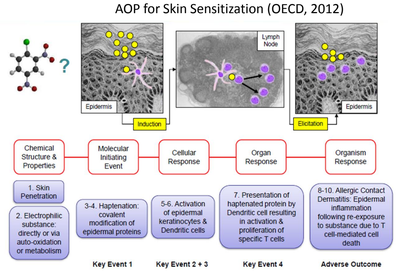
Effects of Skin Sensitization

- Chemicals classified as contact skin sensitizers have the capacity to cause allergic contact dermatitis (ACD) (see photos above).
- 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)

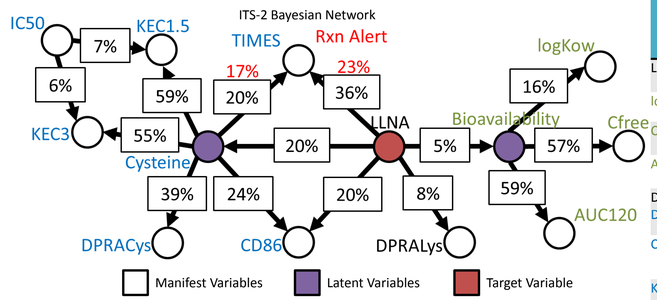


We evaluated the ITS-2 model and attempted to replace one of the key components of the model the TIMES-SS predicted score, with a reaction alert prediction generated using the OECD QSAR Toolbox. We also examined two key factors that are thought to be important to skin penetration, molecular weight and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

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 alert 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 alerts in place of the TIMES-SS predictions was comparable to the original ITS-2 model.



Components of the ITS2 Model

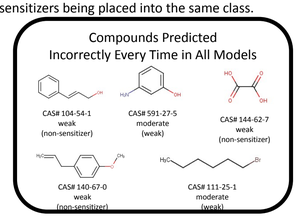
LLNA	Skin sensitization result from the local lymph node assay
logKow	Octanol/water partition coefficient
Cfree	Maximum free concentration in the mid-epidermis
AUC120	Amount absorbed by the epidermis over 120 hours
DPRALys, DPRACys	Percentage of peptide remaining after reaction in the DPRA assays
CD86	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 Alert	Binary for if a reaction alert was found in the OECD QSAR toolbox or not.

Stratified 10-fold Cross Validation

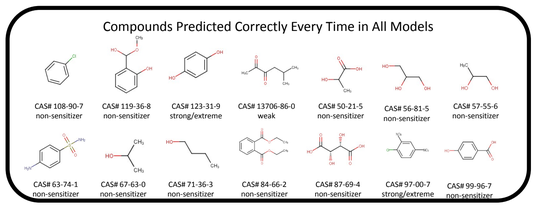
Test Set	Correct for Sensitization	Correct LLNA Value
ITS-2 Network	89%	65%
ITS-2 Network without TIMES-SS	79%	49%
ITS-2 Network with Rxn Alert Replacing TIMES-SS	84%	54%

"Correct for sensitization," indicates how often a given model correctly predicted that a compound was a sensitizer in the LLNA. "Correct LLNA value" indicates how often the network predicted the correct score for a skin sensitizer, with strong and extreme sensitizers being placed into the same class.

Compounds Predicted Incorrectly Every Time in All Models



Compounds Predicted Correctly Every Time in All Models



Compounds that had their skin sensitization class predicted correctly or incorrectly every time by all three networks. For those incorrectly predicted, the most commonly predicted class is given in parenthesis. Note that the model places strong and extreme skin sensitizers in the same class for prediction.

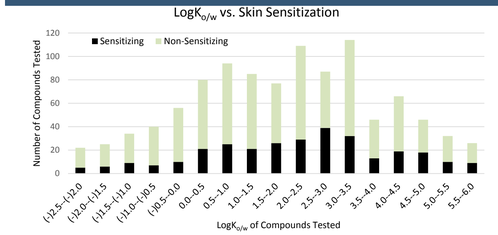
Bioavailability

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 one.

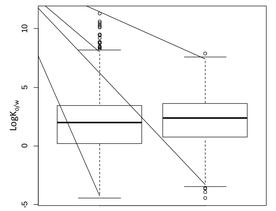
Using the OECD eChemPortal which allows the ECHA REACH dissemination database to be searched, we collected a large dataset of compounds tested for their skin sensitizing ability. We also collected data for compounds with experimentally determined $LogK_{o/w}$ values.

Bioavailability: $LogK_{o/w}$

$LogK_{o/w}$ vs. Skin Sensitization

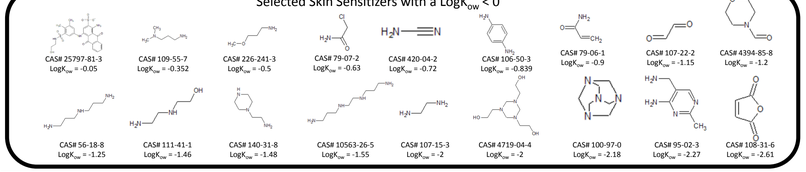


Box Plot of Skin Sensitization



Data on 1155 compounds was collected, however due to the wide range of $LogK_{o/w}$ values only 1039 of the compounds are 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 $LogK_{ow}$ < 0

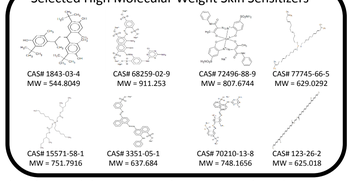


Bioavailability: Molecular Weight

Skin Sensitization Information From ECHA

	MW > 500		MW ≤ 500	
	# of Compounds	% of Compounds	# of Compounds	% of Compounds
Sensitizers	31	17.6%	646	26.9%
Non-Sensitizers	145	82.4%	1753	73.1%
Total Compounds	176		2399	

Selected High Molecular Weight Skin Sensitizers



We gathered the largest data set of skin sensitizers with a MW >500 to be reported to date. 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.

Conclusions

Aim 1: ITS-2

- Adding reaction alerts to the ITS-2 network restores some, but not all of the predictive value of TIMES-SS.

Aim 2: Bioavailability

- Being hydrophilic does not rule out the possibility of a compound being a skin sensitizer, nor does high molecular weight.
- Chemical skin sensitizers may enter the viable epidermis via alternative routes to the stratum corneum, such as hair shunts or pores.

References

References

Jaworska J., Dancik Y., Kern P., Gerberick F., Natsch A., Bayesian integrated testing strategy to assess skin sensitization potency: from theory to practice. Journal of Applied Toxicology. 2013; 33, 11:1353-1364

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EC. (2009). Regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009 on cosmetic products. Off. J. Eur. Union L342: 59-209

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Images

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

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

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

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

http://mymakeupblog.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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