Towards AOP application – implementation of an integrated approach to testing and assessment (IATA) into a pipeline tool for skin sensitisation

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G. Patlewicz[1*], C. Kuseva[2‡], A. Kesova[2], I. Popova[2], T. Zhechev[2], T. Pavlov[2], D. W. Roberts[3], O. Mekenyan[2]

[1]DuPont Haskell Global Centers for Health & Environmental Sciences, Newark DE, USA; [2]Laboratory of Mathematical Chemistry, Prof "As Zlatarov", Bourgas, Bulgaria; [3]Liverpool John Moores University, School of Pharmacy and Biomolecular Sciences, Byrom Street, Liverpool, L3 3AF, UK

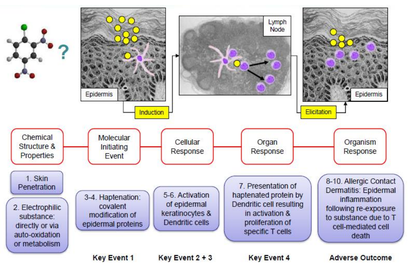
[‡]Presenting author:chanita_k@btu.bg, *Corresponding author: patlewig@hotmail.com

ABSTRACT

Since the publication of the Adverse Outcome Pathway (AOP) for skin sensitisation by the OECD in 2012 [1], a number of activities were initiated on how best to integrate and interpret non-standard information generated for key events (KEs) in a manner that can be practically useful for decision making. The types of frameworks to facilitate these processes are known as Integrated Approaches to Testing and Assessment (IATA). Here we have outlined an IATA for skin sensitisation which focuses on existing information including non testing approaches such as QSAR and read-across. The IATA was implemented into a Pipeline tool using OASIS technology to provide a means of systematically collating and compiling relevant information which could be used in an assessment of skin sensitisation potential. A test set of substances with available skin sensitisation information taken from Teubner et al (2013) [2] was profiled using the Pipeline IATA. For the majority of test set chemicals, *in silico* and *in chemico* profiling information was found to be sufficient to conclude on likely skin sensitisation potential, with a preliminary accuracy of 73.85%. Information from other relevant endpoints (e.g. Ames mutagenicity) was found to improve the accuracy further (to 87.6%) when coupled with a reaction chemistry mechanistic understanding. This Pipeline platform could be useful in the assessment of skin sensitisation potential and marks a step change in how non testing approaches can be practically applied.

INTRODUCTION: Development of an AOP

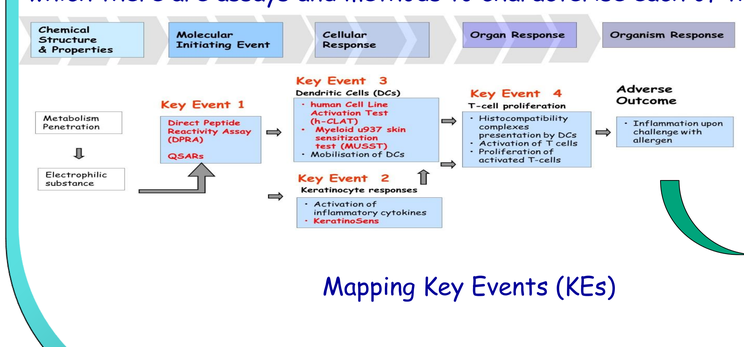
Skin sensitisation is a well studied endpoint – well characterised at all levels of biological organisation . The OECD published the AOP for skin sensitisation in 2012 [1].



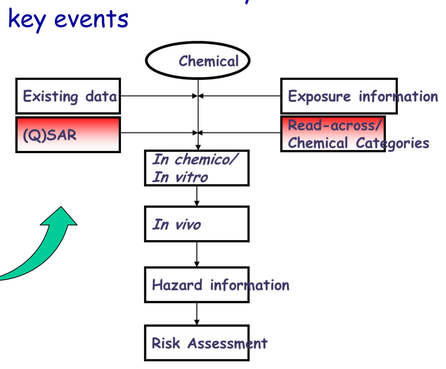
1	Develop the AOP
2	Develop new (or map existing) specific assays to key events within the AOP
3	Conduct (or document) **Analytical Validation** of each assay
4	Develop new (or map existing) models that predict a specific key event from one or more pre-cursor key events. (The input data for the prediction models comes from the assays described in Steps 2 and 3 above.)
5	Conduct (or document) **Qualification** of the prediction models
6	**Utilisation**: defining and documenting where there is sufficient scientific confidence to use one or more AOP-based prediction models **for a specific purpose** (e.g., priority setting, **chemical category** formation, integrated testing, predicting *in vivo* responses, etc.)
7	Dissemination of all necessary datasets, model parameters, algorithms, etc. to enable fully independent verification and peer review. This will also enable other investigators to more readily add datasets and improve the AOP.

However to consider the practical application of the AOP, its scientific confidence needs to be evaluated in the context of use. The following scientific confidence framework has been proposed [3].

The extent to which the AOP can be practically exploited into IATA will be defined by the extent to which there are assays and methods to characterise each of the key events



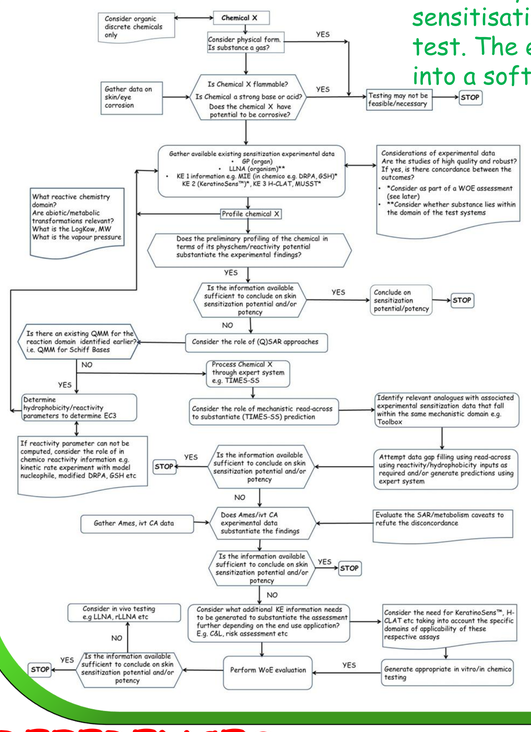
Mapping Key Events (KEs)

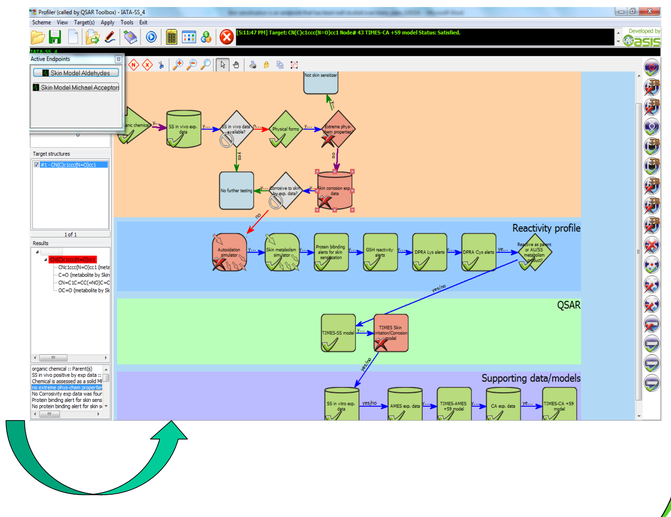


Translating into IATA

METHODS: Developing an IATA for SS and translating it into a practical Pipeline

An IATA was constructed with a strong focus on mechanistic chemistry considerations for the interpretation of existing sensitisation information and to inform the generation of new test. The elements of the conceptual IATA were then translated into a software tool using OASIS Pipeline technology.





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1. OECD. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins Part 1: Scientific Evidence. Series on Testing and Assessment No. 168 ENV/JM/MONO(2012)10/PART1., **2012**.
2. Teubner, W et al. *Regul Toxicol Pharmacol. 67*, **2013**, 468-485.
3. Cox LA et al *Reg. Toxicol. Pharmacol.* **2014** *in press*
4. Natsch A. et al *J Appl Toxicol.* **2013** doi: 10.1002/jat.2868.
5. Patlewicz et al. *Reg Toxicol Pharmacol.* **2014** *in press*

METHODS – Translating the IATA into a practical Pipeline

Components of the IATA represented in the Pipeline

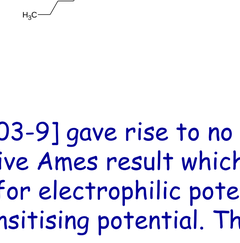
1. Docked to the OECD Toolbox to access available in vivo sensitisation data
2. Physical form – what are the relevant physicochemical properties – such as vapour pressure, pKa, LogKow, MW that could play a role in limiting testing from a practical perspective?
3. Skin irritation/corrosion experimental data and predictions from specific TIMES model – is the substance corrosive – and will this impact its sensitisation potential?
4. Protein binding alerts from the OECD Toolbox and from specific profilers developed on KE data from the DRPA, GSH which characterise electrophilic reactivity?
5. Simulation of potential degradates formed from autoxidation or through metabolism
6. TIMES-SS predictions
7. Experimental data from *in vitro* chromosomal aberration and Ames tests – provides complementary MIE information
8. TIMES models for the *in vitro* chromosomal aberration and Ames tests
9. Other KE information from *in chemico/in vitro* assays

To test out the practical utility of the IATA-SS Pipeline, a dataset of 100 substances taken from ref [2] were taken and profiled within the Pipeline.

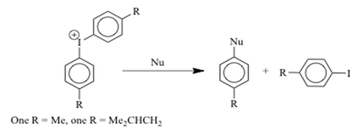
RESULTS

Of the 100 substances, 3 were found to be inorganic and out of scope of the AOP and its associated IATA, 24 were found to have experimental in vivo data within the OECD Toolbox and 8 were flagged as having physicochemical properties (LogKow) that were "extreme" [-3< or >8]. The remaining 65 substances were processed through the remainder of the pipeline. Using the components characterising reactivity and the TIMES-SS model itself – a sensitivity of 74.1%, specificity of 73.7% and accuracy of 73.85% resulted for the 65 substances. There were 17 incorrect predictions with 7 apparent false negatives and 10 false positives. Each of these were evaluated in turn to identify what refinements were merited within the IATA-SS pipeline.
A handful of examples are provided below:

Tetradecylchloroformate [56677-60-2] was one FN identified. This could react via an acylation mechanism. The alert was modified to accommodate this type of "haloester" structure.



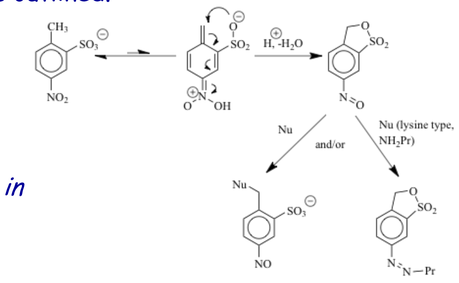
iodonium, (4-methylphenyl)[4-(2-methyl-propyl)phenyl]-, hexafluorophosphate(1-) (1:1) [344562-80-7] was another FN. The positive iodine suggests electrophilic potential, possibly similar to how aryl diazonium salts couple with nucleophiles (see scheme).



One R = Me, one R = Me_2CHCH_2

4-Nitrotoluene-2-sulphonic acid [121-03-9] gave rise to no alerts but was associated with a positive Ames result which does lend credibility to the potential for electrophilic potential and hence might be an indicator of sensitising potential. The following scheme was outlined.

From such evaluations, the number of FNs was reduced to 3 and the number of FPs to 5. This resulted in an improved accuracy of 87.6%.



None of the 65 substances were associated with any specific *in vitro/in chemico data*, as such several examples were taken from Natsch et al (2013) [5] to illustrate how the IATA-SS could be used to guide a WoE for skin sensitisation.

CONCLUSIONS

Evaluating the skin sensitisation potential of a substance relies on different information. Here an IATA has been developed to mimic a work flow of how available existing information for the substance under consideration in concert with data from appropriate analogues or based on QSAR approaches can be used. A software pipeline using OASIS technology named IATA-SS has been created to mirror many of the workflow components [6]. Each of the components is underpinned by a strong mechanistic basis. Using the dataset published by Teubner et al (2013), based on the protein binding alerts and TIMES-SS predictions, correct predictions of likely sensitisation potential was possible for the majority of substances. There were a number of apparent false positives and false negatives and these were considered in turn to determine to what extent further refinements were needed in the IATA-SS itself. The exercise demonstrated that the use of non testing approaches whether it be entirely *in silico* based or using rules extracted from MIE assay information do go some way to conclude on sensitisation potential and highlights how MIE information is a valuable and reasonable predictor of skin sensitisation hazard. A handful of additional substances were taken from Natsch et al (2013) [5] to illustrate how the information from IATA-SS pipeline components can be evaluated in a guided WoE approach.