

Towards a testing strategy for the identification of respiratory sensitizing chemicals

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VALORISATION PARAGRAPH TO THE PHD THESIS

Towards a testing strategy for the identification of respiratory sensitizing chemicals

Occupational asthma, asthma obtained through exposure to a chemical at the workplace, is a prevalent condition among workers. Compared to most other types of work related diseases, occupational asthma has very severe consequences for a worker. When a worker is exposed and sensitized to a specific chemical, the worker usually is not able to come near the workplace and potentially not even near the building itself. Compared to for example a work-related injury such as breaking a leg at a construction site, the socioeconomic consequences are much harsher for occupational asthma. It is therefore highly desirable to identify chemicals that can cause occupational asthma and take precautionary measures to prevent exposure to those chemicals. The problem is that prospective identification of these chemicals is currently not possible due to the lack of widely accepted testing methods (both animal and animal-free methods). In addition, with the current mindset of replacing, reducing and refining animal testing, the challenge to develop a method to identify these chemicals has not become easier. Nevertheless, this thesis aims to evaluate and develop different testing methods that are specifically designed to identify chemicals that cause occupational asthma.

The first type of methods evaluated are (quantitative) structure activity relationship models (QSARs). These *in silico* methods provide predictions on respiratory sensitization potential of chemicals based on their chemical structure. They roughly apply two different approaches to interpret the query chemical. Some *in silico* models evaluate the query chemical structure and search for similarities between the query chemical and a database of chemical structures known to cause occupational asthma. The other type searches the query chemical structure for specific chemical subdomains known to be protein reactive. Both applications are deemed valid, although a slight emphasis on the latter approach can be found in recent publications regarding respiratory sensitization as protein binding is known to be the molecular initiating event of respiratory sensitization that leads to occupational asthma. Further development of such *in silico* models, or at least, more insight in the actual chemistry involved in respiratory sensitization can result in highly efficient (time-wise and money-wise) tools for this toxicological end-point.

The second method evaluated in this thesis is the Direct Peptide Reactivity Assay (DPRA). This method measures the binding potential of chemicals to heptapeptides that mimic human proteins to which chemicals can bind. This method was originally designed to identify chemicals that cause skin sensitization, but it can also be applied to the field of respiratory sensitization as covalent binding between chemicals and proteins is the molecular initiating event for respiratory sensitization as well. The published methodology was evaluated and improved upon to increase the reliability of predictions and to obtain information on reaction chemistry. This information can be directly used by QSAR developers to improve their *in silico* models. In addition, the DPRA can provide predictions for chemicals that are (currently) not able to be predicted by

QSARs due to issues in their applicability domain. Together, QSARs and the DPRA can provide a chemically relevant reliable prediction on the respiratory sensitization potential of chemical. However, the process that ultimately leads to occupational asthma involves many biological processes as well.

The third method developed in this thesis specifically addresses a small part of these biological processes. Specifically, an *in vitro* method was developed based on the first cell layer a chemical encounters when it enters the lungs, the epithelial lung barrier. It was found that several different genes were affected by respiratory sensitizers that are shown to be involved in the process of respiratory sensitization. However, these processes are mostly associated with the barrier function of the cells themselves, and less involved in immunological processes. Although this is not a major issue in terms of predictivity and biological relevance, it can be argued that an *in vitro* model based on more immunological relevant genes might be more appropriate, especially when it comes to applying the obtained gene set for a patent.

This thesis shows that by combining the abovementioned methods for the prediction of respiratory sensitization potential of chemicals, a prediction accuracy higher than any individual method can be obtained. This application, generally called a testing strategy, is becoming to be more relevant in the scientific community in general. The general consensus is that focus should shift to the development of these types of strategies instead of focusing solely on development of individual methods. Obviously, testing strategies only function when individual methods are combined, so sufficient attention should be given to that aspect as well. Increasing predictive performance of a model or strategy for respiratory sensitization is required as it will lead to better protection of workers. It is also important for industries working with potential respiratory sensitizers as it is more economical to adapt a manufacturing process beforehand than afterwards. One specific branch that could benefit from the developments in testing strategies for respiratory sensitization is contract research laboratories, as they could provide a prediction service based on a respiratory sensitizer testing strategy for affected industries.

It is concluded in this thesis that combining different individual testing methods is the way forward to allow for fast evaluation of chemicals that can potentially cause occupational asthma. This is deemed highly necessary as many workers are affected by this disease and therefore the impact on society is high. With the advancement of individual methods, the development of a knowledgebase regarding this toxicological endpoint in the form of an adverse outcome pathway, and the insights that the individual methods can be combined according to the different aspects of the AOP, it is likely that a strategy for the prospective identification of low molecular weight respiratory sensitizers is to be validated and accepted.

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