1. Introduction

The adverse outcome pathway (AOP) provides a framework to encapsulate the chemical and biological processes that can lead to toxicological outcomes [1]. The molecular initiating event (MIE) is a chemical interaction which starts an AOP [2,3]. Some examples of MIEs include receptor activation, DNA modification and enzyme inhibition. Chemistry is key to understanding the MIE. What is it about these compounds that allow them to do this?

2. Transition State Modelling

The Ames mutagenicity assay is a long established in vitro test for compound mutagenicity potential. One of the MIEs that can lead to mutagenicity is the covalent binding of electrophilic chemicals to DNA nucleobases. To explore this we modelled reactions between electrophilic α,β unsaturated carbonyls and a model ethylmethylnucleophile using quantum mechanical density function theory calculations.

3. Artificial Intelligence

Machine learning algorithms such as neural networks have found use in a number of branches in computer science due to their high predictivity. Their use in toxicology has so far been limited, but they are gaining much attention [8]. One drawback of such complex methods is difficulty in understanding their predictions - making them less attractive in risk assessment procedures. A cartoon of a neural network that might be used for MIE prediction is shown below.

![Neural network cartoon](image)

Inputs are fed into the left hand side of the network through the input neurons. Each layer of neurons is connected to the adjacent layers by synapses. When the network is trained on known data it adjusts the weights of each synapse to alter how signals propagate through the network. Output nodes on the right correspond to desired predictions. In the case of MIE prediction we can feed a neural network chemical information to its input layer and predict biological activity at its output layer. The types of input information, the number of neurons and hidden layers, and the mathematical relationships contained within neurons and synapses can all be changed before training to investigate which set of parameters provide the highest levels of predictivity.

The calculations allow us to separate the graph into three regions. Above 25.7 Kcal/mol all the compounds calculated are Ames negative as they cannot bind directly to DNA. Below 22.0 Kcal/mol they are all Ames positive and between 22.0 and 25.7 Kcal/mol there are examples of both. These results suggest a clear link between activation energy and mutagenicity potential. This methodology allows us to calculate an energy barrier for this process, and other covalent bond forming MIEs and compare them to experimentally tested compounds.

4. Chemical and Biological Similarity

To better understand how neural networks “think” we can explore the similarity between network signals propagating through different chemical compounds. Once a network is trained compounds can be fed in and provide different numerical values at each individual node. These values can be thought of as coordinates in n-dimensional space, and the distance between them calculated.

![Similarity calculation](image)

Compounds that are close have a high similarity in the neural network. Because the network links chemical information to biological predictions the compounds can be thought of as being both chemically and biologically similar. When making predictions the neural network could provide a prediction and list of most similar compounds, increasing confidence in its predictions in risk assessment.

5. Conclusions

- MIEs are important chemical-biological interactions in toxicity that can be modelled in silico.
- Quantum mechanical transition state modelling and activation energy calculations have been used to investigate DNA binding as an MIE leading to genotoxicity.
- Activation energy was found to correlate well with Ames mutagenicity studies for α,β unsaturated carbonyls, with high activation energies corresponding to negative Ames test results.
- Neural networks have been investigated for the prediction of receptor binding MIEs, with promising results reported for the Androgen receptor.
- Future work will involve attempting to better understand neural network predictions, using a combination of chemical and biological similarity between novel and training compounds.

References


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