Examining the myths and realities of aromatic amine mutagenicity.

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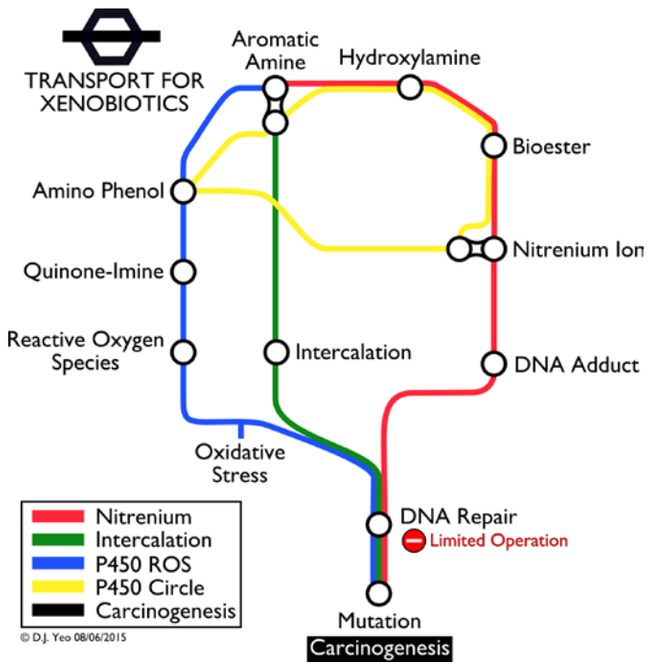
Introduction

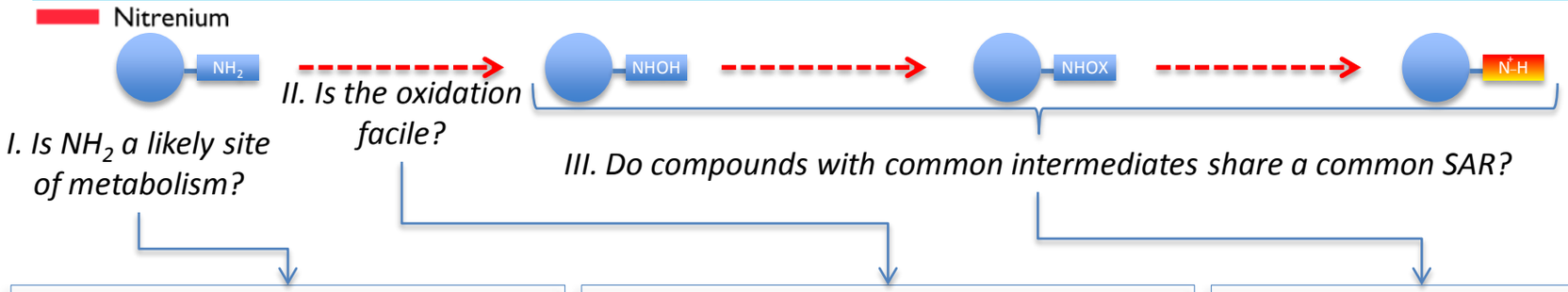
Aromatic amines are notorious mutagens, not least because predicting their activity in the Ames test has proven a difficult task (1). With increasing reliance on *in silico* models for mutagenicity (e.g. 2) it has become clear that where aromatic amines are concerned, extra care should be taken in the interpretation of predictions (3). However, we should also ask why predictions for the mutagenicity of aromatic amines require additional scrutiny. Here, some of the underlying assumptions are re-evaluated.

Our work started with a review of the evidence in the published literature for aromatic amine mutagenicity, which is summarised below:

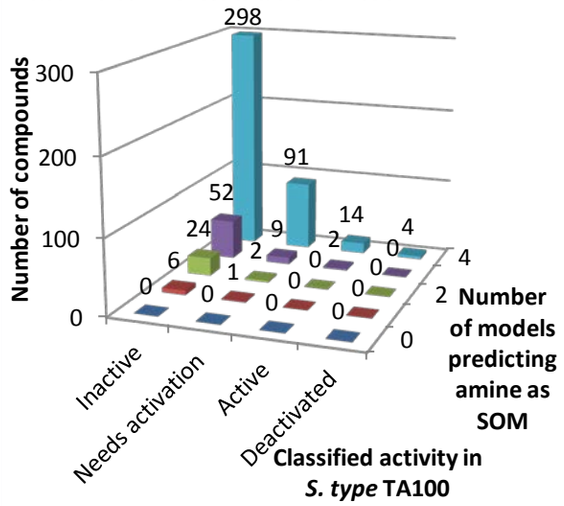


For the purposes of modelling it is assumed that the journey to mutations requires travel along the nitrenium ion line only (e.g. 4-6). However, the support for this is largely indirect, e.g. by density functional theory analysis of nitrenium ion formation (7,8) or *in vivo* studies of aromatic amines that are atypical of those commonly experienced during day-to-day genotoxicity testing and risk assessment. Hence in this work three questions were posed to further interrogate the nitrenium ion hypothesis.

Methods and Results

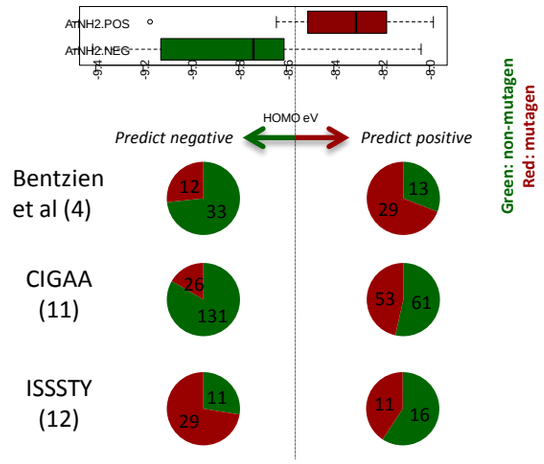


A data set of 503 primary aromatic amines that contained no additional Derek Nexus alerts for mutagenicity was prepared. *In silico* site of metabolism (SOM) studies were conducted using SmartCYP (9) and an in-house technique based on Meteor Nexus. The results are shown below:



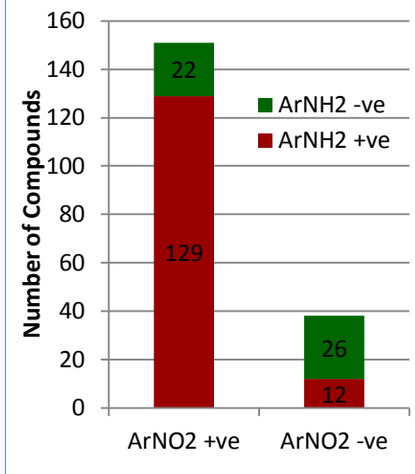
In summary, there are 103 mutagens that require metabolic activation and in 91 of these the amine group is predicted to be a SOM in all four models. There are 94 compounds where one or more models do not predict the amine to be a SOM and 82 of these are non-mutagens. Similar results were found for other strains.

In chemical terms oxidation is loss of electrons. The strength by which the amine can hold onto its electrons can be measured by the HOMO (highest occupied molecular orbital) energy. Higher energies mean that oxidation is more facile. These were calculated using the MOPAC2012 package within KNIME for a data set of primary aromatic amines from the Hansen data set (10) and plotted below:



The boxplot indicates that mutagenic and non-mutagenic aromatic amines have different median HOMO energies. A decision tree classifier produced a model from this separation: compounds with HOMO>-8.588 eV were predicted mutagenic (and vice versa). The results in the pi charts demonstrate the predictivity against a public data set (Bentzien et al), a data set generated through data sharing between pharmaceutical companies (CIGAA) and secondary aromatic amines extracted from a large public data set (ISSSTY).

A data set of 189 aromatic scaffolds where there was Ames test data for both primary amine and nitro substituents was prepared and the activity of analogues compared. The results are shown below:



Where an aromatic nitro compound is mutagenic, based on this analysis there is an 85% chance of the corresponding amine also showing mutagenic activity. Where the aromatic nitro compound is non-mutagenic, the probability of the amine analogue being non-mutagenic is 68%.

Conclusions

All three methods returned evidence supportive of the hypothesis that the formation of nitrenium ions promotes the mutagenicity of aromatic amines in the Ames test, but each with a caveat: (I) There was a relationship between predicted SOM and mutagenic activity (although many non-mutagenic compounds are also predicted to be metabolised at the amine); (II) There is a relationship between HOMO energy and mutagenicity, however thresholds learnt from one data set may not apply to more challenging data sets (e.g. that described proprietary or more complex chemical space; (III) The activity of aromatic amines can be read-across from the corresponding aromatic nitro compound, but this is less reliable where the aromatic nitro compounds are non-mutagenic (suggesting that there are additional factors to consider, such as other pathways leading to aromatic amine mutagenicity). Overall, it's reasonable to take nitrenium ions at face value, but useful to remember that it's just the most likely hypothesis not an experimental certainty.

References and PubMed IDs (unless otherwise stated): (1) Sutter et al 23669331; (2) ICH M7 guidance bit.ly: 1JrkpF4; (3) Greene et al 25980641;(4) Bentzien et al 20078034; (5) McCarren et al 21524589); (6) Shamovsky et al 22946514; (7) Leach et al 19225647 ; (8) Borosky 17261035; (9) Rydberg et al 22631565 ; (10) Hansen et al 19702240; (11) Elder et al DOI: 10.1021/acs.oprd.5b00128 ; (12) Downloaded from Vitic Nexus.