The Role of Expert Assessment and *In Silico* Predictions in Determining Genotoxic Risk of N-Nitrosamine Impurities

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Introduction

N-nitrosamine compounds are generally considered highly potent genotoxic carcinogens, whereby even an intake below the conservative threshold of toxicological concern (TTC) for impurities in pharmaceutical products under the ICH M7 guideline has the potential to be of risk to patients. The N-nitrosamine class is therefore included in the 'cohort of concern' within ICH M7 and such compounds must be controlled to a significantly lower acceptable limit.[1]

Investigation of historical Ames assay and rodent carcinogenicity data indicates that using very general alerting features to identify cohort of concern compounds may be overly conservative. Although there are exceptionally potent (Ames positive) carcinogens within this class, such as N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA), this does not appear to be a universal property of all nitrosamines when the available evidence is assessed.[2] Analysis of an Ames data set of 282 nitrosamines found 22% were judged to be non-mutagenic.

By identification of molecular features that eliminate mutagenic activity or reduce carcinogenic potency, and consideration of potential mechanistic rational, it may be possible to de-risk certain sub-classes of N-nitrosamines that are deemed to be of lower genotoxic risk compared to NDMA and NDEA.

Objective

The aim of this study is to demonstrate that a combination of ***in vitro*** data, ***in vivo*** data and ***in silico*** tools may aid expert review for ICH M7 impurity assessment of N-nitrosamine compounds.

Method

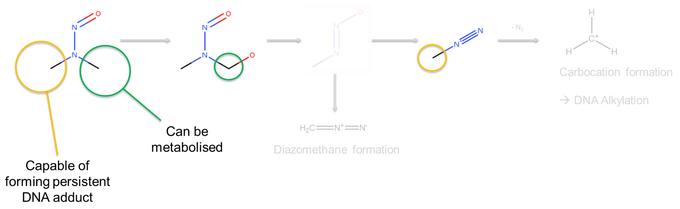
- A data set of 131 public and proprietary nitrosamine compounds with associated Ames test and rodent carcinogenicity study data was collated.
- Compounds were processed using the ICH M7 Batch Prediction function in Nexus (2).
- Compounds predicted to be positive by the "ICH M7 Expert Review *In Silico* Overall Call" were processed against the carcinogenicity endpoint in Derek Nexus (3).
- Considering carcinogenicity *in silico* predictions and alert comments, expert review was carried out to identify compounds containing deactivating features and, where appropriate, a positive call was either overturned or deemed positive with a lower carcinogenic potency compared to NDMA.
- The results before and after expert assessment were analysed and compared to the predictive performance of the Ames test for carcinogenic potential (1).

References: **1.** ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk | European Medicines Agency (2015) https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential. **2.** Thresher et al, Reg. Tox. Pharmacol. (2020) 116, 104749. **3.** Thomas et al, Reg Tox Pharmacol (2021) 121, 104875. **4.** www.lhasalimited.org/products/lhasa-carcinogenicity-database.htm

Expert Review – what to consider?

Mechanistic implications for the SAR

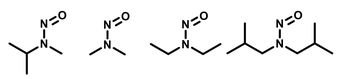
- Requirement for two suitable alkyl substituents:



Structural features that eliminate mutagenicity/carcinogenicity

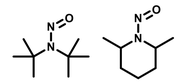
- *In vitro* mutagenicity (Ames test) and carcinogenicity (rodent) data from historical literature, extracted from Vitic, was analysed.
- SAR for substructural features identified:

Metabolism possible



Carcinogenic and mutagenic

Metabolism partially blocked



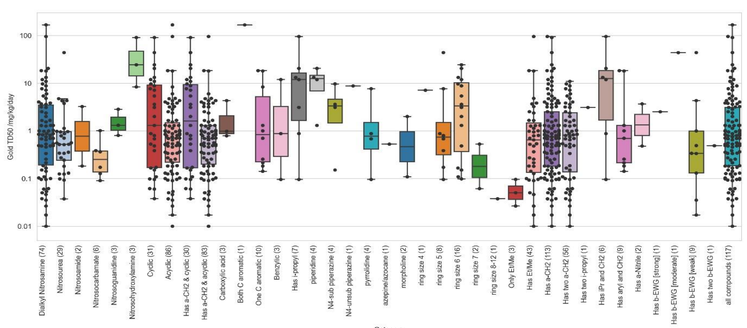
Non-mutagenic and non-carcinogenic

Weakly carcinogenic and mutagenic

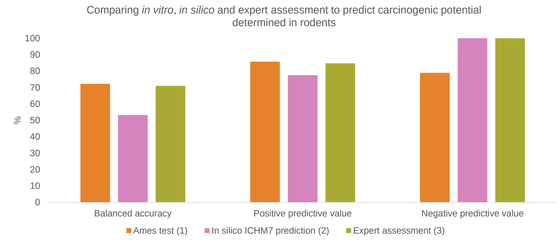
Non-carcinogenic
(mutagenicity unknown)

Categorisation of carcinogenic potency by structural features[3]

- Deactivating features and supporting data are described within the Derek Nexus carcinogenicity alert to aid expert review.



Comparing the predictive performance *in vitro*, *in silico* and expert assessment methods for identification of carcinogenic potential



Ames test (1)

- 112 compounds were positive in the Ames test, of which 98 are carcinogenic.
- 19 compounds were negative, of which 15 are non-carcinogenic.

In silico ICH M7 assessment (2)

- 129 compounds were predicted to be Ames positive by *in silico* ICHM7 assessment, of which 100 are carcinogenic.
- 2 compounds were predicted to be Ames negative and are non-carcinogenic.

Further expert assessment of carcinogenic potential (3)

- 5 compounds were not predicted to be carcinogenic by Derek Nexus and are non-carcinogenic in rodent assays.
- 8 compounds were predicted to be positive; however, alert comments describe deactivating features present, likely to render such compounds non-carcinogenic, therefore the result was overturned. Of these, 6/8 are known Ames negative and 8/8 non-carcinogenic.
- A further 39 compounds were identified to have deactivating features which are likely to reduce carcinogenic potency compared to NDMA and NDEA. Of these, 4 are non-carcinogenic. All 7 compounds with available Lhasa TD50 values in the Lhasa Carcinogenicity database[4] are less potent than NDMA (by ~3-34-fold).

Conclusions

- There is a good correlation between the Ames test and rodent carcinogenicity data; however, expert review is required to ensure *in vivo* relevance.
- *In silico* alerts for ICH M7 derived from public and proprietary Ames test data provide a conservative prediction of mutagenic potential.
- While the *in silico* ICH M7 prediction correlates well with carcinogenic potential, predictions may be refined further by consideration of the SAR derived from rodent carcinogenicity studies, as described within the Derek Nexus alert.
- Alongside *in silico* predictions, expert review is important to achieve a more refined prediction of carcinogenic potential and risk.