

# Are All Nitrosamines Concerning?

R. V. Williams, M. Coombs, R. Foster, D. J. Ponting, R. E. Tennant, R. Thomas, A. Thresher

Granary Wharf House, 2 Canal Wharf, Leeds, LS11 5PS, United Kingdom

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## ■ Introduction

The assessment and control of mutagenic impurities is outlined in the ICH M7 guideline<sup>1</sup>. This describes how theoretically acceptable levels of human exposure (e.g. threshold of toxicological concern (TTC)) can be derived from carcinogenic potency data (e.g. TD<sub>50</sub> – the dose at which the probability of remaining tumour-free after chronic administration for the standard lifespan would be halved)<sup>2</sup>. The recent discovery of nitrosamine impurities in several marketed pharmaceuticals has led to increased interest in their mutagenic and carcinogenic activity. Regulatory authorities have requested that marketing authorisation holders review all synthesised active pharmaceutical ingredients for nitrosamine impurities, using a risk-based approach to prioritise evaluations and subsequent testing. However, the ICH M7 guideline includes nitrosamines in a ‘cohort of concern’, where acceptable levels of exposure are likely to be significantly lower than those defined in the guidelines. In this sense, the presence of a nitrosamine must be controlled on a case-by-case basis using carcinogenicity data for closely-related compounds. Whilst some nitrosamines, for example nitrosodiethylamine (NDEA), are exceptionally potent carcinogens it is unclear whether this is a universal property of all members of this class.

## ■ Vitic Data

Mutagenicity and carcinogenicity data was extracted from published literature to expand the coverage of nitrosamines in the Vitic toxicity database<sup>3</sup>. The database now contains a total of 518 nitrosamines; 411 with Ames test data and 234 with rodent carcinogenicity data. 184 contain both Ames and rodent carcinogenicity data. The increase in data compared to the previous version of the Vitic database (2018.1) is shown in Figure 1. In addition to the new compounds, data for 150 (39%) of the existing nitrosamines was expanded to include a broader range of strains and study protocols.

Vitic includes an overall Ames call for each compound, derived from the individual study results<sup>4</sup>. This workflow was expanded to also derive a carcinogenicity call from the rodent carcinogenicity studies. Ames calls were compared to carcinogenicity calls for both the nitrosamines and the remaining, non-nitrosamine, compounds in Vitic as an indicator of how well the Ames test predicts carcinogenic potential (Figure 2).

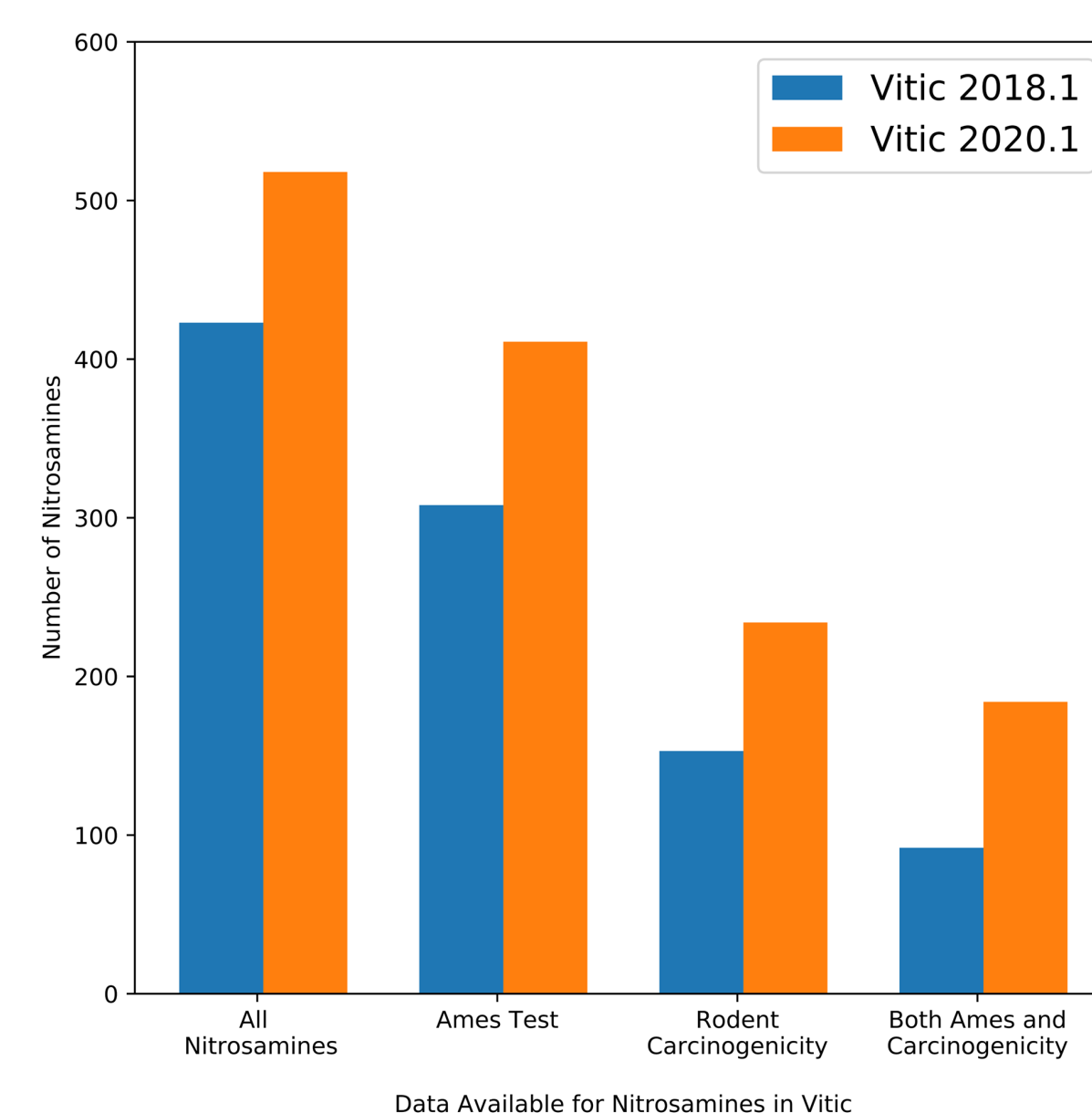


Figure 1: Number of nitrosamines with Ames and/or rodent carcinogenicity data in Vitic 2018.1 and 2020.1

## ■ References

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, 2017, M7.

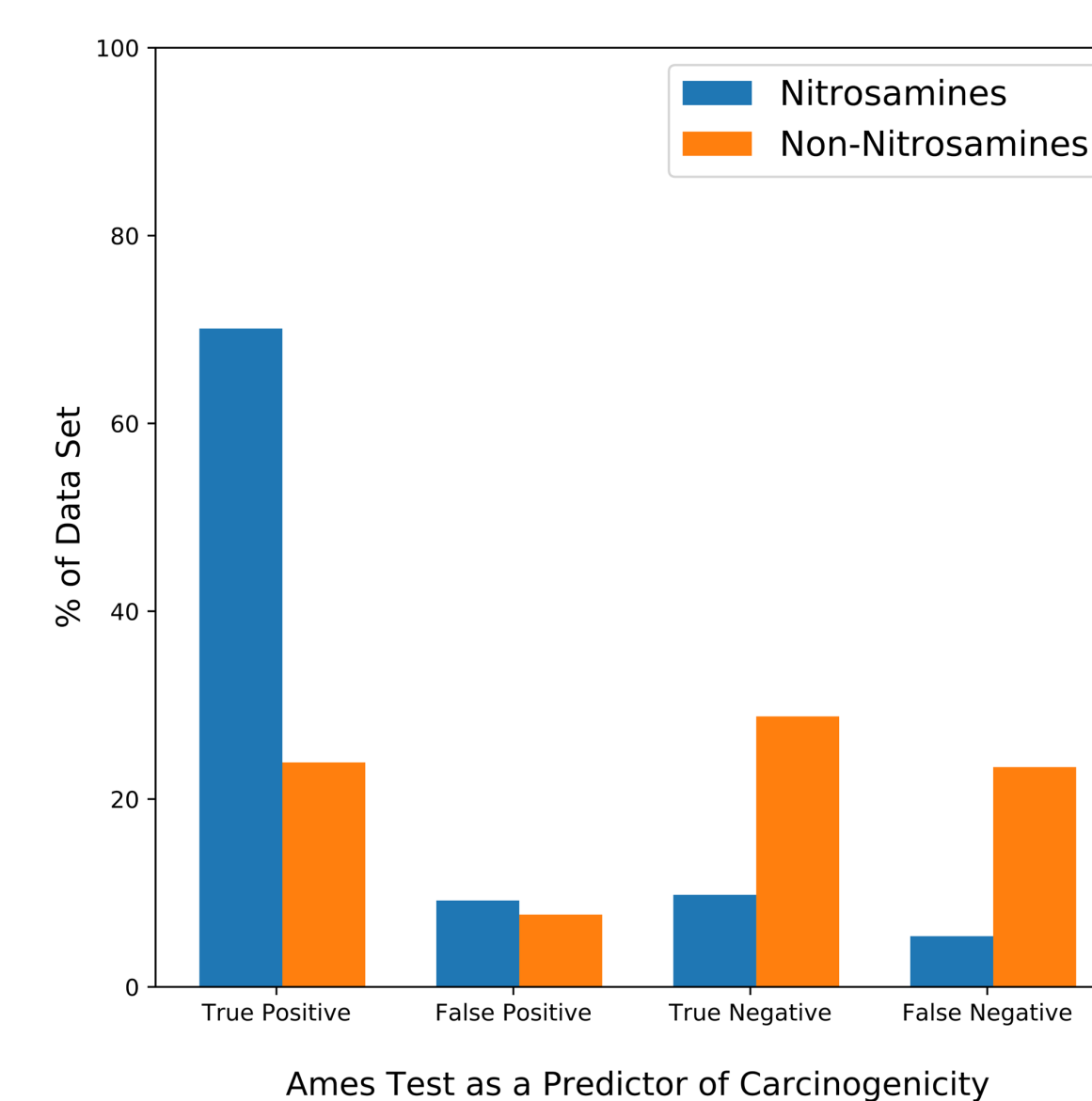


Figure 2: Correlation of Ames and carcinogenicity calls for nitrosamines and non-nitrosamines

## ■ Lhasa Carcinogenicity Database (LCDB)

The LCDB<sup>5</sup> was created to safeguard the data in the Carcinogenic Potency Database (CPDB)<sup>6</sup> originally created by Gold *et al.*, but not updated since 2007. LCDB contains both the CPDB TD<sub>50</sub> values plus Lhasa generated TD<sub>50</sub> values created using a script based on the original CPDB methodology<sup>7</sup>. It contains a total of 139 nitrosamines, of which 119 (86%) were considered positive by the original study authors and 48 (35%) contained both Lhasa and CPDB TD<sub>50</sub> values. The correlation between the two sets of values is shown in Figure 3 and demonstrates the concordance of the two calculations.

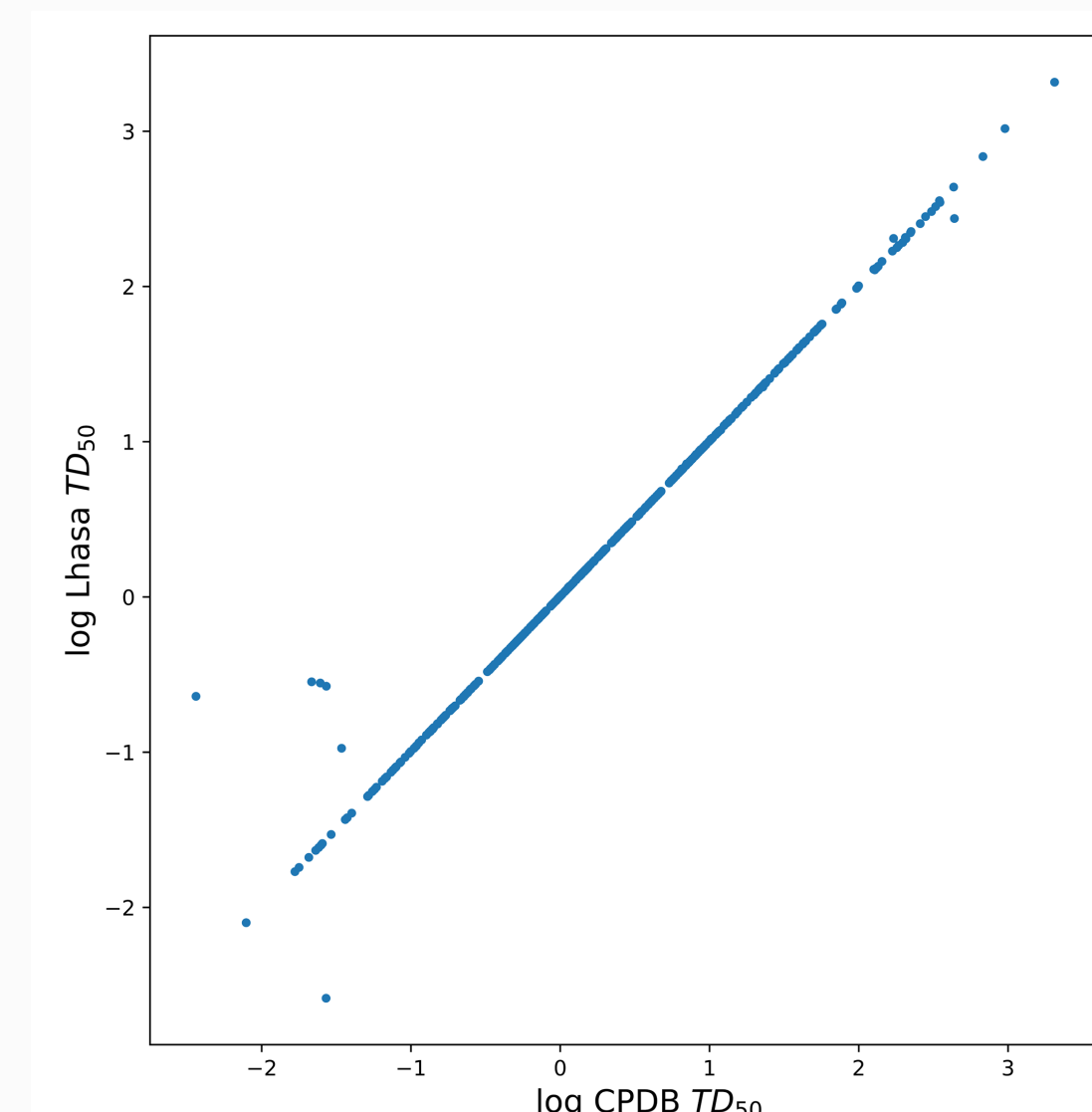


Figure 3: Correlation of log Lhasa and log CPDB TD<sub>50</sub> values

Figure 4 shows the distribution of log Lhasa and log CPDB TD<sub>50</sub> values for both nitrosamines and non-nitrosamines. The Lhasa and CPDB log TD<sub>50</sub> values are comparable across both data sets, although CPDB contains a higher number of outliers. For many of these outliers the Lhasa model did not calculate a TD<sub>50</sub> value as the available dose-response data was considered unsuitable for linear TD<sub>50</sub> modelling.

The distribution of the nitrosamine log TD<sub>50</sub> values is lower than that of the non-nitrosamines, highlighting that, as a chemical class, they are more potent than other carcinogens within the LCDB. This is further illustrated by Figure 5, which shows the distribution of log Lhasa TD<sub>50</sub> values for the nitrosamines and non-nitrosamines as a proportion of the respective data sets, together with the median log Lhasa TD<sub>50</sub> values for each data set. While there is substantial overlap in potency between the two data sets, the median nitrosamine log TD<sub>50</sub> value (-0.334) is considerably lower than that of the non-nitrosamine compounds (1.654).

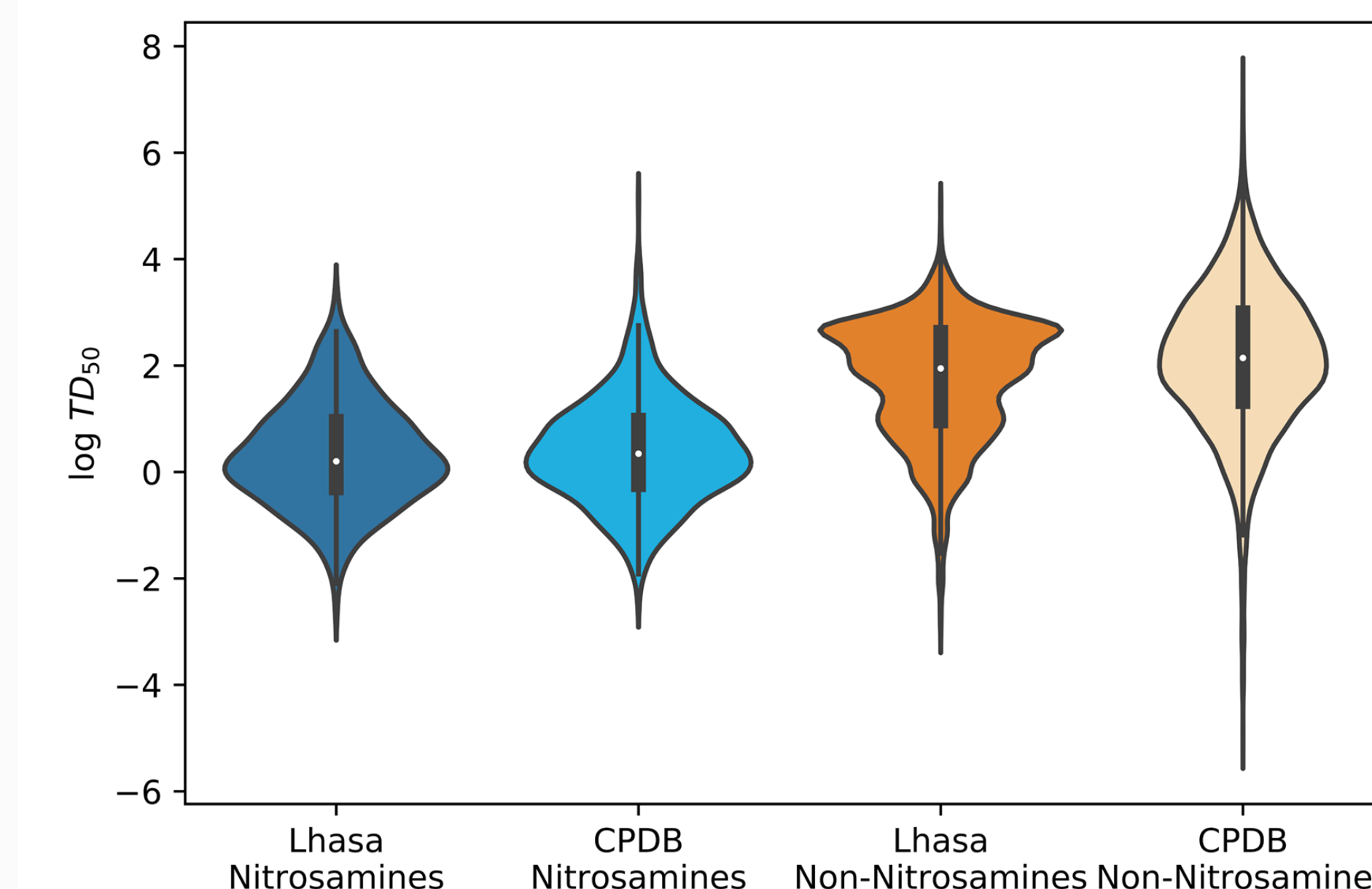


Figure 4: Distribution of log TD<sub>50</sub> values for nitrosamines and non-nitrosamines

NDEA is the most potent nitrosamine for which carcinogenicity data is available. Although it is commonly used to illustrate the carcinogenic potential of nitrosamines, Figure 5 shows that NDEA is exceptionally potent compared to most other nitrosamines. The log TD<sub>50</sub> value for NDEA (-2.585) is considerably lower than the class median (-0.334), and is comparable to aflatoxin B1 (-2.458) in the non-nitrosamine data set, which is also present in the ‘cohort of concern’.

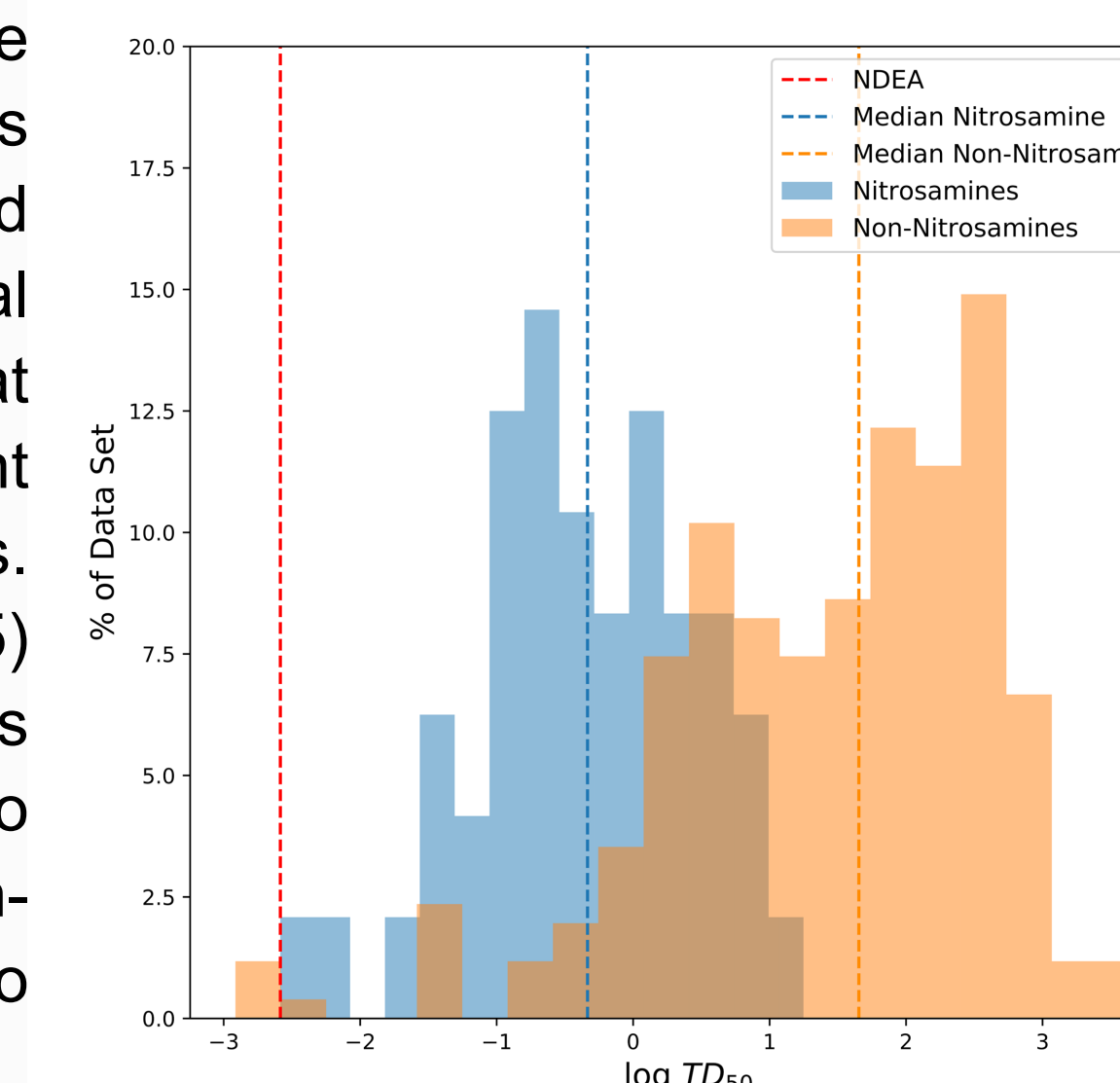


Figure 5: Distribution of log TD<sub>50</sub> values

## ■ Conclusion

A considerable amount of mutagenicity and carcinogenicity data for nitrosamines has now been added to that already available in Vitic and the LCDB. Figure 2 shows strong correlation between the Ames and carcinogenicity calls, showing the Ames test is highly predictive of carcinogenic potential. The data shows that a significant proportion of the data set (19%) tested negative for carcinogenic activity. However, as nitrosamines, these compounds are still present in the ‘cohort of concern’, and so will be considered as posing a significant risk to human health below the acceptable intakes defined in the ICH M7 guideline. Further analysis of these non-carcinogenic nitrosamines is needed to determine any structural features common to this subgroup that prevent carcinogenicity. Although carcinogenic nitrosamines, as a class, are typically more potent than other carcinogens there is still a large distribution in the TD<sub>50</sub> values suggesting that it is unfair to judge all by the most potent in class (Figure 5). Together, this suggests that NDEA may not be an exemplar of the potency of this chemical class and that more mechanistically similar compounds should be considered when performing an ICH M7 assessment.

5. Lhasa Limited Carcinogenicity Database. <https://carcdb.lhasalimited.org/>
6. Carcinogenic Potency Project (CPDB). <https://files.toxplanet.com/cpdb/cpdb.html>
7. Thresher A, Gosling J.P., Williams R., (2019), *Toxicol. Res.*, **8**, 696-703.