Quantifying the nitrosamine potency distribution

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1. Introduction

N-Nitrosamines have recently been the subject of intense regulatory scrutiny, including the setting of low exposure limits (18 ng/day)[1]. We evaluated different methodologies to determine statistically robust bounds on the carcinogenic potency of chemical classes, using historic TD50 data and exemplified for N-nitrosamines. Initially, the distribution of TD50 values (TD50s) for N-nitrosamines of known potency was characterised. From this, it is possible to compare parametric and non-parametric methods to obtain percentiles of interest from the distribution of TD50s, which are shown to be robust to uncertainty in the initial TD50 estimates. These methods may then be applied to different chemical subclasses. The values obtained may be of use in refining acceptable intakes for N-nitrosamines and their subclasses.

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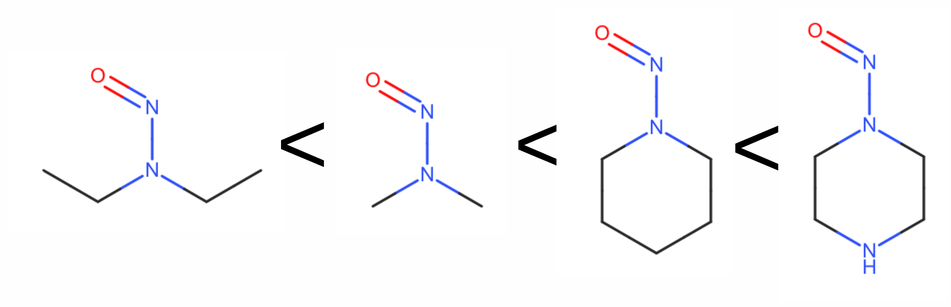
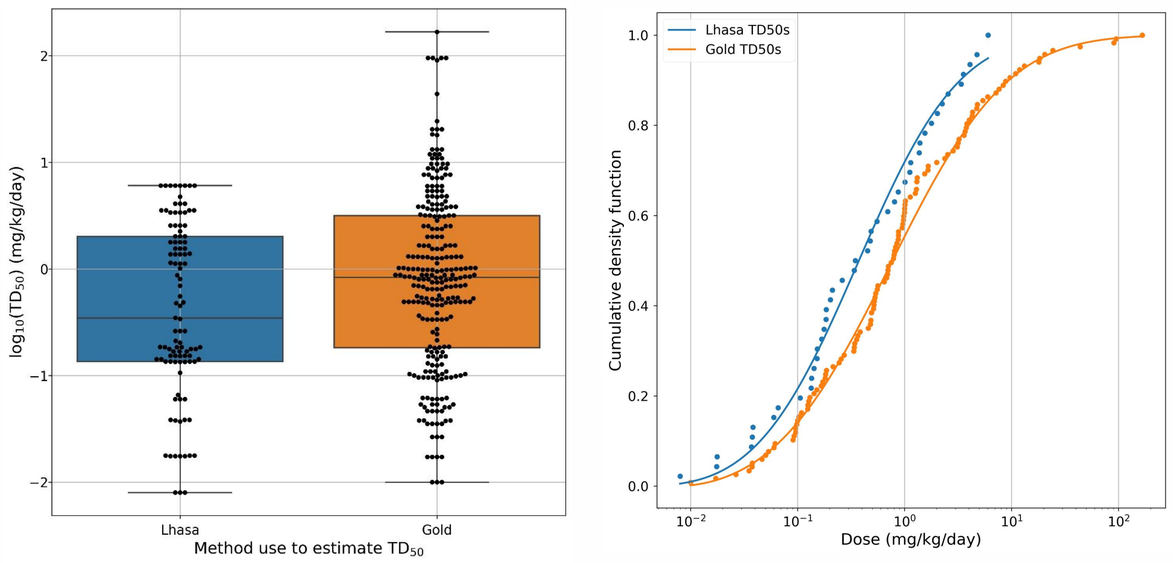


2. The TD50 distribution

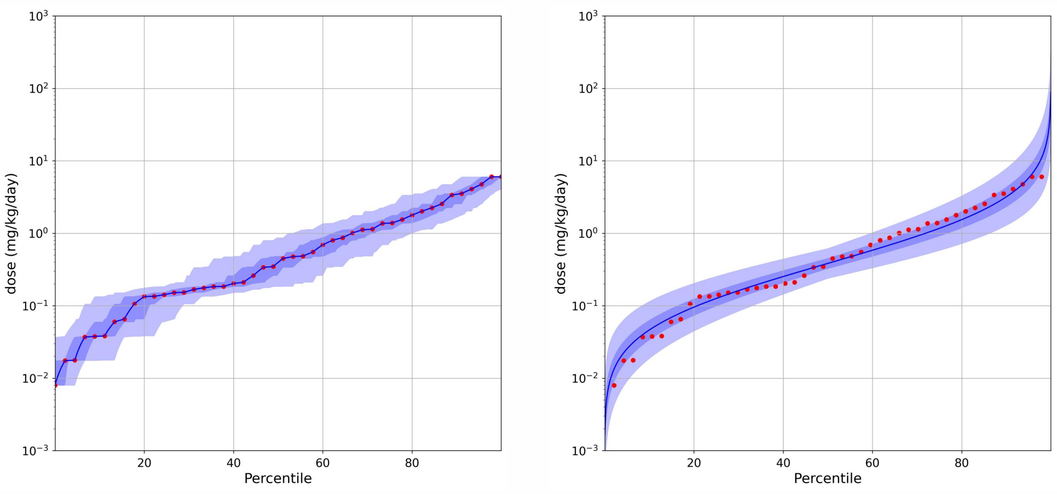
- Tumorigenic dose 50 (TD50) is the dose which will cause tumors in 50% of cases. The Carcinogenic potency database (CPDB) is a widely used database containing TD50s for over 1700 compounds, including approximately 120 nitrosamines.
- CPDB contains 2 estimates of the TD50. The original Gold values calculated in the 80s, and an updated Lhasa value with more stringent data quality requirements[2]. 117 Gold TD50s and 46 Lhasa TD50s belonging to nitrosamines were identified.
- Under examination it is apparent that the nitrosamine TD50s follow a log-normal distribution. Characterising this distribution allows calculations using the TD50s to be performed with increased statistical power.



The distribution of nitrosamine percentiles is log-normal.

3. Estimating percentiles

- Recently published acceptable intake limits were based on estimates of the 5th percentile of the TD50 distribution. This was done without characterising the distribution, we compared this estimate with those obtained from the characterised distribution.
- If a distribution is not known percentiles can be calculated non-parametrically. This involves lining the values up from lowest to highest and picking the value a desired distance along the line (e.g., the 10th value for the 5% of 200 values). This is easy to do but lacks statistical power, especially when values far from the middle of the distribution are estimated.
- If the distribution is known it is possible to estimate the values directly from the distribution providing much more reliable estimates.
- Although the recent limits fall within the range of values we obtain, they are on the lower end of what we predict. Differences in non-parametric estimates are likely due to differences in interpolation, but the exact method used for the limits has not been released.



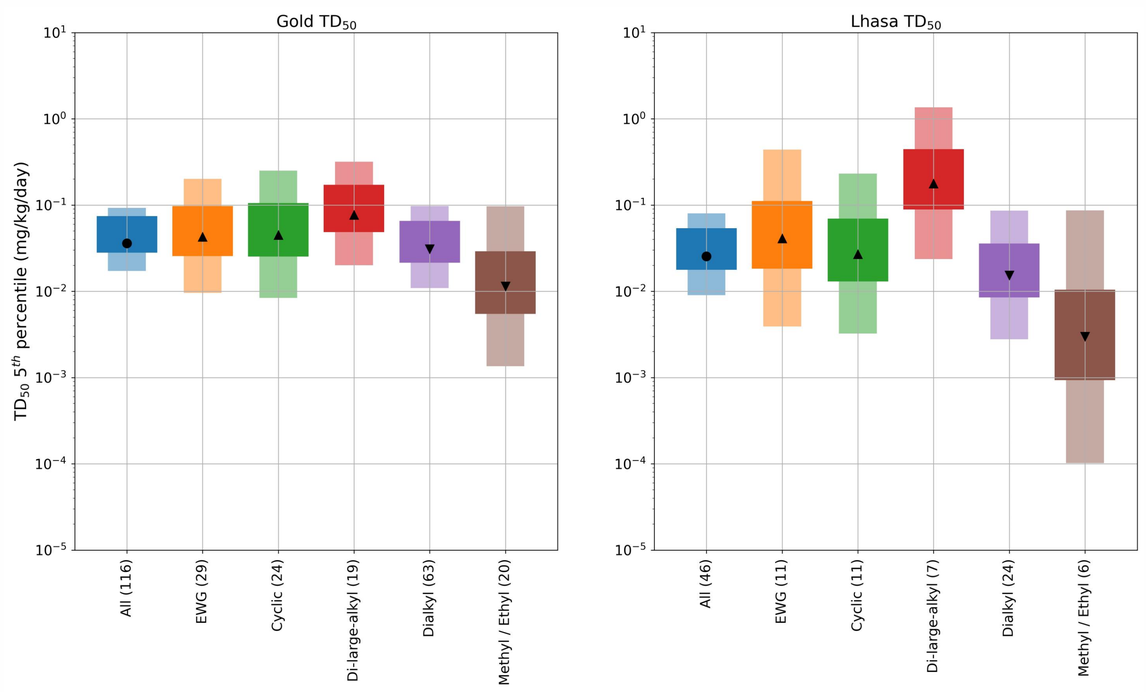
Non-parametric (left) and parametric (right) percentile estimates. Shaded regions represent 50% and 95% confidence intervals.

5th percentile estimates (mg/kg/day)	Non-parametric estimate	Parametric estimate
Gold TD50	0.045 (0.037-0.053)	0.036 (0.028-0.047)
Lhasa TD50	0.023 (0.018-0.037)	0.025 (0.018-0.036)
Current limit estimate	0.018 (no interval given)	-

Comparison of 5th percentile estimates using parametric and non-parametric methods.

4. Structural subclasses

- Identification of more or less potent structural subclasses is hampered by the small number of compounds with potency data available. The increased power of parametric methods allows differences to be investigated on these small datasets.
- The nitrosamines were grouped into 5 subclasses (a single compound may belong to multiple classes). The parametric methods discussed previously were applied to estimate the equivalent 5th percentile.
- The results indicate that there is a difference in potency between these subclasses, but more work is needed to obtain reliable subclass limits.



5th percentile estimates for 5 nitrosamine subclasses. Overall the subclasses show significant differences but further work is needed to define a structure-activity relationship.

1. European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP), “Procedure under Article 5(3) of Regulation EC (No) 726/2004: Nitrosamine impurities in human medicinal products,” 2020.
2. A. Thresher, J. P. Gosling and R. V. Williams, “Generation of TD50 values for carcinogenicity study data.,” *Toxicology Research*, vol. 8, pp. 696-703, 2019.