# Addressing the global challenge of N-nitrosamine impurity assessment

Abstract 273

**David J. Ponting, Michael J. Burns, Rachael E. Tennant, Andrew Thresher**
Lhasa Limited, Granary Wharf House, 2 Canal Wharf, Leeds, LS11 5PS, UK

## OBJECTIVES

- Software tools can assist with the process of ICH M7 impurity assessment in a number of areas
  - Expectation they are regularly updated
- This work aims to ensure the continued relevance of Lhasa Limited QSAR models, purge predictions and databases for M7 assessment of N-nitrosamine compounds

## APPROACH

- Toxicity data for N-nitrosamine compounds was curated from the public literature and proprietary sources
  - This data was made available in Vitic
  - Updated QSAR tools Derek and Sarah Nexus
- Data for N-nitrosamine formation and purge was curated
  - Updated purge analysis tool Mirabilis

## MAIN RESULTS

- Significant increase in coverage in Vitic
- Updated Derek alerts show improved specificity without decrease in sensitivity
- Sarah has learned that there are areas of negative chemical space within the overall class of N-nitrosamines
- Mirabilis contains predictions for the purge of N-nitrosamines and can support ICH M7 Option 4 control

## IMPACT

- Evaluate Ames reliability (see poster #274)
- Roll out nitrosamine-relevant data across products
- Nitrites in Excipients data-sharing group established
- Additional experimental data being generated
- More reliable predictions = better submissions
- **For more information, contact:**
  Dr David J Ponting, david.ponting@lhasalimited.org

# Addressing the global challenge of N-nitrosamine impurity assessment

Lhasa Limited

## OBJECTIVES

- ICH M7 allows the use of in silico tools in a variety of ways
  - Lhasa products impacted are shown at right
  - Those discussed in this presentation highlighted
- There is a requirement that QSAR models are up-to-date
- N-Nitrosamine impurities are a significant challenge for pharmaceutical industry
  - Critical to ensure that they are accurately predicted

- Questions to ask included:
  - What is known about nitrosamine toxicity and reactivity?
  - Which nitrosamines have been studied in the past?
  - Where are the data gaps?
  - Is the experimental data reliable?
  - Are all nitrosamines hazardous?
  - Can nitrosamines form in different ways to nitrite + amine?
  - How can a nitrosamine be purged once formed?

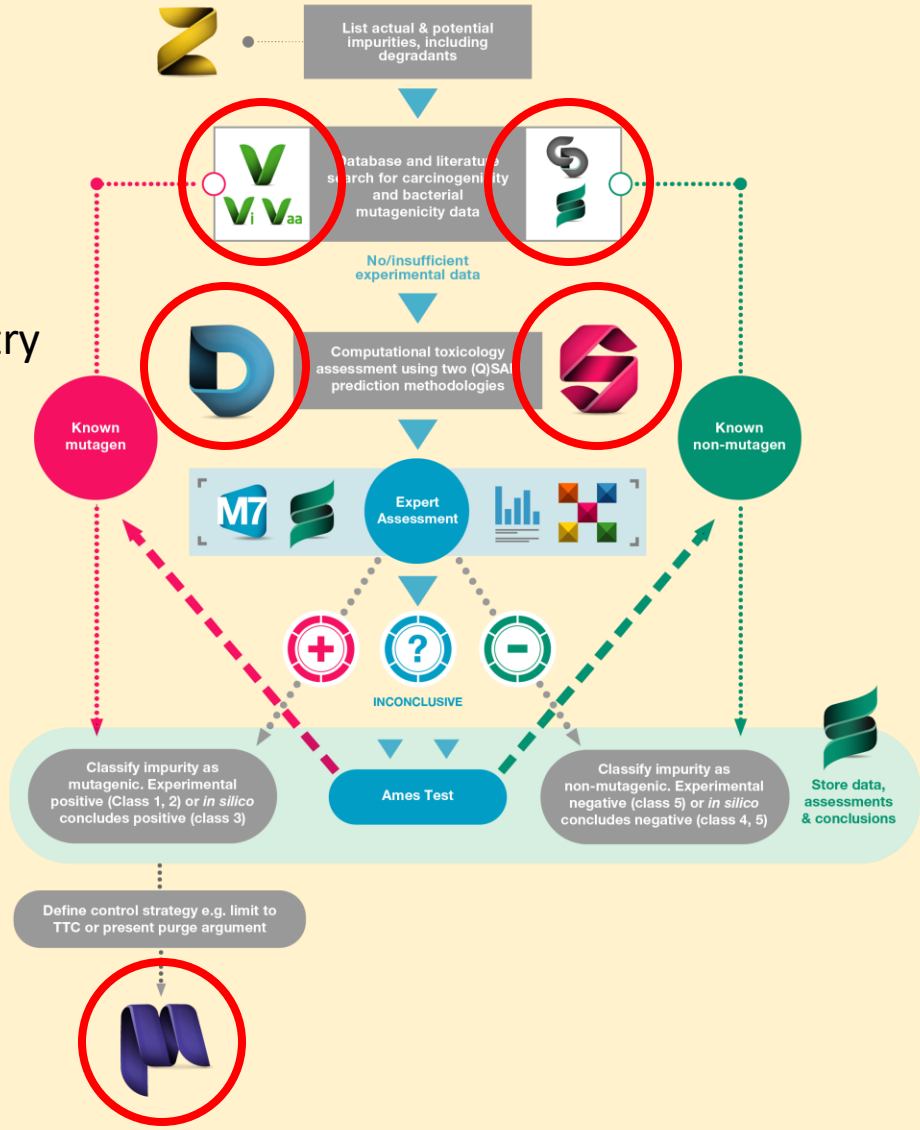


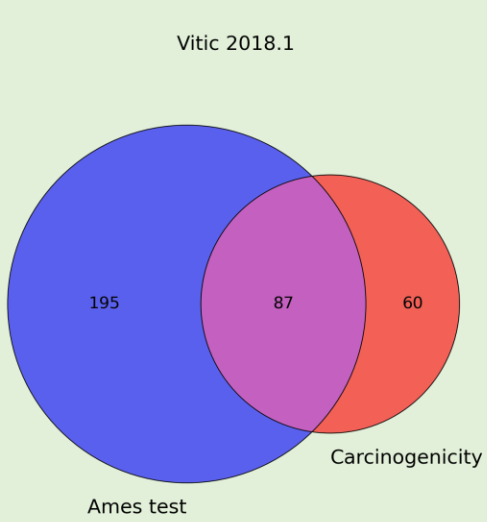

Figure from: Ponting *et al*, "Use of Lhasa Limited products for the *in silico* prediction of drug toxicity", ch. 17 of *In Silico Methods for Predicting Drug Toxicity*, ed. Benfenati (2nd Edition), Springer, *in press*

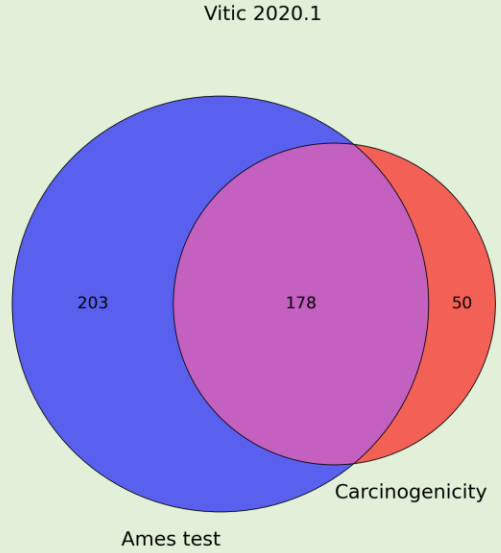
# Addressing the global challenge of N-nitrosamine impurity assessment

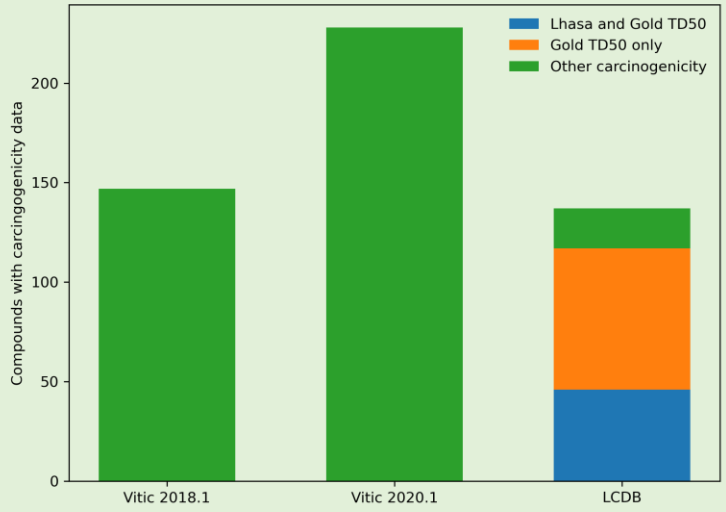
Lhasa Limited

## APPROACH – TOXICITY DATA

- An extensive search of the public literature was performed, resulting in a significant increase in the number of N-nitrosamines in the Vitic database, especially in the set of compounds with both Ames test and rodent carcinogenicity data.
- This dataset was then used to update the statistical Sarah Nexus model
- Data was also provided to expert scientists to investigate for potential updates to the Derek Nexus alerts
- The freely-available Lhasa carcinogenicity database was also updated where the newly-added data was suitable
  - 117 compounds have Gold TD50s
  - 46 of these also have Lhasa TD50s[1]



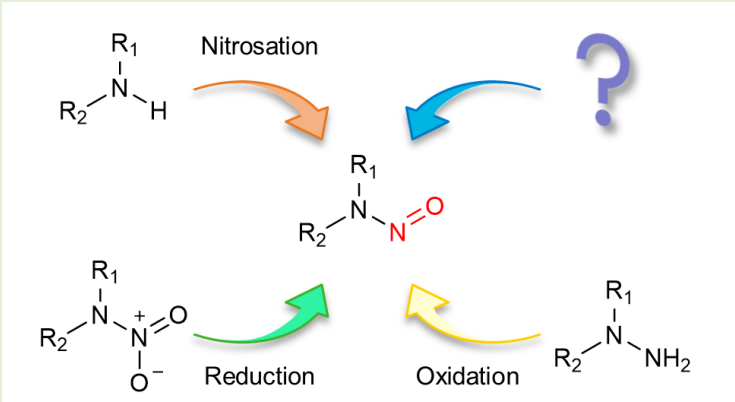




[1] Thresher *et al* (2019), *Toxicol Res*, **25**, 696-703

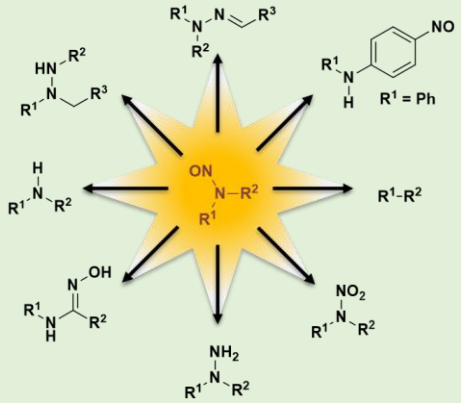
# Addressing the global challenge of N-nitrosamine impurity assessment

Lhasa Limited

## APPROACH – REACTIVITY DATA

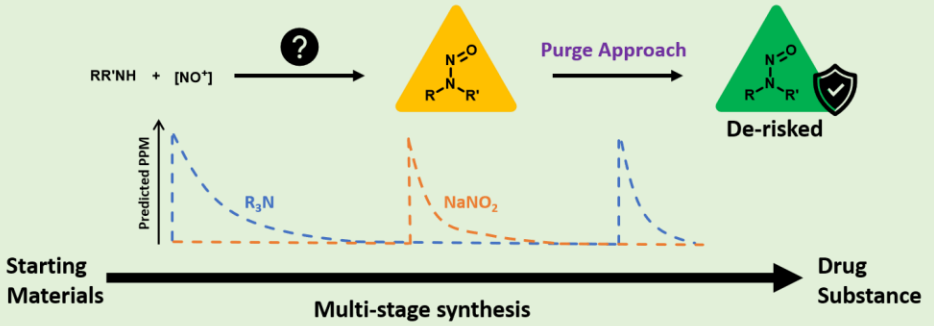



- How are nitrosamines formed? - Understanding conditions necessary for formation informs the risk assessment
  - A thorough review of conditions and mechanisms of formation[1]
  - Developing Mirabilis alerts where formation could occur
    - Provide a warning to ensure the risk is considered




- Identify knowledge of nitrosamine reactivity and identify gaps
  - A thorough review of conditions and mechanisms of reactions[2]
  - Update Mirabilis to reflect current knowledge of reactivity

- Purge assessments can de-risk formation and carryover of nitrosamines[3]
  - Component parts (e.g. Amine and nitrite) must be present together under appropriate conditions to pose a risk
  - Where risk exists, process controls may still allow the risk to be managed

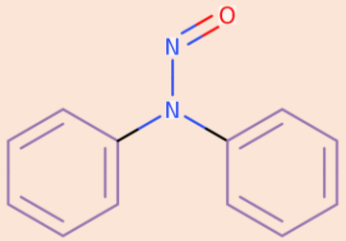



[1] Lopez-Rodriguez *et al* (2020), *Org. Process Res. Dev.*, **24**, 9, 1558–1585
[2] Borths *et al,* N-Nitrosamine Reactivity: A Survey of Reactions and Purge Processes. *Org. Process Res. Dev.* - Manuscript submitted
[3] Burns *et al* (2020), *Org. Process Res. Dev*, **24**, 8, 1531-1535

# Addressing the global challenge of N-nitrosamine impurity assessment

Lhasa Limited

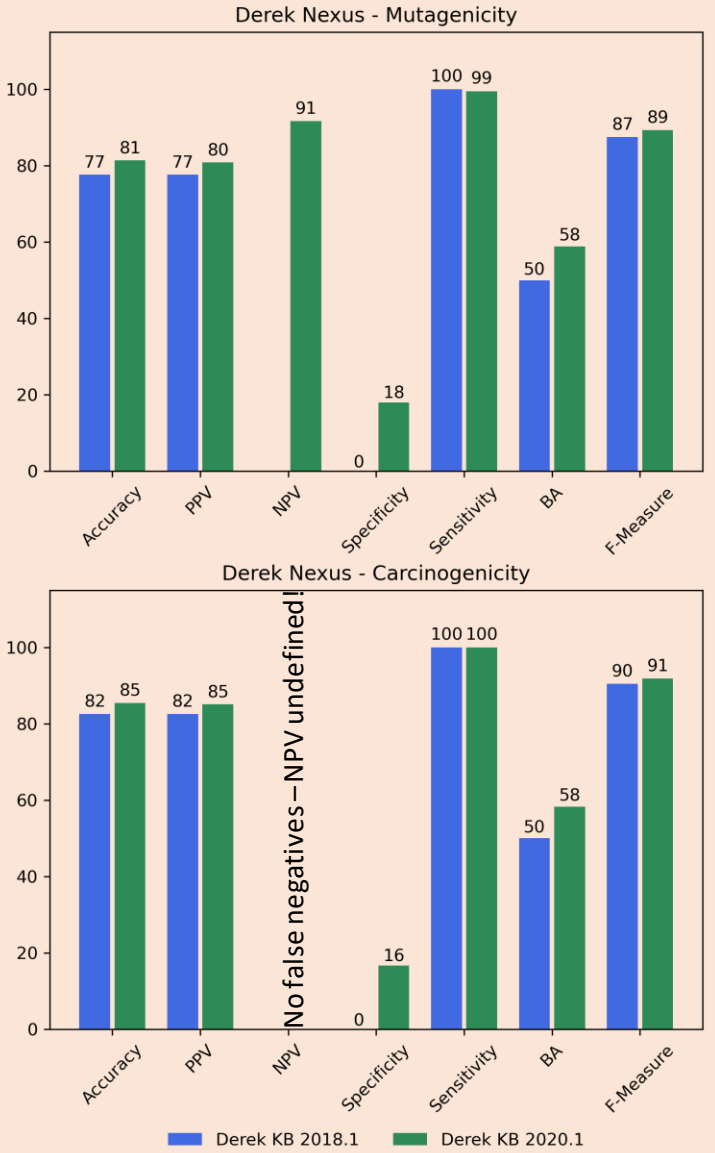
## MAIN RESULTS – UPDATES TO QSAR TOOLS




- Nitrosamines without α-$CH_2$ groups are typically negative
  - Excluded from Derek alert for mutagenicity if dialkyl/aryl
  - *Cyclic* without α-$CH_2$ excluded from carcinogenicity alert
  - Dialkyl where one side is *tert*-butyl derivative also excluded
- Descriptions updated to support expert review of other N-nitrosamines
  - e.g. which features reduce carcinogenic potency?
- Derek Nexus specificity has been improved with negligible cost to sensitivity
  - *One* false negative introduced for Ames – unusual strain, potential alternative mechanisms, positive carc and in Sarah Nexus thus expert review needed
  - Specificity is still low since very conservative approach taken

- Further experimental data will allow further refinement of the alerts
- Results of working group (see talk: Cross and Ponting "*Predicting N-Nitrosamine Activity from Structure-Activity Relationships*") will be incorporated

- Performance statistics for Sarah Nexus not shown since are a statistical tool self-predicting a training set
  - Running updated training set through older model gave 34 FPs and 9 FNs
  - All except 6 FP excluded from training set compounds now report correctly

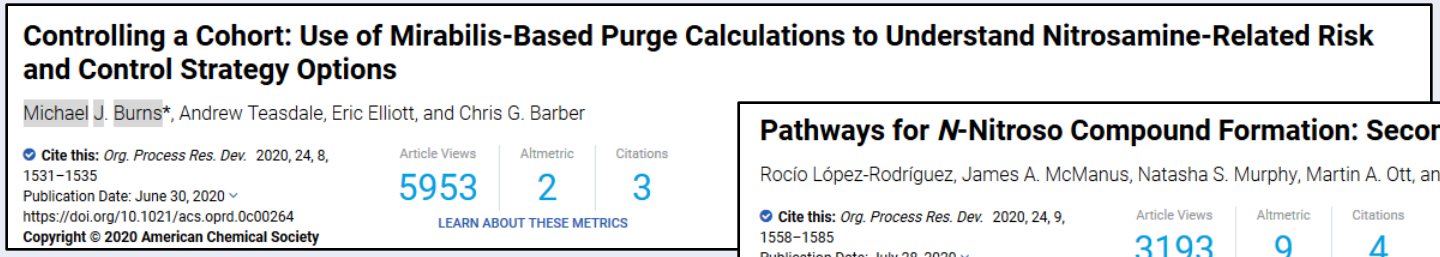


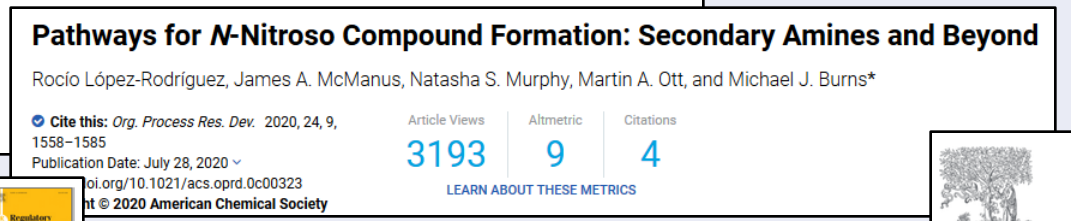
# Addressing the global challenge of N-nitrosamine impurity assessment

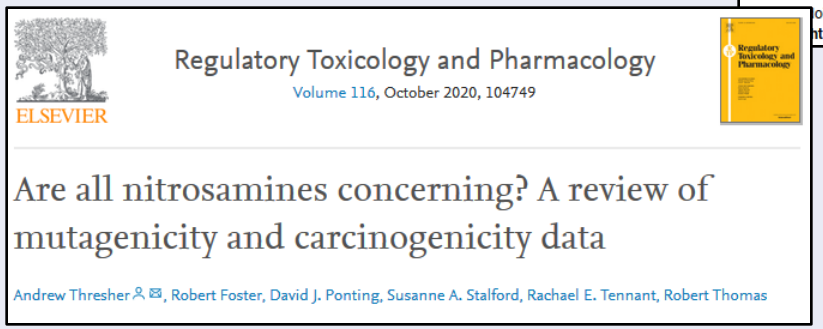
Lhasa Limited

## IMPACT/SIGNIFICANCE

- Curated dataset allows investigation of the reliability of the Ames test (poster #274)
- Updated QSAR tools give higher performance but still err on the side of conservatism
- Significantly expanded Derek alert descriptions aid expert review
- Large dataset based on public data now available in Vitic
- Nitrites in Excipients data-sharing group established to better understand that source of N-nitrosamine contamination
- Additional work being undertaken on both quality and safety aspects
- This work is described in a number of publications




doi.org/10.1016/j.yrtph.2020.104749
doi.org/10.1016/j.yrtph.2021.104875
doi.org/10.1021/acs.oprd.0c00264
doi.org/10.1021/acs.oprd.0c00323