Why do some mutagenic N-nitrosamines not require metabolic activation?

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Introduction

The detection of N-nitrosamines in pharmaceuticals resulted in industry-wide efforts to better understand their carcinogenic potential. The OECD-471 bacterial reverse mutation (Ames) assay is the standard primary screen to assess drug impurities for potential mutagenic risk and forms a core component of the safety assessment data required by regulatory agencies for acceptance of new drug substances. It is therefore important to understand how metabolic conditions in the Ames test may affect its ability to identify potentially carcinogenic N-nitrosamines.

Although it is widely understood that the primary mechanism of mutagenicity for N-nitrosamines usually requires an initial metabolic activation step to form a DNA-reactive diazonium ion (Figure 1), investigation of historical Ames assay data indicates that some sub-classes of N-nitrosamine compounds may undergo spontaneous decomposition to the reactive species or an alternative mechanism of mutagenic activity [Tennant et al., 2023].

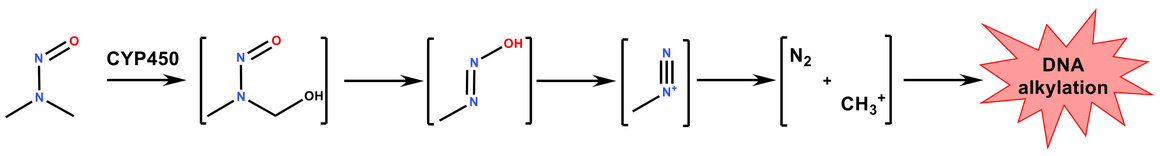


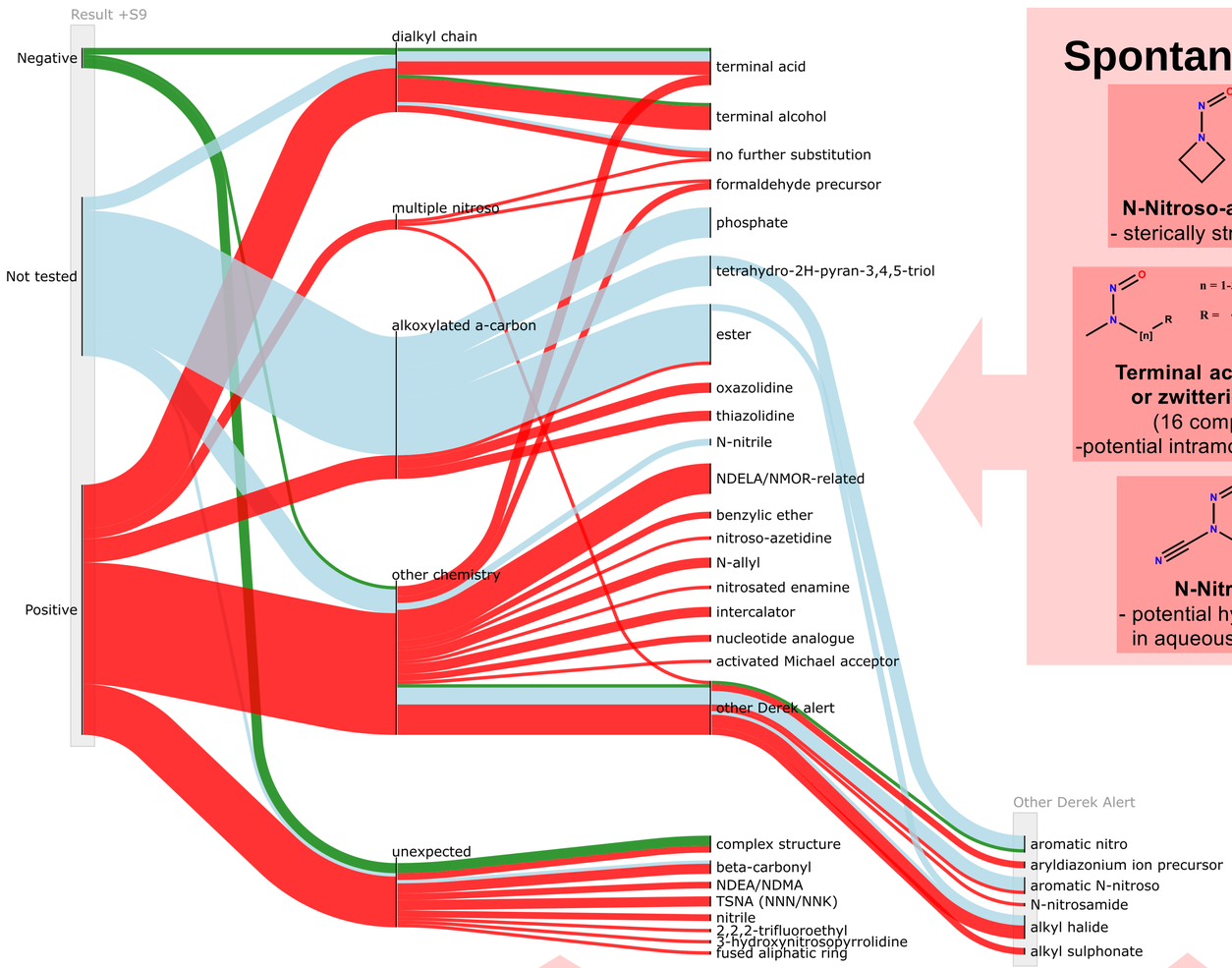
Figure 1. Metabolic alpha-hydroxylation mechanism of mutagenicity for N-nitrosamines.

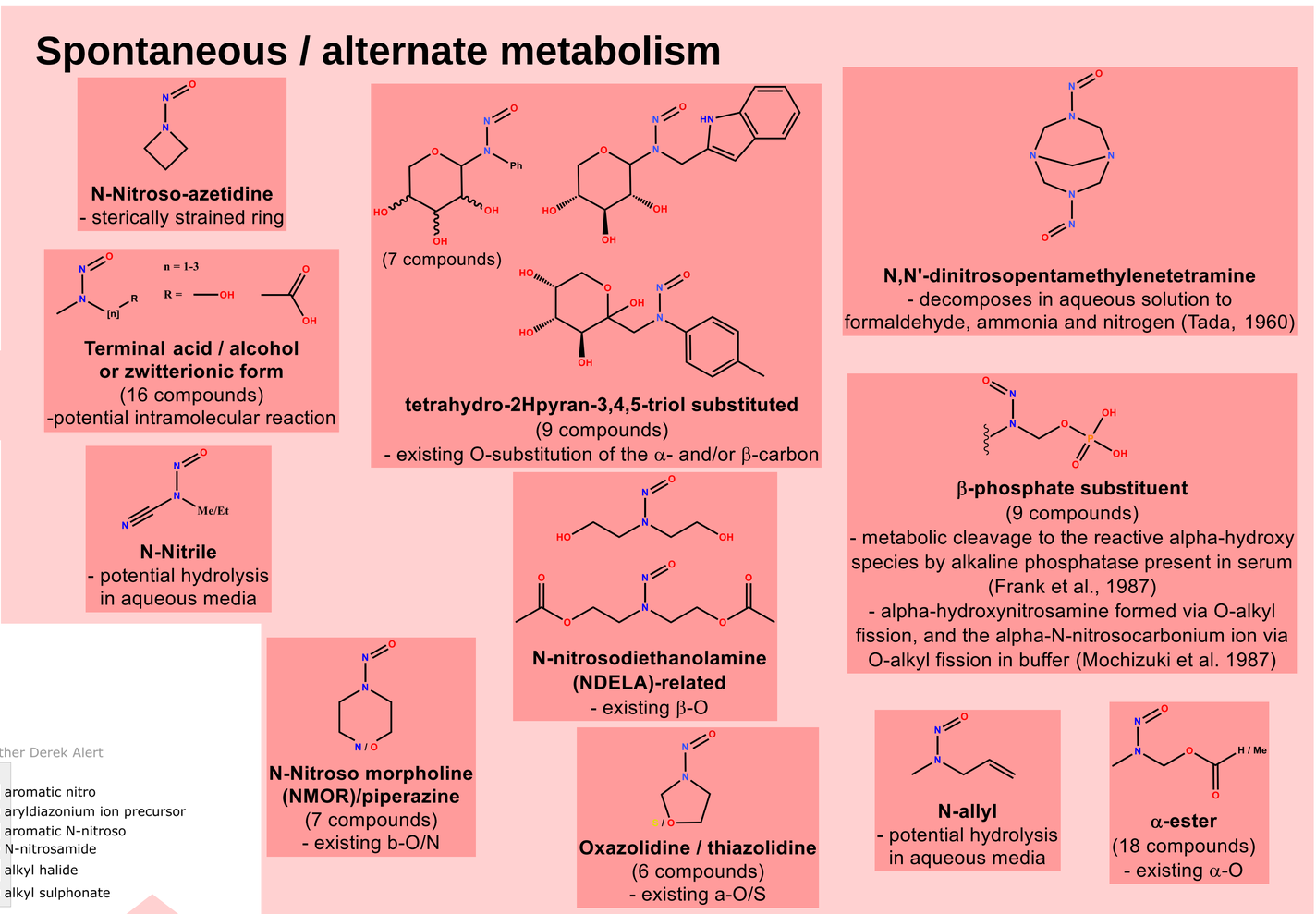
Method

Studies related to alkyl, cyclic, heteroaromatic and phenyl N-nitrosamines, excluding direct-acting mutagens - nitrosocarbamates, nitrosoureas, nitrosoamides and nitrosohydroxylamines - were extracted from the Lhasa Vitic database version 2022.1 and the Vitic Complex Nitrosamines database version 2023.2.0. Data related to the Ames test were subsequently filtered and analysed using KNIME Analytics Platform. Structural analysis was conducted by processing through Derek Nexus (Derek KB 2022 2.0) to identify additional toxicophores, and by expert assessment to identify potentially relevant chemical features.

Results

A data set of 391 N-nitrosamine compounds was collated, of which 126 demonstrated a positive response in the Ames test without metabolic activation. When tested both in the presence and absence of S9, analysis found that a significant proportion (75/80; 94%) demonstrate mutagenic activity in both cases. Molecular features have been identified alongside potential mechanistic rationale for sub-classes that are likely to result in mutagenicity *via* a pathway other than the traditional activation route (Figure 2), either due to the presence of additional toxicophores or properties that allow for spontaneous decomposition or alternate metabolism. A small proportion of compounds remain for which the behaviour cannot yet be explained.





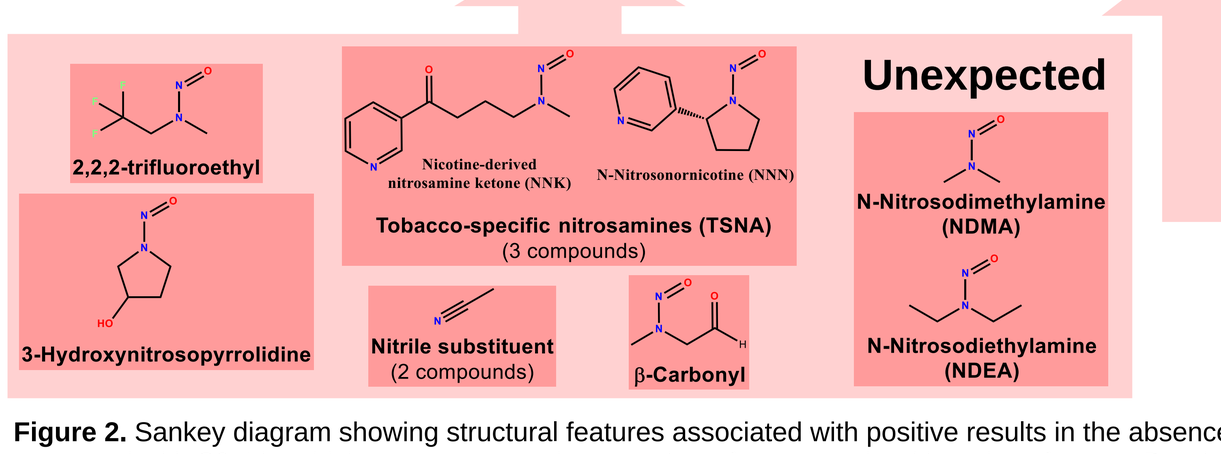


Figure 2. Sankey diagram showing structural features associated with positive results in the absence of S9 activation. Green = negative when tested with S9; red = positive when tested with S9; blue = not tested with S9. Line thickness corresponds to number of compounds with a given feature. Derek alerts evaluated using Derek Nexus v6.2.1, Derek KB 2022 2.0.

Discussion

A large proportion of the compounds identified as active in the absence of S9 bear an existing heteroatom substituent at the alpha carbon (45/126, 36%). These compounds were direct-acting in N-nitrosamine-sensitive strains, TA100, TA1535, WP2 and WP2 hcr−, except for one compound bearing an alpha-tertiary-butyl group. Furthermore, reduced activity was seen for compounds bearing a sterically hindered alpha substituent such as an iso-propyl or sec-butyl group, suggesting that spontaneous decomposition to the reactive alkyl diazonium ion is likely to be responsible for the activity of these compounds.

Twenty-two compounds (17%) contain additional toxicophores which may be responsible for direct-acting mutagenicity. For example, aromatic N-nitroso compounds such as N-nitrosotryptophol were active in TA98 or TA100, indicating the involvement of an alternative mechanism.

Nineteen compounds (15%) demonstrated unexpected activity in the absence of S9, where an alternate mechanism could not be identified. It is possible that these positive results could be due to impurities in the test compound. For example, NDEA and NDMA cannot have any mechanism of activation other than metabolic activation, thus should be negative without S9. Other studies in the relevant strains were negative, suggesting the rare positives may be due to impurities.

Conclusion

Generally, historical studies show that metabolic activation is required for the activation of an N-nitrosamine compound to a mutagen *via* the proposed alpha-hydroxylation mechanism. Where activity is seen in the absence of an exogenous source of metabolic activation, potential for spontaneous decomposition to the mutagen or an alternative mechanism of action can generally be identified. However, activity only in the absence of S9 is very rarely observed. Activity in the absence of S9 is an indication that the compound may undergo an alternate mechanism; however, this often does not preclude the possibility of the alpha-hydroxylation pathway occurring simultaneously in the presence of S9. It is therefore recommended that the Ames test is conducted both with and without S9 activation, as per OECD guidance, to cover either eventuality. This knowledge can be used for better interpretation of Ames test results for N-nitrosamines during chemical safety assessments.

References

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