

# A knowledge based *in silico* system to aid prediction of N-nitrosamine degradants in organic compounds that contain a vulnerable amine moiety



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## Introduction

Nitrites ( $\text{NO}_2^-$ ) and nitrates ( $\text{NO}_3^-$ ) can react with secondary and tertiary amines to generate N-nitroso compounds (NOCs).<sup>1</sup> NOCs are part of the cohort of concern since they are considered potential human carcinogens (Figure 1).<sup>2</sup> Many drug substances and drug products contain secondary/tertiary amine functionality and if contaminated with a source of nitrites/nitrates (from excipients/containers/packaging material) could result in a nitrosation reaction taking place. The industry guidance issued by the FDA<sup>3</sup> stipulates that a risk assessment of degradation pathways should be considered to assess the risk of nitrosamine impurity formation (Scheme 1). As such, our objective has been to capture knowledge of potential N-nitrosation degradation pathways as a collection of transformations that can be queried against a relevant drug substance or drug product.

Chemistry for the N-nitrosation of various types of amines was identified from a combination of pharmaceutical reports, chemistry literature and consultation with experts. A suite of new transformations was then implemented into a knowledge base embedded in the software program Zeneth,<sup>4,5</sup> which has the capacity to be queried to generate prediction results.

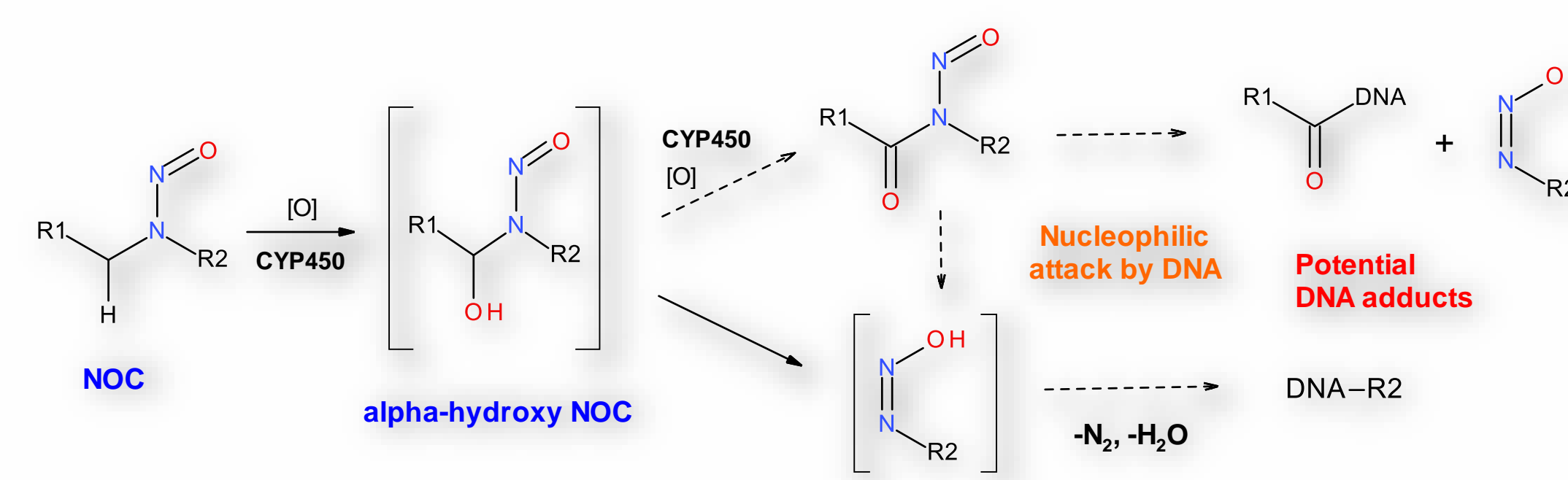
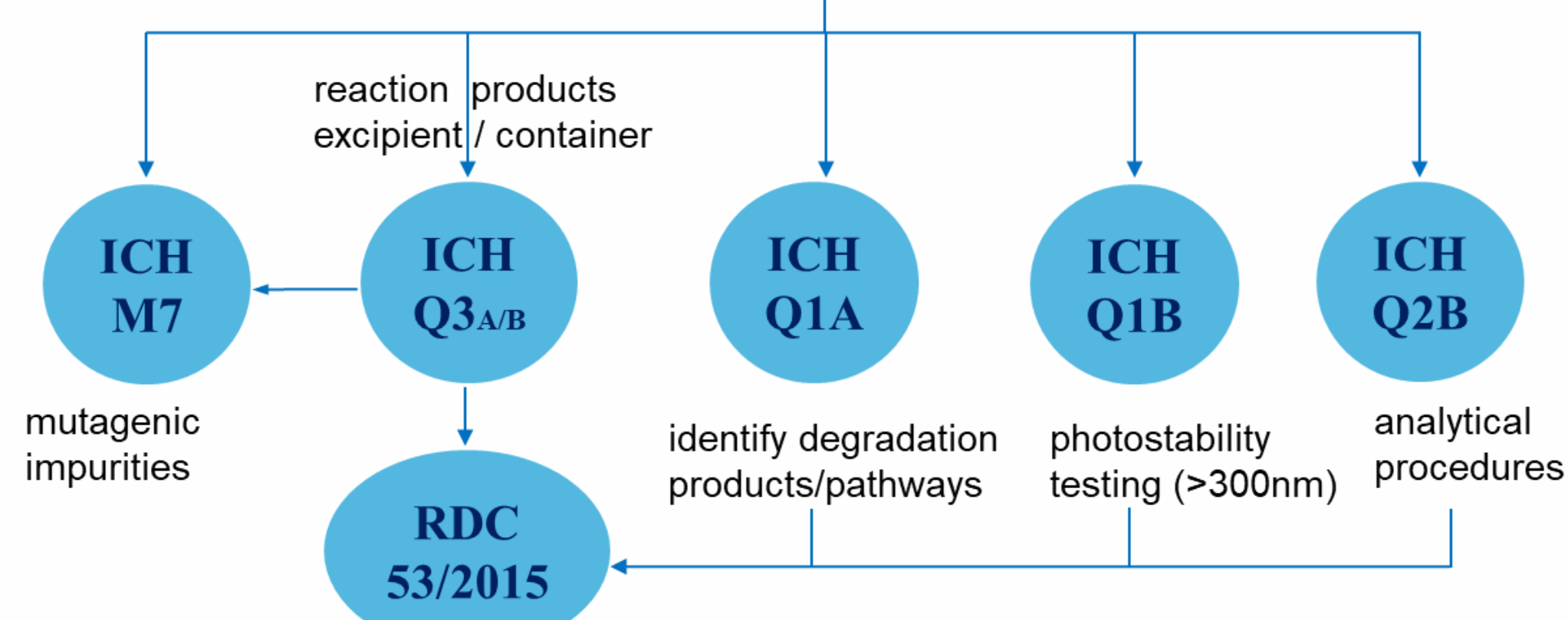


Figure 1: Cytochrome P450-catalysed metabolism of NOCs producing DNA-damaging intermediates.

**Degradation Product:** A molecule resulting from a chemical change in the drug molecule brought about over time and/or by the action of light, temperature, pH, water, or by reaction with an excipient and/or the immediate container/closure system.

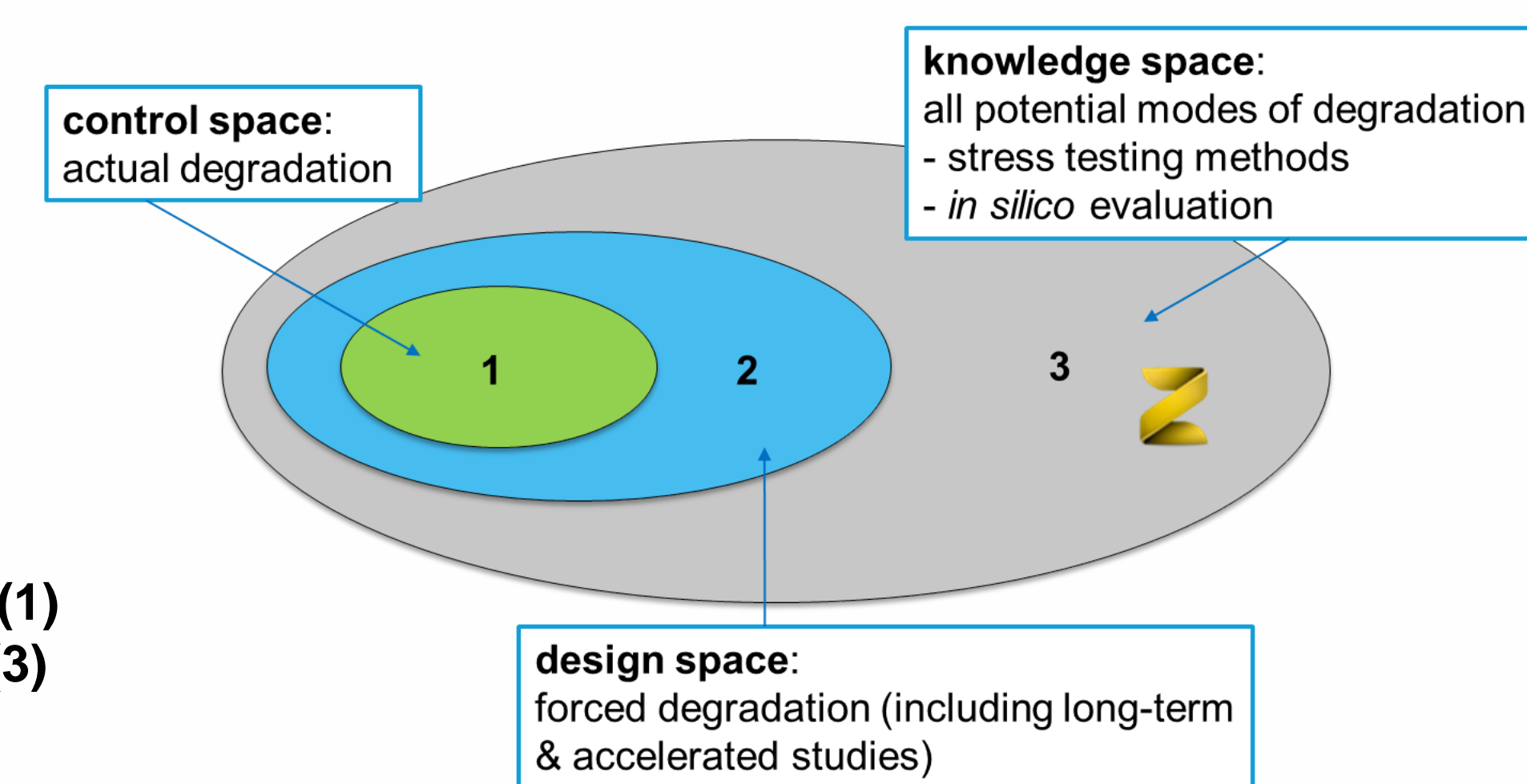


Scheme 1: International regulatory guidance of a new degradation product (including types of studies to be conducted and characterisation of the degradation products).

**Forced degradation studies on the API:**

Ensure all relevant degradation products are "known" by applying stress conditions

Increase the confidence of the control space (1) by having a well-designed knowledge space (3)



## Formation of N-nitroso compounds

Nitrites and nitrates can react with secondary and tertiary amines to generate NOCs.<sup>1</sup> Many API's contain secondary/tertiary amine functionality and if contaminated with a source of nitrites/nitrates (from excipients or container/packaging) then a nitrosation reaction could take place.

A nitrosating agent is regarded as a nitrosonium ion carrier ( $[\text{NO}^+]$  e.g., dinitrogen trioxide, protonated nitrous acid), of electrophilic character, which is prone to attack nucleophiles such as amino compounds.<sup>7</sup> The most general nitrosating reagent is nitrous acid formed by reaction of a mineral acid, with sodium nitrite in water or in a mixture of water and an organic solvent (Figure 2).

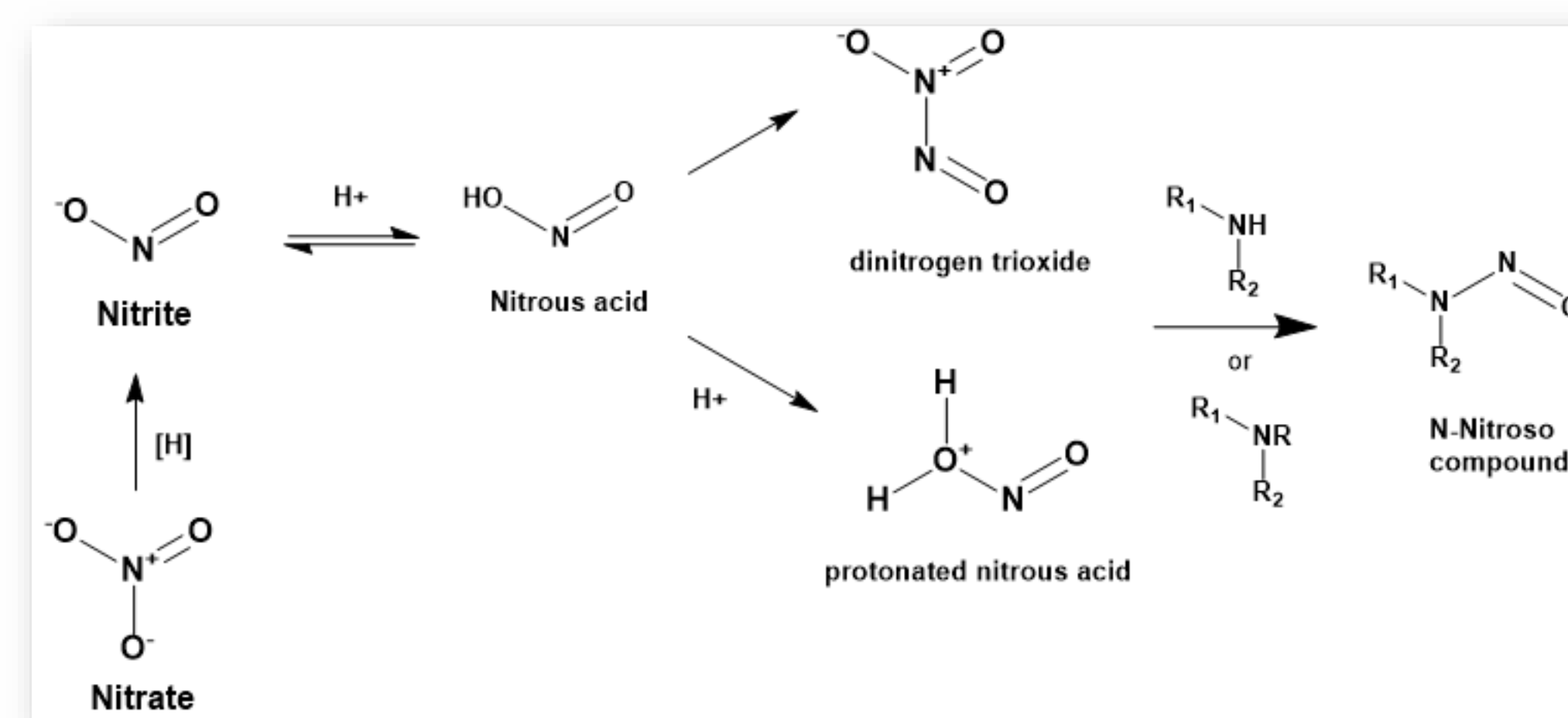


Figure 2: N-Nitrosation of a secondary amine by  $[\text{NO}^+]$  carrier.

## Results and discussion

In order to evaluate the chemistry that should be covered by the knowledge base within Zeneth, a thorough investigation of the literature was conducted and a total of eight new transformations were developed, covering both secondary and tertiary amines. Tertiary amines were found to be significantly less reactive than secondary amines.

Classes of secondary amines (and analogues)	Classes of tertiary amines
<p><b>Secondary amines</b></p> <p><math>\text{R}_1-\text{N}(\text{H})-\text{R}_2</math> R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon</p>	<p><b>1. Aliphatic type bearing an alpha hydrogen and undergoing an N-dealkylation step:<sup>1</sup></b></p> <p><math>\text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{R}_3</math> <math>\xrightarrow{\text{HNO}_2/\text{H}^+}</math> <math>\text{R}_1-\text{N}(\text{R}_2)-\text{N}=\text{O}</math> + <math>\text{R}_3-\text{CHO}</math></p> <p>proposed mechanism:</p> <p><math>\text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{R}_3 \xrightarrow{[\text{NO}^+]} \text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{N}=\text{O} \xrightarrow{\text{NOH}} \text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{OH} \xrightarrow{\text{H}_2\text{O}} \text{R}_1-\text{N}(\text{R}_2)-\text{N}=\text{O} + \text{R}_3-\text{CHO}</math></p> <p>R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon R3, R4 = aliphatic carbon (not multiply bonded or attached to a heteroatom) or hydrogen</p>
<p><b>Hydroxylamines</b></p> <p><math>\text{R}_1-\text{N}(\text{OH})-\text{R}_2</math> R1 = aliphatic carbon or hydrogen R2, R3 = aliphatic carbon, aromatic carbon or hydrogen R4 = aliphatic carbon (not multiply bonded or attached to another heteroatom), aromatic carbon or hydrogen</p>	
<p><b>Secondary amides, carbamates, ureas</b></p> <p><math>\text{R}_1-\text{N}(\text{H})-\text{C}(=\text{O})-\text{R}_2</math> X = C(R3)-R4, N-R5, O or S (divalent) R1, R2 = aliphatic carbon (not multiply bonded) or aromatic carbon R3-R5 = aliphatic carbon (not multiply bonded), aromatic carbon or hydrogen R2 may also be hydrogen in the case of amides.</p>	
<p><b>Hydrazines</b></p> <p><math>\text{R}_1-\text{N}(\text{H})-\text{N}(\text{H})-\text{R}_2</math> R1 = aliphatic carbon or aromatic carbon R2 = aliphatic carbon, aromatic carbon or hydrogen R3 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon</p>	
<p><b>Guanidines</b></p> <p><math>\text{R}_1-\text{N}(\text{R}_2)-\text{C}(=\text{NH})-\text{R}_3</math> R1 = any atom R2 = aliphatic carbon or aromatic carbon R3 = aliphatic carbon, aromatic carbon or hydrogen R4 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon</p>	<p><b>2. Benzylic type (beta carbon is aromatic):<sup>6</sup></b></p> <p><math>\text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{R}_3</math> <math>\xrightarrow{\text{HNO}_2/\text{H}^+}</math> <math>\text{R}_1-\text{N}(\text{R}_2)-\text{N}=\text{O}</math> + <math>\text{R}_3-\text{CHO}</math></p> <p>proposed mechanism:</p> <p><math>\text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{R}_3 \xrightarrow{[\text{NO}^+]} \text{R}_1-\text{N}(\text{R}_2)-\text{CH}_2-\text{N}=\text{O} \xrightarrow{\text{H}_2\text{O}} \text{R}_1-\text{N}(\text{R}_2)-\text{N}=\text{O} + \text{R}_3-\text{CHO}</math></p> <p>R1, R2 = aliphatic carbon (not multiply bonded or attached to another heteroatom) or aromatic carbon R3 = aliphatic carbon (not multiply bonded or attached to a heteroatom) or hydrogen R4 = aromatic carbon</p>
<p><b>Aromatic nitrogen (pyrrole)</b></p> <p><math>\text{R}_1-\text{N}(\text{H})-\text{R}_2</math> R1, R2 = carbon or nitrogen The bonds R1-N-R2 must be aromatic.</p>	

Table 1: New transformation classes for secondary and tertiary amines (nitrosation taking place with nitrous acid under acidic conditions).

## Conclusions

In total, eight transformations have been developed and incorporated into the knowledge base within Zeneth. Generally, tertiary amines are much less reactive than secondary amines because they require an additional dealkylation step (Table 1); the  $\alpha$ -CH bond breakage deemed to be the rate determining step. This difference in reactivity is accounted for when a prediction is made. The addition of this chemistry allows the knowledge base to be used to assess the formation of NOCs for compounds such as norfloxacin, ranitidine, doxylamine and gramine, when the presence of nitrous acid and acidic conditions are indicated.

## References

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