CBG MEB
MEDICINES EVALUATION BOARD

Regulatory experience with nitrosamine impurities in pharmaceuticals

NVT Utrecht 24 April 2024

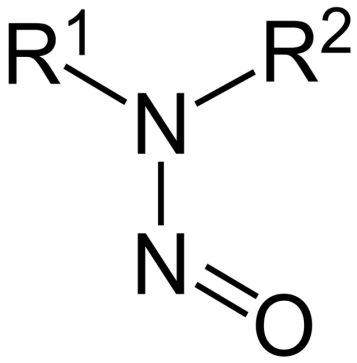
Leon van Aerts

GOOD MEDICINES USED BETTER

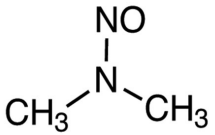
Disclaimer

The views expressed in this presentation are those of the author and do not necessarily reflect those of the MEB, the EMA or any other regulatory authority

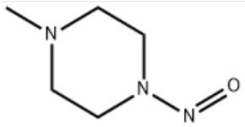
What is a N-nitrosamine?



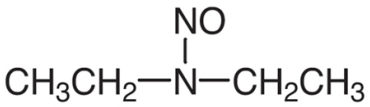
N-nitrosodialkylamine
(N-nitrosamine)



N-nitrosodimethylamine
(NDMA)

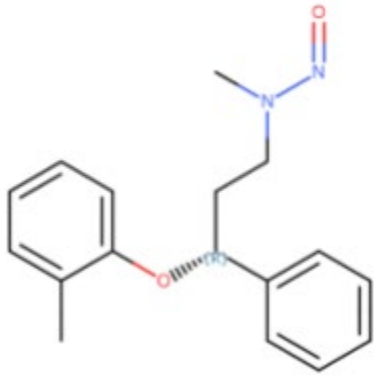


1-methyl-4-
nitrosopiperazine

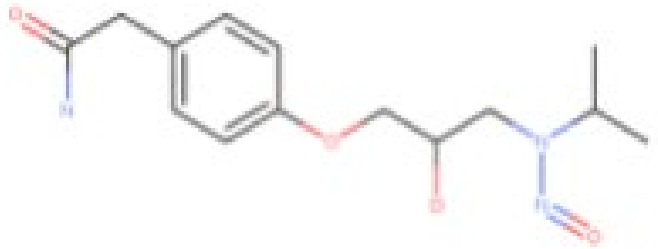


N-nitrosodiethylamine
(NDEA)

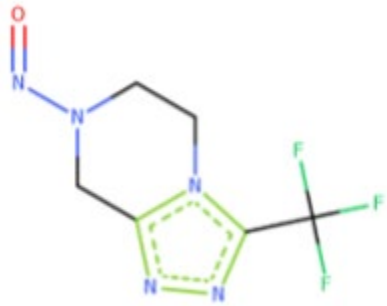
Nitrosamine Drug Substance Related Impurity (NDSRI)



N-nitroso-atomoxetine



N-nitroso-atenolol



Nitroso-STG-19 (NTTP)

Why are N-nitrosamines a problem?

- Most N-nitrosamines have mutagenic and carcinogenic properties.

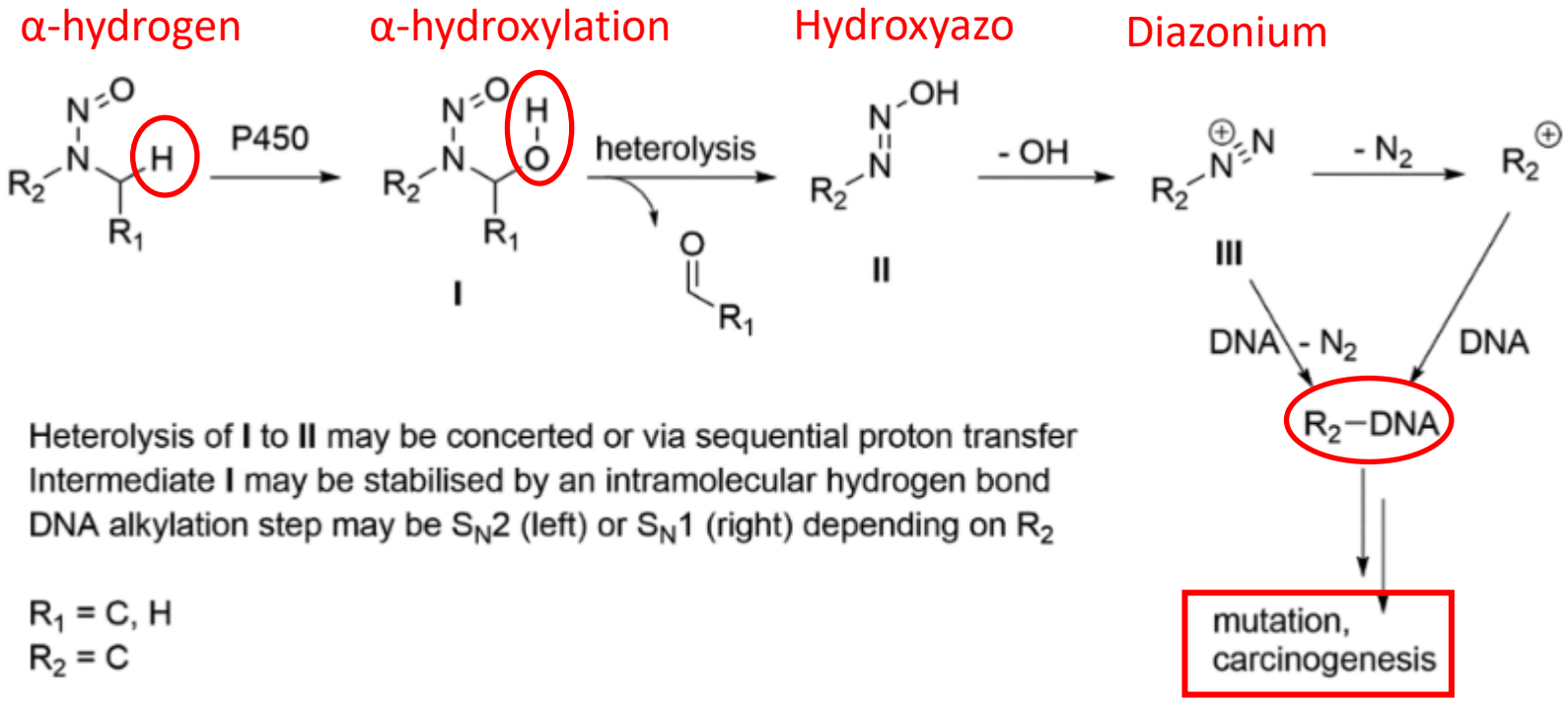


Fig. 1. α-carbon hydroxylation of dialkyl *N*-nitrosamines.

Figure taken from Cross, K. P., Ponting, D., 2021. Developing Structure-Activity Relationships for N-Nitrosamine Activity. Computational Toxicology, 20, 100186.

Why are N-nitrosamines a problem?

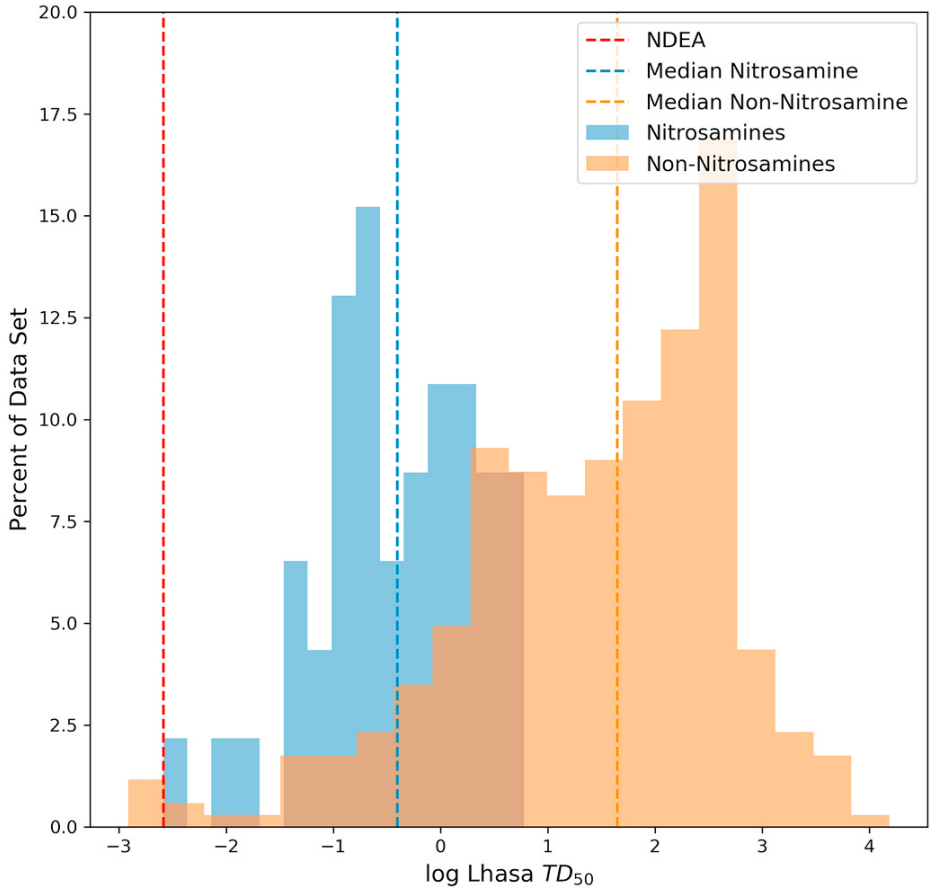
- Genotoxic impurities in pharmaceuticals regulated in ICH M7 guideline

18 July 2023
EMA/CHMP/ICH/83812/2013
Committee for Medicinal Products for Human Use

ICH M7(R2) Guideline on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk
Step 5

- N-nitrosamines belong to the so-called '**cohort of concern**' compounds, meaning that the mutagenic and carcinogenic potency is high compared to the average potency of mutagenic carcinogens and require compound-specific assessment. TTC not applicable.

Why are N-nitrosamines a problem?



- *Median log TD_{50} value for non-nitrosamines 1.654 (equivalent to 45 mg/kg/day)*
- *Median log TD_{50} value for nitrosamines -0,334 (equivalent to 0.46 mg/kg/day)*
- *Yet, carcinogenic potency of N-nitrosamines stretches 4 orders of magnitude*

Figure taken from Thresher A. et al., 2020. Are all nitrosamines concerning? A review of mutagenicity and carcinogenicity data. Regulatory Toxicology and Pharmacology 116 (2020) 104749.

Why are N-nitrosamines a problem?

- Actual presence was previously not well-recognised

of substances that would be impossible to generate unknowingly as synthetic impurities (i.e. aflatoxins, benzidines, nitroso compounds, α-nitrofuryls, azo dyes, endo-

- From: Delaney et al. 2007. An impact analysis of the application of the threshold of toxicological concern concept to pharmaceuticals

- Currently multiple root causes have been identified where a range of nitrosamine precursors can be nitrosated by many nitrosating agents. (Horne S. et al. 2023. Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals. Journal of Pharmaceutical Sciences 112 (2023) 1166–1182)

- An in silico analysis of more than 12,000 small molecule drugs and drug impurities showed that a large proportion of drugs contain secondary or tertiary amine moieties making them susceptible to nitrosation.
  - 40.4% of APIs and 29.6% of API impurities
  - Schlingemann et al., The landscape of potential small and drug substance related nitrosamines in pharmaceuticals. J. Pharm. Sci. 112, 1287–1304

Nitrosamine contaminations – Timeline of events (1/4)

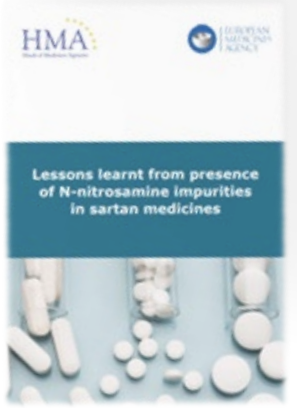
- June 2018:
  - NDMA detected in valsartan
- July 2018:
  - Art 31 referral triggered
- September 2018:
  - Scope extended to include other sartans
  - Other nitrosamines detected in sartans: NDEA, DIPNA, EIPNA and NMBA
- May 2019:
  - Art 31 referral on sartans opinion (updated March 2021)
  - Lessons Learnt Exercise initiated to consider ways to prevent unexpected impurities such as N-nitrosamines from being present in human medicines and to better manage such cases should they occur in the future
- September 2019:
  - NDMA detected in ranitidine – Art 31 referral triggered
  - 'Call for review': EMA advises companies on steps to take to avoid nitrosamines in human medicines

Nitrosamine contaminations – Timeline of events (2/4)

- September 2019:
  - Procedure under Article 5(3) of Regulation EC (No) 726/2004 Nitrosamine impurities in human medicinal products initiated
    - to provide guidance on avoiding presence of nitrosamine impurities to MAHs to consider alongside their knowledge of the manufacturing processes of their products (Nitrosamines call for review).
    - evaluate all available scientific knowledge on the presence of nitrosamines in medicines and advise regulatory authorities on actions to take if companies find nitrosamines in their medicines.
- April 2020:
  - Ranitidine Art 31 referral opinion → suspended
- June 2020:
  - Lessons Learnt Exercise published.
- July 2020:
  - Assessment report on Article 5(3) procedure on nitrosamine impurities in human medicinal products
- August 2020:
  - Q&A on outcome of Art 5.3 procedure (20th update 15 January 2024)

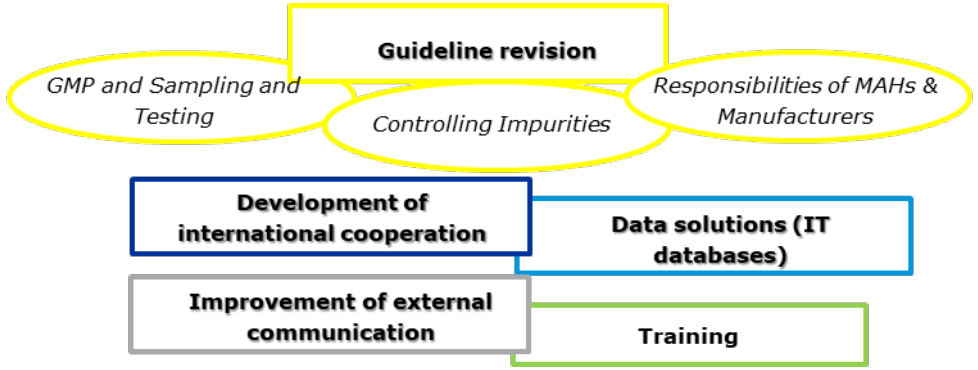
Sartans lessons learnt exercise (1/2)

- **EMA and EU regulatory network Lessons Learnt Exercise** on cases of Sartans with N-Nitrosamine impurities
  - To improve the way impurities in medicines are identified and handled
  - To consider how to prevent such incidents in future
  - To see if the management of such incident can be improved should they occur



- **Report published on 23rd June 2020, implementation plan on 23rd October 2020**

- **Recommendations:** 40 recommendations for the regulatory network



Sartans lessons learnt exercise (2/2)

- **Guideline revision**
  - Clarifying the responsibilities of marketing authorisation holders and manufacturers
  - Publication of detailed information about potential sources of N-nitrosamine impurities and other cohort-of-concern compounds including requirements in order to mitigate the risks of N-nitrosamines

- **Improvement of external communication**
  - Providing more context when explaining risks

- **International cooperation**
  - Consider routinely creating a strategic group once a major incident comes to light

- **Data solutions**
  - Develop or acquire a data tool for mutagenicity assessments for use by assessors at national competent authorities and EMA

Article 5(3) scientific review

Aspects considered by the CHMP during review

- **Root causes** for the presence of N-nitrosamines in human medicines, and measures to be taken to mitigate this
- **Calculation of risk** for exposed patients in case of detection of N-nitrosamines in medicines
- Methodology for **defining limits** for N-nitrosamines in medicines
- **Analytical methods** to identify and quantify N-nitrosamines in active substance and finished products
- **Need to extend the scope** to cover other human medicines than those containing chemically synthesised active substances
- **Need for further studies** to be conducted

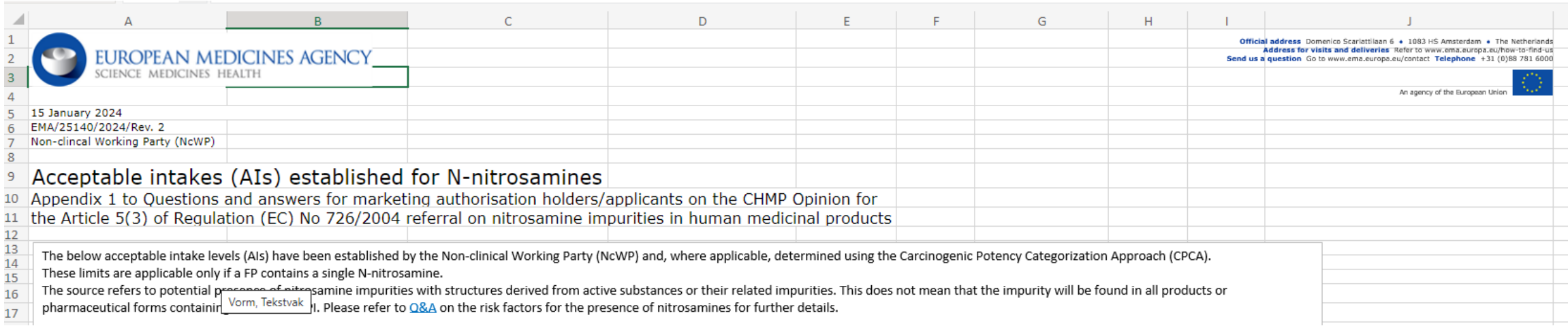
Nitrosamine contaminations – Timeline of events (3/4)

- 2021-2022:
- Nitrosamines call for review led to identification of increasing number of medicines containing nitrosamines
- Same and new small nitrosamines identified, and subsequently also API-derived nitrosamines (NDSRIs), e.g.
  - Metformin: NMDA → temporarily higher limits accepted while CAPs were implemented
  - Rifampicin: MeNP → temporarily higher limits accepted
  - Varenicline: Nitroso-varenicine→ suspended until CAPs implemented

N-Nitrosamine (CAS number)	ng/day*
N-Nitrosodimethylamine, NDMA[1] (62-75-9)	96.0
N-Nitrosodiethylamine, NDEA[1] (55-18-5)	26.5
N-Nitrosoethylisopropylamine, EIPNA[2] (16339-04-1)	26.5
N-Nitrosodiisopropylamine, DIPNA[2] (601-77-4)	26.5
N-Nitroso-N-methyl-4-aminobutyric acid, NMBA[3] (61445-55-4)	96.0
1-Methyl-4-nitrosopiperazine, MeNP[2] (16339-07-4)	26.5
N-Nitroso-di-n-butylamine, NDBA[2] (924-16-3)	26.5
N-Nitroso-N-methylaniline, NMPA[1] (614-00-6)	34.3
N-nitroso-morpholine, NMOR[4] (59-89-2)	127
N-nitroso-varenicline, NNV [5]	37.0
N-nitrosodipropylamine, NDPA (621-64-7)[2]	26.5
N-nitrosomethylphenidate[6]	1300
N-nitrosopiperidine (100-75-4)	1300
N-nitrosorasagilene[7]	18
7-Nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3- a]pyrazine[8]	37
N-nitroso-1,2,3,6-tetrahydropyridine (55556-92-8)	37
N-nitrosonortriptyline[9]	8
N-methyl-N-nitrosophenethylamine, NMPEA (13256-11-6)	8

Nitrosamine contaminations – Timeline of events (4/4)

- 2023:
  - Enhanced Ames test (EAT) protocol
  - Carcinogenicity Potency Categorisation Approach (CPCA)
  - In vivo mutagenicity studies derisking some nitrosamines
  - >100 NDSRIs detected and reported



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact Telephone +31 (0)88 781 6000
An agency of the European Union

15 January 2024
EMA/25140/2024/Rev. 2
Non-clincal Working Party (NcWP)

Acceptable intakes (AIs) established for N-nitrosamines

Appendix 1 to Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products

The below acceptable intake levels (AIs) have been established by the Non-clinical Working Party (NcWP) and, where applicable, determined using the Carcinogenic Potency Categorization Approach (CPCA). These limits are applicable only if a FP contains a single N-nitrosamine.
The source refers to potential presence of nitrosamine impurities with structures derived from active substances or their related impurities. This does not mean that the impurity will be found in all products or pharmaceutical forms containing the AI. Please refer to Q&A on the risk factors for the presence of nitrosamines for further details.

Vorm, Tekstvak

- Appendix 1 contains currently (01-05-2024) 150 nitrosamines

Tools to derive an Acceptable Intake (AI) for N-nitrosamines

Carcinogenicity data providing TD50-derived AI

- ICH M7: AI = TD50/50000
- For many nitrosamines no robust carcinogenicity data are available

Read across

- Use surrogate nitrosamine with sufficiently reliable carcinogenicity data
- Few nitrosamines with robust data
- Similarity too limited

Nitrosamine-specific TTC

- 18 ng/day
- Difficult to comply for some nitrosamines

Carcinogenic Potency Categorisation Approach (CPCA)

- 18, 100, 400 or 1500 ng/day depending on potency category
- Can be applied without experimental data based on SAR principles
- Not applicable for other nitroso compounds or nitrosamines with N in indole ring structure

Enhanced Ames Test (EAT)

- Negative study allows an AI of 1500 ng/d

***In vivo* mutagenicity assays**

- Negative study allows control as non-mutagenic impurities in accordance with ICH Q3A/B

Carcinogenic Potency Categorisation Approach (CPCA)

- Appendix 2 to Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products Carcinogenic Potency Categorisation Approach for N-nitrosamines

- Kruhlak et al 2024. Establishing Recommended Acceptable Intake Limits for N-Nitrosamine Impurities in Pharmaceuticals: Development and Application of the Carcinogenic Potency Categorization Approach (CPCA). *Regulatory Toxicology and Pharmacology*.

Mechanism of Reactivity

- Most potent mechanism of nitrosamine carcinogenicity is through metabolic activation of an α-hydrogen

α-hydrogen α-hydroxylation Hydroxyazo Diazonium

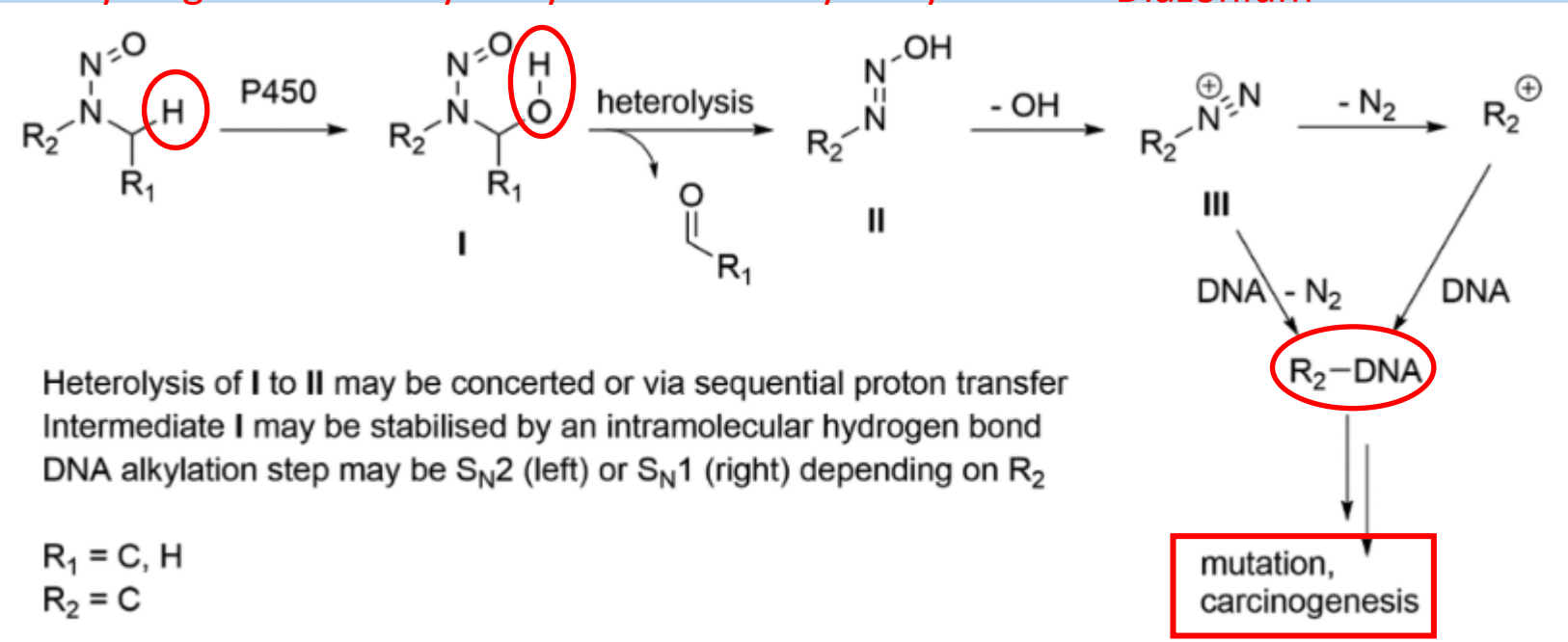


Figure taken from Cross, K. P., Ponting, D., 2021. Developing Structure-Activity Relationships for N-Nitrosamine Activity. Computational Toxicology, 20, 100186.

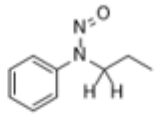
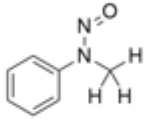
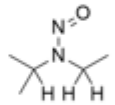
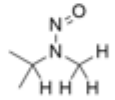
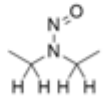
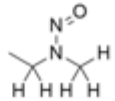
- Metabolic activation and carcinogenesis dependent on nitrosamine structural environment
- Carcinogenic potency decreases if:
  - α-hydrogen abstraction is electronically and sterically unfavored
  - The resulting DNA-reactive species are unstable
  - The impurity and/or metabolic products are rapidly cleared

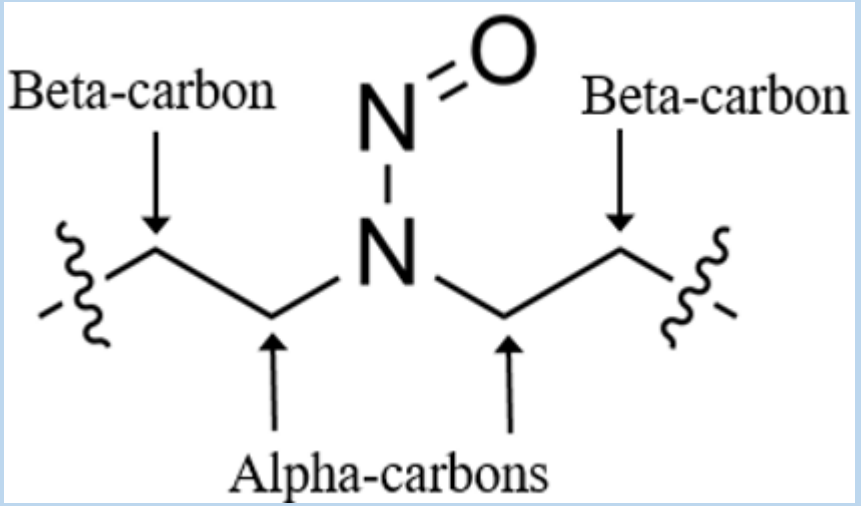
SAR patterns and potency score

- Review of 84 *N*-nitrosamines with either rat TD50 values from the Carcinogenic Potency Database (CPDB) and/or the Lhasa Carcinogenicity Database (LCDB), relative potency classifications as defined by Rao et al. (1979), and/or AI limits based on previously-conducted surrogate analyses.
- (SAR) concepts described in recent scientific publications for *N*-nitrosamine compounds (e.g. Cross and Ponting 2021; Thomas et al. 2022; Ponting et al 2021)
- Potency score leads to assignment to one of 5 different potency categories.

Potency Score =
Alpha-Hydrogen Score + Deactivating Feature Score (sum all relevant features) + Activating Feature Score (sum all relevant features)

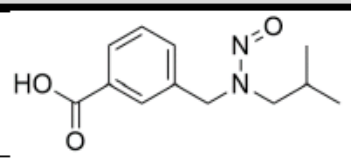
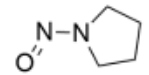
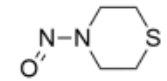
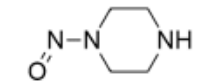
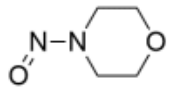
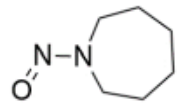
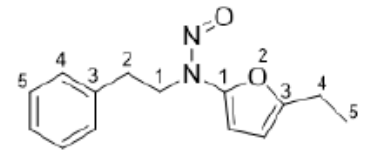
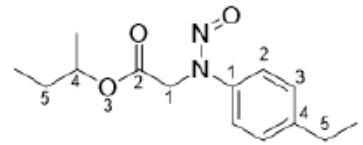
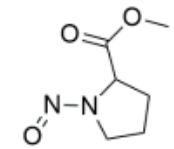
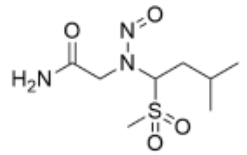
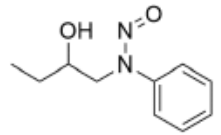
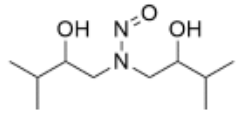
Alpha-Hydrogen Score

Count of hydrogen atoms on each α-carbon, lowest first	Example	α-hydrogen Score
0,2		3[$]
0,3		2
1,2		3
1,3		3
2,2		1
2,3		1

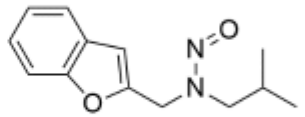
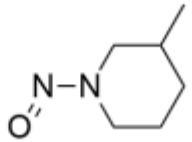


[$]A score of 3 applies when the methylene alpha-carbon is not part of an ethyl group. If the methylene alpha-carbon is part of an ethyl group, a score of 2 should be applied.

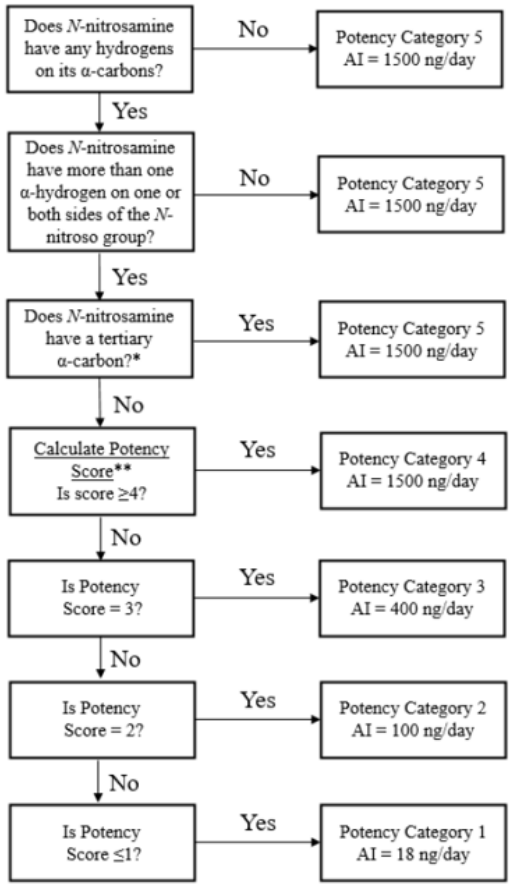
Deactivating Feature Score

Deactivating Feature	Example	Individual Deactivating Feature Score
Carboxylic acid group anywhere on molecule		+3
N-nitroso group in a pyrrolidine ring		+3
N-nitroso group in a 6-membered ring containing at least one sulfur atom		+3
N-nitroso group in a 5- or 6-membered ring*		+2
N-nitroso group in a morpholine ring		+1
N-nitroso group in a 7-membered ring		+1
Chains of ≥5 consecutive non-hydrogen atoms (cyclic or acyclic) on both side of acyclic *N*-nitroso group. Not more than 4 atoms in each chain may be in the same ring.	 	+1
Electron-withdrawing group** bonded to α-carbon on only one side of *N*-nitroso group (cyclic or acyclic)		+1
Electron-withdrawing groups** bonded to α-carbons on both sides of *N*-nitroso group (cyclic or acyclic)		+2
Hydroxyl group bonded to β-carbon*** on only one side of *N*-nitroso group (cyclic or acyclic)		+1
Hydroxyl group bonded to β-carbon*** on both sides of *N*-nitroso group (cyclic or acyclic)		+2

Activating Feature Score

Activating Feature	Example	Individual Activating Feature Score
Aryl group bonded to α-carbon (i.e., benzylic or pseudo-benzylic substituent on *N*-nitroso group)		-1
Methyl group bonded to β-carbon (cyclic or acyclic)		-1

Flow chart to predict Potency Category



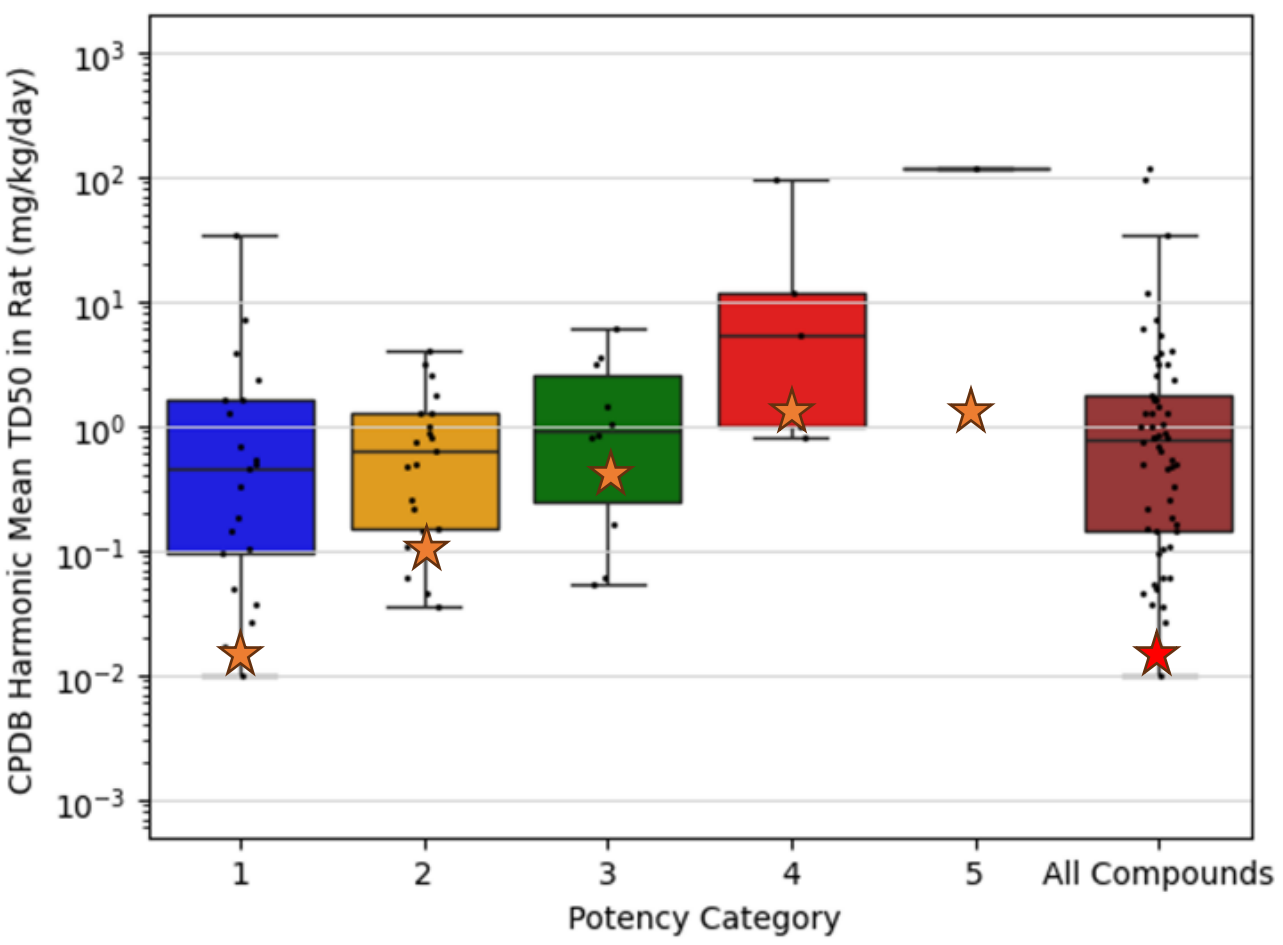
* A tertiary α-carbon is defined as an α-carbon atom in an sp^3 hybridization state, bonded to three other carbon atoms.
** To calculate Potency Score, see Appendix A.

Performance of CPCA

CPCA Potency Category (PC)	Experimental Potency Classification			
	Strong Carcinogen	Carcinogen	Weak Carcinogen	Non-Carcinogen
1	4	6	2	2
2	0	1	0	4
3	0	0	2	3
4	0	0	1	4
5	0	0	1	2

Confusion matrix for 32 training set compounds comparing counts of the predicted CPCA PC to the carcinogenic potency classification as determined by Rao et al. (1979) and experimental negatives reported in the CPDB and by Cross and Ponting (2022) based on rat carcinogenicity data (Data taken from Kruhlak et al. 2024).

Performance of CPCA



Box plots illustrating harmonic mean CPDB rat TD_{50} values against predicted PC for 58 training set compounds (modified from Kruhlak et al. 2024).

Benefits of the Categorization Approach

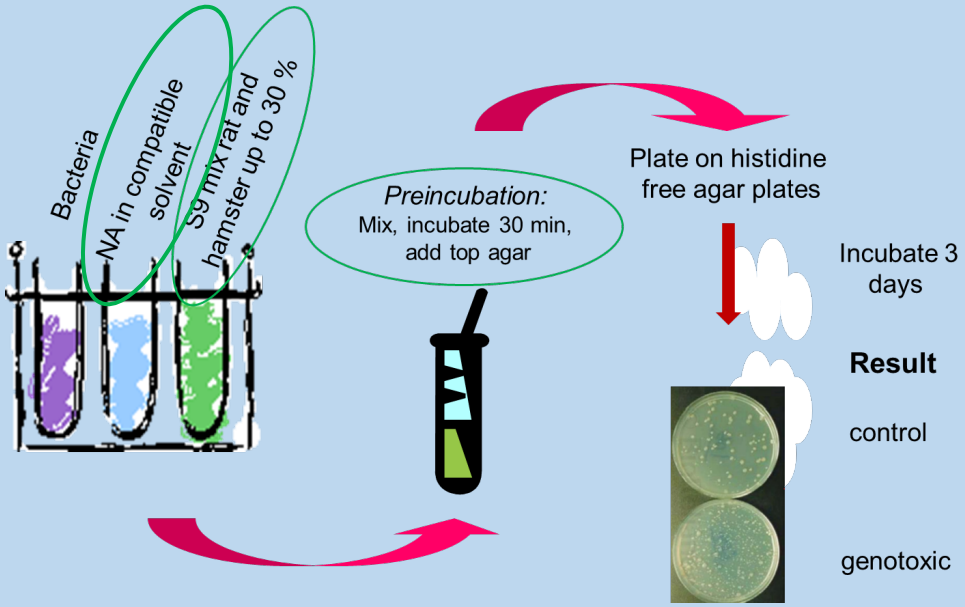
- Applicable to all types of nitrosamines*, including the NDSRIs
- Internationally harmonised approach allowing for faster assessment
- More transparent approach based on SAR principles
- Can be adapted with new data in future amendments
- Leverages larger body of carci data than surrogate analysis—less dependent on a single robust study
- Has been/will be implemented in prediction tools from Lhasa, Instem and Multicase to help establishing limits

*not for nitrosamines with N in indole ring

Enhanced Ames test (EAT)

Enhanced Testing principle: Conditions developed to enhance sensitivity to nitrosamines to minimise possibility of false negative results. The EAT relies on the TG 471 for the principles of the Test, but requests additional test parameters to be added and additional nitrosamine positive controls :

- Solvents to be compatible with the Ames and metabolic systems used
  - Same **solvent volume** for tested NA and positive control
  - Solvent **concentration** as low as possible
- Test with metabolic activation **up to 30% rat and hamster S9** if positive at a certain concentration of S9 no need to test beyond that S9 concentration
- Inclusion of **two nitrosamines known to be positive with metabolic activation as positive controls**.
  N-nitrosamine positive controls needs to be **justified** based on the **anticipated metabolism** of the tested N-nitrosamine (NDSRI)



Future developments

- **More NDSRIs to come**: In an *in silico* analysis of over 12000 drugs and impurities herein it was shown that a large proportion of these molecules contain secondary or tertiary amine moieties as structural features that make them susceptible to nitrosation ,i.e., 40.4% of the analyzed APIs and 29.6% of the API impurities, resulting in thousands of different potential nitrosamine impurities (Schlingeman et al 2022).

- **Other N-nitroso compounds**: E.g. nitrosamides, nitrosoguanidines.

- **Results from research efforts**: Mutamind; HESI.

- **Further development of test paradigms**: *In vivo* mutagenicity assays (protocols, endpoints, interpretation of data); *in vitro* assays (format and applicability)
  - ecNGS
  - BMD – derive AI based on mutagenicity data

- **Regulatory updates**: Nitrosamines Q&A; ICH M7 addendum

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Thank you!
Questions?

GOOD MEDICINES USED BETTER

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Back-up slides

Regulatory guidance

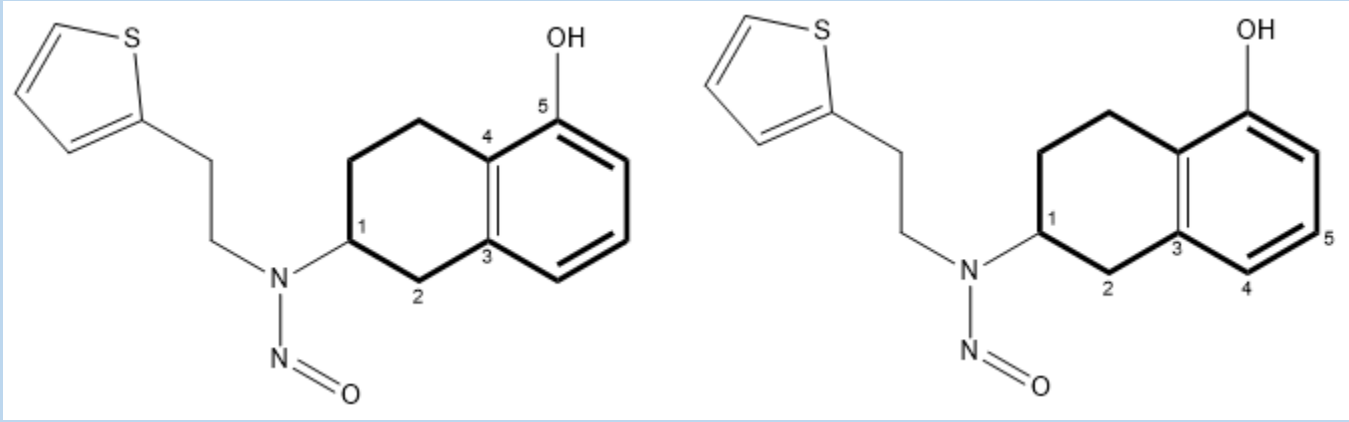
- EMA webpage on Nitrosamine impurities
- Regulatory process described in European Medicines Regulatory Network approach for the implementation of the CHMP Opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 for nitrosamine impurities in human medicines.
- Further details described in Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products EMA/409815/2020 Rev.20.
- Appendixes to Q&A describe EAT protocol, CPCA methodology and list of established AIs

Regulatory network for Nitrosamines

- Rapid Alert Network (RAN): quick exchange of information; determine criticality of a product
- Incident Review Network (IRN): facilitate the exchange of information and to evaluate whether additional measures are needed or whether a different regulatory pathway is warranted
- Nitrosamine Multidisciplinary Expert Group (NMEG): establish a temporary higher limit for critical products
- Nitrosamine Safety Operational Expert Group: NS OEG: Establish an AI for a new NNA; advise on other nitrosamine related safety issues; exchange with interested parties
- Nitrosamines International Steering Group (NISG): Exchange on policies between regulators from different regions
- Nitrosamines International Technical Working Group (NITWG): Exchange on safety and quality issues between regulators from different regions
- Nitrosamine Implementation and Oversight Group (NIOG): oversight and implementation of LLE and Art 5.3 procedure recommendations; exchange with interested parties

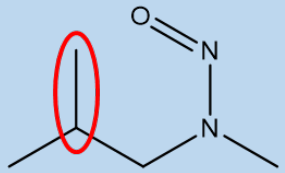
5-atom chain on both sides feature

- Chains of ≥5 consecutive non-hydrogen atoms (cyclic or acyclic) on both side of acyclic *N*-nitroso group. **Not more than 4 atoms in each chain may be in the same ring**.
- A fused ring system/enveloped ring is not viewed as “the same ring”, and the feature will still apply for the structures below, counting through the separate rings
- The feature takes the bulkiness of the compound into consideration

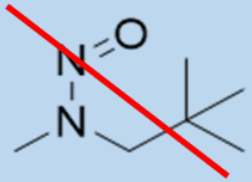


Methyl in beta position

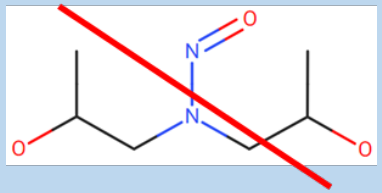
- Positive inductive effect from the methyl group may have a stabilizing effect on the carbocation formed at the alpha-carbon
- Limited to cases where the beta-carbon is sp3 hybridized and a beta-hydrogen atom is present. The methyl should be on a branch point on the backbone



- Quaternary carbon excluded, as the steric hindrance from the beta-carbon substituents are considered to offset the positive inductive effect on the methyl group

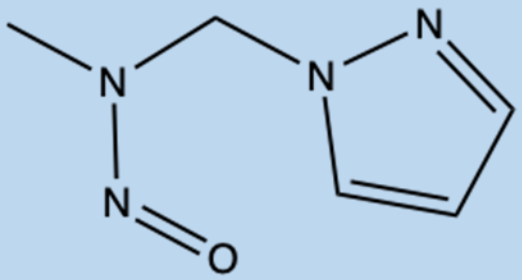


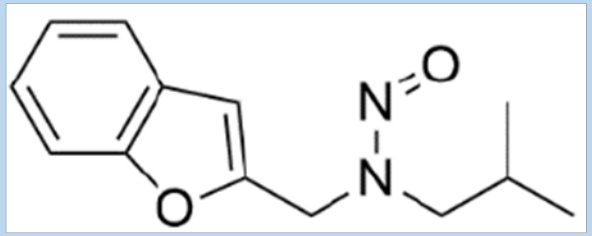
- Cases containing a hydrogen and a hydroxyl group on the beta-carbon are excluded as the methyl group is the part of the backbone and is not on a branch point

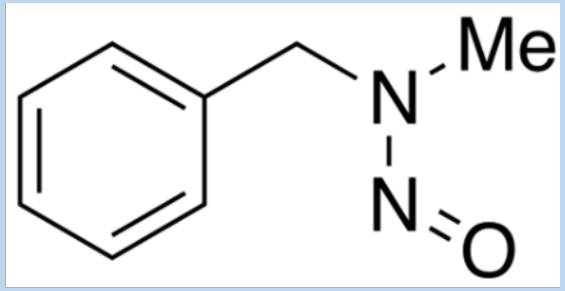


Benzylic/pseudo-benzylic feature

- Applies to any aromatic or heteroaromatic system, including fused rings.







- It does not apply to conjugated acyclic systems.