Using Structure-Activity Relationships to Inform Setting Acceptable Intakes for Nitrosamine Impurities

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CRCG-FDA Workshop on NDSRIs

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Outline

- (Quantitative) Structure-Activity Relationship terminology
- FDA guidance, "Control of Nitrosamine Impurities in Human Drugs"
  - Use of surrogates for setting AI limits
  - Challenges associated with setting limits for nitrosamine drug-substance related impurities (NDSRIs)
- Research on nitrosamine SAR at FDA/CDER
  - Local similarity method to identify nitrosamine surrogates
  - Current research on predicting carcinogenic potency using weighted features and structural fingerprinting
- Future directions

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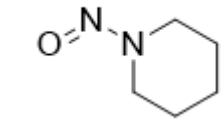
(Q)SAR Terminology

(Q)SAR Terminology

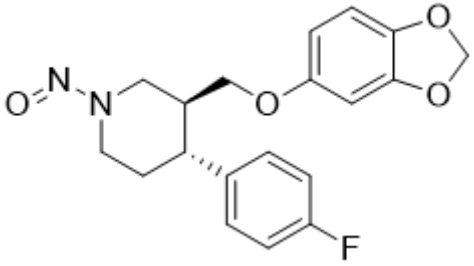
- (Q)SAR = (Quantitative) Structure-Activity Relationship
  - Modeling identifies associations between attributes of chemical structures and biological activity (e.g., toxicity)
  - General assumption: Similar molecules exhibit similar chemical and biological properties

  ⇨ Toxicity can be explained by chemical structure
- QSAR and SAR collectively referred to as (Q)SAR
  - QSAR: Quantitative, often developed through machine-learning, "statistical-based"
  - SAR: Qualitative, human expert derived, "expert rule-based"
- (Q)SAR models used to make a prediction of a chemical's toxicity based on (multiple aspects of) its structure
- Read-across: Use of a structurally similar analog, or "surrogate," and SARs to make a prediction of biological activity for a (data-poor) target molecule



N-Nitrosopiperidine (NPIP)

Read-across



N-Nitrosoparoxetine

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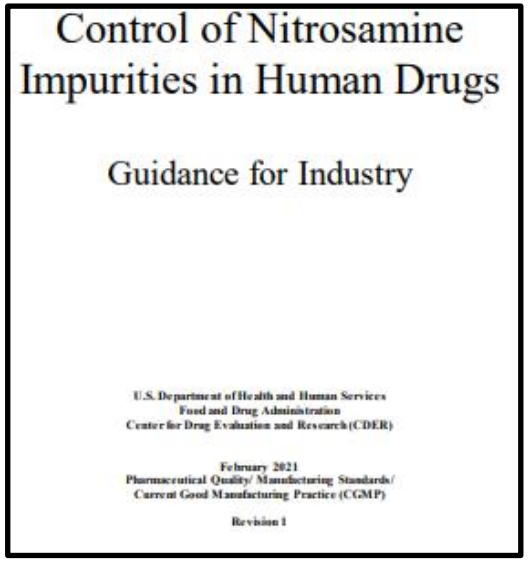
FDA Nitrosamine Guidance

FDA Nitrosamine Guidance

- *Control of Nitrosamine Impurities in Human Drugs*, published September 2020, revised February 2021[1]
- Appendix B: A human acceptable intake (AI) limit can be calculated by linear extrapolation from a rodent TD50 value, which represents the dose at which 50% of animals in a long-term, repeat-dose carcinogenicity study exhibit tumors
  - Convert TD50 (1:2 tumor incidence in rats) to AI (1:100,000 excess cancer risk in humans)
  - Lower TD50 or AI = greater carcinogenic potency
- TD50 values for ~140 nitrosamines reported in the Carcinogenic Potency Database (CPDB)
  - Data available through the Lhasa Carcinogenicity Database (LCDB) (https://carcdb.lhasalimited.org/)
  - Mostly small molecule nitrosamines: Carcinogenic potency ranges over 4 orders of magnitude—some nitrosamines are non-carcinogenic
  - Many studies are not robust
- In the absence of robust empirical carcinogenicity data for an impurity, an AI can be calculated using TD50 value from a structurally related analog, or surrogate


Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

February 2021
Pharmaceutical Quality/ Manufacturing Standards/
Current Good Manufacturing Practice (CGMP)

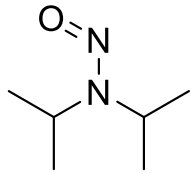
Revision 1

[1]FDA, 2021. FDA Guidance for Industry: Control of Nitrosamine Impurities in Human Drugs (Feb 2021, available at https://www.fda.gov/media/141720/download)

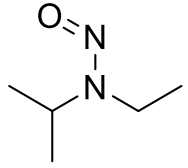
Examples of Nitrosamine Impurities in Pharmaceuticals

- Nitrosamine impurities can be small molecules or nitrosated forms of an API or its fragments (Nitrosamine Drug Substance Related Impurity, or NDSRI)

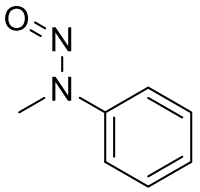
Small Molecule Nitrosamine Impurities



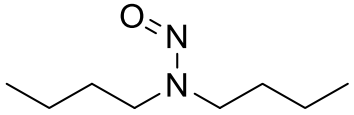
N-Nitrosodiisopropyl amine (NDIPA)



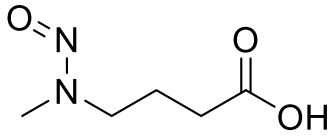
N-Nitrosoisopropyl ethylamine (NIPEA)



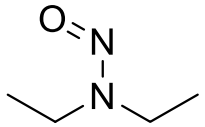
N-Nitrosomethylphenyl amine (NMPA)



N-Nitrosodibutyl amine (NDBA)

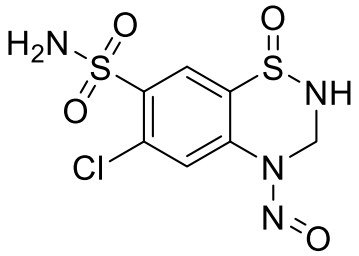


N-Nitroso-*N*-methyl-4-aminobutyric acid (NMBA)

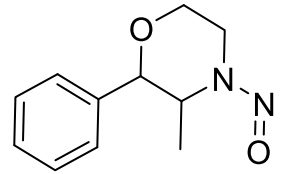


N-Nitrosodiethyl amine (NDEA)

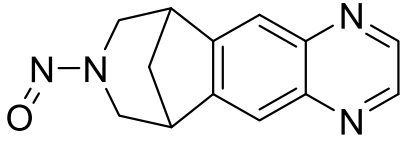
NDSRIs



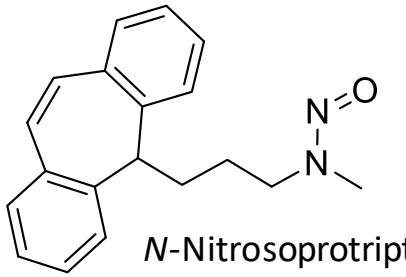
N-Nitrosohydrochlorothiazide



N-Nitrosophenmetrazine



N-Nitrosovarenicline



N-Nitrosoprotriptyline

- Typically, no experimental mutagenicity or carcinogenicity data available for NDSRIs; no new carci data expected
- Often unique to drug substance
- For risk assessment, need to determine 1) mutagenic potential and 2) carcinogenic potency

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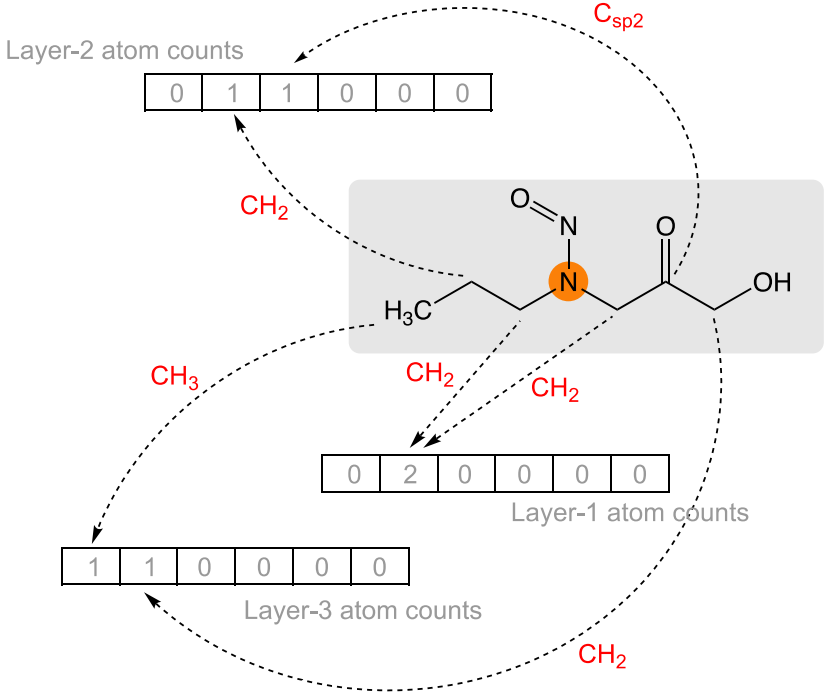
Research Efforts at FDA/CDER

Local Similarity Assessment

- Initial FDA research efforts focused on developing methods to more systematically identify a nitrosamine surrogate for read-across based on local similarity
- Collaborated with MultiCASE Inc. on development of a structural fingerprint to calculate local similarity of *N*-nitroso group environment to guide surrogate candidate identification[1]



Concatenate elements from 5 layers

Fingerprint with encoded information on structural environment around *N*-nitroso group

0	2	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0

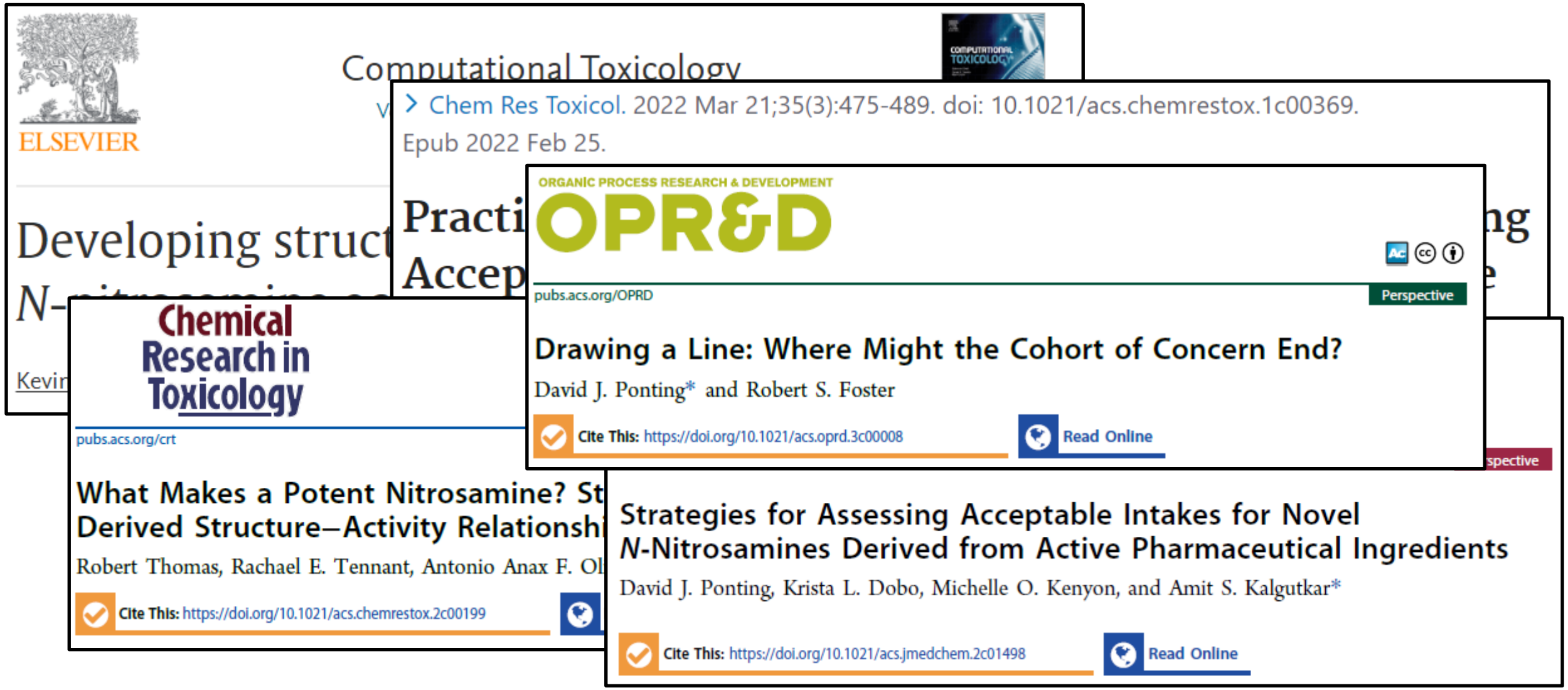
[1]Kruhlak et al., 2022. A New Structural Similarity Method to Identify Surrogate Compounds for Assessing the Carcinogenicity of Nitrosamine Impurities. Society of Toxicology Annual Meeting and Expo, March 2022, virtual poster presentation.

Limitations of Read-Across

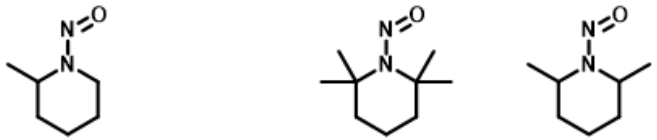
- Limited number of adequately tested surrogates—some areas of chemical space have many studies where none are highly robust (e.g., nitrosated piperazines)
  - Prefer a prediction method that is not reliant upon a single compound
  - Prefer a method that is less subjective (Problem: How similar is similar enough?)
- Develop new prediction methods that consider multiple structural, metabolic and/or physicochemical factors
  - Can leverage a larger body of "training" data for identification of SAR patterns, even if individual studies are not highly robust
  - Can use relative carcinogenic potency data even if no TD50s are available
  - Can integrate chemical reactivity or ADME considerations

A significant body of work has already been published on SAR patterns for nitrosamines…

SAR Patterns Described in Recent Publications

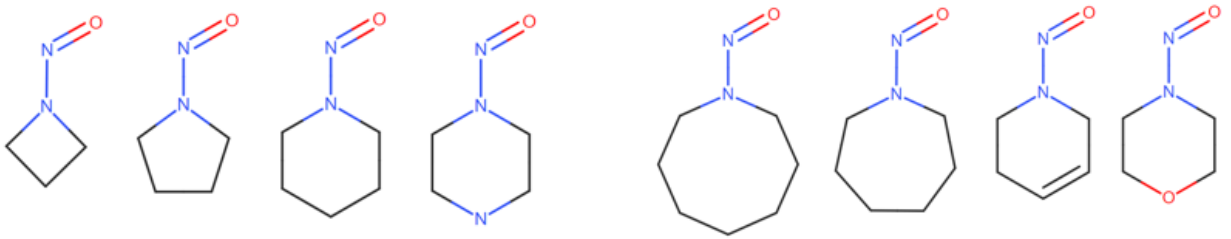


Examples of SAR Patterns for Nitrosamines[1,2]



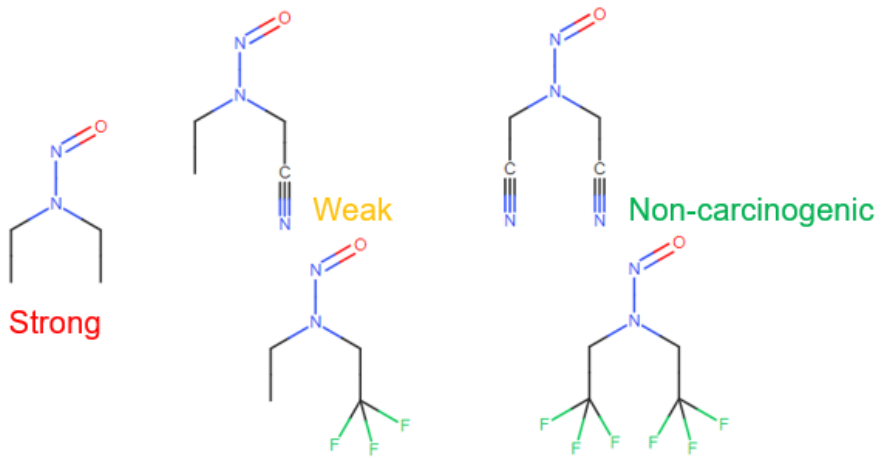
Mutagenic, Carcinogenic

Non-mutagenic, Non-carcinogenic



Weak

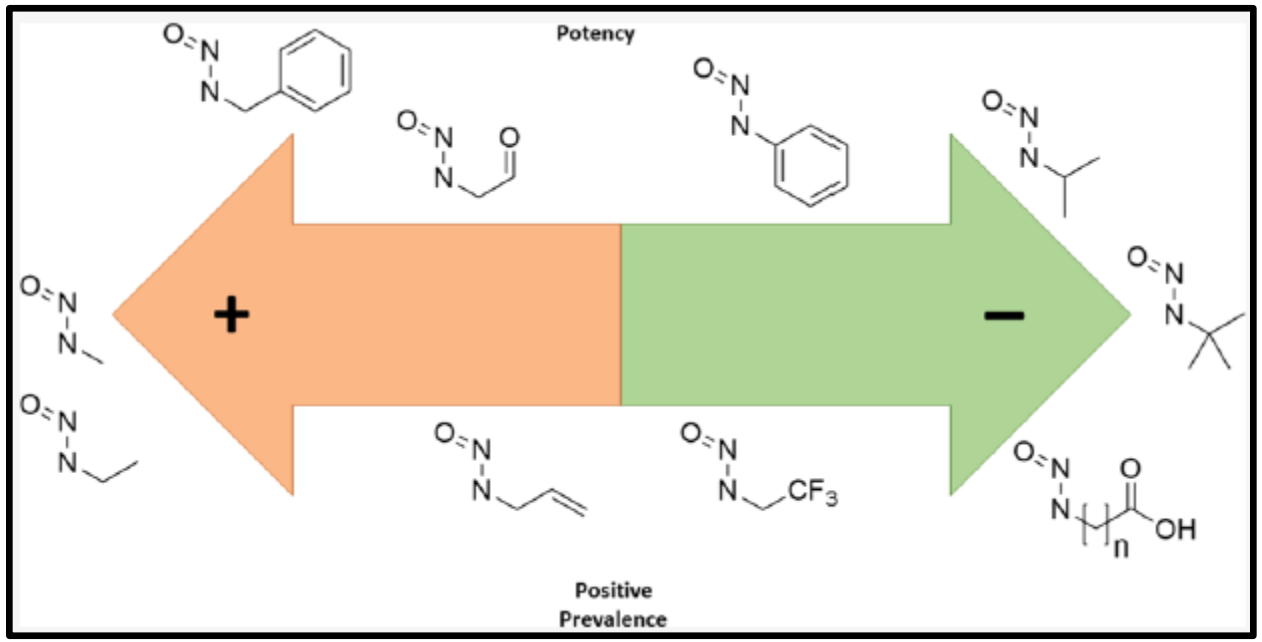
Strong



Strong

Weak

Non-carcinogenic

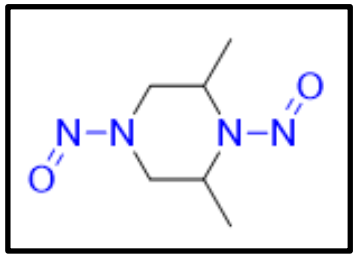


1) Slide adapted and used with permission from presentation by David Ponting, Lhasa Limited; 2) Inset figure reproduced from Thomas, R., Tennant, R.E., Oliveira, A.F., and Ponting. D. J., 2022. Chem. Res. Toxicol. 2022, 35, 1997−2013.

Exploring Relative Potency of Structural Features

- Collaboration with international drug regulatory authorities through Nitrosamines International Technical Working Group (NITWG)
  - Goal of setting an AI by predicting potency using activating and deactivating structural features present in nitrosamine
  - Categorical prediction shows good promise
- Created a data set of 84 nitrosamines with rat TD50s from CPDB/LCDB and/or relative potency classifications from Rao et al. (1979)[1]
  - Molecular weight range of 74 to 278 g/mol (described as "small molecules" on later slides)
  - Corresponds to 90 individual *N*-nitroso groups (some molecules have two groups)

Examined each *N*-nitroso group environment separately



2,6-Dimethyldinitrosopiperazine

[1]Rao et al., 1979. Mutagenicity of Aliphatic Nitrosamines in Salmonella typhimurium. *Mutation Research,* 66, 1—7.

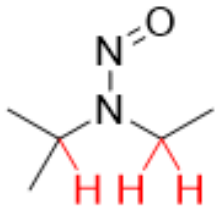
Exploring Relative Potency of Structural Features

- Assigned relative weights to activating and deactivating features based on:
  - SAR trends reported in the published literature (examples on Slides 12 and 13)
  - SAR trends observed in CPDB/LCDB TD50 dataset
  - Mechanistic rationale
- Assessed prevalence of features using molecular fingerprints across 90 *N*-nitroso group environments in small molecule dataset
- Applied the same fingerprints to drugs marketed in US with nitrosatable amines
  - Secondary amines and tertiary dimethyl amines
  - Each *N*-nitroso center evaluated separately

Parent Drug MW (g/mol)	Prevalence (%)
0-250	18
251-500	66
501-750	10
750-1000	3
>1000	3

Alpha-Hydrogen Counts

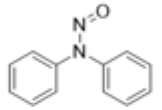
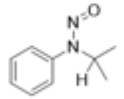
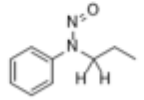
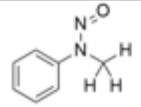
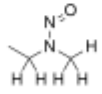
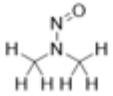
- Hydrogen distribution across alpha-carbon atoms can indicate potential for metabolic activation



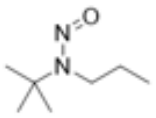
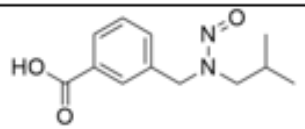
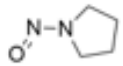
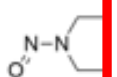
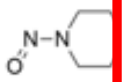
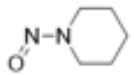
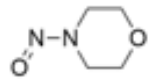
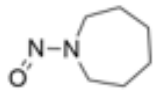
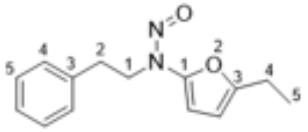
1,2

- Most prevalent category in small molecule dataset is 2,2 (high potency) compared to NDSRIs, which have broader distribution

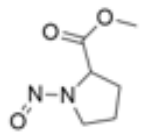
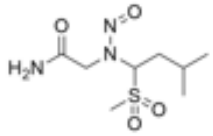
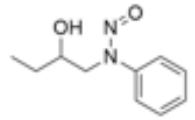
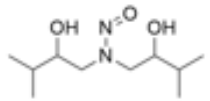
*Activity is very low when tertiary alpha-carbon is present

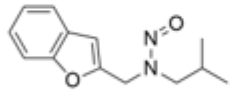
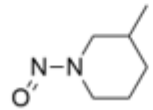
Count of hydrogen atoms on each alpha-carbon, lowest first	Example	Relative Carcinogenic Potency	Prevalence in small molecule dataset (%)	Prevalence in drug dataset (%)
0,0		Low	1	14
0,1		Low	0	5
0,2		Medium/Low*	0	14
0,3		Medium*	10	6
1,1		Low	3	3
1,2		Low	4	13
1,3		Low	2	12
2,2		High	58	13
2,3		High	20	20
3,3		High	1	0

Deactivating and Activating Features

Feature	Example Structure	Deactivating Effect	Prevalence in small molecule dataset (%)	Prevalence in drug dataset (%)
Tertiary alpha-carbon		Very Strong	1	6
Carboxylic acid group anywhere		Strong	4	13
Pyrrolidine		Strong	2	≤2
6-Membered ring with sulfur				≤2
Piperazine				≤2
Piperidine		Medium	4	4
Morpholine		Weak	1	≤2
Azepane		Weak	1	≤2
Two chains of ≥5 atoms		Weak	4	25

17% prevalence of N-nitroso group in a 5 to 7-membered ring

Electron-withdrawing group on one alpha-carbon		Weak	1	9
Electron-withdrawing group on both alpha-carbons		Medium	2	≤2
Hydroxyl group on one beta-carbon		Weak	12	20
Hydroxyl group on both beta-carbons		Medium	6	≤2

Feature	Example	Activating Effect	Prevalence in small molecule dataset (%)	Prevalence in drug dataset (%)
Benzylic-like		Weak	1	10
Methyl on beta-carbon		Weak	3	≤2

Future Directions

- Combined weights of features may be used to generate a prediction of the carcinogenic potency of an untested NDSRI
  - Considers the effects of multiple features in the molecule—important for complex nitrosamines such as NDSRIs
  - Leverages larger body of publicly available carci data than surrogate analysis—less dependent on robust data for a single compound
  - Can refine features and weights with new testing data generated over time, similar to evolution of ICH M7 (Q)SAR models used for other classes of mutagenic impurities
  - Can integrate additional data types and chemical/biological considerations as they emerge (e.g., quantum mechanical descriptors, metabolic insights)
- Structural fingerprinting of NDSRIs can inform future testing and modeling
  - NDSRI chemical space differs substantially from that of small molecule nitrosamines
  - Use identified data gaps to inform compound selection for NDSRI testing initiatives
  - New data and knowledge will improve model performance over time and lead to greater prediction accuracy and coverage

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