Predicting *N*-Nitrosamine Activity from Structure-Activity Relationships

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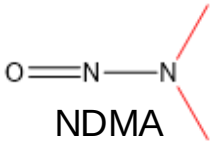
Goals of this Presentation

- It has been observed that nitrosamines vary significantly in carcinogenic potency. This presentation identifies those features that have been observed to lead to a reduction or elimination of potency of dialkyl-*N*-nitrosamines.

- This presentation will illustrate how such features can be used to assist in predicting *N*-nitrosamine potency using read-across analogs.

Background – the problem



- Detection of *N*-nitrosodimethylamine (NDMA) in pioglitazone and ranitidine, led to the European Medicines Agency (EMA) in 2019 requiring that *N*-nitrosamine risk assessments be performed on every marketed product in an aggressive timeline[2] .

- Given the immediate need to address the risk of *N*-nitrosamine impurities in marketed pharmaceuticals (most recently Metformin recalled on August 21, 2020), regulatory agencies have provided provisional Acceptable Intake limits for *N*-nitrosamine impurities based on its structure activity relationship (SAR) with "close" analogs[1,2,3,4]

[1]European Medicines Agency (EMA), 2020b. 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.

[2]European Medicines Agency (EMA), 2019c. Temporary interim limits for NMBA, DIPNA, EIPNA, impurities in sartan blood pressure medicines. EMA/351053/2019 rev 1.

[3]European Medicines Agency (EMA), 2019a. Assessment Report: Referral under Article 31 of Directive 2001/83/EC: Angiotensin-II-receptor antagonists (sartans) containing a tetrazole group. EMA/217823/2019.

[4]Swissmedic, 2019. Potential nitrosamine contamination: Request to perform a risk evaluation. https://www.swissmedic.ch/swissmedic/en/home/news/mitteilungen/aufforderung-zlinhaberinnen-ham.html.

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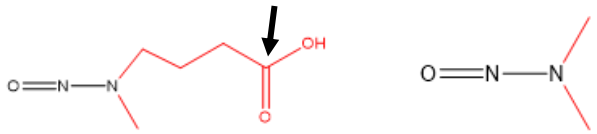
Regulatory limits for *N*-nitrosamine compounds

Example: NMBA

Temporary acceptable Intake limit (AI) for 4-[Methyl)(nitroso)amino]butanoic Acid (NMBA) determined by comparing the Structure-Activity Relationship with *N*-nitrosodimethylamine (NDMA)[1,2]:



NMBA

??

NDMA

AI 96 ng/day[2]

CPDB TD_{50} 0.096 mg/kg/day

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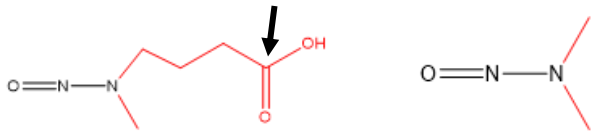

NMBA
AI 1 ug/day
bladder-only
Carcinogen
(recalculated)

NDMA
AI 96 ng/day[2]
CPDB TD_{50} 0.096 mg/kg/day

The carboxylic acid groups tend to reduce carcinogenic potency via α-carbon hydroxylation mechanism, phase 2 conjugation instead[5,6,7]

[5]Helguera, A. M., et al., 2007. Toxicol Appl Pharmacol. 221, 189-202.
[6]Helguera, A. M., et al., 2008. Toxicol Appl Pharmacol. 231, 197-207.
[7]Helguera, A. M., et al., 2010. QSAR Env. Res. 21, 277-304.

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Can we do better at predicting *N*-nitrosamine carcinogenicity potency?

1. Can we define less potent subclasses?
2. Start with analysis of the data
3. Investigate reaction mechanisms (based upon metabolic activation mechanisms) for potency
4. Define patterns for classification by mechanism
5. Categorize patterns to predict broad carcinogenic potency categories (low, med, high, very high)
6. Apply patterns to classify a test compound by mechanism **prior to** finding structure-similarity analogs during read-across.

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Background Work

- Original work performed during development of Genotoxic Impurities book (version 2) Edited by Andrew Teasdale, Ph.D., chapter on, “Compound- and Class-Specific Limits for Common Impurities in Pharmaceuticals”
  – Joel Bercu, Ph.D. Gilead Sciences, primary author

- More scientific investigation was needed specific to *N*-nitrosamine SAR activity
- More *N*-nitrosamine testing was needed and investigation of appropriate conditions for the Ames test within OECD 471

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The Nitrosamine SAR Workgroup

- 23 companies and universities participating
- 52 members
- Led by Instem (Kevin P. Cross, Ph.D.) and
  Lhasa Limited (David J. Ponting, Ph.D.)

- Abbvie, Ache, AstraZeneca, Bayer, Boehringer-Ingelheim, Bristol Myers Squibb, Genentech, Gilead Sciences, GSK, Instem, Janssen, Lhasa Limited, Lilly, Novartis, Merck & Co, Merck KGaA, Pfizer, Sanofi, Servier, Swansea University, Takeda, Teva, Xiphora.

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The Nitrosamine SAR Workgroup (5 Teams)

1. Mutagenicity – Dr. Krista Dobo, Pfizer

- Evaluate alternative mechanisms for mutagenicity based upon metabolic activation
- Discuss the impact of features on mechanism and potency
- Evaluate compounds with mis-matched mutagenicity/carcinogenicity data

2. Carcinogenicity – Dr. Susanne Stalford, Lhasa Limited

- Determine historical (negative) carcinogenicity studies that are reliable
- Determine organ, sex, and species differences in carcinogenicity
- Evaluate in vivo biological effects on carcinogenicity data (adducts, DNA repair)
- Evaluate compounds with mis-matched mutagenicity/carcinogenicity data

3. Data acquisition and sharing – Dr. David Ponting, Lhasa Limited

- Curate public and donated data to create best possible dataset
- Investigate the impact of varying species, sexes, tissues, routes of administration

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The Nitrosamine SAR Workgroup (5 Teams)

4. SAR development – Dr. Kevin P. Cross, Instem

- Improve *N*-nitrosamine structure-activity relationships
- Create categories based on structural features and reaction mechanism
- Identify structural mitigators of metabolic activation pathways affecting potency
- Determine how to select the best read-across analog for a novel *N*-nitrosamine

5. Risk assessment – Dr. Melisa Masuda-Herrera, Gilead Sciences

- Create AI/PDE monographs for 8 reference compounds with reliable carcinogenicity data to reduce the need to read-across only to NDMA or NDEA.
- Determine if the Less-than-Lifetime approach for Acceptable Intake limits is applicable
- Identify nitrosamines that can be mechanistically excluded from the Cohorts of Concern

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The Nitrosamine Ames Testing Workgroup

- Alejandra Trejo-Martin, Gilead Sciences - workgroup leader
  - Evaluating Ames/Carcinogenicity correlation
  - Testing appropriate *N*-nitrosamines supporting this goal and the SAR workgroup
  - A separate workgroup with large overlap of members

  - See:
    - Trejo-Martin, "*N*-Nitrosamines and Bacterial Reverse Mutation Assay as a Predictor for Carcinogenicity, Genetic Toxicity Association meeting, May 3-6, 2021.
    - Trejo-Martin *et al,* (2021) *"*Use of the Bacterial Reverse Mutation Assay to Predict Carcinogenicity of *N*-Nitrosamines", EMM, *in-preparation.*

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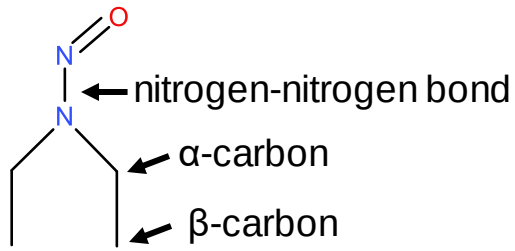
Reaction Mechanisms for *N*-nitrosamines for Mutagenicity

Multiple competing mechanisms of reactivity for dialkyl-*N*-nitrosamines[15]



α-carbon hydrogen mechanism of action dominates high carcinogenicity potency

EMA Assessment Report, Nitrosamine impurities in human medicinal products, June 25, 2020[1]. "It is therefore prudent to consider all *N*-nitrosamines containing a α-hydrogen that can be metabolically activated as potentially mutagenic and carcinogenic to humans, however with different potencies depending on nature of the functional group, specifics of metabolic activation and repair efficiency and capacity."

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[15]Lijinsky, W., 1987, Cancer Metastasis Rev. 6, 301-56.

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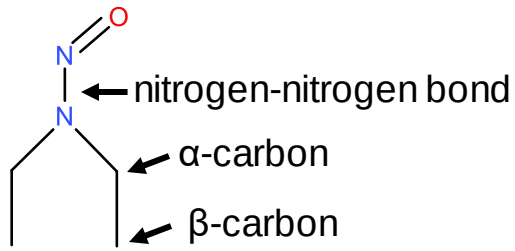

- Structure-Activity for mutagenicity depends upon dominant mechanism
- Slight structure variations change dominant mechanism[16]
  - Steric hinderance – branching, chain length
  - Atom types, bond strengths
  - Local electrostatics (electrophilicity)
- Required metabolism enzymes affects dominant mechanism[16]

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[16]Helguera, A. M., et al., 2008, Toxicol Appl Pharmacol. 231, 197-207.

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Finding Patterns for Nitrosamine Structure-Activity

- Identify the many chemical characteristic patterns affecting potency.
  - Use best carc data to establish trends then reinforce with other data
  - Characteristics overlap and interfere with each other

- First identify classes to explain the data, then patterns to predict potency
  - *N*-nitrosamine TD_{50} potency values span several orders of magnitude.
  - Consider 4 logarithms of potency categories[17]

- How do address the potency multi-functional compounds?
  - Identify dominant mechanisms, their potency class, then assess analogs

[17]Bercu, Compound- and Class-Specific Limits for Common Impurities in Pharmaceuticals in Genotoxic Impurities version 2, Teasdale, Ed. 2020

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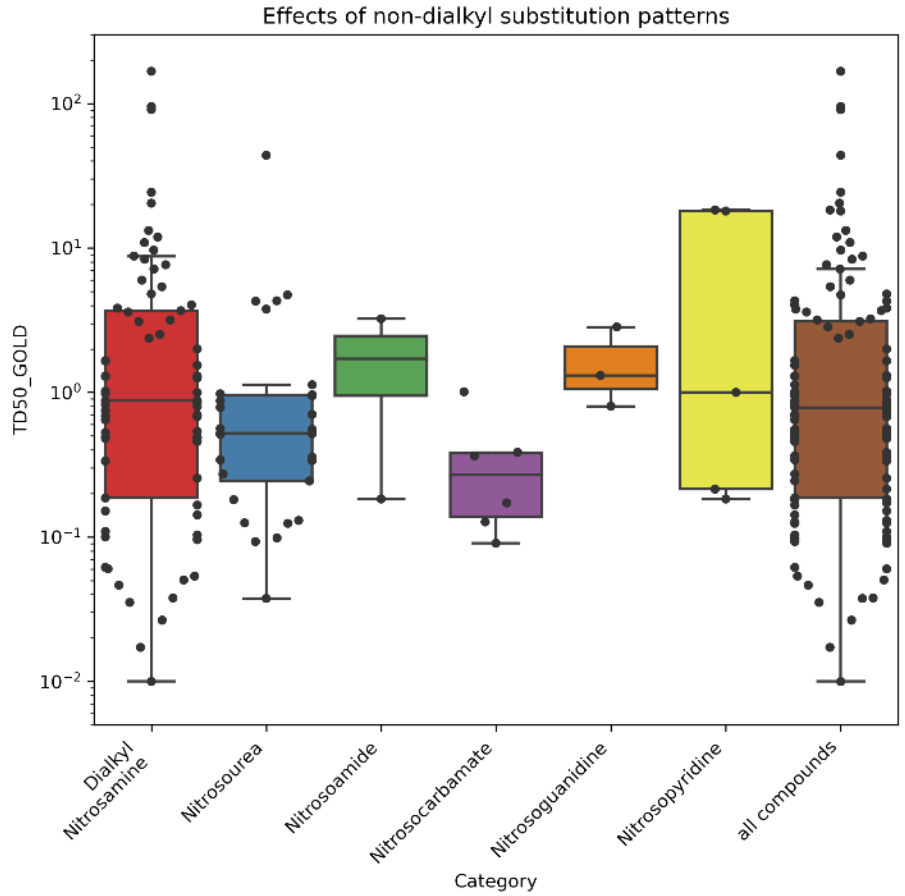
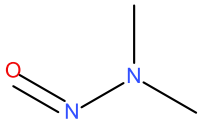
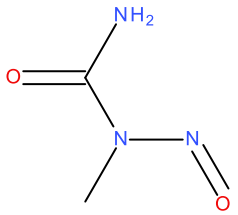
Nitrosamine Potencies Amongst Non-Alkyl Nitrosamines



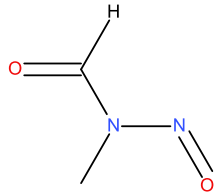
- Some classes are direct-acting mutagens
- Nitrosoureas, nitrosamides and nitrosocarbamates
- Class potency ranges vary
- Fewer high potency non-dialkyl nitrosamines
- Subsequent analysis only on dialkyl nitrosamines
  - Large potency ranges
  - Multiple metabolic pathways



NDMA



methylnitrosourea



methylnitrosamide

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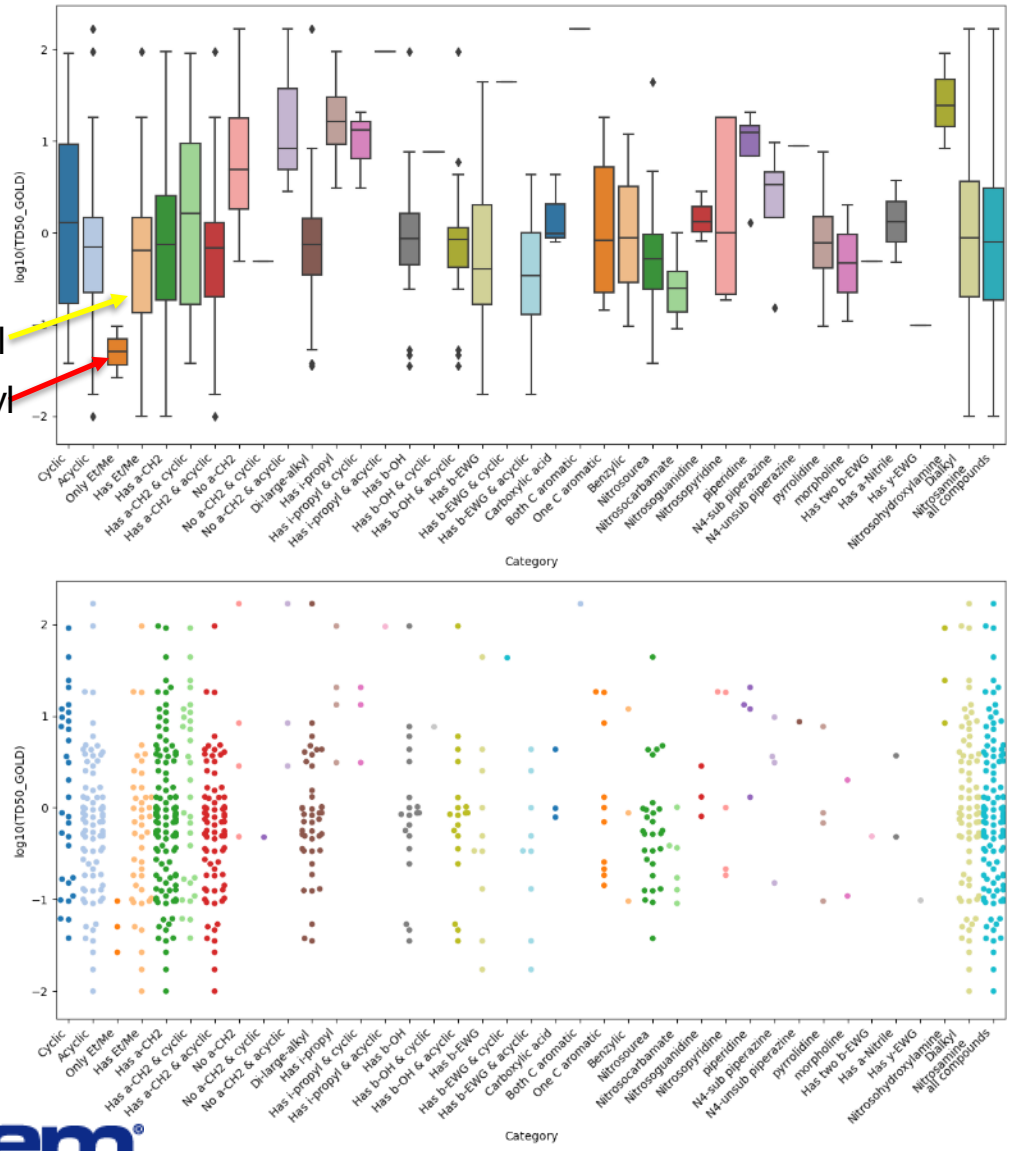
Nitrosamine Features Affecting Potency – Gold TD_{50} values

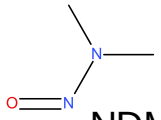


Has **one** methyl/ethyl

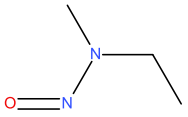
Has **only** methyl/ethyl

Many different features can affect potency

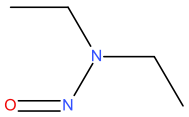
- 4 orders magnitude range of TD_{50} values
- Having only methyl and ethyl groups is very potent
- Methyl or ethyl group plus other group has a large range
- Chain lengths
- α-carbon substitution
- Acyclic vs cyclic compounds (ring size)
- β–electron-withdrawing groups (frequency and type)
- β–carbon hydroxylation
- Multiple substituents and low observations complicate analysis
- New 2-year rodent bioassay data unlikely



NDMA



NMEA



NDEA

Has **only** methyl/ethyl

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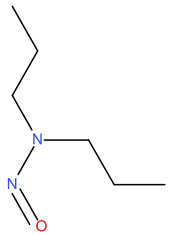
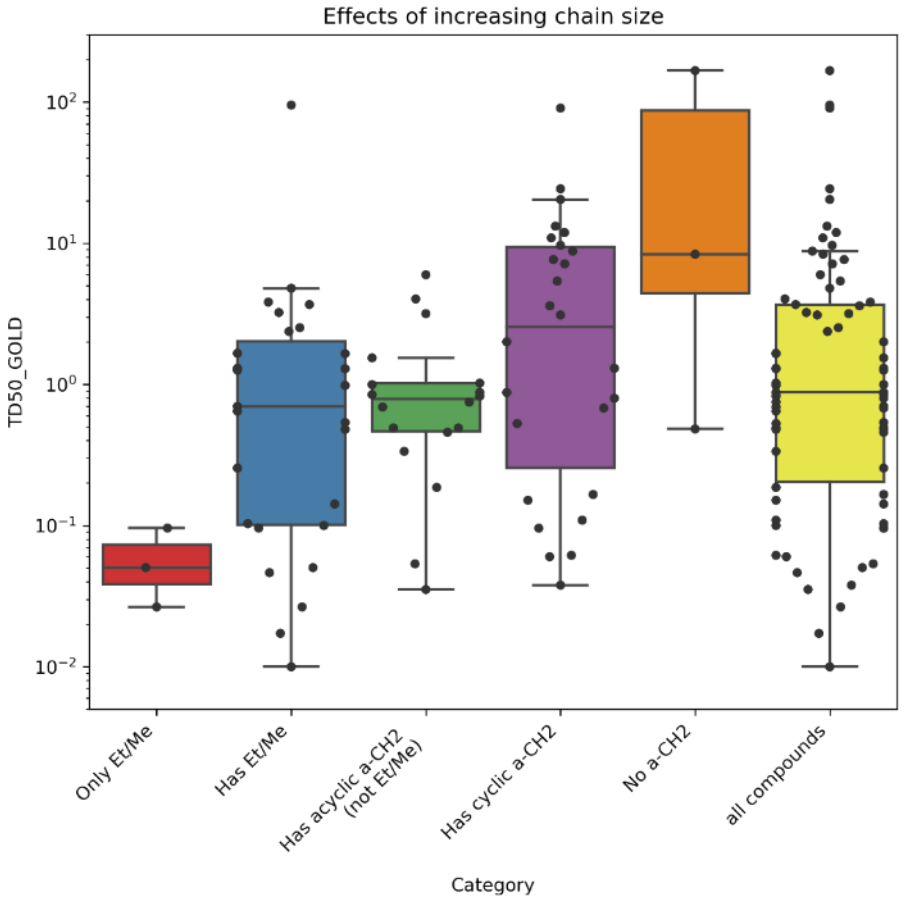
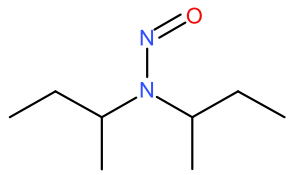
Effects: Increasing Chain Length



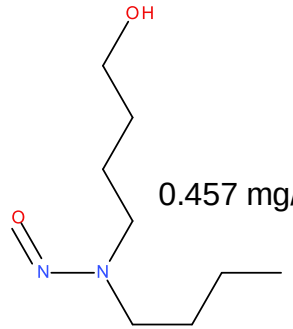
0.186 mg/kg/day



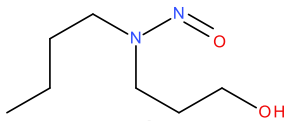
- Methyl and ethyl groups are very potent
- Longer chain lengths can decrease potency
- Cyclic compounds potency depends on ring size
- 0 or 1 α-carbon hydrogens compounds lack potency



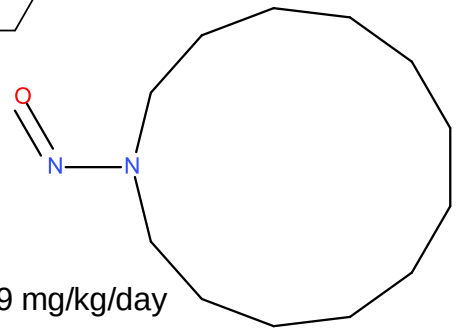
Negative



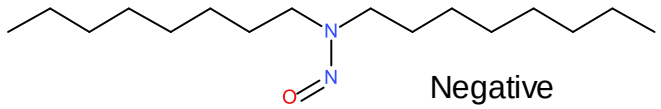
0.457 mg/kg/day



Negative



10.9 mg/kg/day



Negative

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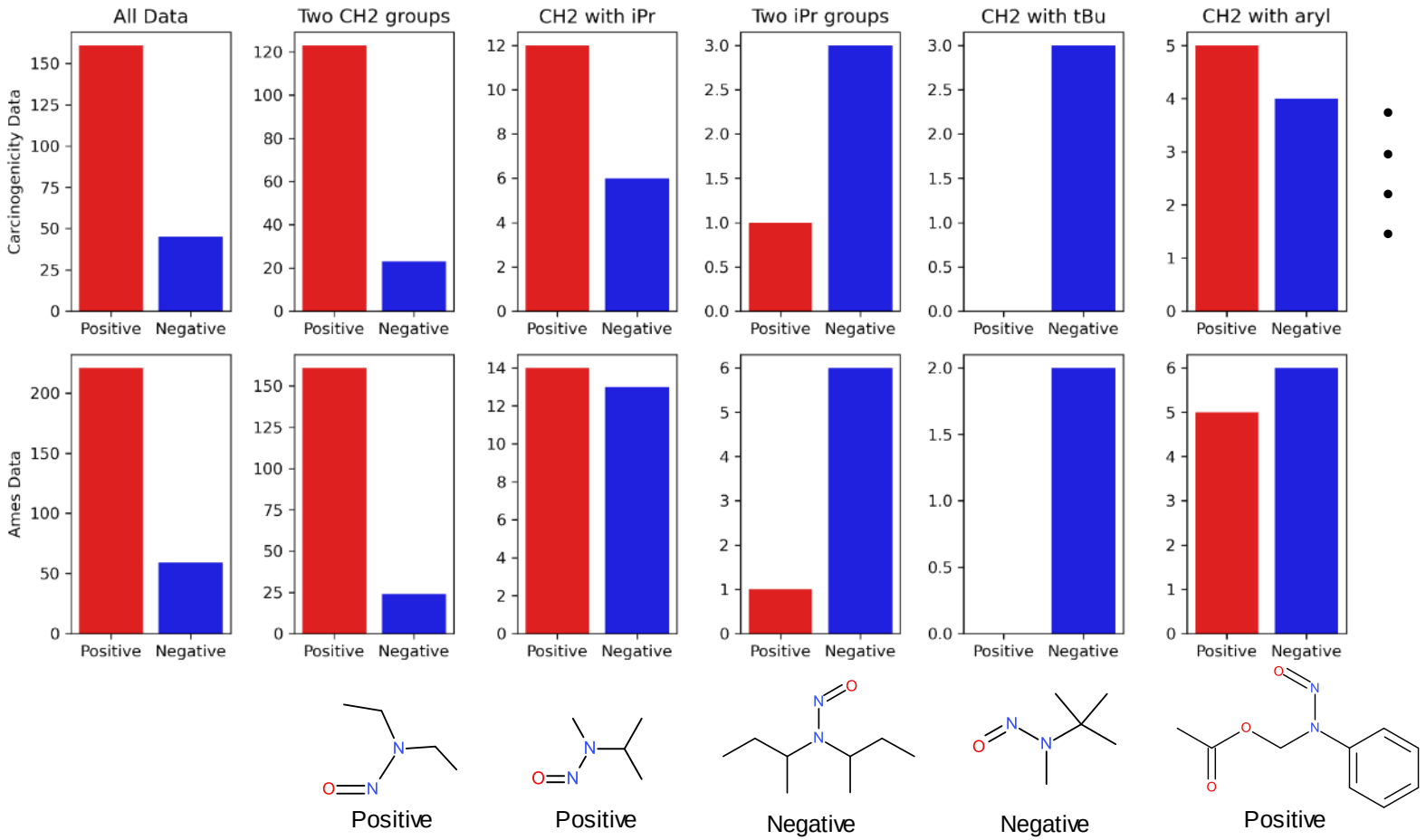
Effects: Degree of α-carbon substitution

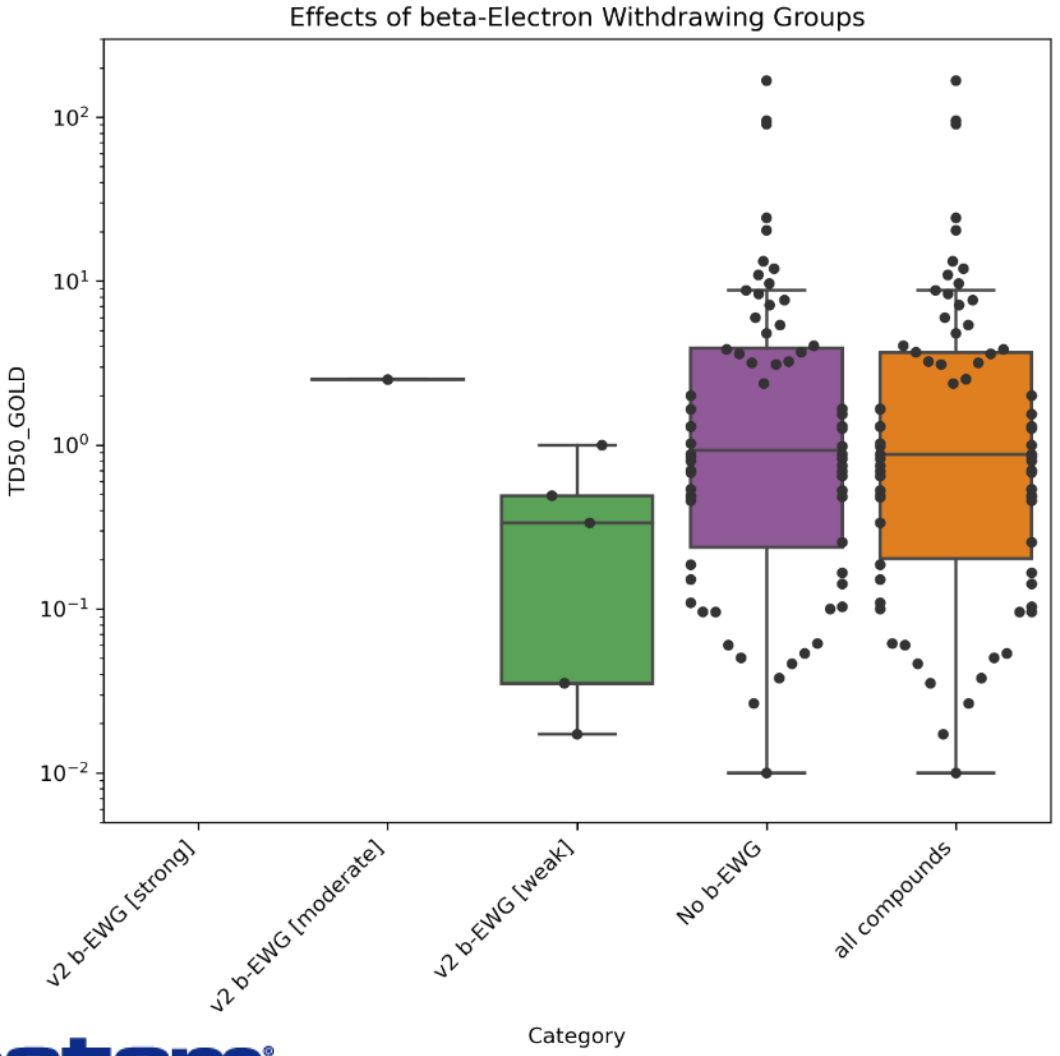


- α-carbon substitution substantially lowers positive rate
- Substitution on both sides is significant
- Bulk on one side can negate mutagenicity
- No α-carbon hydrogens negates mutagenicity

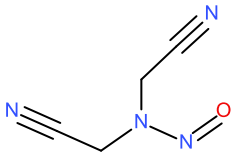
Comparison with additional Ames data concurs with this effect

Includes negative carc compounds as well as those with TD_{50} values

Effects: Electron Withdrawing Groups (EWG)

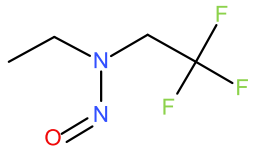


- Strong EWGs reduce mutagenicity potency
- Moderate EWGs reduce mutagenicity potency
- Weak EWGs have little effect



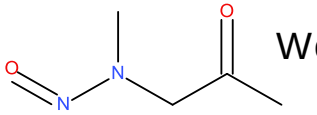
Strong EWG

Negative



Moderate EWG

2.52 mg/kg/day



Weak EWG

0.0172 mg/kg/day

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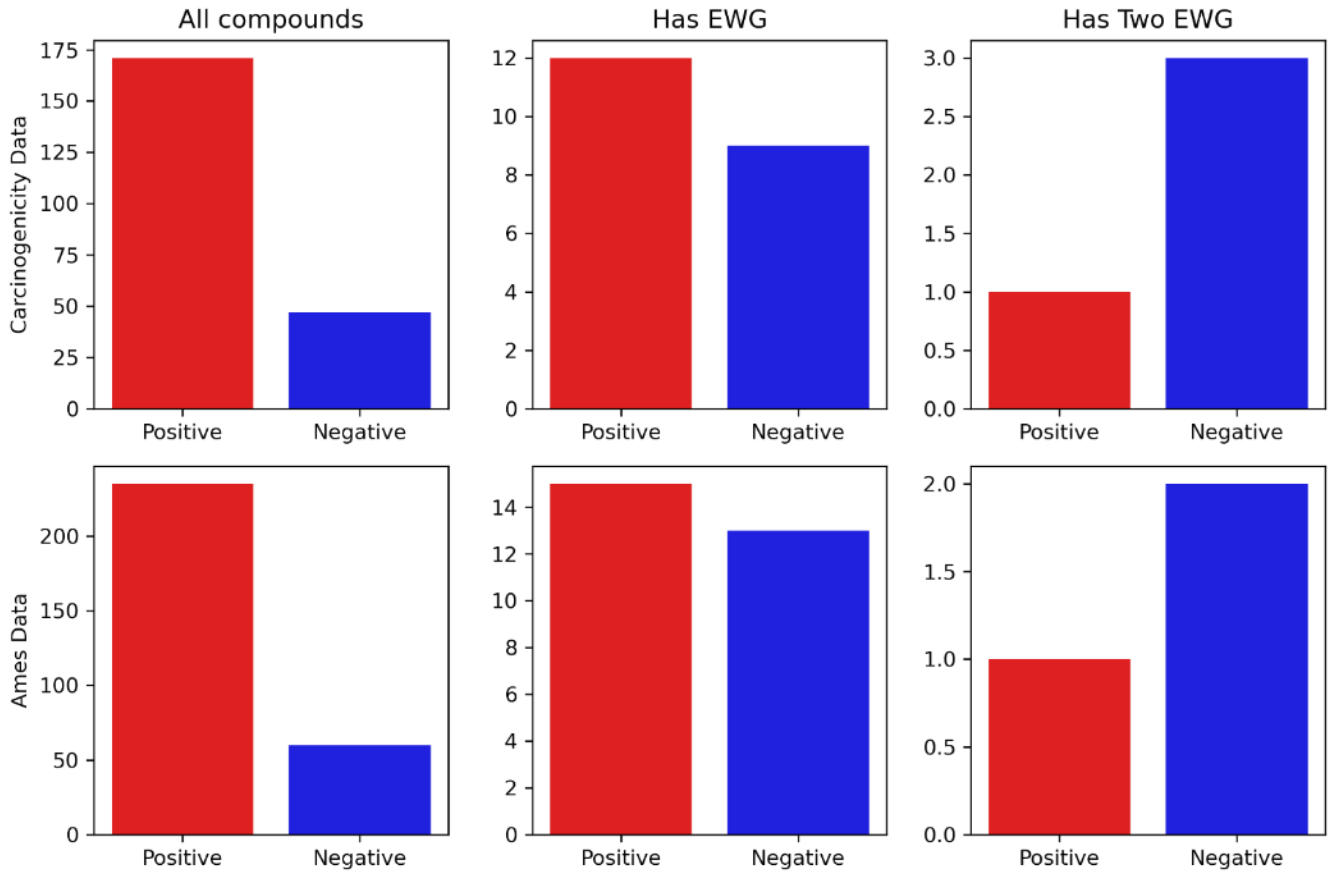
Effects: Electron Withdrawing Groups (Carc and Ames)



- Electron-withdrawing groups have a large effect on decreasing mutagenicity and increasing negative carcinogenicity prevalence
- Compounds with two groups have fewer mutagenic compounds than those with one
- But there are still positive exceptions

Comparison with additional Ames data concurs with this effect

Includes negative carc compounds as well as those with TD_{50} values

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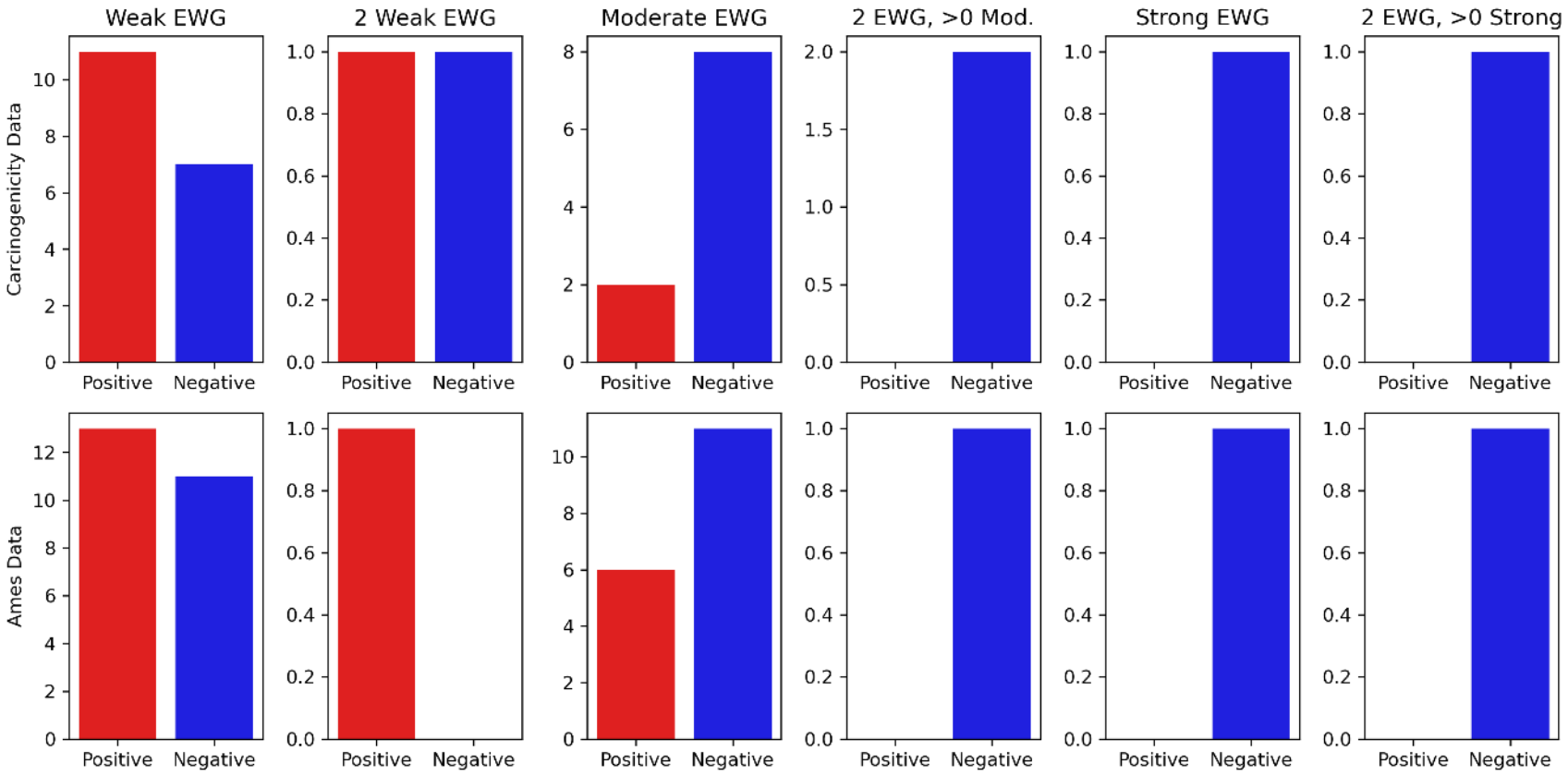
Effects: Electron Withdrawing Group Strength



- Strong EWGs negate mutagenic potential
- Moderate EWGs reduce mutagenic potential
- 2 Moderate EWGs negate mutagenic potential
- Weak EWGs have little effect
- Two (weak) groups reduce mutagenic potential more than one group

Comparison with additional Ames data concurs with this effect

Includes negative carc compounds as well as those with TD_{50} values

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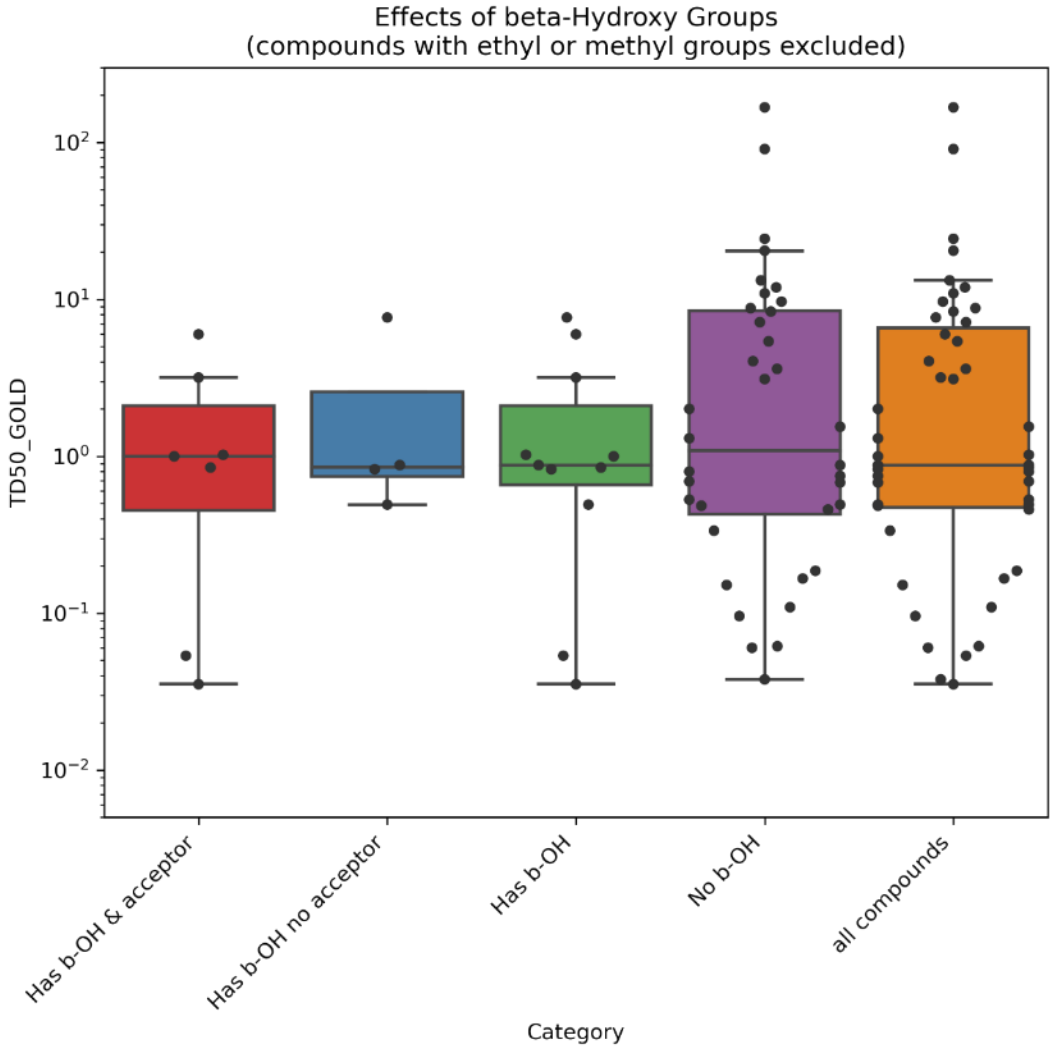
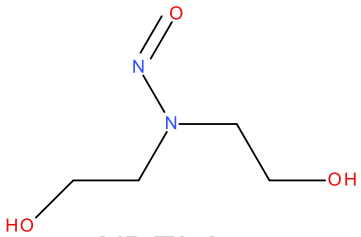
Effects: β-hydroxyl Groups – no α-carbon hydroxylation



- β-OH groups eliminates extreme potency compounds
- Division into potency subclasses (H acceptor/donors)
- Steric and competing metabolic activation electronic effects
- Quantum mechanical analysis underway:
  See Shu Yu, Pfizer, *in-preparation*



NDELA
3.17 mg/kg/day

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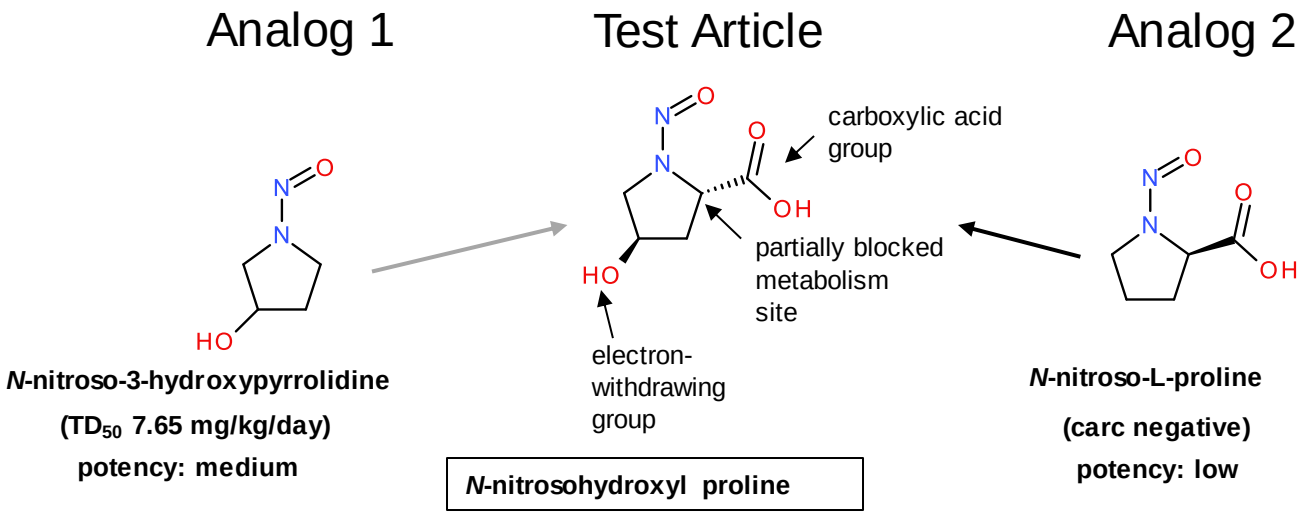
An Example Assessing Analogs to Predict *N*-nitrosamine Potency

- Multiple features can affect α-carbon metabolism



- Identify the feature most affecting (reducing) potency
- Analog 2 is a better structure for comparison
- Test article is indeed carcinogenicity negative

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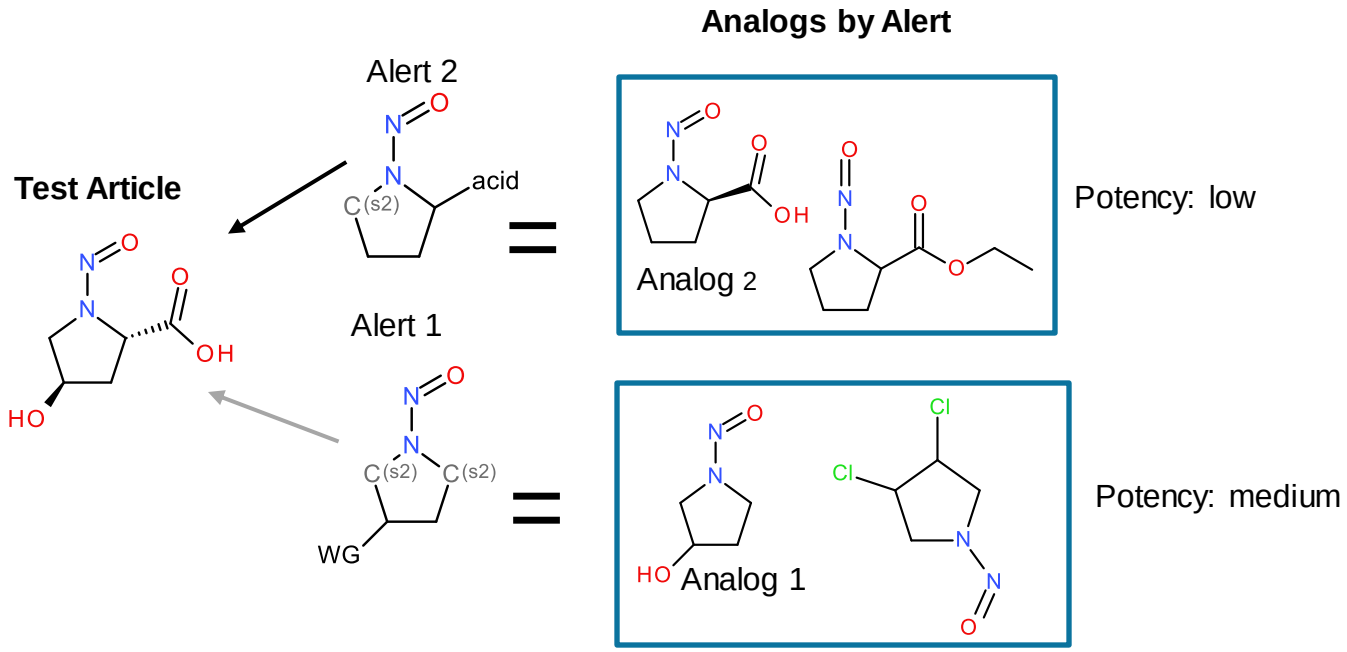
Applying Categorial Alerts to Support Read-Across



1. Select Alert based upon mechanistic relevancy
2A. Select potency TD_{50} range (i.e. lowest value)
or
2B. Select TD_{50} of nearest analog in group (i.e. Analog 2)

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Conclusions

- The effect on both potency and prevalence of various structural features was demonstrated
- Some structural features may lead to a reduction or elimination of potency
- Metabolism mechanisms drive potency for dialkyl-*N*-nitrosamines
- This approach supports read-across assessments by identifying mechanistically-relevant analogs for structural similarity consideration

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Publication Status

- Published and/or accepted:
  1. Cross K.P., (2021) “Predicting *N*-Nitrosamine Activity from Structure-Activity Relationships”, Genetic Toxicity Association Meeting, May 3-6, 2021.
  2. Cross K.P. (organizer), Ponting D.J. (chair) (2021) “N-Nitrosamine Impurities in Pharmaceuticals – potency, prediction, and acceptable limits”, Toxicology Forum August 2, 2021.
  3. Ponting D. J., Cross K.P., (2021) “Predicting *N*-Nitrosamine Activity from Structure-Activity Relationships”, American College of Toxicology Meeting, November 14-17, 2021.
  4. Bercu *et al* (2021) “Compound- and Class-Specific Limits for Common Impurities in Pharmaceuticals”, in *Mutagenic Impurities*, 2nd Edition, *ed.* Teasdale.
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Publication Status

- Manuscripts in preparation:
  1. Cross, Ponting, et al – Predicting N-Nitrosamine Activity from Structure-Activity Relationships
  2. Trejo-Martin *et al* – "Use of the Bacterial Reverse Mutation Assay to Predict Carcinogenicity of *N*-Nitrosamines", EMM.
  3. Stalford *et al* – extracting valuable knowledge from older carcinogenicity data
  4. Cross *et al* - Technical letter: NDMA and NDEA Activity and their Relevance to other N-Nitrosamine Potencies
  5. Snodin *et al* – Should all Nitrosamines be part of the Cohort of Concern?
  6. Masuda-Herrera *et al* – Acceptable Intake monographs on the big 8 nitrosamines
  7. SAR publication, workgroup members as authors; title to be determined
  8. Additional papers from workgroup(s) members,

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