# Exploring Local Structural Environments of Nitrosamine Analogs to Improve Carcinogenicity Read-Across Assessments

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## Abstract

Assessing the carcinogenic potency of N-nitrosamines, especially nitrosamine drug substance-related impurities (NDSRIs), is crucial as these compounds have been identified as potent carcinogens in animals and are suspected to be carcinogenic in humans.

One way to assess N-nitrosamine's carcinogenic potency is to use structural analogs with known carcinogenic data. However, the current database of N-nitrosamines with known carcinogenic data is limited, hindering the ability to assess their carcinogenic potency accurately. Enhancing the chemical space of the N-nitrosamine database by including more structurally diverse compounds will improve the assessment of their carcinogenic potency.

In this study, we evaluated the effect of adding additional N-nitrosamines to the existing QSAR Flex N-nitrosamine potency database of 135 compounds. We identified 59 additional N-nitrosamines with reported experimental data in the public literature. In addition, we explored the structural environment of the newly added compounds compared to the previously compiled dataset and the effect of the expansion of the chemical space on our ability to predict the carcinogenic potency of NDSRIs more efficiently.

Our results showed that the expanded chemical space of the QSAR Flex N-nitrosamine carcinogenic potency database coupled with enhanced algorithms for calculating similarity and with the improved workflow of selecting the proper structural analogs improves the quality of the carcinogenic potency assessments. These findings, as well as the workflow, are illustrated in several case studies.

Our study highlights the importance of expanding the chemical space of the database for a more accurate assessment of carcinogenicity and improving our ability to protect human health.

## Data

### NitrosoDB release version 2022

153 N-Nitrosamines 138 positives, 15 negatives.

**Data sources:**

**CPDB[1]:** 153 Chemicals

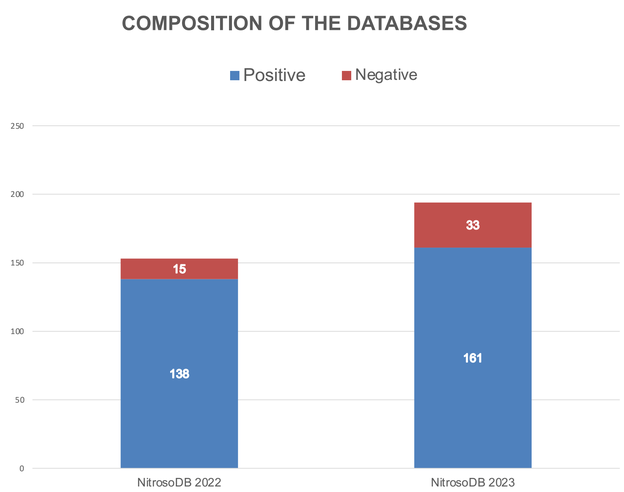
**LHASA Carcinogenicity Database[2]:** 49 Chemicals

### NitrosoDB release version 2023

**New data sources, additional chemicals**

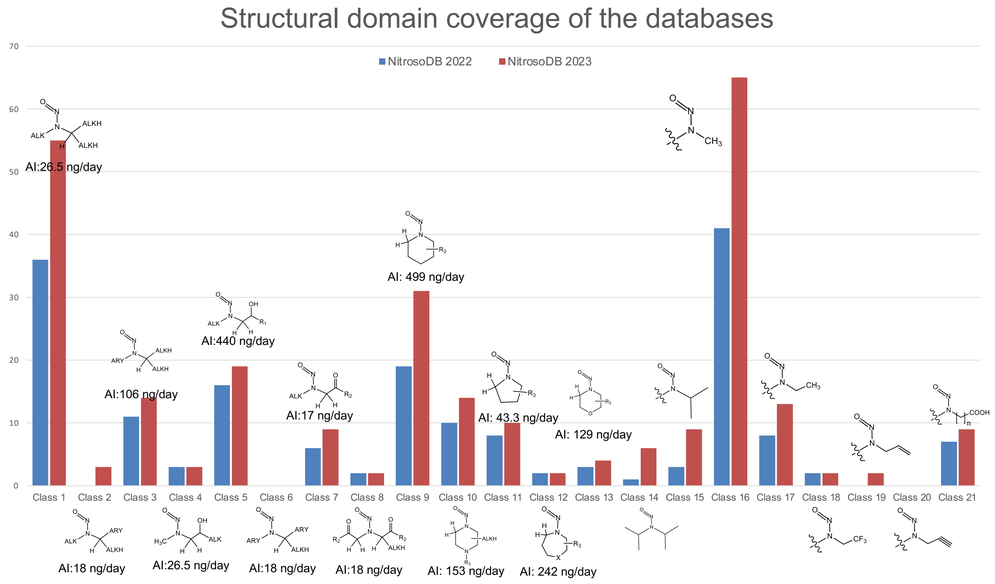
**Druckrey at al[3]:** 41 Chemicals

**Dobo at al[4]:** 16 Chemicals




## Results

There is an improvement in coverage for 15 structural types of N-Nitrosamines (NA) out of 21. The biggest improvement is observed for the structural types, potentially decreasing the carcinogenicity of NAs.

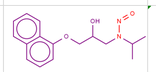
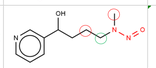
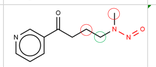
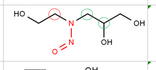
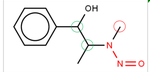
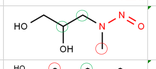
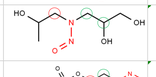
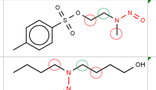
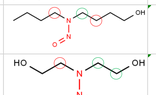
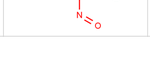


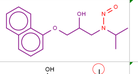
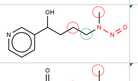
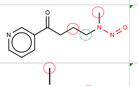
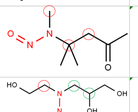
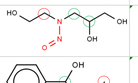
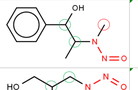
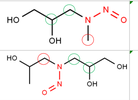
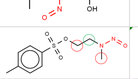
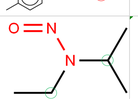
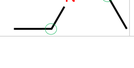

| | |
|---|---|
| Class 1 | Dobo's group 1 |
| Class 2 | Dobo's group 2 |
| Class 3 | Dobo's group 3 |
| Class 4 | Dobo's group 4 |
| Class 5 | Dobo's group 5 |
| Class 6 | Dobo's group 6 |
| Class 7 | Dobo's group 7 |
| Class 8 | Dobo's group 8 |
| Class 9 | Dobo's group 9 |
| Class 10 | Dobo's group 10 |
| Class 11 | Dobo's group 11 |
| Class 12 | Dobo's group 12 |
| Class 13 | Dobo's group 13 |
| Class 14 | Sterically hindered both sides |
| Class 15 | Sterically hindered one side |
| Class 16 | Methyl group on either side |
| Class 17 | Ethyl group on either side |
| Class 18 | Beta CF3 |
| Class 19 | Beta allyl group |
| Class 20 | Beta propargyl group |
| Class 21 | Carboxilic acid anywhere |

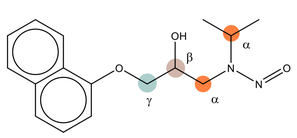
Structural classes identified based on Dobo et al[4] and Pointing et al[5]

## Effect on assessing the potential carcinogenicity of NDSRIs

With the increased coverage of the NitrosoDB database, more relevant surrogates are identified by the enhanced read-across technique.

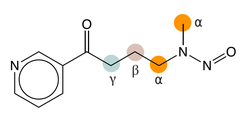
| Registry Number | Chemical | Alert Env. Similarity | Whole Structure Similarity | Name | Mol. Wt. | CPDB TD50 (mg/kg/day) | LHASA TD50 (mg/kg/day) |
|---|---|---|---|---|---|---|---|
| 0 |  | 1.000 | 1.000 | *N-Nitrosopropranolol* | 288.347 | - | - |
| 76014-81-8 |  | 0.517 | 0.231 | *4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANOL* | 209.249 | 0.103* | - |
| 64091-91-4 |  | 0.517 | 0.185 | *4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-(BUTANONE)* | 207.233 | 0.100* | 0.142* |
| 89911-78-4 |  | 0.483 | 0.238 | *3-((2-hydroxyethyl)nitrosamino)-1,2-propanediol* | 164.161 | 5.980* | 6.040* |
| 17608-59-2 |  | 0.473 | 0.250 | *N-Nitrosoephedrine* | 194.234 | 95.200* | - |
| 86451-37-8 |  | 0.467 | 0.259 | *N-NITROSOMETHYL(2,3-DIHYDROXYPROPYL)AMINE* | 134.135 | 0.646* | - |
| 89911-79-5 |  | 0.433 | 0.272 | *N-NITROSO(2,3-DIHYDROXYPROPYL)(2-HYDROXYPROPYL)AMINE* | 178.188 | 0.054* | - |
| 66398-63-8 |  | 0.417 | 0.173 | *N-Nitrosomethyl-(2-tosyloxyethyl) amine* | 258.299 | 4.800* | - |
| 3817-11-6 |  | 0.383 | 0.140 | *N-BUTYL-N-(4-HYDROXYBUTYL)NITROSAMINE* | 174.244 | 0.457* | 0.261* |
| 1116-54-7 |  | 0.383 | 0.138 | *N-NITROSODIETHANOLAMINE* | 134.135 | 3.170* | 3.380* |

| Registry Number | Chemical | Alert Env. Similarity | Whole Structure Similarity | Name | Mol. Wt. | CPDB TD50 (mg/kg/day) | LHASA TD50 (mg/kg/day) |
|---|---|---|---|---|---|---|---|
| 0 |  | 1.000 | 1.000 | *N-Nitrosopropranolol* | 288.347 | - | - |
| 76014-81-8 |  | 0.517 | 0.231 | *4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANOL* | 209.249 | 0.103* | - |
| 64091-91-4 |  | 0.517 | 0.185 | *4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-(BUTANONE)* | 207.233 | 0.100* | 0.142* |
| 16339-21-2 |  | 0.500 | 0.101 | *N-methyl-N-(2-methyl-4-oxopentan-2-yl)nitrous amide* | 158.201 | 10000.000* | - |
| 89911-78-4 |  | 0.483 | 0.238 | *3-((2-hydroxyethyl)nitrosamino)-1,2-propanediol* | 164.161 | 5.980* | 6.040* |
| 17608-59-2 |  | 0.473 | 0.250 | *N-Nitrosoephedrine* | 194.234 | 95.200* | - |
| 86451-37-8 |  | 0.467 | 0.259 | *N-NITROSOMETHYL(2,3-DIHYDROXYPROPYL)AMINE* | 134.135 | 0.646* | - |
| 89911-79-5 |  | 0.433 | 0.272 | *N-NITROSO(2,3-DIHYDROXYPROPYL)(2-HYDROXYPROPYL)A* | 178.188 | 0.054* | - |
| 66398-63-8 |  | 0.417 | 0.173 | *N-Nitrosomethyl-(2-tosyloxyethyl) amine* | 258.299 | 4.800* | - |
| 16339-04-1 |  | 0.400 | 0.276 | *Ethylisopropylnitrosamine* | 116.164 | 1.580* | - |



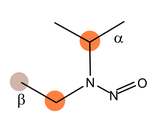
Query NDSRI

| Position | $R_1$ | $R_2$ |
|---|---|---|
| α | $CH_2$ | $CH(CH_3)_2$ |
| β | CH-OH | - |
| γ | $CH_2$ | - |
| Cyclic? | No | |



Surrogate ($TD_{50}$ = 0.142 mg/kg/day)

| Position | $R_1$ | $R_2$ |
|---|---|---|
| α | $CH_2$ | $CH_3$ |
| β | $CH_2$ | - |
| γ | $CH_2$ | - |
| Cyclic? | No | |



Surrogate ($TD_{50}$ = 1.580 mg/kg/day)

| Position | $R_1$ | $R_2$ |
|---|---|---|
| α | $CH_2$ | $CH(CH_3)_2$ |
| β | $CH_3$ | - |
| γ | - | - |
| Cyclic? | No | |

## Conclusions

Our study highlights the importance of expanding the chemical space of the N-nitrosamine carcinogenic potency database for a more accurate assessment of carcinogenicity and improving our ability to protect human health

## References

[1] CPDB database , https://files.toxplanet.com/cpdb/index.html

[2] Thresher, T., Gosling J, P., Williams, R. Generation of TD50 values for carcinogenicity study data. Toxicology Res; 8: 696-703; (2019).

[3] Drukcrey, et al "Organotropic carcinogenic effects of 65 various N-nitroso- compounds on BD rats." Z Krebsforsch.; 1967; 69; 2; 103-201.

[4] Dobo, et al. "Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities." Chem Res Toxicol . 2022 Mar 21;35(3):475-489

[5] Ponting, et al. "Strategies for Assessing Acceptable Intakes for Novel N -Nitrosamines Derived from Active Pharmaceutical Ingredients." Journal of Medicinal Chemistry, November 28, 2022.