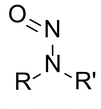
A New Structural Similarity Method to Identify Surrogate Compounds for Assessing the Carcinogenicity of Nitrosamine Impurities

Naomi L. Kruhlak[1], Suman Chakravarti[2], Govindaraj Kumaran[1], Roustem Saiakhov[2]

[1]FDA Center for Drug Evaluation and Research (CDER), 10903 New Hampshire Ave., Silver Spring, MD, 20993; [2]MultiCASE Inc., Beachwood, OH, 44122.

Abstract

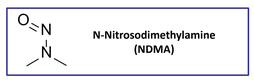
N-nitroso compounds, which include *N*-nitrosamines (NAs), can be formed during the manufacture and storage of human drugs and can pose a safety risk since many are mutagens and potent rodent carcinogens. Ideally, empirical mutagenicity and carcinogenicity assay data are used to establish an acceptable intake (AI) for an NA in a drug product; however, many NAs have recently been identified in regulated pharmaceutical products and have not been robustly tested in standard mutagenicity and/or carcinogenicity assays. In these cases, AIs may be based on data from structurally-related, surrogate NAs, which are selected based on factors such as their local NA environment, potential for metabolic activation, and physicochemical properties. Identifying the most similar and relevant surrogate NAs can be subjective and potentially lead to differences in calculated AIs due to the selection of different surrogates. Therefore, the current study sought to develop a computational local similarity method to more objectively and efficiently calculate the most relevant surrogates to a data-poor NA. Circular fingerprints up to 5 bonds from the nitroso group were built using factors known to modulate carcinogenic potential, including the degree of substitution at the alpha-carbon position, the presence of the nitroso group in a ring, and the proximity of bulky or electron donating/withdrawing substituents. Separate smaller fingerprint segments were generated for every bond depth and concatenated to produce the final 990-element fingerprint. The fingerprint was then used to calculate a local similarity index for a data-poor NA relative to the 139 experimentally tested N-nitroso compounds in the database, rank-ordered by relevance. The method was tested using 6 recently reported data-poor NA impurities identified in regulated pharmaceuticals. The results showed that when compared to the top ranked structural surrogate identified through visual inspection by a human expert, the method correctly proposed the same surrogates within the top 9 hits. The results support the conclusion that this new method for calculating local NA similarity can provide a semi-automated approach to more efficiently and objectively identify the most structurally relevant surrogates for setting an AI for an untested NA impurity than by visual inspection alone.



N-nitrosamine

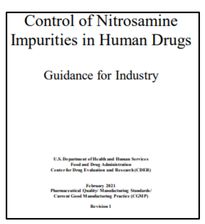
Background

- Nitrosamine impurities were unexpectedly found in human drugs in June 2018[1]
  - NDMA discovered in valsartan, an angiotensin II receptor blocker (ARB)
  - Contamination subsequently confirmed in other ARBs, including irbesartan and losartan, and other drug classes, such as in the H_2-blocker, ranitidine



N-Nitrosodimethylamine (NDMA)

- Nitrosamine impurities are members of the ICH M7 "cohort of concern" and are regulated more tighter than typical mutagenic impurities because of their high carcinogenic potency[2]
- FDA Guidance for Industry—*Control of Nitrosamine Impurities in Human Drugs*—published September 2020, and revised in February 2021[1]
- Appendix B: A human acceptable intake (AI) 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[1]
  - TD50 in mg/kg/day
  - lower TD50 = greater carcinogenic potency
  - TD50s for drug-like nitrosamines span 4 orders of magnitude; some nitrosamines are reported as non-carcinogenic[3]



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

- In the absence of robust empirical carcinogenicity data, an AI can be calculated using TD50 values from structurally related analogs, or surrogates

Read-Across: Use of structurally similar analogs and structure-activity relationships (SARs) to make a prediction of biological activity of a (data-poor) target molecule

SAR: A qualitative relationship between chemical structure and biological activity (e.g., carcinogenicity)

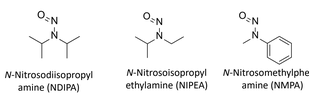
TD50 Values

- TD50 values were initially compiled in the Carcinogenicity Potency Database (CPDB)[4] for ~1500 compounds, including ~140 nitrosamines. The CPDB is no longer updated.
- Thresher et al. recalculated and reported revised TD50 values in the Lhasa Carcinogenicity Database (LCDB) using a standardized method[5]
  - Some values could not be recalculated as studies were conducted with only a single dose group
  - The most robust studies have both CPDB and LCDB TD50s values

Examples of Nitrosamine Impurities

- Nitrosamine impurities can be small molecules, but can also be a nitrosated form of an API, called a Nitrosamine Drug Substance Related Impurity (NDSRI)
  - Nitrosamine impurities may be formed by reaction between a secondary or tertiary amine and nitrite under acidic conditions during the manufacturing process or through degradation[1]
  - NDSRIs can be formed by reaction between the API and nitrite in an excipient in the drug product formulation[6]

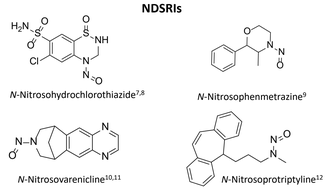
Simple Nitrosamine Impurities[1]



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[7,8] | *N*-Nitrosophenmetrazine[9]

N-Nitrosovarenicline[10,11] | *N*-Nitrosoprotriptyline[12]

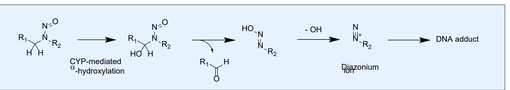
Similarity Assessment – Global vs Local

- Structural similarity searching can be used to identify surrogates
- Problem: Global similarity assessment (e.g., Tanimoto coefficient) compares all features in a molecule, not just those of greatest relevance to endpoint of concern
  - Often inadequate for identifying surrogates for NDSRIs
- Substructure searching can be effective, but requires multiple searches with manual refinement based on expert knowledge—inefficient and subjective
- Nitrosamine mutagenicity and carcinogenicity are more heavily dependent on immediate nitrosamine environment due to need for metabolic activation—consider **local similarity searching**

Considerations for selecting appropriate surrogates for a read-across analysis to derive an AI for structurally complex nitrosamines include:
- the degree of substitution
- steric bulk
- electronic influences
- potential for metabolic activation
- stability/reactivity of the resulting metabolites
- overall molecular weight and physicochemical parameters

Need a method to efficiently and objectively assess nitrosamine local similarity

Metabolic activation of alkyl nitrosamines by α-hydroxylation, adapted from Hecht, 1997[13]

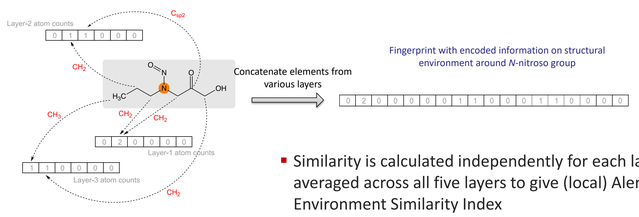


New Structural Fingerprint for Local Similarity

- A new circular fingerprint was developed from atom type elements and ring counts based on nitrosamine metabolism and known SARs. Data elements include:
  - Periodic table element
  - Hybridization state
  - Number of hydrogens on hetero and unsaturated atoms
  - Aromaticity
  - Formal charge
  - Stereochemistry
  - 3- or 4-membered ring
  - Number of double bonds for P and S
  - Indication of junction of two aromatic rings
  - Count of 5, 6 or greater ring size containing nitrosamine
- SAR trends observed to modulate nitrosamine carcinogenicity[14]

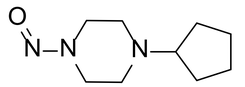
Deactivating Features		Activating Features
Substitution at α-carbon	Presence of carboxylic acid group	Unsaturated carbon-carbon bond at α-position
Nitrosamine in smaller than 6-membered ring	Electron withdrawing group in β-position	Methyl group directly bonded to one side of nitrosamine
Presence of hydroxyl groups	Increasing alkyl chain length	β-methyl group

- Fingerprint considers up to 5 bonds (layers) from the nitrosamine
- Each layer is composed of 198 bits
- Fingerprint is 990 (198 x 5) bits



- Similarity is calculated independently for each layer then averaged across all five layers to give (local) Alert Environment Similarity Index

Example - 1-Cyclopentyl-4-nitrosopiperazine (CPNP)



CPNP

Screenshot of the most locally similar nitrosamine analogs in the database as determined using the nitrosamine fingerprint and (local) Alert Environment Similarity.

Chemical	Alert Env. Similarity	Whole Structure Similarity	Name	Mol. Wt.	CPDB TD50 (mg/kg/day)	LHASA TD50 (mg/kg/day)	Ames Mutagenicity (binary)	LogP	Water Solubility (gm/L)
	1.000	1.000	mol-1	183.255	-	-	-	0.927	14.857
X	0.617	0.326	*1-Nitroso-3,4,5-trimethylpiperaz*	157.217	0.151*	0.153*	-	0.657	91.851
X	0.567	0.221	*1-Nitroso-4-benzoyl-3,5-dimeth*	247.298	9.660*	-	1.000*	1.528	11.156
X	0.517	0.163	*N-Nitrosothialdine*	192.309	0.483*	-	-	1.795	1.206
	0.500	0.548	*N-Nitrosopiperidine*	114.148	1.430*	1.120*	1.000*	0.360*	76.501*
	0.500	0.417	*4-nitroso-thiomorpholine*	132.188	5.390*	3.540*	1.000*	0.400*	43.967
	0.500	0.417	*1-Nitrosopiperazine*	115.136	8.780*	6.040*	1.000*	0.180*	117.239
	0.500	0.417	*N-NITROSOMORPHOLINE*	116.120	0.109*	0.135*	1.000*	-0.440*	1000.016*
X	0.488	0.316	*1-nitroso-alpha-phenyl-2-Piperi acid, methyl ester*	262.309	10000.000*	-	1.000*	2.348	0.535
X	0.480	0.375	*N-NITROSO-2-HYDROXYMORPH*	132.119	14.900*	-	1.000*	-0.783	567.609

*Experimental values

Increasing Alert Environment Similarity

Carci data robust enough for Lhasa calculated TD50

All structures with an unhindered α-carbon are reported to be mutagenic

Excluded: Contains β-methyl groups, which are activating features not present in target molecule

Excluded: Contains two thioether groups and substituted α-carbons, which are not present in target molecule

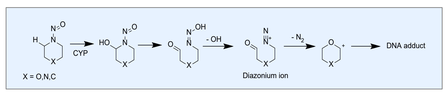
Excluded: Contains bulky β-electron withdrawing group, which is deactivating feature not present in target molecule

Excluded: Contains hydroxyl group, which is deactivating feature that increases solubility and is not present in target molecule

Target molecule

Negative response in carcinogenicity assay

- The most structurally relevant analogs are 6-membered cyclic alkyl nitrosamines
  - Cyclic alkyl nitrosamine are expected to undergo metabolic activation to form a cyclic intermediate (see below, adapted from Dobo et al., 2022)[15]



- Mutagenic nitrosamines are expected to pose the greatest risk to human health
- Surrogates can be further refined by visual inspection
  - For CPNP, surrogates with β-methyls, thioethers, hydroxyls, α-substituents and β-electron withdrawing groups are excluded
- Robustness of carcinogenicity data heavily influences the acceptability of a surrogate
  - Surrogates with both CPDB and LCDB TD50 are prioritized
- In cases where multiple surrogates are available, the conservative approach is to derive an AI based on the lowest TD50.

Local similarity searching can guide the selection of surrogates but does not provide an absolute measure of acceptability

Conclusions

- A new circular fingerprint was developed to facilitate structure-based searching for potential nitrosamine surrogates.
  - Fingerprint considers structural environment up to 5 bonds (layers) from nitrosamine, consistent with most common mechanism of metabolic activation, α-hydroxylation.
- Local similarity calculation can provide a more efficient and objective means to search for potential surrogates than other structure-based search methods.
  - Streamlines search and data visualization process to enable review of potential surrogates with their associated carcinogenicity and mutagenicity data and calculated physicochemical properties.
  - More efficient than iterative substructure searching
  - More structurally relevant than global similarity searching for NDSRIs

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