A Quantitative Read-Across Approach to Address the N-Nitrosamine Compound Class as Impurities

Abs# 4275 / Board# Q792

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MOTIVATION

Nitrosamines are genotoxic carcinogens and are known to be unintentionally present in foods and drugs as processing or production impurities. Leveraging the large amount of carcinogenicity data, this study applies quantitative approaches to capture structural group effects on the point of departure and potency categories. Ensemble searching for a POD (*e.g.*, BMD10 or TD50) allows estimation of potency categories and approximate accepted intake limits of impurities in drugs (NDSRIs) and foods (NIASs).

I. NITROSAMINE KNOWLEDGEBASE

1.1 Establishing the Underlying Knowledgebase

- Carcinogenicity database
  - Source: CPDB, NTP, EFSA, FDA CDER (appr. 2,500 compounds)
  - POD: BMD10/BMDL10, TD50s, hazard, NOAEL/LOAEL
- 160 nitrosamines in the Carcinogenicity database
- 256 potential drug-nitrosamines as NDSRIs (FDA database)[1]
- EFSA opinions for 32 nitrosamines related to NIASs of foods[2]

[1]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits
[2]EFSA Journal 2023 Scientific Opinion, DOI: 10.2903/j.efsa.2023.7884

1.2 Compound Classes of Nitrosamines

- Major structure groups are similar to other published reports[3,4] (*e.g.*, [3]Dobo *et al.* Chem. Res. Tox. 2022, 35(3), 475; [4]2024, 37(2), 181)
- This study evaluated five major groups: short aliphatic, functionalized aliphatic (OH), cyclic, piperazine, aromatic

Group	ToxPrint Chemotypes	Z-scores* pTD50	Z-scores* pBMD10
alkyl, aliphatic	bond:N=O_N-nitroso_dialkyl	63	47
	chain:alkaneLinear_ethyl_C2(H_gt_1)	50	32
	chain:alkaneLinear_ethyl_C2_(connect_noZ_CN=4)	21	14
	chain:alkaneCyclic_ethyl_C2_(connect_noZ)	11	8
	chain:alkaneLinear_propyl_C3	22	15
	chain:alkaneBranch_isopropyl_C3	13	7
	chain:alkaneLinear_butyl_C4	17	12
	chain:alkeneLinear_mono-ene_ethylene_generic	7	7
functionalized aliphatic (Urea, Guanidine)	bond:C=O_carbonyl_generic	46	27
	bond:C(=O)N_carboxamide_generic	39	19
	bond:C=O_acyl_hydrazide	33	17
	bond:COH_alcohol_diol_(1_2-)	7	5
	bond:COH_alcohol_pri-alkyl	19	13
	bond:COH_alcohol_sec-alkyl	16	9
	bond:CN_amine_alkyl_ethanolamine	10	6
	group:ligand_path_4_bidentate_aminoethanol	21	13
	bond:CC(=O)C_ketone_methyl_aliphatic	8	8
	bond:CC(=O)C_ketone_generic	9	9
	bond:COC_ether_aliphatic	5	3
	bond:CX_halide_alkyl-X_generic	8	4
aromatic	ring:aromatic_benzene	15	8
	ring:aromatic_phenyl	8	6
	chain:aromaticAlkane_Ph-C1_acyclic_generic	7	5
heterocycle	ring:hetero_[6]_N_piz+pid	9	6
	ring:hetero_[6]_Z_1-	14	10
	ring:hetero_[6]_Z_1_4-	8	4
	ring:hetero_[5]_Z_1-Z	4	4
	ring:hetero_[5]_Z_1_3-Z	8	3

*pTD50 (-logTD50) and pBMD10 (-logBMD10) are color coded by Z scores. **Orange: Z>=2; Tan: 1<Z<2; Gray: -1<Z<1; Blue: -2<Z<-1; Navy: Z<=-2.** Numeric values represent the hit counts.

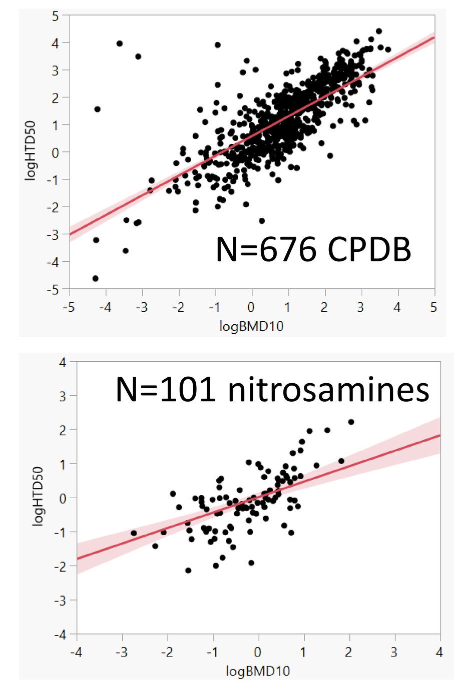
II. BMD10 VS. TD50

2.1 BMD10/BMDL10

- Dose-response curve selection criteria
  - BMDL10 was determined for tumors in liver (rat oral studies)
  - Counts from all tumor bearing animals were excluded.
  - Dose-response model must yield BMDL < BMD < BMDU
  - To increase the BMD counts, single dose studies where the control has acceptably low counts were included in this study (For Cancer TTC dataset, single dose studies were not included)[5]
- Post modeling exclusions
  - log(BMDU/BMDL) ≥ 3
  - BMDL10, BMD10 values ≤ 1E-6 were excluded from this analysis

[5]Yang C *et al.* Food Chem. Tox. 182 (2023) 114182

BMD10 vs. Harmonic TD50 Correlation



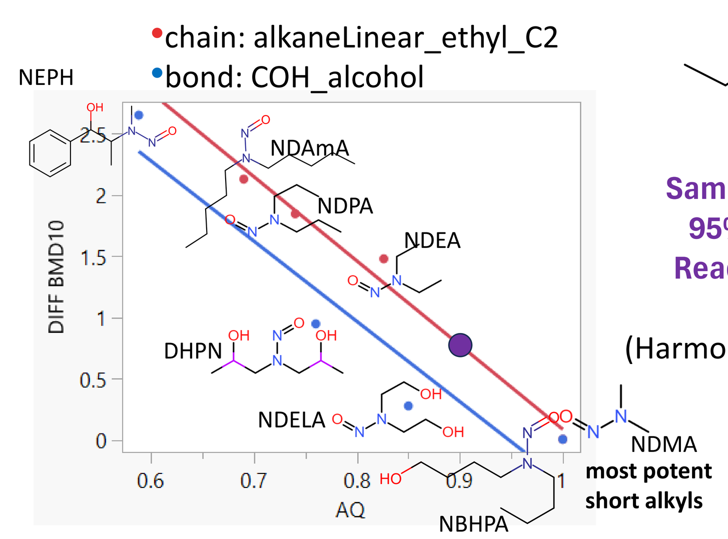
Harmonic TD50 and benchmark dose modeling (BMD) PODs show moderate correlations.

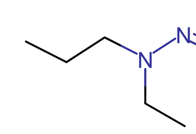
2.2 Harmonic TD50 from the Original CPDB Database

- Harmonic TD50 is an average of TD50 values from all tumor types and animals
- Legacy values available in the CPDB database (120) were used for this nitrosamine analysis to evaluate results from US FDA CDER

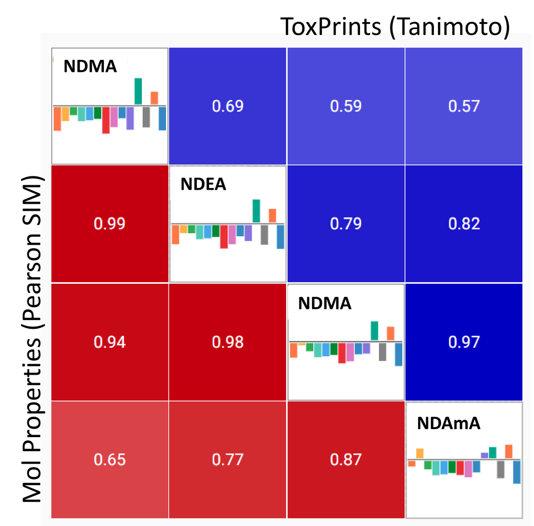
III. CLASS-SPECIFIC READ-ACROSS

3.1 Local Similarity – Short Alkyl Chain Neighbors





NEPA
Sample mean: 0.25 mg/kg/day
95% CI: 0.02 ≤ BMD10 ≤ 3.2
Read-Across Reliability: 78 %
Hazard: carcinogenic
(Harmonic TD50 sample mean=0.075)

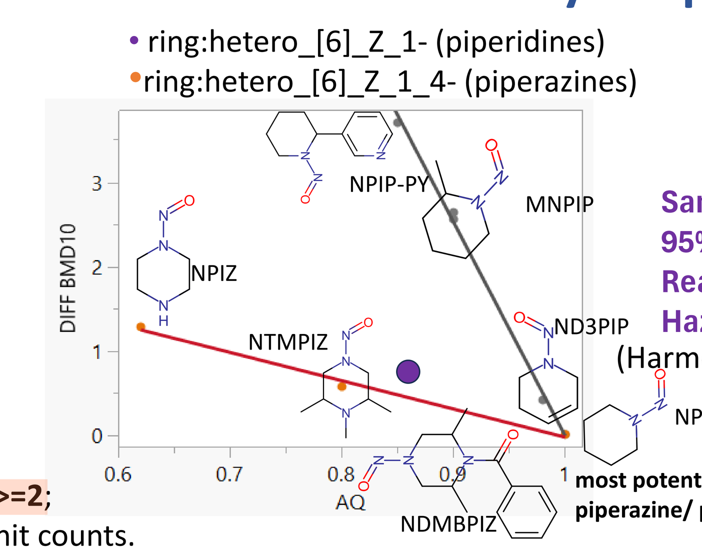


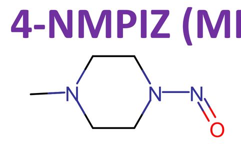
# of Atoms, # Rotatable bonds, H-acceptors, H-donors, Rule-of-5 violations, # Stereocenters, MW, Complexity, TPSA, LogP, Asphericity, Heats Formation, HOMO, LUMO, HOMO/LUMO

DIFF BMD10[6]	difference of logBMD10 values of the pair	$Diff_{AB}(BMD10)=\log\left[\frac{BMD10_A}{BMD10_B}\right]$
AQ (analogue quality)[6]	defined by structural features and physicochemical properties	$AQ=\sqrt[N]{\prod_{i=1}^{N}(SimilarityMeasure)_i}$

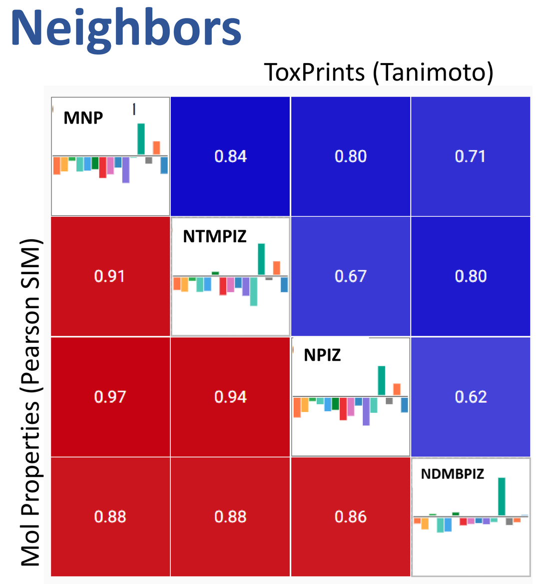
[6]Yang *et al.* Chem. Res. Toxicol. 2021, 34, 616-633. "Do Similar Structures Have Similar NOAEL Values?"

3.2 Local Similarity – Piperazine/Piperidine Neighbors





4-NMPIZ (MNP)
Sample mean: 2.11 mg/kg/day
95% CI: 0.11 ≤ BMD10 ≤ 41.0
Read-Across Reliability:83%
Hazard: Carcinogenic
(Harmonic TD50 sample mean = 1.27)



IV. REGULATORY IMPURITIES

4.1 NIAS (Foods) & NDSRI (Drugs)

- Non-Intentionally Added Substances (NIAS)[2]
  - Out of 32 nitrosamines, 23 were carcinogenic
  - 10 were detected in food: NDMA, NMEA, NDEA, NDPA, NDBA, NMA, NSAR, NMOR, NPIP and NPYR (TCNAs)
  - N-nitrosamines with a TD50 below 1.5 mg/kg/day (cohort of concern chemicals): NMPEA > **NDEA** > **NDMA** > **NMEA** > **NNK** > NNN > **NMOR** > **NMA** > NDPA > **NDBA** > **NPYR** > MNNG > **NMBA** > **NPIP** (from higher potency to lower)
- Nitrosamine Drug Substance Related Impurities (NDSRI)[1]
  - As of January 2025, potency categories and acceptable intake limits (AIL) for 272 potential drug nitrosamines were reported by FDA CDER
  - Possible drug impurities (7): **NDMA**, **NDEA**, NMPA, NDIPA, NIPEA, **NDBA**, **NMBA**
  - In several cases, compound data for **NNK**, **NPIP**, **NDMA**, **NDEA** were used for specific compound or read-across assessment. Bold font denotes impurities found either in food or drugs.

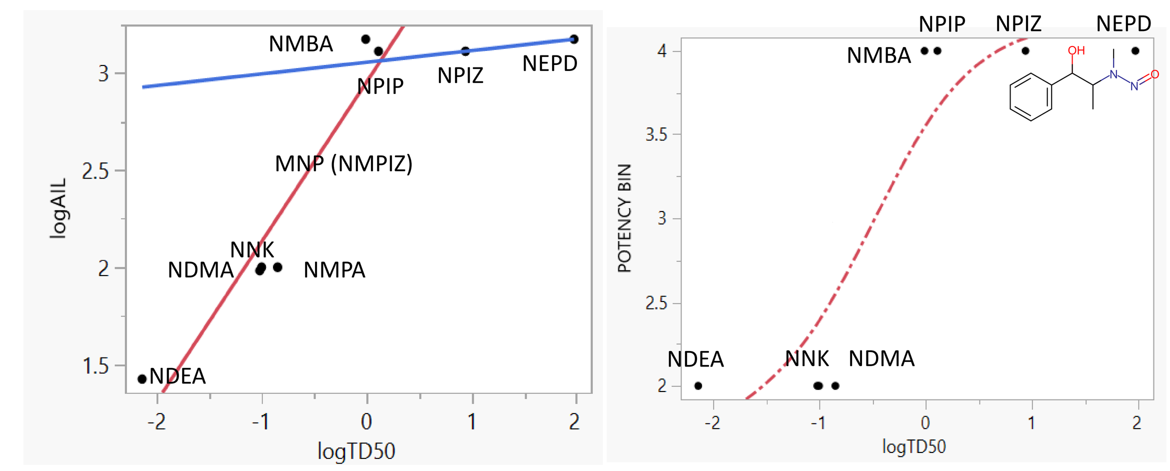
4.2 Profiles: BMD10, TD50, Potency, AI Limits

N-Nitroso	NIAS; NDSRI	BMD10 (mg/kg bw/day)	TD50 (mg/kg bw/day)	Potency Category	AI Limit (ng/day)
NDMA	EFSA; FDA	0.00828	0.0959	2 (2)	96 (146)
NDEA	EFSA; FDA	0.0289	0.00725	2 (1)	26.5 (18)
NMPA	FDA	0.240	0.142	2 (2)	100 (202.7)
NDIPA	FDA	NA (0.063)	NA (0.205)	5 (3)	1,500 (1,036)
NIPEA	**FDA**	NA (0.145)	NA (0.042)	3 (3)	400 (942)
NDBA	FDA	0.233	0.691	NA (3)	NA (742)
NMBA	FDA	1.442	0.982	4 (3)	1,500 (989)
NNK	EFSA; FDA	0.103	0.0999	2 (2)	100 (152)
NPIP	EFSA; FDA	0.0133	1.3	4 (3)	1,300 (1245)
NPIZ	EFSA	19.0	8.78	4 (4)	1300 (1,300)
MNP	FDA	NA (8.67)	NA (3.63)	3 (4)	400 (1,232)
NMOR	EFSA	2.678	2.37	NA (2)	NA (163)
NMEA	EFSA	0.0895	0.0503	NA (2)	NA (86.6)

Compounds with values in blue or purple font do not have experimental data. Purple indicates read across examples from 3.1 & 3.2. Values in the parentheses are estimated by applying the relationships established in this study: TD50 values were obtained by read-across; AI Limits were calculated by equation (1); potency categories were assigned by TD50 values in table in Section 5.1.

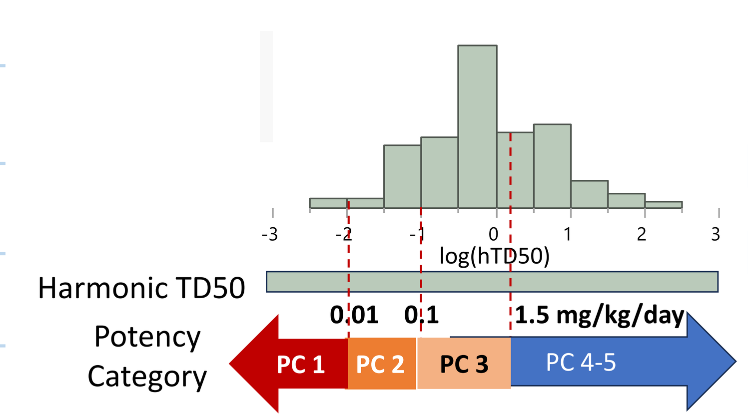
V. TD 50, POTENCY CATEGORY, AI LIMIT

5.1 TD50, AI Limit, Potency Category



- log(AIL) vs log (TD50) relationship shows two distinct regions for nine nitroso compounds in Table 4.2
  - Linear region for log (TD50) < 0
  - Plateau region: log(AIL) values level off at log (TD50) > 0
  - These regions were modeled to a fitting equation and used to estimate the log(AIL) from log (TD50)
- Similar leveling-off relationship is observed in relationship between Potency Categories and log (TD50) values

$$\log(AIL)=3.029+\begin{Bmatrix}-0.028_{linear}\\+0.028_{plateau}\end{Bmatrix}+\left(0.440+\begin{Bmatrix}+0.380_{linear}\\-0.380_{plateau}\end{Bmatrix}\right)\log(TD50) \qquad (1)$$



TD50 (mg/kg/day)	Potency Category	AI Limit (ng/day)* min	AI Limit (ng/day)* max	AI Limit (ng/day)* avg	US FDA*
>15	5	1,500	66,700	4,581	≥ 1,500
1.5 < TD50 <=15	4	1,300	8,330	2,120	1,500
0.15 < TD50 <=1.5	3	100	1,215	570	400
0.015<TD50 <= 0.15	2	26.5	230	109	100
<=0.01	1	26	17.7	26.9	26.5

* These values were taken from the nitrosamine guidelines from US FDA CDER.

- This approach enables systematic estimation of the TD50, AI Limits, and Potency Categories, and is generally a more robust but comparable method to the empirical relationship used by ICH M7 (R1): AI (mg/day) = [TD50 (mg/(kg day))/50 000] 50 kg human

ICH M7 (R1): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, M7(R1), Step 4; ICH, 2017.

CONCLUSIONS

- For read-across, local similarity based on defined structural classes can help identify better analogues than relying on global similarities
- Read-across predicts BMD10 more accurately than TD50; however, since classifications of potency categories and AI Limits are approximate assessments, the use of TD50 was adequate to estimate them
- The approach was applied to nitrosamines having no reliable data, *e.g.*, NEPA, MNP, NDIPA, and NIPEA; examples for two structure groups are presented
  - local read-across of TD50 (ng/kg-bw/day) → AI Limit (ng/day) and Potency Category
  - results comparable to Group AI approach by Dobo *et al.*[3]
- The approach is easily applicable to ureas and guanidines (Table 1.2)
- The approach is applied in ChemTunes·ToxGPS® Express Cross.Road

MN-AM Hosted Session: Wednesday 3:15-4:15 pm (Room W209A) **Contact on ChemTunes.CrossRoad: info@mn-am.com (Booth 530)** **Scientific Contact: chihae.yang@mn-am.com**