EUROTOX
Vienna, 2026
P18-83 #1282

In Silico Assessment of Relative Metabolic α-Hydroxylation and Detoxification Potential of Nitrosamine Drug Substance-Related Impurities

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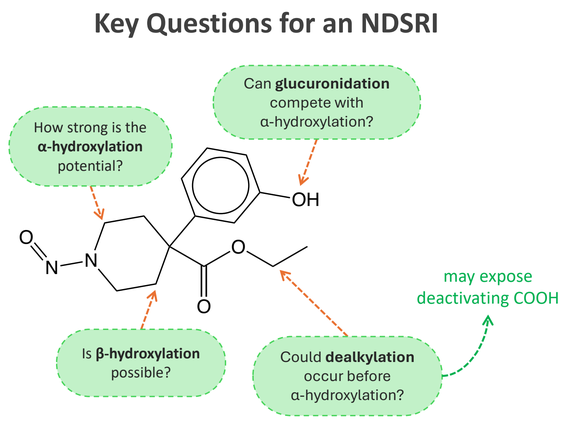
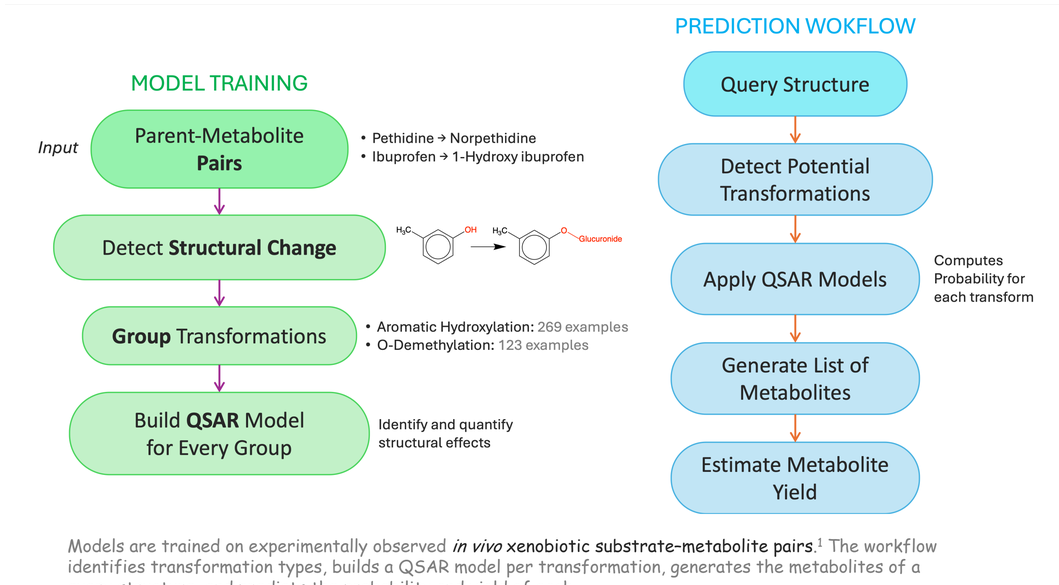
Background and Purpose

- Carcinogenic potency of dialkyl N-nitrosamines is governed by in vivo metabolism, above all by **α-hydroxylation**, which generates DNA-reactive intermediates.
- Competing routes — **phase II conjugation, N/O-dealkylation, ester hydrolysis** — modulate that potential by promoting clearance or by altering the structure itself.
- **NDSRIs** are far more structurally diverse than the small nitrosamines for which carcinogenicity data exist, so read-across from those data is weak.
- **Aim:** quantify the balance between activation and detoxification, rather than scoring activation alone.

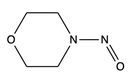
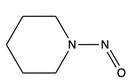
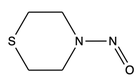
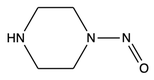


End-to-End Machine-Learning Framework



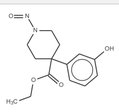
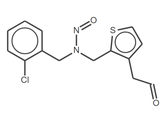
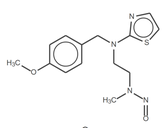
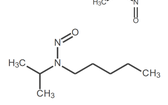
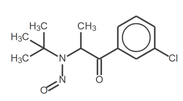
Models are trained on experimentally observed *in vivo* xenobiotic substrate-metabolite pairs.[1] The workflow identifies transformation types, builds a QSAR model per transformation, generates the metabolites of a query structure, and predicts the probability and yield of each.

Predicted α-Hydroxylation Aligns With Known Carcinogenic Potency

Nitrosamine	Structure	Predicted α-Hydroxylation probability	α-Hydroxylation (% of conversion)		TD_{50} (mg/kg/day)[2] lower = more potent
N-Nitrosodiethylamine		0.87		87%	0.027
N-Nitrosomorpholine		0.58		64%	0.109
N-Nitrosopiperidine		0.77		61%	1.43
4-Nitrosothiomorpholine		negligible		~0%	5.39
1-Nitrosopiperazine		negligible		~0%	8.78

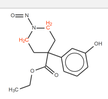
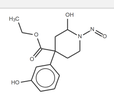
Predicted α-hydroxylation orders these five nitrosamines as their measured carcinogenic potency does: the two compounds with no predicted α-hydroxylation are the two least potent.

For NDSRIs, Clearance Competes with Activation

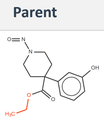
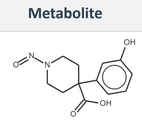
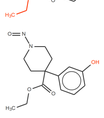
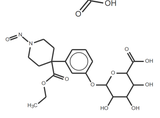
NDSRI	Structure	Predicted Metabolites	α-Hydroxylation (% of conversion)		Leading competing pathways (prob.)
N-Nitroso Hydroxypethidine		36		14%	O-Dealkylation (0.38) O-Glucuronidation (0.36)
N-Nitroso Tenidap analog		40		14%	C-Oxidation (0.33) Reduction (0.25)
N-Nitroso Zolamine		21		36%	O-Demethylation (0.52) Glucuronidation (0.13)
N-Nitroso Pentyl-isopropylamine		9		54%	β-C-Hydroxylation (0.14) Dehydrogenation (0.13)
N-Nitroso Bupropion		16		~0%	Aromatic Hydroxylation (0.21) Reduction (0.13)

Across NDSRIs, a-hydroxylation is rarely the dominant route: clearance and structure-altering pathways carry 46–100 % of predicted conversion. For N-nitroso-bupropion, both a-sites are non-productive — one carries no a-hydrogen (structurally blocked), the other falls below threshold.

N-Nitroso Hydroxypethidine

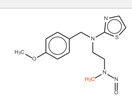
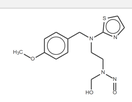
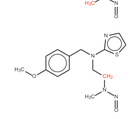
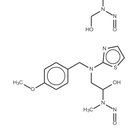
Parent	Metabolite	Reaction	Prob.	Moles
		α-Hydroxylation (CH2)	0.41	12

Competing pathways (86.5%)

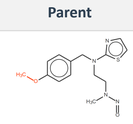
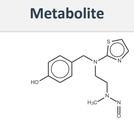
Parent	Metabolite	Reaction	Prob.	Moles
		O-Dealkylation	0.38	11
		O-Glucuronidation	0.36	11

The leading clearance route rivals α-hydroxylation. O-Dealkylation hydrolyses the ester to expose a deactivating carboxylic acid; O-glucuronidation conjugates the phenol and is likely to eliminate the NDSRI intact. Ranking activation against clearance — not activation alone — is what the method adds.

N-Nitroso Zolamine

Parent	Metabolite	Reaction	Prob.	Moles
		α-Hydroxylation (CH3)	0.56	26
		α-Hydroxylation (CH2)	0.11	5

Competing pathways (64.4%)

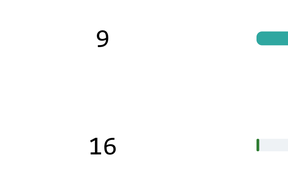
Parent	Metabolite	Reaction	Prob.	Moles
		O-Demethylation	0.52	24

A γ-tertiary amine suppresses the neighbouring α-site five-fold. The CH_3 α-carbon is hydroxylated with p = 0.555; the CH_2 α-carbon — the one bearing the γ-tertiary amine — falls to p = 0.108. CPCA scores the molecule as a whole; the model resolves the individual site.

Case Study — N-Nitrosoparoxetine: Predicted Pathways Against Experiment

Heck et al. (2025) Xenobiotica 55 (10), 737–752

N-Nitrosoparoxetine was non-mutagenic, with CYP metabolism favoring benzodioxole oxidation and Phase II conjugation rather than DNA-reactive α-carbon oxidation of the piperidine ring.[3]



Predicted α-hydroxylation probabilities at the two piperidine α-carbons

Metabolite	Reaction	Prob.	Moles	% of conversion
	α-Hydroxylation (CH2)	0.41	21	36%
	α-Hydroxylation (CH2)	0.27	14	23%
	Ring Opening	0.15	8	13%

Ring opening opens the gate. The 1,3-benzodioxole ring opening (p = 0.15) is the highest-probability competing pathway route, and it unmasks the catechol that the predicted phase-II conjugations then act on (glucuronidation p = 0.098 and 0.097) — the sequence Heck et al. observed, recovered from structure alone.

α-Hydroxylation yields no stable product. Among the metabolites, phase-II conjugates of the catechol carry 53% of the flux, matching a profile in which virtually all recovered species were phase-2 conjugates.

Conclusions

- An automated ML framework predicts **α-hydroxylation and its competing pathways quantitatively**, for structures with no carcinogenicity data of their own.
- Predicted α-hydroxylation **reproduces the potency ranking** of some small nitrosamines with measured TD_{50} values.
- For NDSRIs the predictions **agree with CPCA's activating and deactivating features** and additionally resolve **site-level** effects — a γ-tertiary amine suppresses the adjacent α-carbon five-fold.
- The method identifies cases where **an alternative pathway may compete with activation** — for N-nitrosoparoxetine, 1,3-benzodioxole ring opening followed by phase-II conjugation, consistent with the observed metabolite profile — so weighing activation against detoxification supports **mechanistic evaluation of NDSRI carcinogenic risk**.

References

1. Chakravarti, S. Computational Prediction of Metabolic α-Carbon Hydroxylation Potential of N-Nitrosamines: Overcoming Data Limitations for Carcinogenicity Assessment. Chem. Res. Toxicol. 2023, 36, 959–970.
2. Kruhlak, Naomi L. et al. Determining Recommended Acceptable Intake Limits for N-Nitrosamine Impurities in Pharmaceuticals: Development and Application of the Carcinogenic Potency Categorization Approach (CPCA). Regulatory Toxicology and Pharmacology 150 (June 2024): 105640.
3. Heck, D. E. et al. Studies addressing potential bioactivation and genotoxicity liabilities of the N-nitroso derivative of the antidepressant paroxetine. Xenobiotica 2025, 55(10), 737–752.

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