Defining boundaries: which *N*-nitroso compounds might not belong in the cohort of concern?

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Cohort of concern: a vague definition

N-Nitroso compounds belong to the cohort of concern due to some members of the chemical class having significant carcinogenic potential below the general 1.5 µg/day threshold of toxicological concern (TTC) limit. Structurally-defined by name only, *N*-nitroso compounds are broadly represented by N-N=O; however, around half the *N*-nitroso compounds analysed have lower carcinogenic potency than the TTC [1]. In fact, high potency is attributed to those capable of undergoing facile α-hydroxylation & subsequent formation of a diazonium ion in proximity to react with DNA. An understanding of the structural features which mitigate activity has been curated [2, 3] & used to suggest refinement of the structural definition of the cohort of concern to allow *N*-nitroso compounds with low carcinogenic risk to be assessed in the same manner as other impurities under ICH M7 without increasing the risk to human health [4].

α-Hydroxylation: the potent mechanism

The carcinogenic activity of *N*-nitroso compounds is typically attributed to metabolic formation of an alkyldiazonium ion *via* α-hydroxylation (**Fig. 1**). Although it is possible for carcinogenicity to occur *via* other mechanisms (e.g., nitrosoureas may react directly with DNA, whereas *N*-nitrosodiarylamines may undergo transnitrosation to *N*-(nitrosoaryl)aniline), significant carcinogenic potency is linked to the ability to undergo facile α-hydroxylation.

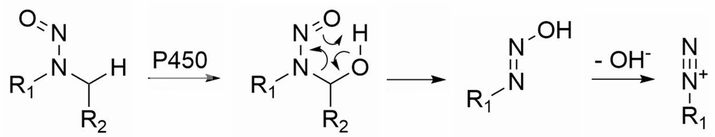
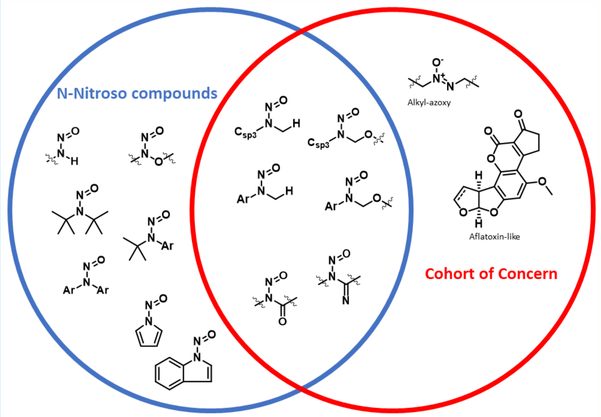


Fig. 1. Alkyldiazonium formation *via* *N*-nitrosamine α-hydroxylation.

N-Nitroso compound subclasses: cohort or not?

The rate at which metabolic activation occurs (if it is even possible) & the stability of the resulting alkyldiazonium ion is not consistent between subclasses of *N*-nitroso compounds [4]. **Fig. 3** briefly highlights those subclasses which may be considered outside the scope of the cohort of concern due to their lack of significant carcinogenicity.

Which *N*-nitroso compounds might not belong in the cohort of concern?

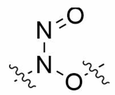


Can we redefine the boundaries of the cohort of concern?

1. Nitrosated primary amines

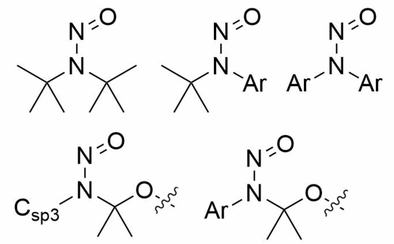
- May form diazonium ion *via* α-hydroxylation; however, it will be inherently unstable *in vivo* & is expected to decompose before reaction with DNA can occur.

2. Nitrosoalkoxylamines



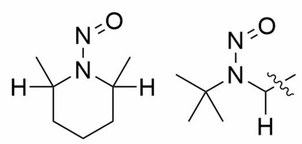
- May undergo α-hydroxylation but are incapable of forming an alkyldiazonium ion & are observed to be weakly carcinogenic or non-carcinogenic [3].

3. Nitrosamines without α-hydrogen



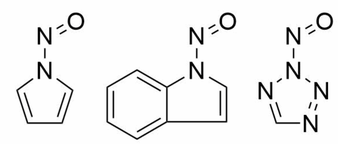
- Cannot undergo α-hydroxylation to form a diazonium ion.
- Aliphatic substituents: negative in carcinogenicity assays.
- Aromatic substituents: weakly carcinogenic *via* other mechanisms (e.g., transnitrosation to *N*-(nitrosoaryl)anilines).
- α-Hydroxy/α-alkoxy: metabolic intermediate in α-hydroxylation mechanism & observed to be carcinogenic.

4. Nitrosamines with α-hydrogen & significant steric hindrance



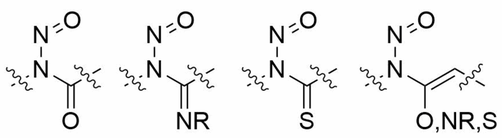
- Mechanistically capable of undergoing α-hydroxylation; however, metabolism it is not facile & these are observed to be non-carcinogenic.

5. Aromatic *N*-nitroso compound



- Unable to form diazonium ion, but mutagenicity may occur *via* *N*-nitroso reduction to a hydroxylamine, hence these have lower carcinogenic potential akin to aromatic amines.

6. Nitrosoureas & analogues



- Direct-acting carcinogens which have potency that conservatively warrants a position in the cohort of concern; however, because no metabolic activation is required, a negative result in the Ames test should be accepted.

Fig. 3. Summary of carcinogenic potential of subclasses of *N*-Nitroso compounds.

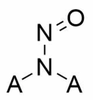
Ponting & Foster

Thresher *et al*. | Cross & Ponting | Thomas *et al*.

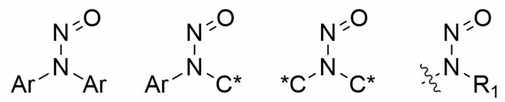
Cohort of concern: redefined

Presence of an α-hydrogen is critical for significant carcinogenic potency. Therefore, it may be reasonable to redefine the cohort of concern to include only dialkyl *N*-nitrosamines bearing an α-hydrogen, or a hydroxy or an alkoxy substituent (as the metabolic products) (**Fig. 2**). Nitrosoureas & analogues may be conservatively included due comparable potency; however, a negative result from the Ames test should be considered acceptable.

Current cohort



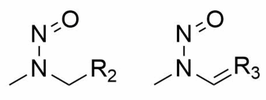
Subclasses to remove from cohort



C* sp^3 and may not be attached to any R_2

R_1 = H, any heteroatom

Proposed cohort



R_2 = H, OH, OC

R_3 = O, N, S

Fig. 2. Current scope of the cohort of concern, subclasses to remove from the cohort of concern & the proposed scope of the cohort of concern.

[1] Thresher *et al*., *Regul*. *Toxicol*. *Pharmacol*., **2020**, *116*, 104749. [2] Cross & Ponting, *Comput*. *Toxicol*., **2021**, 100186.
[3] Thomas *et al*., *Chem*. *Res*. *Toxicol*., **2022**, *35*, 1997-2013. [4] Ponting & Foster, *Org*. *Process Res*. *Dev*., **2023**, Article ASAP.