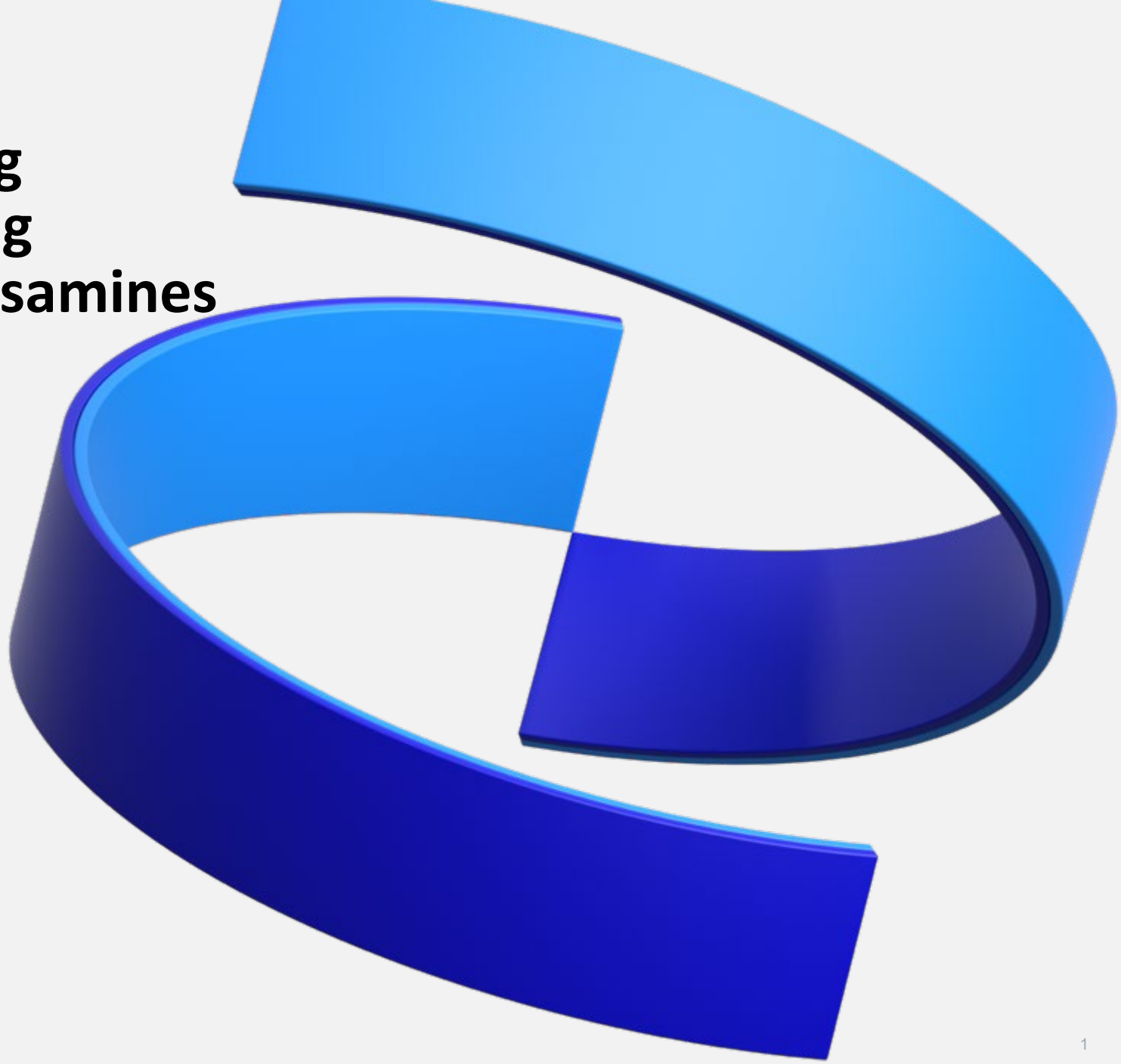
Framework for Establishing Acceptable Intakes for Drug Substance Related *N*-Nitrosamines

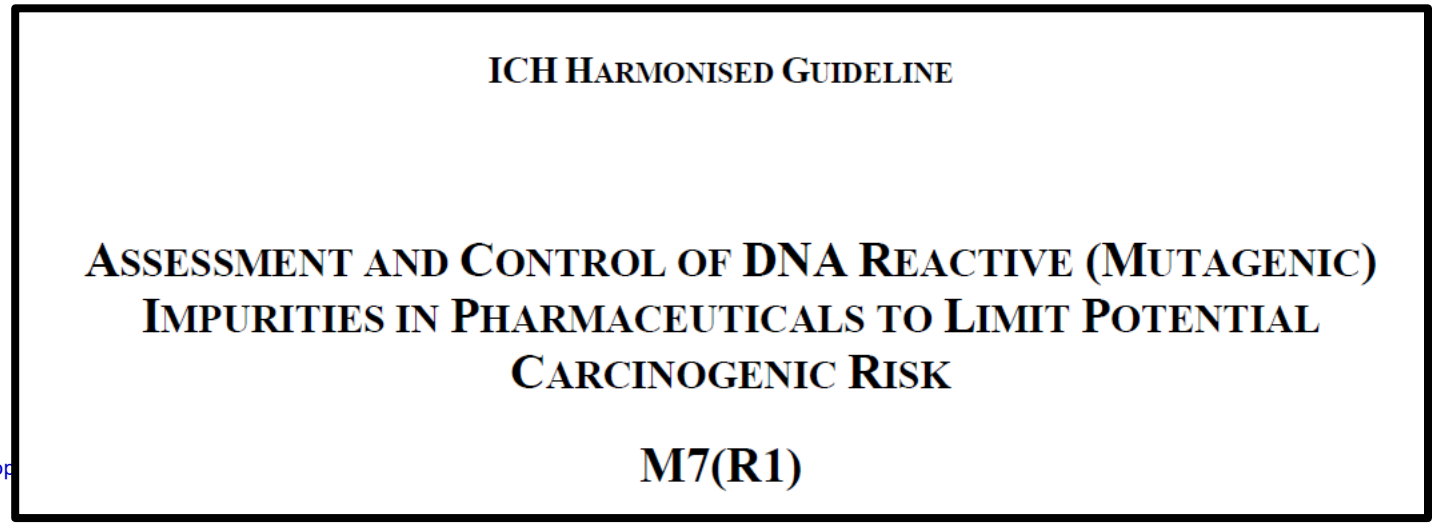


05 May 2023
Genetic Toxicology Meeting
Krista Dobo

Mutagenic Impurities In Pharmaceuticals

Synthesis of Active Pharmaceutical Ingredient

- Utilize reactive intermediates and starting materials
- Chemically reactive materials may also be DNA reactive
- Potential for residual impurities with mutagenic and carcinogenic potential to be present in medicines
- Control to *acceptable limits*


ICH HARMONISED GUIDELINE

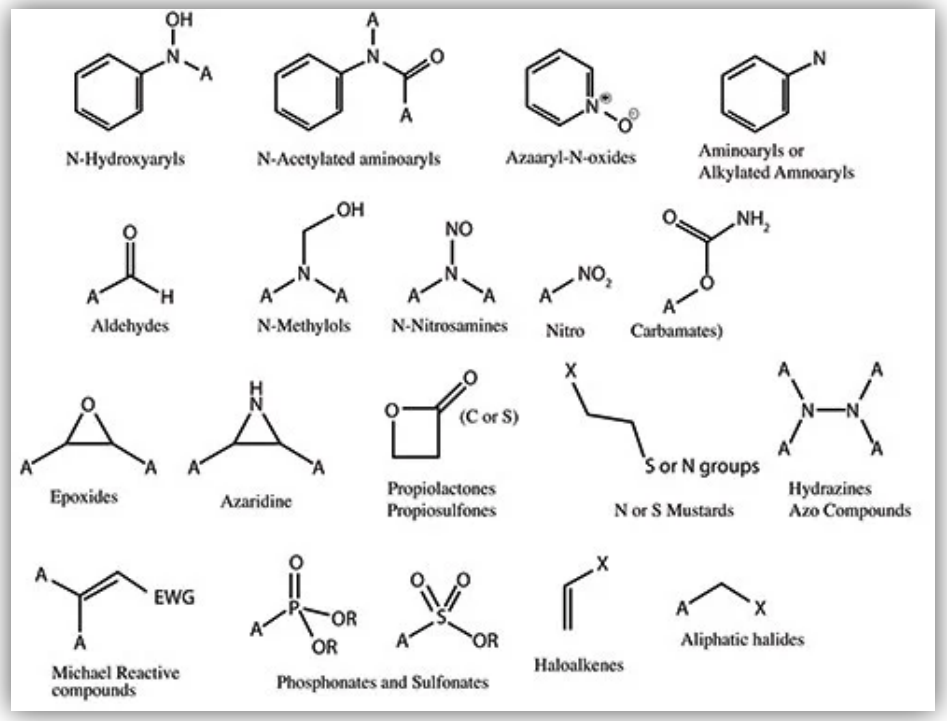
ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK

M7(R1)

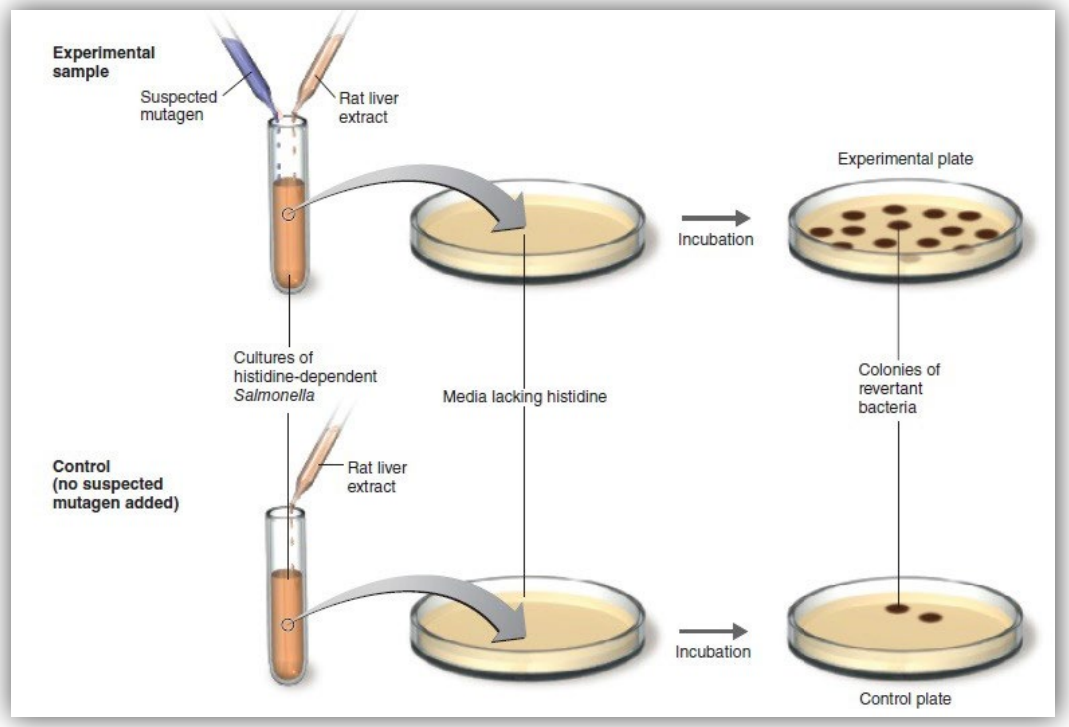
Identification of Mutagenic Impurities

ICH M7 Guideline Recommends Two Approaches

(Q)SAR



Bacterial Mutagenicity Assay (Ames Test)

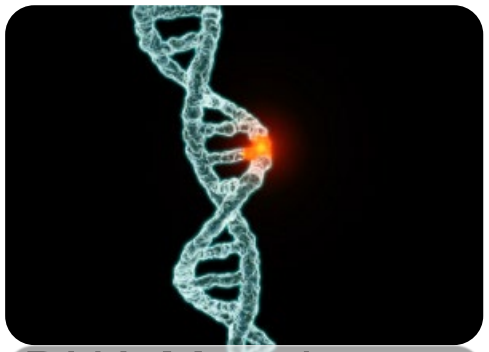


Impurities that are mutagenic in the Ames assay or contain a structurally alerting feature
Align limits such that TTC defined in regulatory guidance not exceeded

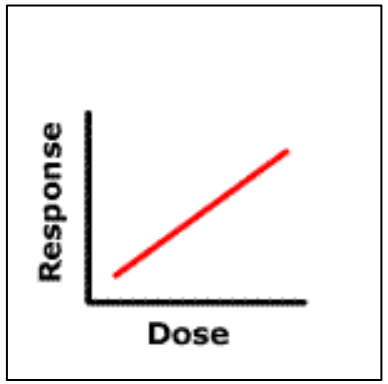
Risk Assessment Approach - Mutagenic Impurity

Method applied in ICH M7 guidance same as used decades earlier by FDA to define “Threshold of Regulation” for foods

Underlying Conservative Assumption:

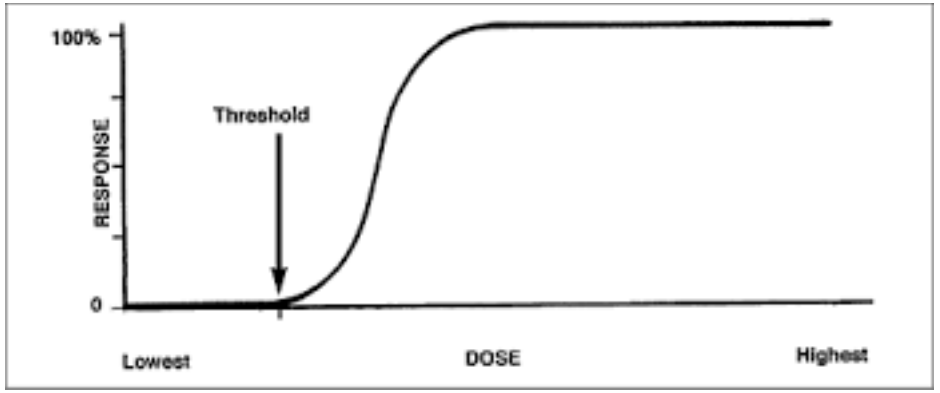


DNA Mutations
Tumor Incidence



Linear Dose Response Relationship

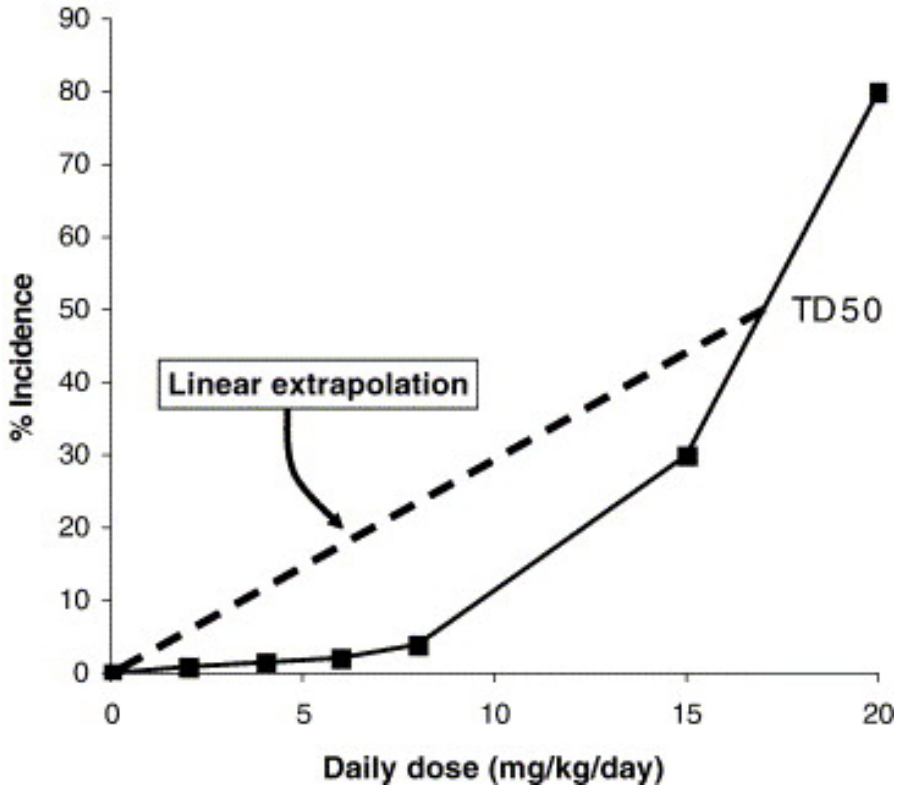
Expected Biological Response:



Threshold Dose Response as a result of Detoxification, DNA Repair, etc

Risk Assessment Approach - Mutagenic Impurity

(ICH M7 (R2), 2023)



Based on 2 Year Rodent Carci Study

- Tumor incidence data used to estimate TD50
- TD50 = Lifetime daily dose associated with a 50% tumor incidence
- Use linear extrapolation to derive the daily dose associated with a ***theoretical lifetime excess cancer risk of 1 in 100,000***
- Assumes all biological processes involved in generation of tumors at high dosages are linear over a 50,000-fold range! Very conservative

Read Across an ICH M7 Principle

ICH M7 (R2) Section 7.5

"..a case by case approach using e.g. carcinogenicity data from closely related structures, if available, should usually be developed to justify acceptable intakes for pharmaceutical development and market products..."

EMA Assessment Report June 25, 2020 (EMA/369136/2020)

"In cases where robust TD50 values as point of departure for excess cancer risk calculations are not available, the SWP recommends using a class specific threshold of theoretical concern (TTC) of 18 ng/d as default option with the possibility to justify a higher limit based on the structure-activity-relationship (SAR) approach described in the ICH M7(R1).

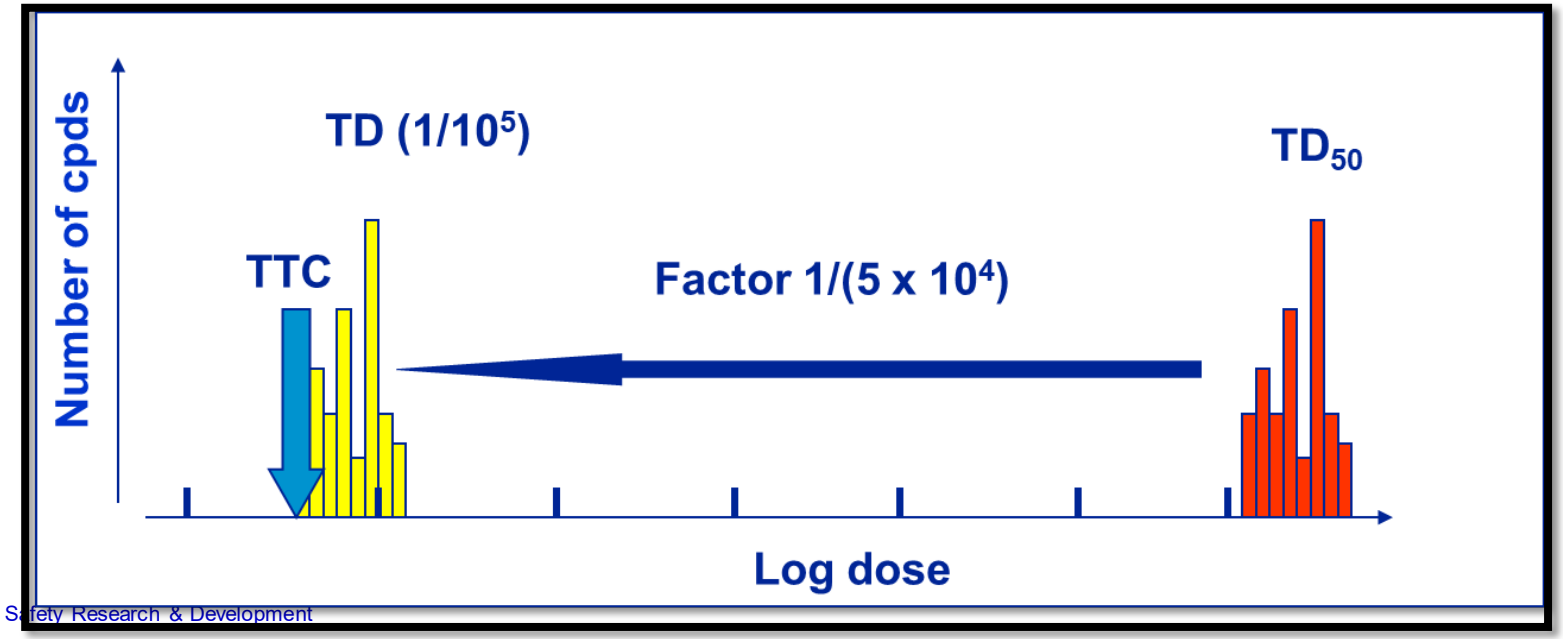
"….consider all N-nitrosamines containing a α-hydrogen that can be metabolically activated as potentially mutagenic and carcinogenic to humans, however with different potencies depending on nature of the functional group, specifics of metabolic activation and repair efficiency and capacity"

Risk Assessment Approach - Mutagenic Impurity

(ICH M7 (R2), 2023)

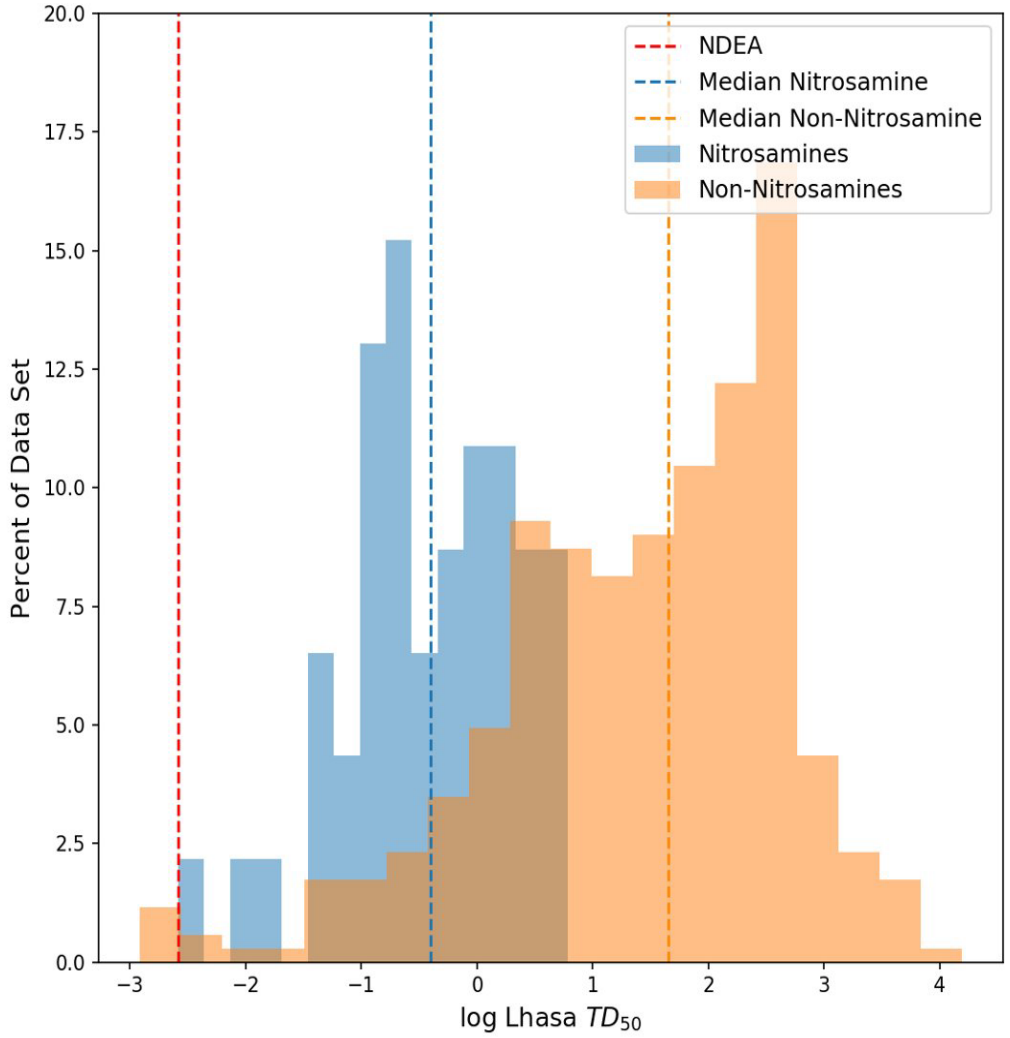
In cases where the mutagenic impurity has not been evaluated in carci study

- Apply a conservative default limit of 1.5 µg/day
- AKA Threshold of Toxicological Concern or TTC
- Also derived using linear extrapolation from TD50s to TD (1 in 100,000)
- From large database of chemicals tested in rodent carcinogenicity studies



N-Nitrosamines

- Some N-nitrosamines are potent mutagenic carcinogens in rodents
- **Cohort of Concern** chemicals in ICH M7 guidance *(ICH M7 (R2), 2023)*
- Acceptable intakes for those with high potency are low
  - NDMA = 96 ng/day*
  - NDEA = 26 ng/day*
- In absence of carcinogenicity data regulators apply default limit of **18 ng/day****
- Translates to ppb limits



Thresher, et al., 2020. Regul. Toxicol. Pharmacol. 116, 104749

*USFDA (2021) *Control of Nitrosamine Impurities in Human Drugs, Guidance for Industry*

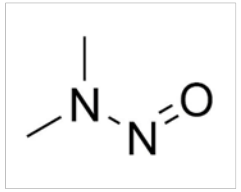
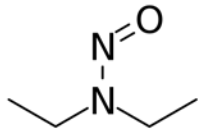
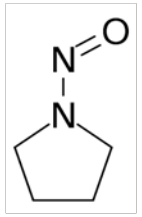
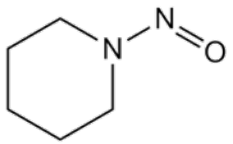
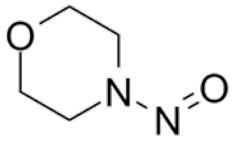
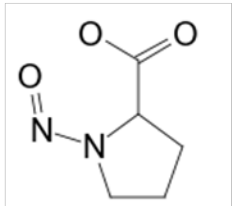
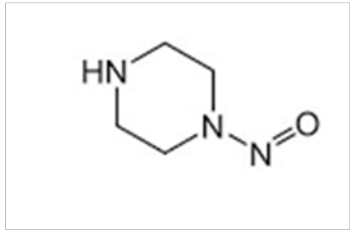
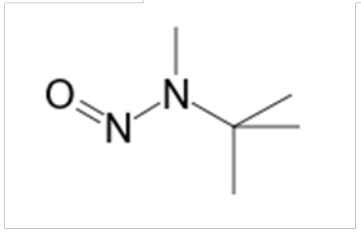
** EMA (2021) *European Medicines Regulatory Network Approach for the Implementation of the CHMP Opinion Pursuant to Article 5(3) of Regulation (EC) No 726/2004 forNitrosamine Impurities in Human Medicines*; 2021.

N-Nitrosamines Related to Drug Substance (NDSRIs)

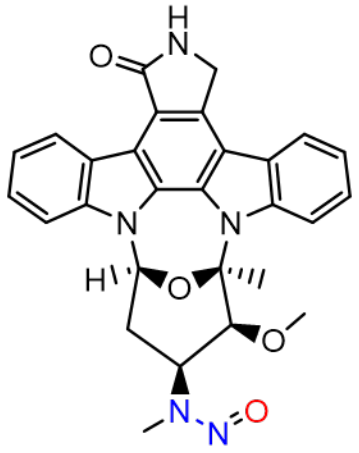
- Possible due to trace nitrite from (e.g.) excipients and secondary / tertiary amine in drug substance structure
- Most have no rodent carcinogenicity data
- Structurally complex in comparison to those tested in carci studies
  - Existing data - simple small molecular weight *N*-nitrosamines
- No consensus regarding:
  - Selection of surrogate structures with rodent carci data for read across
  - Use of other experimental data to inform setting AIs (e.g. Ames, In vivo)
- Regulatory agencies often apply default limit of 18 ng/day
  - Unlikely relevant to NDSRIs

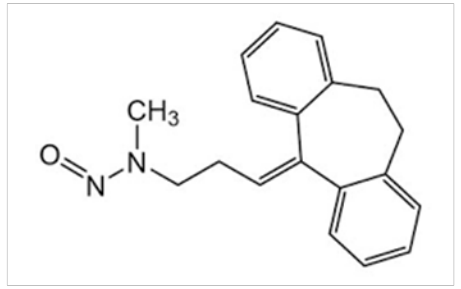
Structural Diversity

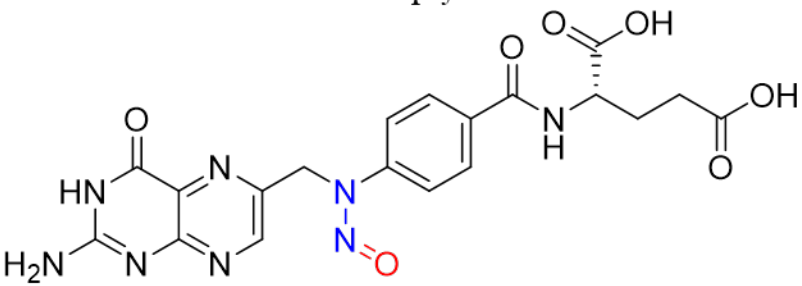
Examples
Tested in Carcinogenicity Studies

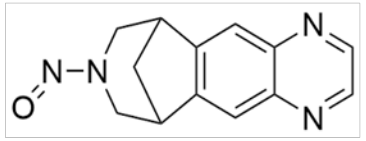









Examples
Complex API-*N*-Nitrosamines

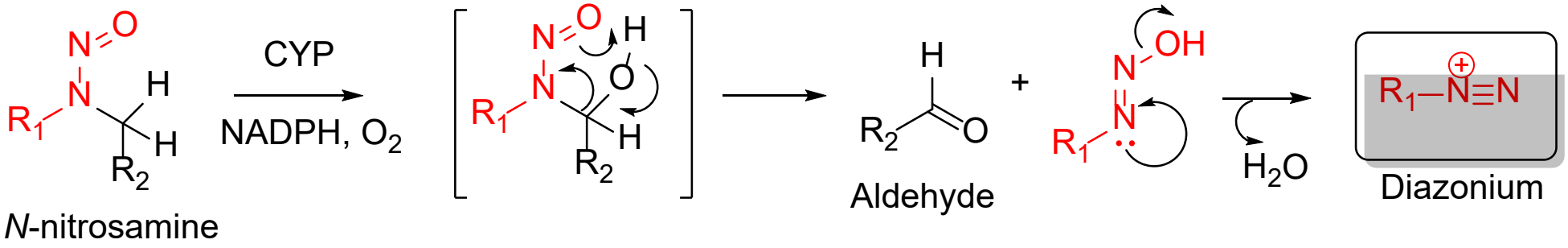

Nitroso-Staurosporin


N-Nitroso-nortriptyline


N-Nitroso-Folic Acid


N-Nitroso-Varenicline

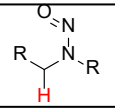
NDSRIs Features That Diminish Rodent Carcinogenic Potency



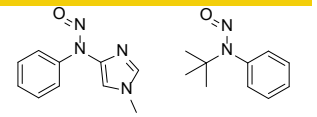
- Decrease or inhibition of α-hydroxylation metabolism
  - Lack of available hydrogens
  - Steric hinderance at the alpha carbon position
  - Competition for other sites of metabolism
  - Competition for detoxification pathways

- Higher molecular weight results in fewer possible diazonium ions on a mg/kg basis.

Temporary AIs for NDSRIs with a MW > 200 Da Nitrosamines

1. Is there at least one alpha carbon that contains at least one abstractable hydrogen atom? e.g. 

No → **Not a Cohort of Concern Out of Scope**

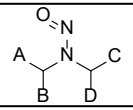
e.g. 

Yes ↓

2. Is the structure mutagenic in an OECD/ICH S2R compliant Ames assay in the presence of S9?

No → **≥ 1500 ng/day***

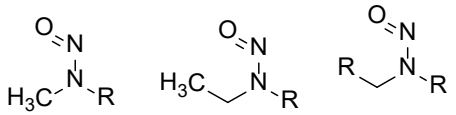
Yes or Ames not conducted ↓

3. Both sides of nitrosamine lack a CH_2 alpha e.g. 

Yes → **≥ 500 ng/day***

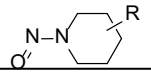
No ↓

4. Is there a methyl or ethyl on at least one side and no aromatic group on the other side?
Or
Is it an acyclic nitrosamine and there is an alpha CH_2 on at least one side and no aromatic group on the other side?



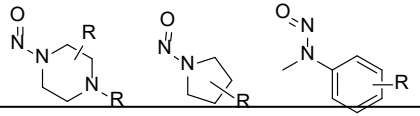
Yes → **≥ 100 ng/day***

No ↓

5. Is the nitrosamine piperidine based ? e.g. 

Yes → **≥ 1300 ng/day***

No ↓

6. All other NDSRIs with MW > 200 AI = **≥ 150 ng/day*** e.g. 

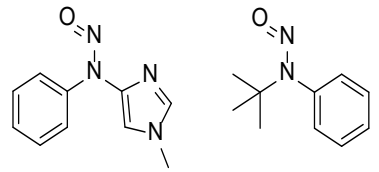
* Higher limits may be justified (SAR, other assays, etc…)

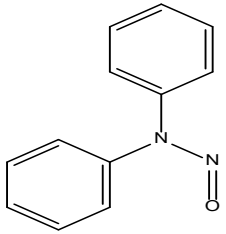
Temporary AIs for NDSRIs (>200 Da)

1. Is there at least one alpha carbon that contains at least one abstractable hydrogen atom?

No

Not a Cohort of Concern
Out of Scope





AI = 116,000 ng/d
198 Da
Both sides lack CH2

Rationale

- The potent mechanism involves alpha-hydroxylation by P450s and ultimate formation of the diazonium ion
- Without an abstractable hydrogen, alpha-hydroxylation is not possible

Note that higher limits may be justified based on SAR or other assays, etc.

Temporary AIs for NDSRIs (>200 Da)

2. Is the structure mutagenic in an OECD/ICH S2R1 compliant Ames assay in the presence of S9?

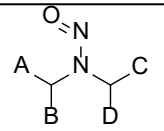
No → ≥ 1500 ng/day*

Rationale

- The Ames is highly sensitive to detect the carcinogenic potential of *N*-nitrosamines (Thresher et al, 2021 Regul Toxicol Pharmacol. 116:104749; Bringezu and Simon, 2022. Toxicol Rep. 9:250-255; Trejo-Martin et al., 2022 Regul Toxicol Pharmacol. In press).
- Currently, groups like HESI and Fraunhofer are looking to improve existing genotoxicity assays for nitrosamines.
- This AI based on ICH M7, is a temporary measure until the assay can be fully optimized and conservatively assumes the nitrosamine is carcinogenic even with an Ames negative response.

Note that higher limits may be justified based on SAR or other assays, etc.

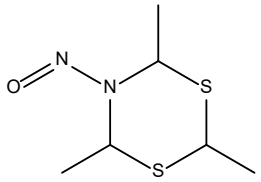
Temporary AIs for NDSRIs (>200 Da)

3. Both sides of nitrosamine lack a CH_2 alpha e.g. 

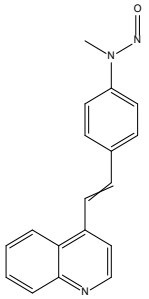
Yes → **≥ 500 ng/day***

Rationale

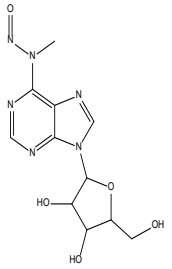
- Substitution reduces metabolism (alpha-hydroxylation) and rodent carcinogenicity



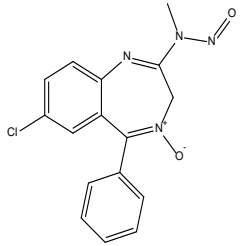
AI = 483 ng/d
192 Da
Both sides lack CH2



AI = 468 ng/d
281.3 Da
One side lacks CH2



AI = 17,400 ng/d
310.3 Da
One side lacks CH2



Neg for Carc.
310.3 Da
One side lacks CH2

Note that higher limits may be justified based on SAR or other assays, etc.

Temporary AIs for NDSRIs (>200 Da)

4. Is there a methyl or ethyl on at least one side and no aromatic group on the other side?

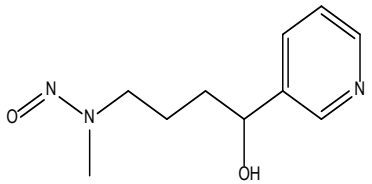
Or

Is it an acyclic nitrosamine and there is an alpha CH_2 on at least one side and no aromatic group on the other side?

Yes → ≥ 100 ng/day*

Rationale

- Methyl or ethyl on one side indicates it may be of higher potency in rodents
- There are 10 *N*-nitrosamines in CPDB with a molecular weight >200 Da (and meet this criteria) with 80% having an ethyl or methyl on one side
- All AIs > 100 ng/day

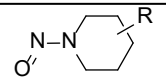


AI = 103 ng/d
209.2 Da

Lowest AI from all compounds (10) with a molecular weight >200 Da

Note that higher limits may be justified based on SAR or other assays, etc.

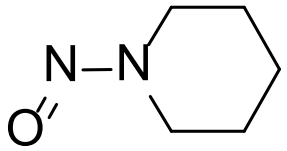
Temporary AIs for NDSRIs (>200 Da)

5. Is the nitrosamine piperidine based ? 

Yes → **≥ 1300** ng/day*

Rationale

- Based on read-across from piperidine and EMA AI
- Increased substitution and molecular weight would likely result in an increased AI

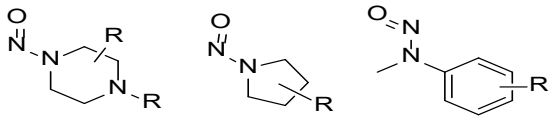


AI = 1300 ng/d (EMA)
114 Da

Note that higher limits may be justified based on SAR or other assays, etc.

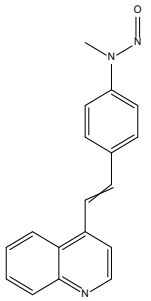
Temporary AIs for NDSRIs (>200 Da)

6. All other NDSRIs with MW > 200 AI = ≥ 150 ng/day*



Rationale

- This part of the decision tree follows filtering out certain *N*-nitrosamines such as methyl, ethyl, acyclic with at least one CH2 (these have an AI ≥ 100 ng/day
- There are 4 *N*-nitrosamines >200 Da that meet this category

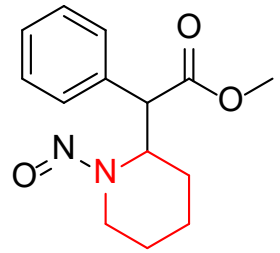


AI = 468 ng/d
281.3 Da
One side with CH2 and the other side aromatic

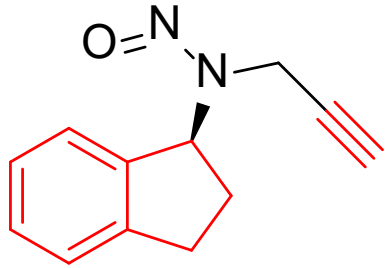
Lowest AI from all compounds (4) with a molecular weight >200 Da and meet this criteria

Note that higher limits may be justified based on SAR or other assays, etc.

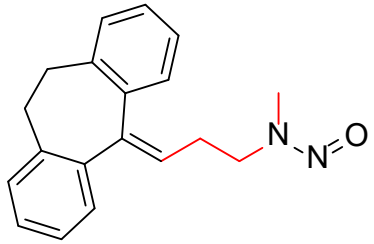
Example AIs for NDSRIs



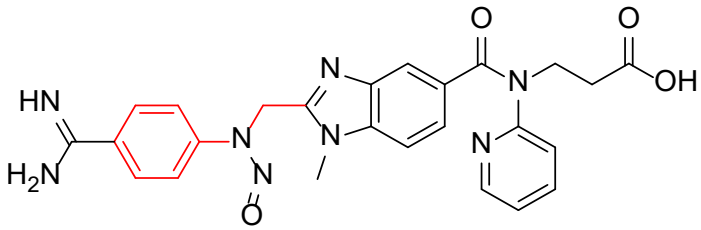
N-Nitroso-methylphenidate
EMA AI – 1300 ng/day
Proposed AI – 1300 ng/day



N-Nitroso-rasagiline
EMA AI – 18 ng/day
Proposed AI – 100 ng/day



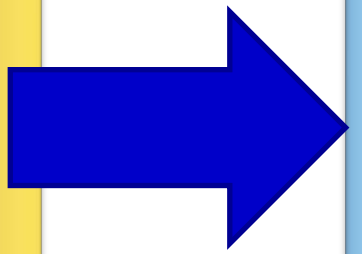
N-Nitroso-nortriptyline
EMA AI – 8 ng/day
Proposed AI – 100 ng/day



N-Nitroso-dabigatran
EMA AI – 18 ng/day
Proposed AI – 150 ng/day

Concluding Thoughts

- ***Current State***
- Derivation of AIs for NDSRIs case by case
- No certainty of outcome when AI greater than default limit of 18 ng/day proposed
  - Based on read across
  - Based on experimental data



- ***Future State***
- Agreed framework for setting AIs
  - Using read across/SAR
  - Experimental data
- Agreed weight of evidence to differentiate
  - CoC *N*-nitrosamines
  - Non-CoC *N*-nitrosamines
  - Non-mutagenic/carcinogenic *N*-nitrosamines