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NITROSAMINES IN PHARMACEUTICALS – IS THERE AN END IN SIGHT?

By

Aloka Srinivasan, Ph.D.

Principal and Managing Partner

Raaha LLC

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DEDICATION

I dedicate my presentation to Dr. Richard N. Loeppky (1937-2012), who taught me everything I know about nitrosamines and devoted his life's work to bringing consciousness amongst the industry members, agencies and public, regarding nitrosamines in drugs and cosmetics.

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TOPICS

- **Introduction**
- **Control of NDSRIs in pharmaceuticals**
  - NDSRIs and the related challenges (how much do we know about them?)
    - Endogenous nitrosation
  - Dilemma with Acceptable Intakes (CPCA or a good surrogate?)
- **What Could Lie Ahead For Nitrosamines?**
- **Conclusion**

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INTRODUCTION TO NITROSAMINES

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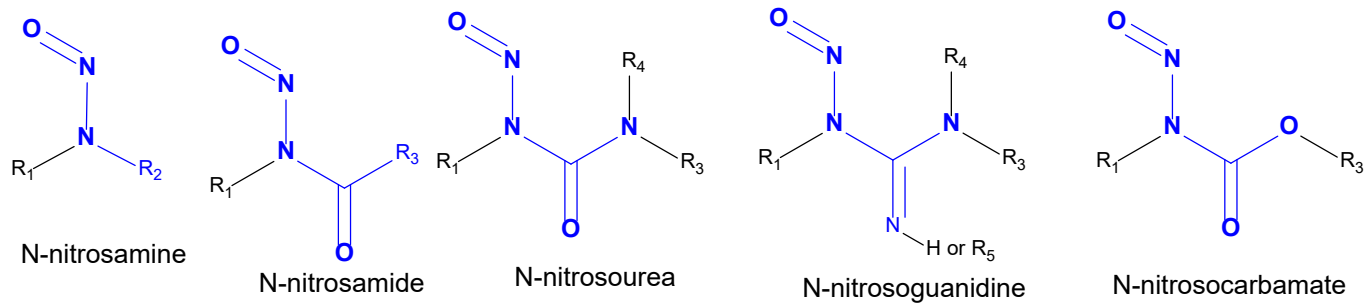
WHAT ARE NITROSAMINES?

- **N-nitroso compounds** are a family of carcinogens which are formed by the reaction of **secondary/tertiary/quaternary amines, alkyl derivatives of amides/ carbamates/urea** with nitrite or other nitrogenous agents with the nitrogen in the +3 state[1].

- **Nitrosamines** are a subset of the N-nitroso compounds (NOCs), formed by the reaction of secondary and tertiary amines with nitrosating agents



R_1, R_2, R_3, R_4, R_5 = alkyl groups

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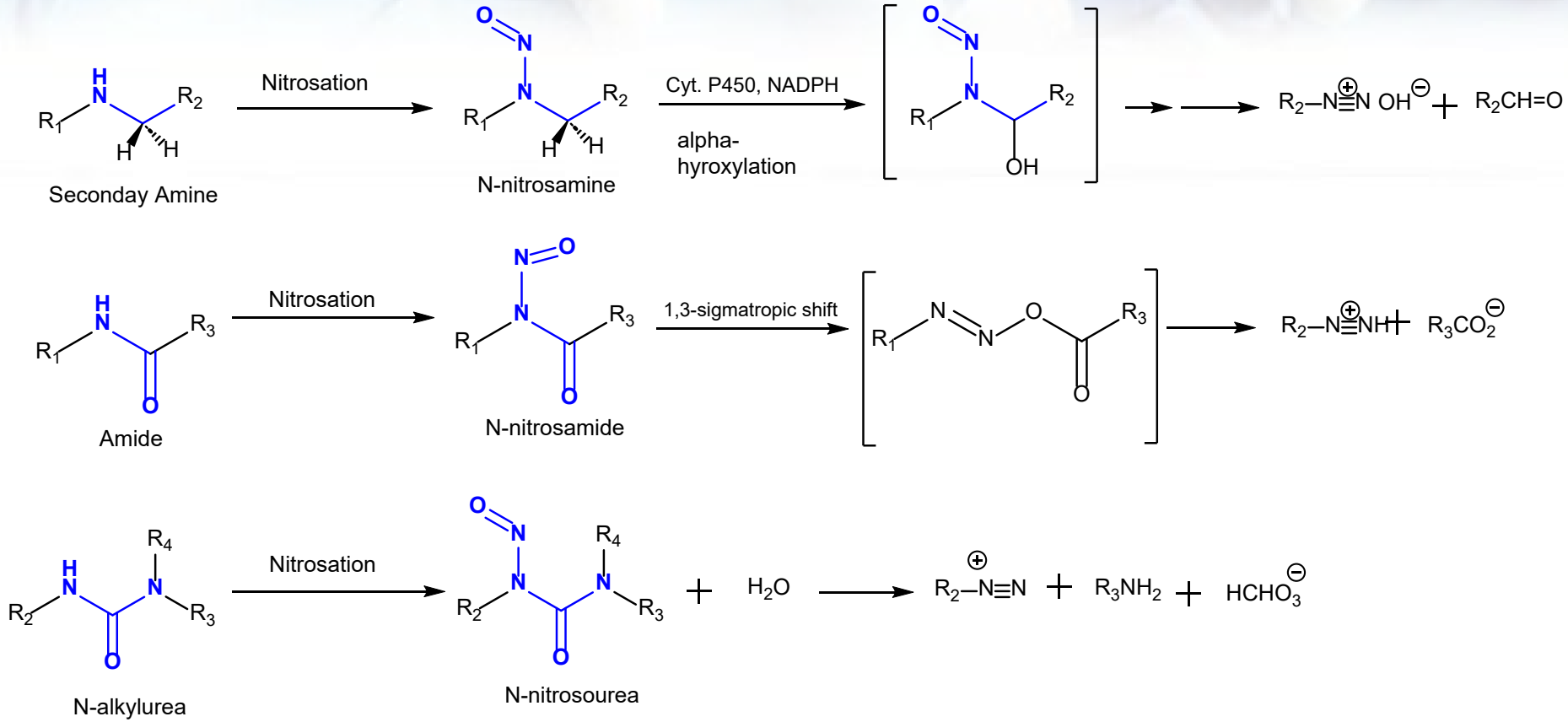
DIFFERENCE IN ACTIVATION OF NITROSAMINES VERSUS NITROSAMIDES



A. Srinivasan and C. Lambert, " Nitrosamides–Should They Be Treated the Same as Nitrosamines?," *Pharmaceutical Technology's Trends in Formulation* eBook (October 2022)

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NDSRIs AND RELATED CHALLENGES

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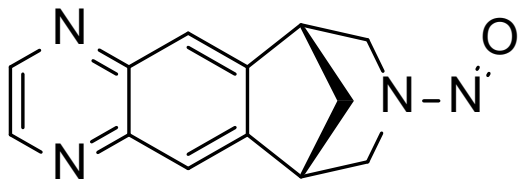
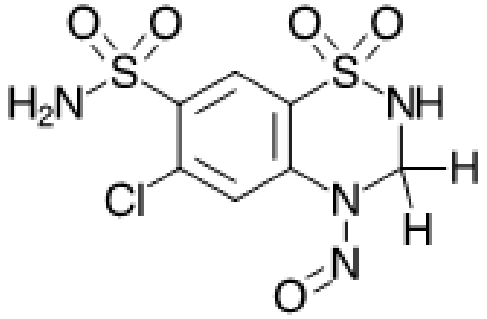
IN CAME THE NDSRIs

What are NDSRIs?

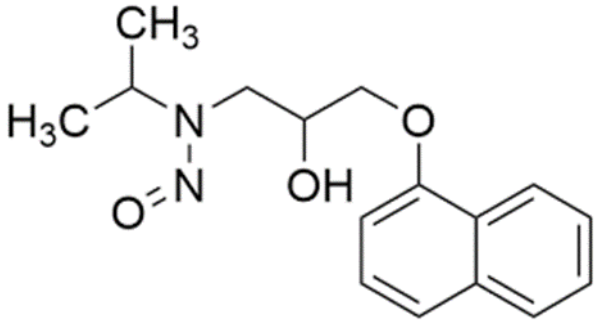
NDSRIs are **n**itrosamine **d**rug **s**ubstance **r**elated **i**mpurities[31] is a term coined by FDA and now used extensively to indicate nitrosamines impurities that are formed when the API itself, an intermediate or impurity is a secondary amine or tertiary amine and capable of nitrosating under suitable conditions and form larger nitrosamines with little or no information in the public domain. Other names include “complex nitrosamine impurities”, “API like nitrosamine impurities”, “novel nitrosamines”. Classic examples are provided below:



N-nitrosovarenicline



N-nitrosohydrochlorothiazide



N-nitrosopropranolol

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THE WORLD IS FULL OF NDSRIs

- Several drug substances are secondary, tertiary amines and capable of forming the corresponding nitrosamines in presence of nitrite/nitrate and other nitrosating agents in the drug product.

- A high-level review of the drug substances present in finished drug products approved by FDA for human use and have monographs of drug substances in the United States Pharmacopeia showed the following (performed by RAAHA, October 2020):
  - Number of Drugs with dimethylamino groups (alerts for NDSRIs & NDMA formation) **~65**
  - Number of Drugs which are secondary amines which can form N-nitrosamines **~95**
  - Number of Drugs which are tertiary amines and thus can form NDSRIs **~135**
  - Number of Drugs which are quaternary amines and may have N-nitrosamines present **~10**

- >6400 NDSRIs are possible – Schlingemann *et. al.* (https://doi.org/10.1016/j.xphs.2022.11.013)
  - A review of **Orange Book** showed that about 1739 nitrosamines are possible based on 512 drugs that are secondary amines and 1227 drugs that are tertiary amines

Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations (https://www.accessdata.fda.gov/scripts/cder/ob/index.cfm)

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ENDOGENOUS FORMATION OF NITROSAMINES INCLUDING NDSRIs

- Endogenous nitrosation of secondary and tertiary amines is a well characterized pathway that occurs for both common dietary components as well as drugs.
- The endogenous production of NDSRIs in the alimentary tract is known to occur and, in some cases, could be orders of magnitude higher than the very low limits set for these impurities in the drug substance under M7 and other guidance.
- Until this endogenous pathway of novel nitrosamine formation is better characterized for various classes of secondary and tertiary amine APIs, setting conservative AIs for API derived nitrosamines is amounting to missing a much larger issue.
- Some well-conducted epidemiological and other related clinical and non-clinical studies for many of these APIs may provide additional insight into the true hazard, if any, of long-term exposure to the endogenous production of novel nitrosamines and help put the exogenous formation in the drug substance in a better perspective.

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NDSRIs: THE KNOWN UNKNOWNs IN THE PHARMACEUTICAL WORLD

Numerous Questions Regarding the NDSRIs and their carcinogenic activation

Is the NDSRI alpha-hydroxylated at all?

- A bulky NDSRI may not be alpha-hydroxylated
- The NDSRI may be activated by pathways other than alpha hydroxylation
- NDSRI may metabolize prior to alpha-hydroxylation

Does the alpha-hydroxylated metabolite react with DNA?

- The reactive metabolite could be detoxified before it reaches the DNA
- The active metabolite may not react with DNA (bulkiness and other substituents or quenched before reacting with DNA)
- Nature of adduct formed by reaction with DNA

Is the DNA adduct carcinogenic?

- The DNA adduct could be repaired
- Possibility that it is benign

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CONTROL OF NDSRIs IN PHARMACEUTICALS

Dilemma with Acceptable Intakes (AIs)

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NDSRIs: THE KNOWN UNKNOWNs

Challenges with controlling NDSRIs

- Most of the NDSRIs are novel nitrosamines with little information in the public domain. Thus, determining an “Acceptable Intake” is challenging.
- FDA has proposed a systematic approach, “Predicted Carcinogenic Potency Categorization Approach” (CPCA) to determine the AI for NDSRIs that have little or no information in the public domain
  - However, many functional groups have not been addressed which may lead to overly conservative AIs
- Industry would like to use the scientifically viable approach of molecular weight adjustments to predict the acceptable intakes of NDSRIs
- Industry would like to use the Less than Lifetime approach stated in ICH M7 to control nitrosamines in drugs used for short term

AGENCIES and NDSRIs

Challenges with controlling NDSRIs

- Almost 30% of the NDSRIs have an AI of 26.5 ng/day (FDA) or 18 ng/day (EMA, HC) even after following the CPCA approach which would make their control in drugs substances and drug products challenging, especially for drugs with high maximum daily dose.

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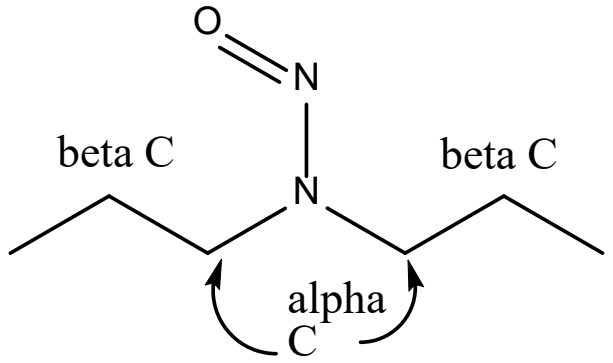
ACCEPTABLE INTAKE (AI) OF DATA POOR NDSRIs

- In recent guidance, FDA has taken an approach, **Predicted Carcinogenic Potency Categorization Approach" (CPCA)**, which broadly categorizes the nitrosamines based on the number of alpha hydrogens and functional groups on the alpha and beta carbon.
- A potency score and a corresponding AI is assigned to an NDSRI based on an empirical assessment of the structure and substitution of the nitrosamines in the CPCA
- Another option that agencies have discussed for establishing AI of data-poor nitrosamines is a **read-across** study to find a suitable **surrogate** with robust data in the public domain.



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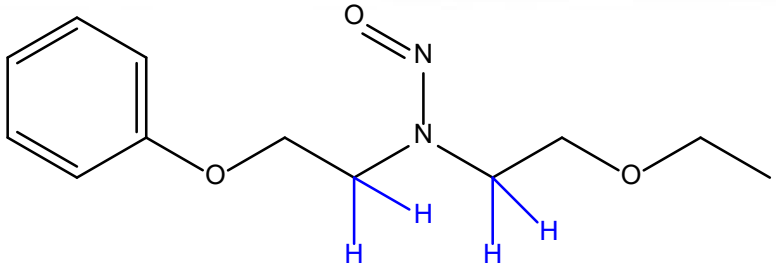
ACCEPTABLE INTAKE OF DATA POOR NDSRIs

Potency Score = α-Hydrogen Score + Deactivating Feature Score (sum all scores for features present in the N-nitrosamine) + Activating Feature Score (sum all scores for features present in the N-nitrosamine)

Category (based on adding the CPCA scores)	Proposed Acceptable Intake (AI)
1	26.5 ng/day
2	100 ng/day
3	400 ng/day
4	1500 ng/day
5	1500 ng/day

ACCEPTABLE INTAKE OF DATA POOR NDSRIs

EXAMPLE 1



N-nitroso Molecule 1

CPCA Category **2 (since CPCA does not address ether groups)**
Proposed limit = 100 ng/day
[2, 2] H = +1
2 chains of 5 non-H molecules = +1

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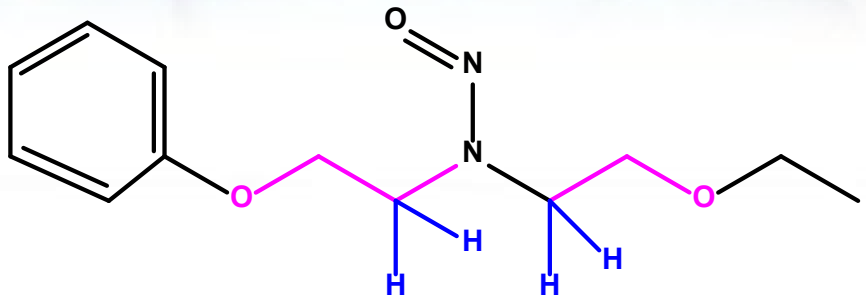
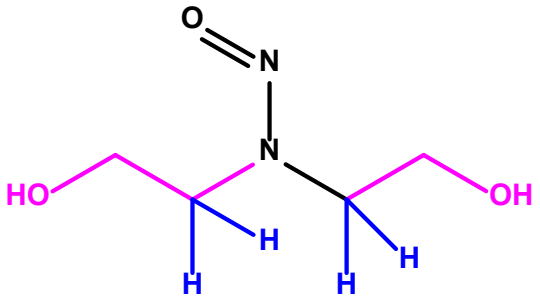
ACCEPTABLE INTAKE OF DATA POOR NDSRIs

CPCA vs SURROGATE



N-nitroso Molecule 1
AI based on CPCA = 100 ng/day



N-nitrosodiethanolamine (NDELA)
AI based on robust studies

Could NDELA be a possible surrogate of N-nitroso Molecule 1?

Ether groups present in the N-nitroso Molecule 1 are considered slightly potency enhancing for nitrosamines compared to hydroxyl groups in beta-postition. But they are not addressed in the CPCA list. Thus, the beta substituents can not be taken into consideration.

Is a limit of 100 ng/day justified for N-nitroso Molecule 1 when NDELA, a good surrogate, has an AI of 1900 ng/day?

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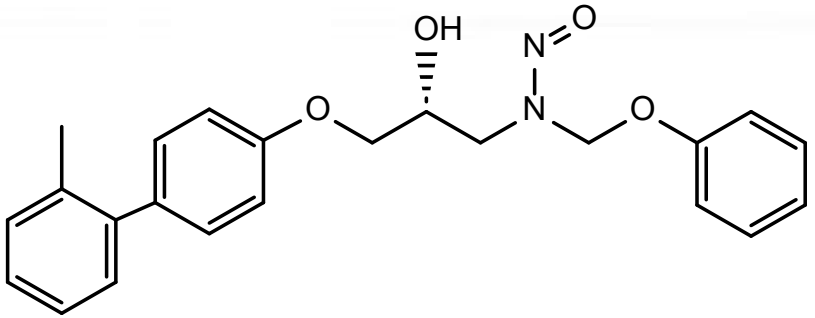
ACCEPTABLE INTAKE OF DATA POOR NDSRIs

EXAMPLE 2

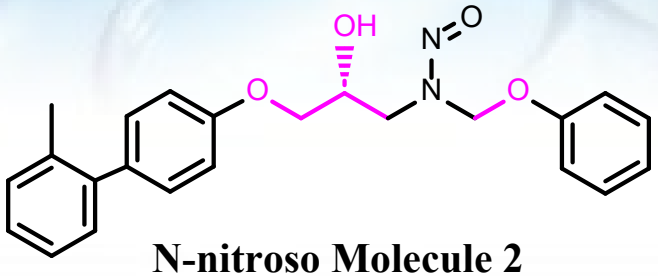


N-nitroso Molecule 2

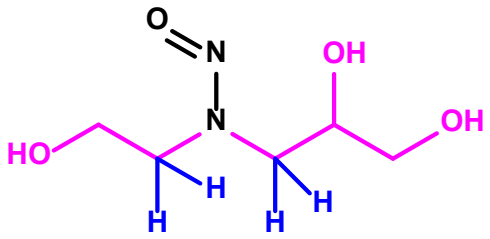
CPCA Category **3 (since CPCA does not address ether groups)**
Proposed limit = 400 ng/day (FDA)
[2,2]H = +1
Beta hydroxy group on one side = +1
5 non-H atoms on both sides of the NNO group = +1

ACCEPTABLE INTAKE OF DATA POOR NDSRIs

CPCA vs SURROGATE



N-nitroso Molecule 2
AI based on CPCA = 400 ng/day



3-[(2-Hydroxyethyl) nitrosoamino]-1,2-propanediol (HNAPD)

-Similar to N-nitroso Molecule 2 based on alert environment similarity

-Has two open alpha methylene carbons and one beta carbon hydroxyl group in common with with N-nitroso Molecule 2.

-TD50 6.04 mg/kg/day (Data from two dose study; Lijinsky 1984)

Lifetime AI = 6,040 ng/day

Could HNAPD be a possible surrogate of N-nitroso Molecule 1?

Ether groups present in the N-nitroso Molecule 2 are considered slightly potency enhancing for nitrosamines compared to beta-hydroxyl nitrosamines.
But they are not addressed in the CPCA list.
Thus, the beta substituents can not be taken into consideration.

Is a limit of 400 ng/day justified for N-nitroso Molecule 2 when HNAPD, a good surrogate, has an AI of 6040 ng/day?

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ACCEPTABLE INTAKE OF DATA POOR NDSRIs

Based on FDA's Guidance, "Recommended Acceptable Intake Limits for Nitrosamine Drug Substance Related Impurities (NDSRIs)":

- **Using read-across assessment to a suitable surrogate, could be used to support a higher AI limit**
- **In certain circumstances, manufacturers or applicants may provide a scientifically justified rationale to pursue an AI limit different than the FDA-recommended limit.**

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ACCEPTABLE INTAKE OF DATA POOR NDSRIs

A read across assessment to a suitable surrogate for an NDSRI is highly recommended as it may be able to support a scientifically justified higher AI limit compared to the CPCA calculation.

Note of caution regarding surrogate studies: A surrogate study needs understanding of the overall structure and chemistry of the nitrosamine molecule. It needs scientific understanding of the salient structural elements of the nitrosamine in question and the surrogate and what could be the active diazonium species. **Read across and surrogate selection is a careful interplay of chemistry and toxicology.**

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ACCEPTABLE INTAKE OF DATA POOR NDSRIs

,

Would you use a duck as a “Surrogate” for a tiger because they both know how to swim?

Let us imagine the nitrosamines to be the “Royal Bengal Tiger” of impurities

Is cat a better “Surrogate” for a tiger even if tigers do not sit on sofas?

When it comes to surrogate selection”, end should not justify the means

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WHAT COULD LIE AHEAD FOR NITROSAMINES?

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SMALLER AND MORE POTENT NITROSAMINES

- The smaller nitrosamines are usually more potent and common:
  - Reagents
  - Container Closures
- We need more work on the TD50 of some of the smaller nitrosamines rather than use 26.5 ng/day as a default value (which is unachievable in many cases)
- We need to develop quick and less expensive analytical methods for analysis of the smaller nitrosamines

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NEAR FUTURE - NDSRIs

- Control the NDSRIs based on AIs calculated by CPCA or surrogate studies
  - Currently, industry is relying heavily on the CPCA and surrogate approach and matching up novel nitrosamines with structurally similar nitrosamine that have already been tested in rodent carcinogenicity assays. *While not accurate, these are helpful*.

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LONG TERM SOLUTION- NDSRIs

- Perform transgenic assays (e.g. BigBlue Assay in Rodents) which are considered sufficient proof of mutagenicity for nitrosamines. These assays can be completed in rats or mice in a few months and provide conclusive evidence of the compound being positive or negative (not a quantitative study).
- Work with the FDA to negotiate if nitrosamine modified bacterial mutagenicity assays, and other *in vivo* genotoxicity assays (e.g., Comet Assay) may, overtime, present a compelling dataset related to the mutagenicity of nitrosamines.
- To derive TD50 data for a NDSRI, rodent carcinogenicity study is required, and these can take about 2 years

Generic Industry Needs More Time and Resources And Support From FDA To Overcome This Hurdle

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ACKNOWLEDGEMENT

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- **QSAR Flex version 2.5 © MultiCASE Incorporated, www.multicase.com**
  - **CPCA Prediction Version 1.0**
  - **Surrogate Search Version 2.0**