NEW APPROACHES TO SET ACCEPTABLE INTAKE LEVELS FOR NDSRIs

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SPEKTRA

AGENDA

- Historic content focus on the acceptablelimit definition for Nitrosamines
- Challenges to set NDSRI acceptable intakes
- New approaches to set AI (Acceptable Intake) CPCA (EMA and FDA)
- Next steps

NITROSAMINES AI BACKGROUND

- NDMA (N-nitrosodimethylamine) ➡ 1st NA (Nitrosamine) found in Valsartan 2018
- Known mutagen and potent carcinogen in animal studies
- Health Authorities (HA) ➡ recalls
- Recalls: Ranitidine, Irbesartan, Metformin, Nizatidine...
- HA ➡ Guidelines to mitigate the presence of NA in pharmaceuticals

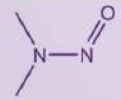
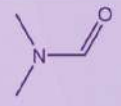
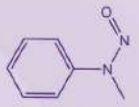
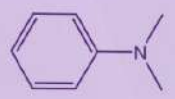
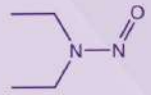
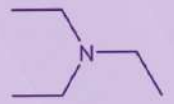
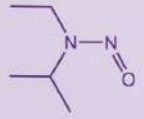
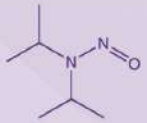
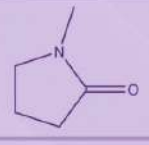
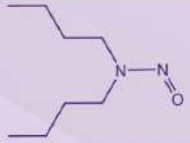
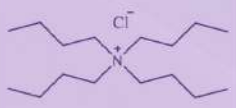
Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

NITROSAMINES AI BACKGROUND

- ✔ **NA are described in ICH M7 as Cohort of Concern group**
  - ➡ TTC (Threshold of Toxicological Concern) does not apply
- ✔ **HA provided Acceptable Intakes (AI) for NA**
  - ➡ Low-molecular-weight NA

Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

NITROSAMINES AI BACKGROUND

N-nitrosamina	Estrutura química	Fonte de agente nitrosante	Fonte de amina	Estrutura química	Limite de exposição (ng/dia)
NDMA		$NaNO_2$	DMF		96.0
NMPA		$NaNO_2$	*N-N*-DMA		34.3
NDEA		$NaNO_2$	TEA		26.5
EPINA		$NaNO_2$	DIPEA		26.5
DIPNA		$NaNO_2$	DIPEA		26.5
NMBA		$NaNO_2$	NMP		96.0
NDBA		$NaNO_2$	TBAB		26.5

Ref.: post Instagram @spektra_soluções_cientificas. HA (EMA, FDA and ANVISA) Guidelines on Nitrosamines

NITROSAMINES AI BACKGROUND

✔ Methods used by HA to derive the NAs AIs

- ➡ Linear extrapolation from TD50 as described in ICH M7
- ➡ Read-across

Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

NITROSAMINES AI BACKGROUND

GAPs: not transparent or crompehensive

Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

NITROSAMINES AI BACKGROUND

- ✔ **2021 ➡ Recall of Chantix (vanericline) ➡ N-nitroso vanericline**
  - ➡ 1st Nitrosamine Drug Substance Related Impurity (NDSRI)
- ✔ **Followed by Propanolol, Quinapril, Orphenadrine**

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

NITROSAMINES AI BACKGROUND

Lack of AI for NDSRI

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

CHALLENGES TO SET AI FOR NDSRI

- ✔ NDSRI ➡ different chemical space
- ✔ Amine α-carbon ➡ α-hydroxylation key metabolic pathway
- ✔ Carcinogenicity studies not available

Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

NITROSAMINES AI BACKGROUND

EMA:

- ✔ TTC 18ng/day
- ✔ 178ng/day–1year

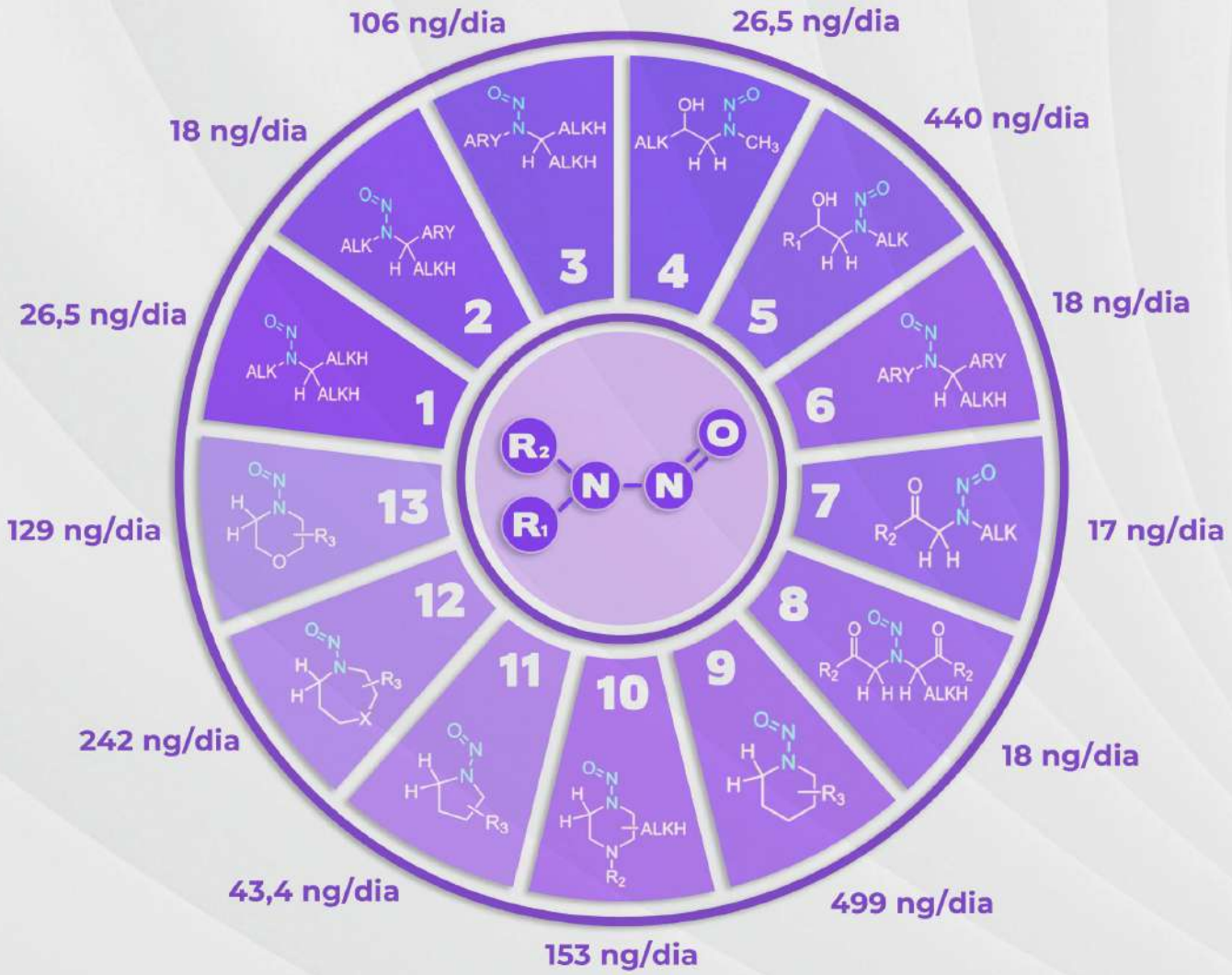
Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

CHALLENGES TO SET AI FOR NDSRI

- ✔ 18ng/day ➡ set based on low-molecular-weight NA
- ✔ 178ng/day ➡ 1 year not sufficient and after that a proper limit should be determine

Ref.: Bercu, J. P. et al (2023) Acceptable intakes (AIs) for 11 small molecule N-nitrosamines (NAs). Regulatory Toxicology and Pharmacology 142.

PROPOSALS FOR AI DETERMINATION



Ref.: Dobo K. L., et al. (2022) Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities. Chem. Res. Toxicol. 35.

PROPOSALS FOR AI DETERMINATION

- ✔ Read-across
- ✔ SAR

Ref.: Cross and Ponting (2021). Comput Toxicol. Thomas R. et al. (2021). Regul. Toxicol. Pharmacol.

READ-ACROSS CHALLENGES

✔ Selection of surrogate compound:

- ➡ Structurally similar around the nitrosamine substructure with similar substitution pattern
- ➡ Robust carcinogenicity data
- ➡ Similar metabolism ⇨ activation
- ➡ Similar DMPK (Drug metabolism and pharmacokinetics)

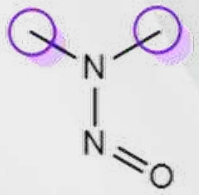
Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

NEW APPROACH TO SET AI

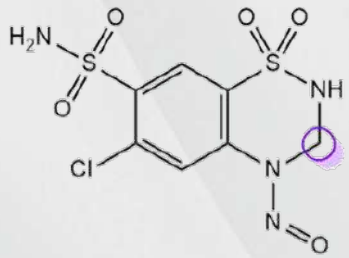
- ✔ **EMA and FDA ⇨ CPCA (Carcinogenic Potency Categorization Approach)**
  - ➡ SAR based
- ✔ **TD50 from surrogate ⇨ Point of Departure to read-across and SAR**
- ✔ **Modified Ames test ⇨ negative result allows control at TTC 1,5µg/day**
- ✔ **Relevant and well-conducted in vivo mutagenicity test ⇨ negative allows control as non-mutagenic**

Ref.: EMA and FDA N-nitrosamine Q&A

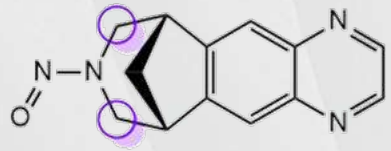
NEW APPROACH TO SET AI



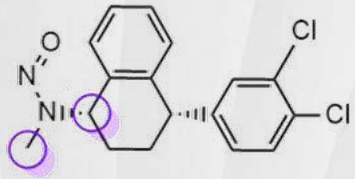
NDMA
(3,3)



N-nitroso-Hydrochlorothiazide
(0,2)



N-nitroso-Varenicline
(2,2)



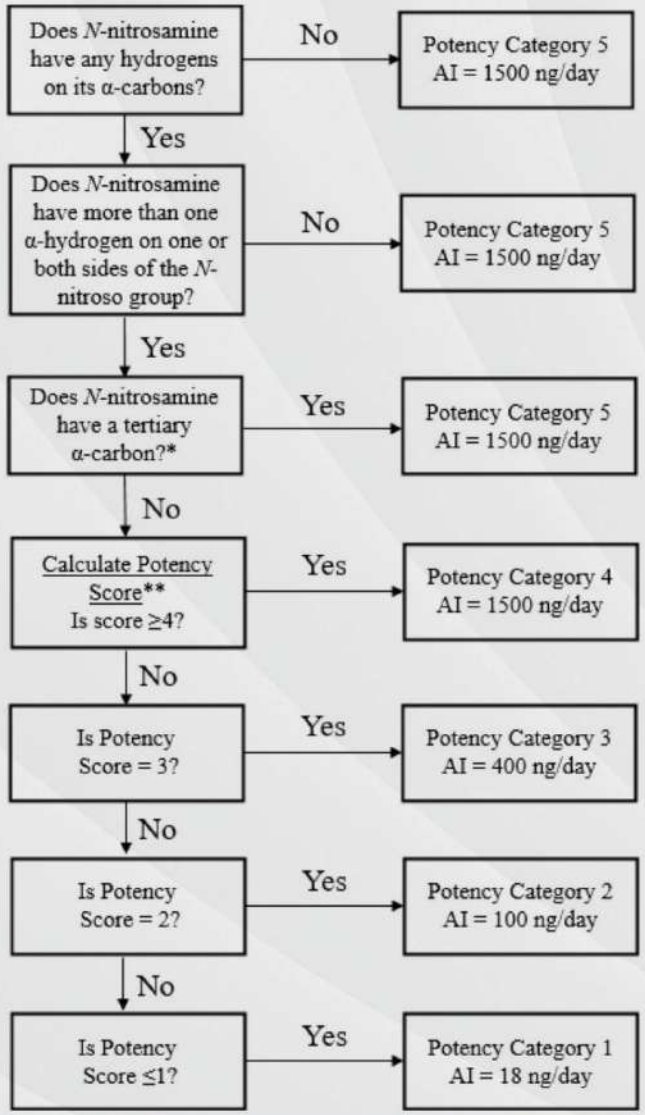
N-nitroso-Sertraline
(1,3)

✔ CPCA

- ➡ Hydrogens on its α-carbon
- ➡ Deactivating and activating features

Ref.: EMA and FDA N-nitrosamine Q&A

NEW APPROACH TO SET AI



Ref.: EMA and FDA N-nitrosamine Q&A

NEW APPROACH TO SET AI

EMA

Table 1. The Five Predicted Potency Categories and Associated AI Limits for *N*-Nitrosamines

Potency Category	Recommended AI Limit (ng/day)	Comments
1	18	The recommended AI limit of 18 ng/day is equal to the class-specific TTC for *N*-nitrosamine impurities.* *N*-nitrosamines assigned to Category 1 are predicted to have high carcinogenic potency; however, the class-specific TTC for *N*-nitrosamine impurities is considered sufficiently protective to patients.
2	100	The recommended AI limit of 100 ng/day is representative of two potent, robustly tested *N*-nitrosamines, *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which have recommended AI limits of 96 ng/day and 100 ng/day, respectively. *N*-nitrosamines assigned to Category 2 are predicted to have carcinogenic potency no higher than NDMA and NNK.
3	400	Compared to Potency Category 2, *N*-nitrosamines in this category have lower carcinogenic potency due to, for example, the presence of a weakly deactivating structural feature. The recommended AI limit was set to reflect a 4-fold decrease in carcinogenic potency from Category 2.
4	1500	*N*-Nitrosamines assigned to Category 4 may be metabolically activated through an α-hydroxylation pathway but are predicted to be of low carcinogenic potency, for example, because the pathway is disfavored due to steric or electronic influences, or because clearance pathways are favored. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7.**
5	1500	*N*-Nitrosamines assigned to Category 5 are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens, or are predicted to form unstable species that will not react with DNA. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7.**

* Assessment report Procedure under Article 5(3) of Regulation EC (No) 726/2004 Nitrosamine impurities in human medicinal products Procedure number: EMEA/H/A-5(3)/1490

** See the International Council for Harmonisation guidance for industry *M7Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk*. Threshold of Toxicological Concern (TTC) of 1.5 µg/day (1500 ng/day) as explained in ICH M7, represents an AI for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effect.

Ref.: EMA N-nitrosamine Q&A

NEW APPROACH TO SET AI

FDA

Table 1. The Five Predicted Carcinogenic Potency Categories and Associated Recommended AI Limits for NDSRIs

Potency Category	Recommended AI (ng/day)	Comments
1	26.5	The recommended AI limit of 26.5 ng/day* is equal to the class-specific limit for nitrosamine impurities based on the most potent, robustly tested nitrosamine, *N*-nitrosodiethylamine (NDEA).** NDSRIs assigned to Category 1 are predicted to have carcinogenic potency no higher than the class-specific limit for nitrosamine impurities.
2	100	The recommended AI limit of 100 ng/day is representative of two potent, robustly tested nitrosamines, *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which have recommended AI limits of 96 ng/day and 100 ng/day, respectively. NDSRIs assigned to Category 2 are predicted to have carcinogenic potency no higher than NDMA and NNK.
3	400	Compared to Potency Category 2, NDSRIs in this category have lower carcinogenic potency due to, for example, the presence of a weakly deactivating structural feature. The recommended AI limit was set to reflect a 4-fold decrease in carcinogenic potency from Category 2.
4	1500	NDSRIs assigned to Category 4 may be metabolically activated through an alpha-hydroxylation pathway but are predicted to be of low carcinogenic potency, for example, because the pathway is disfavored due to steric or electronic influences, or because clearance pathways are favored. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7(R2).***
5	1500	NDSRIs assigned to Category 5 are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens, or are predicted to form unstable species that will not react with DNA. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7(R2).***

AI = acceptable intake; ng = nanogram; NDSRI = nitrosamine drug substance-related impurities; TTC = threshold of toxicological concern.

* For products intended for marketing in the United States, FDA recommends an AI limit of 26.5 ng/day for Category 1, even if a different limit is recommended in other regulatory regions.

** See the guidance for industry *Control of Nitrosamine Impurities in Human Drugs* (February 2021).

Ref.: FDA N-nitrosamine Q&A

NEW APPROACH TO SET AI

✔ ***In vivo*** **mutagenic test:**

- ➡ **Duplex sequencing**
- ➡ **Transgenic mutation in rodent**
- ➡ **Comet assay**

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

NEW APPROACHES TO SET AI

Mutation Research - Genetic Toxicology and Environmental Mutagenesis 891 (2023) 503685

Contents lists available at ScienceDirect

Mutation Research - Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox

Check for updates

Comparison of the transgenic rodent mutation assay, error corrected next generation duplex sequencing, and the alkaline comet assay to detect dose-related mutations following exposure to *N*-nitrosodiethylamine

Joel P. Bercu [a,*], Shaofei Zhang [b,*], Zhanna Sobol [c], Patricia A. Escobar [c], Phu Van [d], Maik Schuler [b]

NEW APPROACHES TO SET AI

- ✓ TGR → OECD 488 most accepted
  - → limited availability of the transgenic animals and qualified laboratories

- ✓ Duplex Sequecing → non-transgenic animals, faster results
  - → comparable to TGR
  - → promising technology but may not be accessible to everyone
  - → mechanistic information > overall toxicological undertanding
- ✓ Comet assay → OECD 489, well-known, sensitive
  - → non-transgenic animals, faster results
  - → comparable to TGR

NEW APPROACH TO SET AI

✔ **GAPs:**

- **Different Molecular Weight not considered**
- **LTL as temporary mesure during CAPA implementation**
  - LTL has proven to be protective
- **Positive results in *in vivo* tests ⇨ no AI defined**

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

NEXT STEPS

- ✔ **Some initiatives happening: HESI, Lhasa, EFPIA**
- ✔ **Prove that Ames test is sufficient to prove mutagenicity of Nitrosamines**
  - ➡ Negative results ⇨ control as non-mutagenic impurities (ICHQ3A/Q3B)
- ✔ **Well-designed in vivo studies ⇨ calculate Benchmark Doses (BMD)**
- ✔ **Use Less-than-lifetime approach to Support the AI**

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

GENERIC COMPANIES PERSPECTIVE

- ✔ **Huge investiment to conduct experimental studies**
- ✔ **INVEST X WITHDRAW**
  - ➡ Dependent on scientific publications
- ✔ **Brazil ⇨ Fase 1 (risk assessment), aligned with new approaches**
- ✔ **ANVISA ⇨ following EMA and FDA**
- ✔ **LATAM ⇨ starting now NA risk assessment**

Ref.: Nudelman R. et al. The Nitrosamine "Saga": Lessons Learned from Five Years of Scrutiny. Org. Process Res. Dev. https://doi.org/10.1021/acs.oprd.3c00100

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