EUROTOX 2023
LJUBLJANA – SLOVENIA
10–13 SEPTEMBER 2023

ToxMinds
THINKING AHEAD IN TOXICOLOGY

NITROSAMINES IMPURITIES CARCINOGENICITY ASSESSMENT – REGULATORY REQUIREMENTS, CHALLENGES AND RECOMMENDATIONS

S. Mishra, M. Autiero, S. Vishnoi, C. De Roy, N. Barai, S. Chakraborty, F. Tencalla, and T. Petry

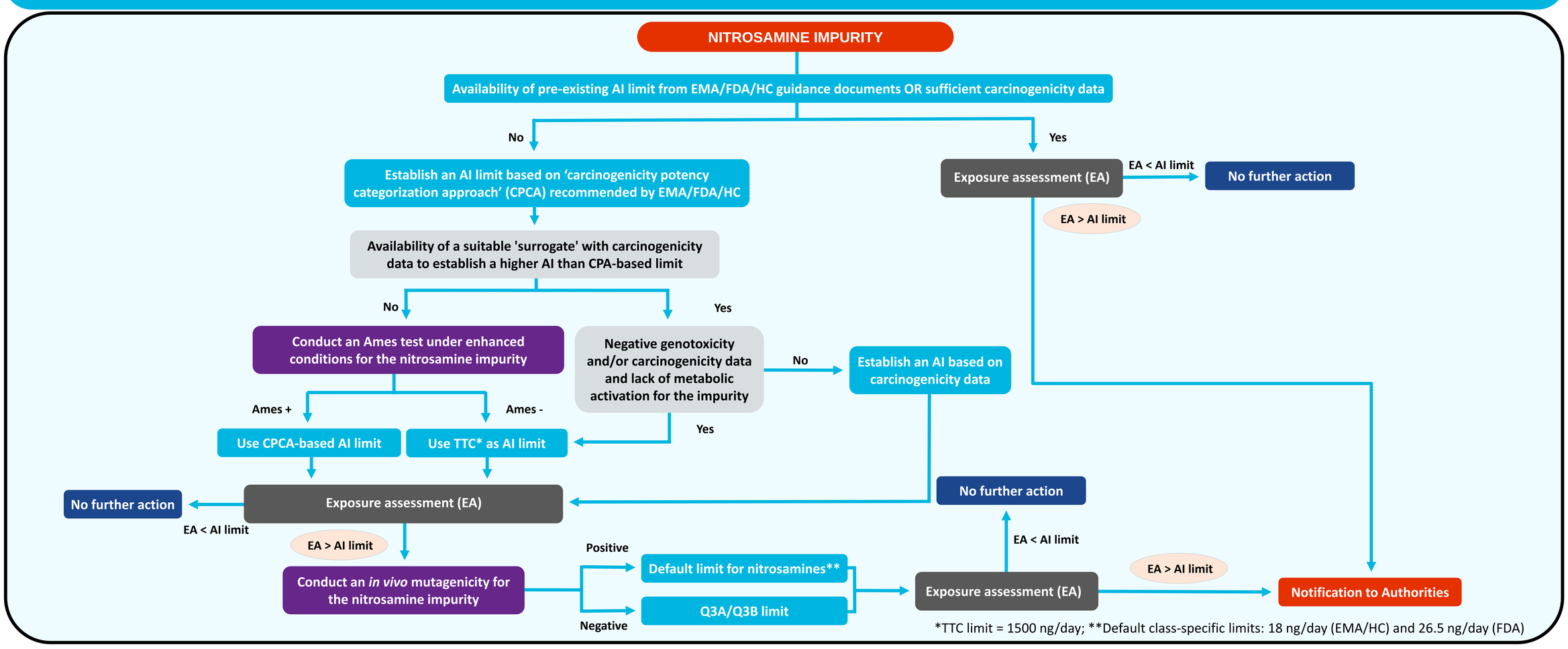
Introduction

Nitrosamines are considered cohort-of-concern compounds according to the ICH M7 guideline due to their suspected genotoxic carcinogenicity potential. Market authorisation holders must review all commercial drug substances/products for potential risk if nitrosamine impurities are detected. A key factor in this review is to evaluate the risk and eventually establish an appropriate, acceptable intake (AI) level for the nitrosamine impurity. In the case of insufficient carcinogenicity data, the recent guidance documents from EMA, FDA and Health Canada recommend using the carcinogenicity potency categorisation approach (CPCA) to establish the AI unless other robust data is available to override this AI.

At ToxMinds, we have established a process to perform the risk assessment of theoretical or identified nitrosamines in drug products. This process includes the following steps: (1) the structural analysis of the impurity according to CPCA; (2) the prediction of the metabolic pathway to determine whether metabolic activation occurs, using the Meteor® Nexus tool from Lhasa; (3) toxicokinetics assessment; (4) the identification of a potential analogue(s) or 'surrogate(s) with robust genotoxicity and/or carcinogenicity data; (5) the evaluation of an optimised Ames test and other genotoxicity data, if available; (5) the evaluation of the robustness of the carcinogenicity study according to the ICH M7 defined criteria; and (6) the proposal of an appropriate AI as control limit.

On the basis of three case studies, the present poster discusses the practical steps that assessors can undertake to de-risk the carcinogenic properties of nitrosamines and/or to establish an AI by using a read across approach.

Workflow for the assessment of nitrosamine impurities



*TTC limit = 1500 ng/day; **Default class-specific limits: 18 ng/day (EMA/HC) and 26.5 ng/day (FDA)

Case study I: Data poor nitrosamine with no suitable surrogate having carcinogenicity data

OBJECTIVE

To establish a higher AI limit for N-nitroso flecainide than the default value based on the carcinogenicity potency categorisation approach.

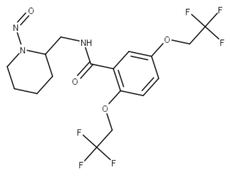
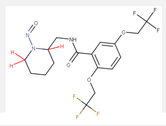
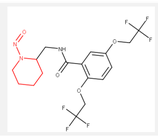


Figure 1: N-Nitroso flecainide

ASSESSMENT OF CARCINOGENICITY POTENCY CATEGORY

Potency score = α-hydrogen score + deactivating feature score (sum of all scores for features present in the N-Nitrosamine) **+ activating feature score** (sum of all scores for features present in the N-nitrosamine)

Count of α-Hydrogens	Score	Feature highlighted in red
1,2	3	

Deactivating features	Score	Feature highlighted in red
N-nitroso group in a 5- or 6-membered ring	+2	
No Activating features present	-	-

Potency score = 3 + 2 = 5	Potency category 5	AI = 1500 ng/day

CONCLUSION

Based on the CPCA flow chart, a 'potency score' of 5 is calculated for N-nitroso flecainide, resulting in its placement in 'potency category' 5 with an associated AI limit of 1500 ng/day.

Case study II: Nitrosamine with a suitable 'surrogate' having carcinogenicity data

OBJECTIVE

To establish an AI limit for N-nitroso desloratadine (NDL) based on the CPCA approach and carcinogenicity data on the 'surrogate'.

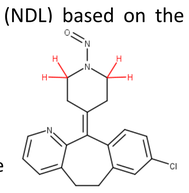


Figure 2: N-Nitroso desloratadine

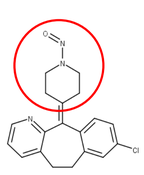
ASSESSMENT OF CARCINOGENICITY POTENCY CATEGORY

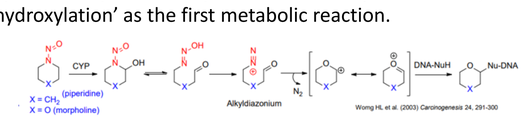
A 'potency category' 3 was assigned to NDL (EMA, 2023), based on an **α-hydrogen score of 1 (**two α-hydrogens on either side of the N-nitroso group) **+ deactivating feature score of +2** (N-nitroso group in a 6-membered ring) **+ activating feature score of 0** (no activating features present).

Potency score = 1 + 2 = 3	Potency category 3	AI = 400 ng/day

AI LIMIT BASED ON CARCINOGENICITY DATA ON 'SURROGATE' NPIP

- N-nitrosopiperidine (NPIP) has an AI limit established by EMA at 1300 ng/day (EMA, 2023).
- **Read across justification:**
  - Overall, NDL and NPIP present a low similarity index with respect to the structure; however, they share the same 'local site of activation';
  - They have the same key functional group (N-Nitroso);
  - They have the same structural alerts for systemic toxicity, sensitisation and genotoxicity, with the 'surrogate' presenting an additional alert for carcinogenicity;
  - They have physico-chemical properties in the same range, with the 'surrogate' having higher bioavailability and therefore representing a worst-case;
  - They are both predicted by Meteor® Nexus (Judson *et al*., 2015), to undergo 'α-hydroxylation' as the first metabolic reaction.





CONCLUSION

Considering the suitability of the surrogate, the AI limit based on CPCA is overridden, and the **AI limit of 1300 ng/day** based on the surrogate is proposed as the control limit for NDL.

Case study III: Nitrosamine presenting genotoxicity and carcinogenicity data

OBJECTIVE

To establish an AI limit for N-nitroso ephedrine (NNE) based on the CPCA approach and its carcinogenicity data.

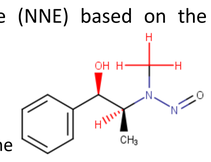


Figure 3: N-Nitroso ephedrine

AI LIMIT BASED ON CARCINOGENICITY DATA

- One 104-week oral carcinogenicity study in rats having a Gold TD50 value of 95.2 mg/kg bw/day (99% confidence interval (CI): 41-332 mg/kg bw/day) was identified from the Lhasa carcinogenicity database.
- The study was not considered to be very robust due to <50 animals (i.e., 32), intermittent dosing of <5 days (i.e., 2 days) and one treated group.
- Therefore, the lower CI was considered, as the PoD (Dobo *et al.,* 2022), followed by correction for continuous dosing (i.e., 41 mg/kg bw/day x 2 days/7 days = 11.7 mg/kg bw/day).
- AI = 0.0117 mg/day (11,700 ng/day)

$$\mathrm{AI} = \frac{11.7\ (mg/kg\ bw\ /day)\ x\ 50\ (kg\ bw)}{50{,}000}$$

- As the available carcinogenicity study was not very robust, the 'potency score' was assessed based on the CPCA approach.
- **Potency score = α-hydrogen score of 3** (one α-hydrogen atom on one side and three α-hydrogen atoms on the other side of the N-nitroso group) **+ deactivating feature score of +1 (**Hydroxyl group bonded to β-carbon on only one side of N-nitroso group) **+ activating feature score of 0 (**no activating features present)

Potency score = 3 + 1 = 4	Potency category 4	AI = 1500 ng/day

METABOLIC ACTIVATION

NNE was predicted by Meteor® Nexus (Judson *et al*., 2015) v.3.1.0 to undergo 'α-hydroxylation and decomposition of N-nitrosamines', as one of the first metabolic reactions.

CONCLUSION

Considering that the available carcinogenicity study on NNE is not very robust and there is an α-hydroxylation metabolism prediction, the **lower AI (1500 ng/day)** based on the CPCA is proposed as the basis for the control limit.

References

EMA. 2018. ICH guideline M7(R1) on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk (Step 5). Report No. EMA/CHMP/ICH/83812/2013, https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m7r1-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit_en.pdf.

EMA. 2023. Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products. Report No. EMA/409815/2020 Rev.16, https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-questions-answers-marketing-authorisation-holders/applicants-chmp-opinion-article-53-regulation-ec-no-726/2004-referral-nitrosamine-impurities-human-medicinal-products_en.pdf.

FDA. 2023. Recommended acceptable intake limits for nitrosamine drug substance-related impurities (NDSRIs). Guidance for Industry. Report No. FDA-2020-D-1530, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), https://www.fda.gov/media/170794/download.

Judson PN, Long A, Murray E, *et al.* 2015. Assessing confidence in predictions using veracity and utility – A case study on the prediction of mammalian metabolism by Meteor Nexus. Mol. Inform. 34 (5):284-291.

www.toxminds.com