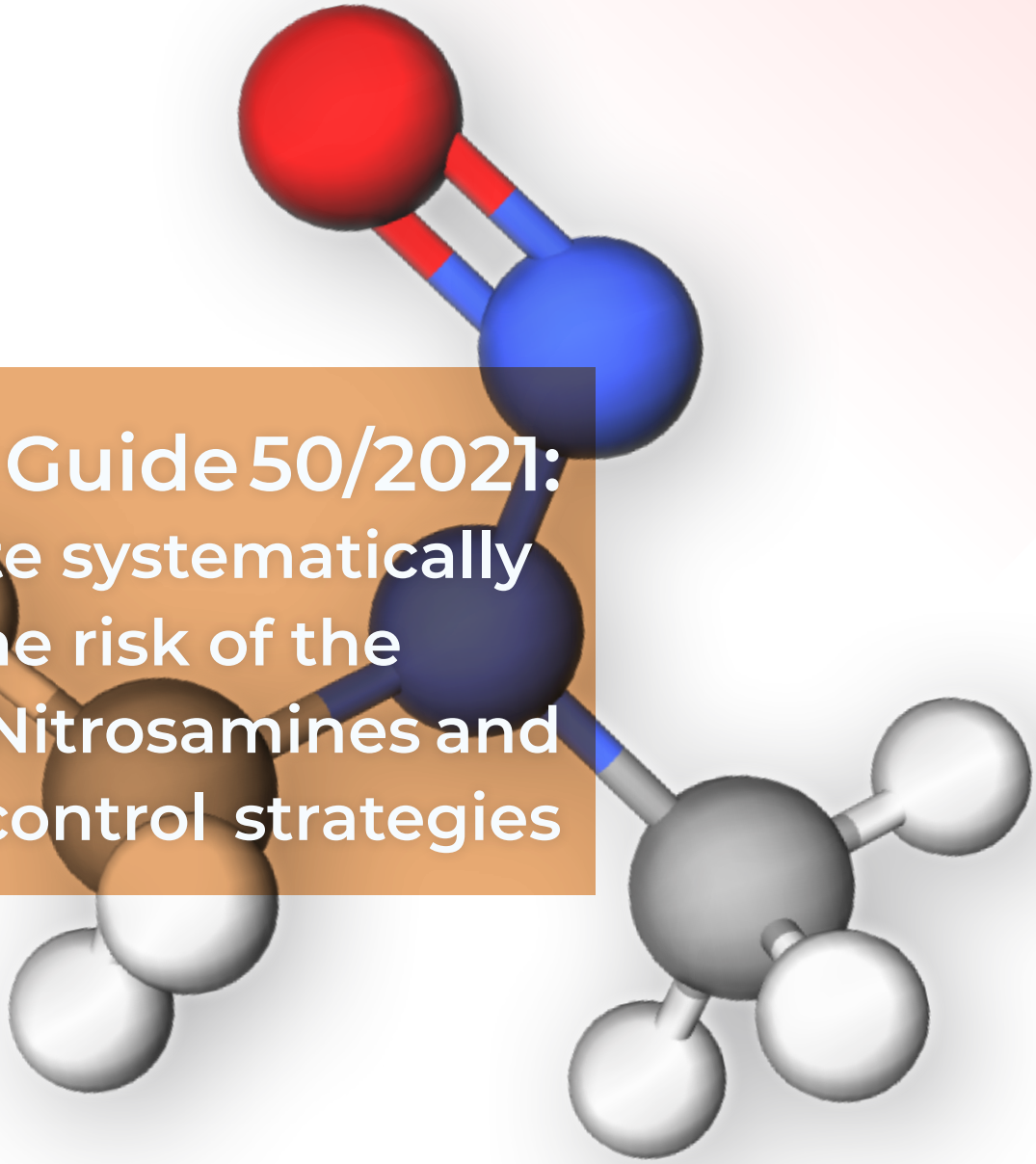
Hub-Nitro™

Hub of technologies for N-nitrosamines risk assessment



RDC 677 and Guide 50/2021:

How to evaluate systematically and robustly the risk of the presence of N-Nitrosamines and to determine control strategies

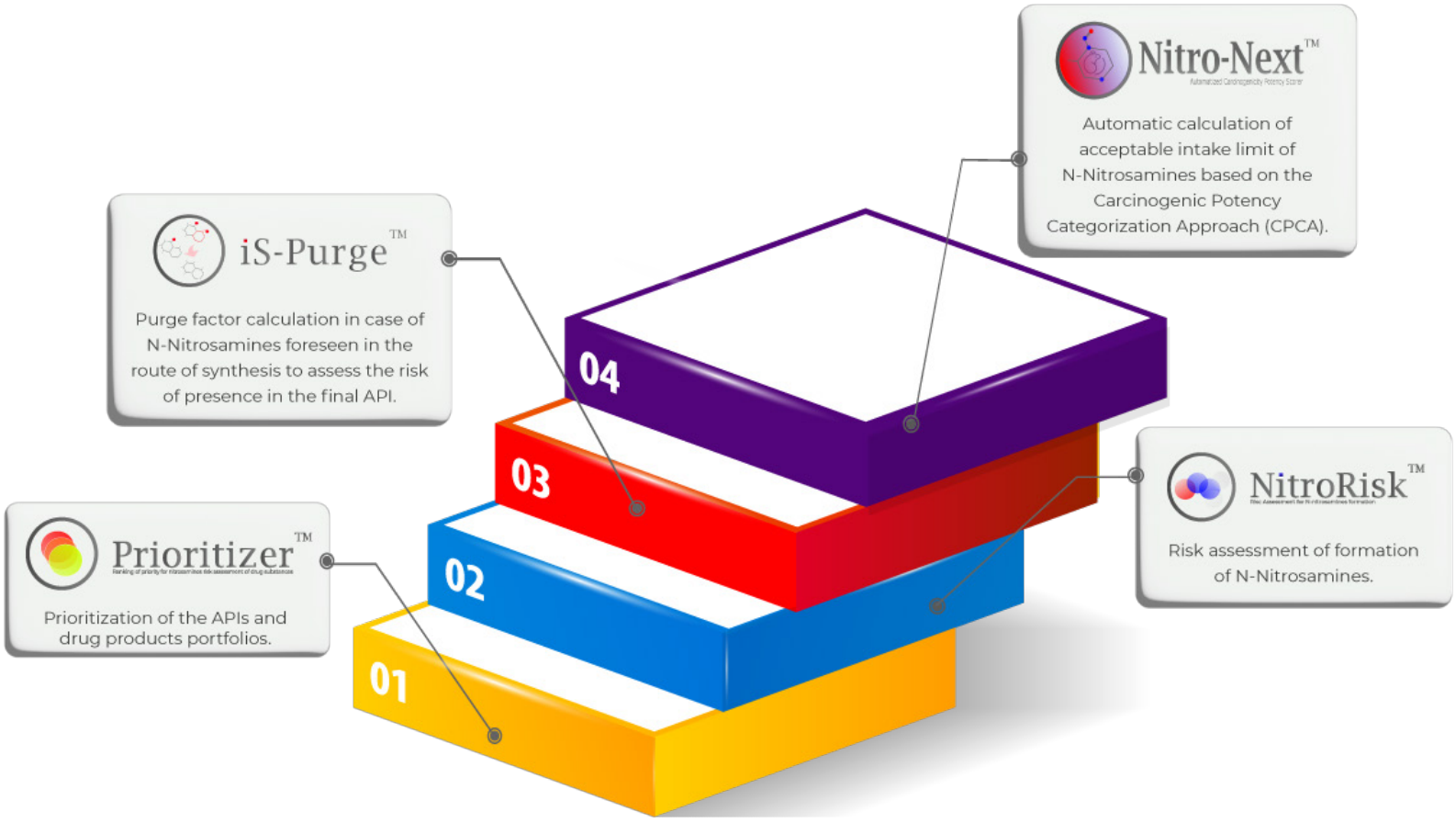
Altox

ALTERNATIVE TOXICOLOGY

The ultimate combination for the evaluation of Nitrosamines

Hub-Nitro© was the first hub launched for risk assessment of N-Nitrosamines in pharmaceutical products. As yet another local innovation project, the tool is the result of experience and technical-scientific collaboration between Altox Ltda and some long data client in the pharmaceutical sector.

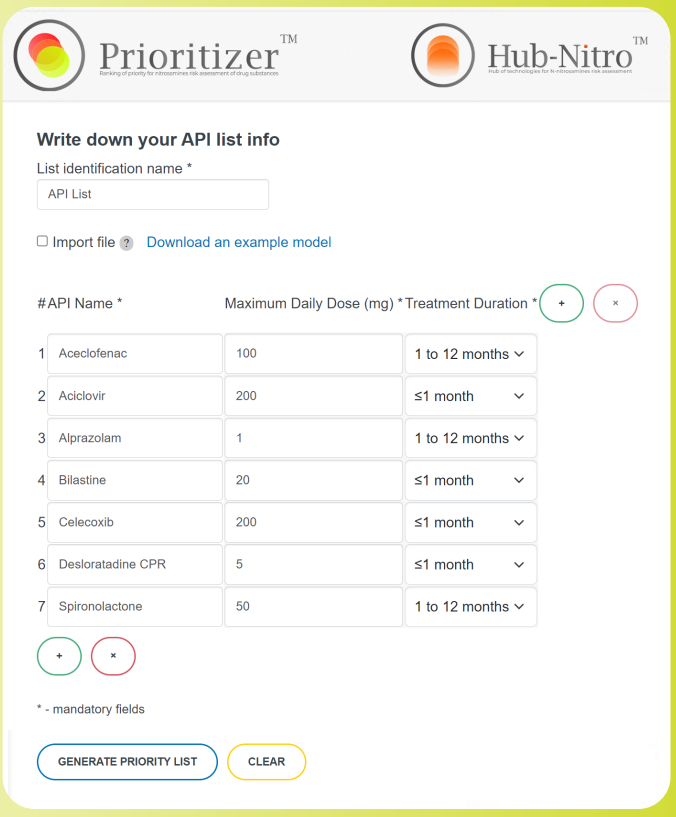
For quick and effective Nitrosamine risk assesments and calculation of acceptable intake limits, with high scientific rigor, the Hub comprises four complementary softwares (Prioritizer, Nitro-Risk, iS-Purge and Nitro-Next), that serve to meet each of the items set forth in ANVISA's Guide 50/2021:



Ranking APIs for evaluation

In compliance with ANVISA's Guide 50/2021, the software performs an evaluation and prioritization of the list of APIs/products in the portfolio, determining the sequence of product evaluation, considering factors such as recommended daily intake, duration of treatment, therapeutic indication, number of patients treated, or others deemed relevant according to the portfolio.

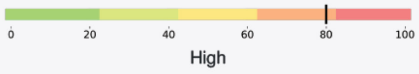
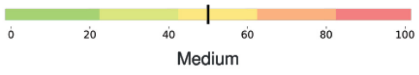
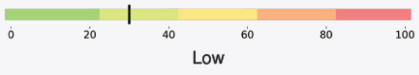
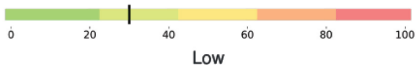
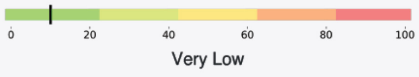
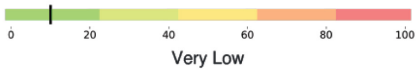
Enter the APIs you want to analyze, the maximum daily doses (mg) and the duration of treatment.



Data provided by Report

API Name	Maximum Daily Dose	Treatment Duration
Ácido zoledronico	5mg	≤1 month
Alprazolam	1mg	1 to 12 months
Ampicilina	500mg	≤1 month
Cabergolina	1mg	≤1 month
Losartana potássica	50mg	>1 year
Hidroclorotiazida	100mg	>1 year

Priority Result

API Name	Score (Percentage)	Risk
Hidroclorotiazida	8 (80.00%)	
Losartana potássica	5 (50.00%)	
Alprazolam	3 (30.00%)	
Ampicilina	3 (30.00%)	
Ácido zoledronico	1 (10.00%)	
Cabergolina	1 (10.00%)	

The first classification is determined by the maximum daily dose and the duration of treatment, as recommended by ANVISA in Guide n° 50/2021.

The second classification is made using a scale (0 to 100%) based on two attributes (maximum daily dose and duration of treatment). Finally, a new risk classification is suggested based on the score resulting from the sum of all priority attributes.

As each attribute contributes to drug priority calculations, the scaling depends on the number of priority factors provided by the user; in the case of three priority attributes, the maximum score is 30 (30 corresponds to 100% in this case), as each component contributes a maximum of 10.

Finally, a new risk classification is suggested based on the score resulting from the sum of all priority attributes, shown in the “Risk” column chart.

Avoid unnecessary tests: Quantitative calculation of the risk of Nitrosamines formation

Attending the ANVISA Guide 50/2021, NitroRisk is designted to guide specialists in the N-Nitrosamine risk assessment formation through the FMEA method (Failure Mode and Effect Analysis), with indication and detection of susceptible amines in the route of synthesis, formation conditions, and different risk factors, quantifying these factors according to failure modes, severity (S) and occurrence (O) variables for the context of nitrosamine formation, including even the prediction of new degradation products with amines susceptible to degradation.

In order to avoid subjective or binary conclusions, the RPN (Risk Priority Number) calculation provides a conclusion if a risk of N-Nitrosamine formation is identified and allows for the quantification of the risk according to the FMEA, determining the need or exemption of confirmatory testing.

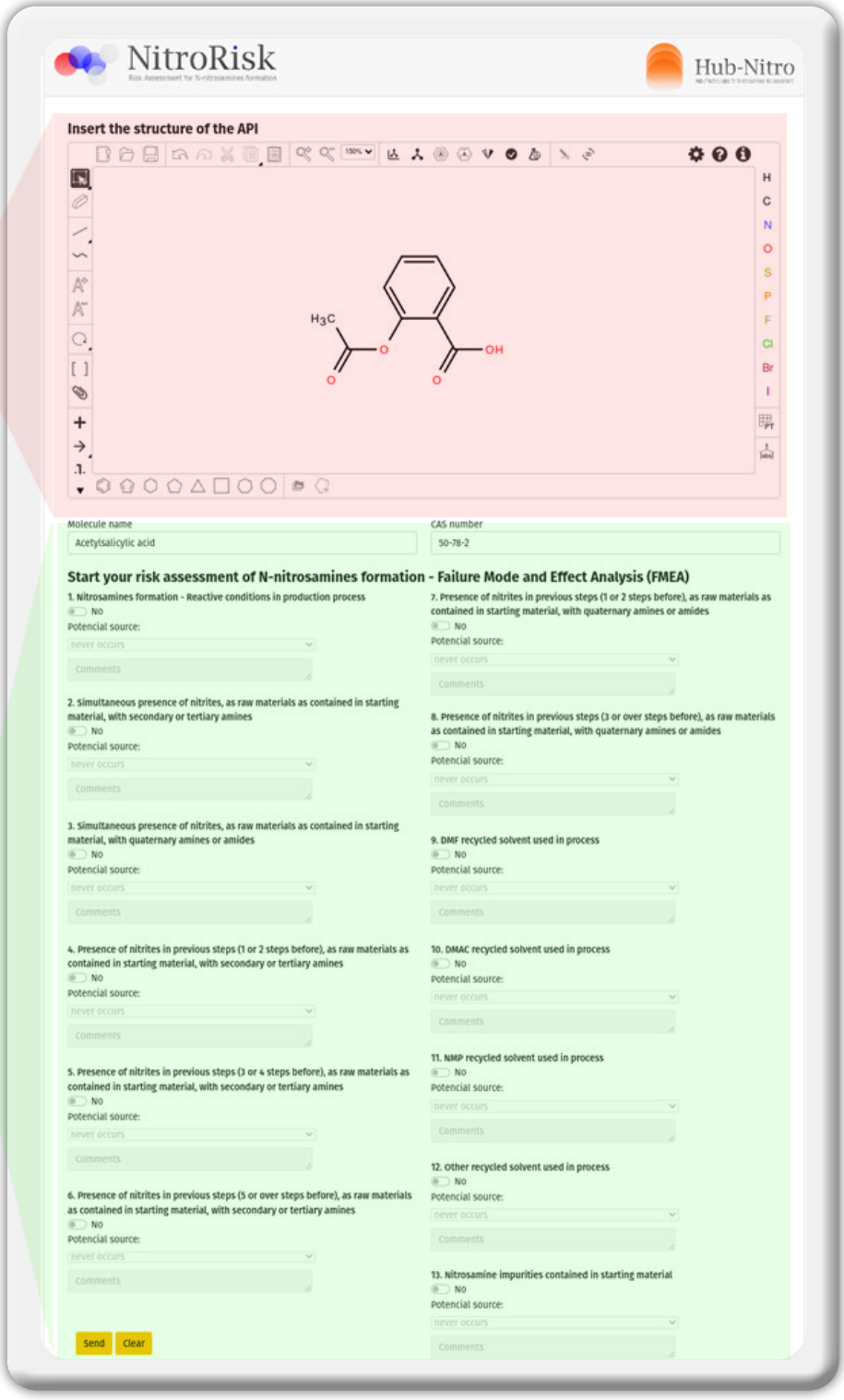
The FMEA is a method that systematizes the process of Risk Assessment, providing quantification for each variable related to the classification and assessment of risk. It reduces subjectivity biases, making it one of the most established methodologies in various contexts.

Custom Risk Analysis

Draw your molecule or insert the structure using the SMILES code.

Select the characteristics previously known regarding raw materials, conditions, and the production process.

These features include:
- Reaction conditions that favors N-Nitrosamines formation;
- The presence of nitrite at various stages of the process;
- The presence of residual solvents.



4 Steps to a Complete Risk Assessment

a

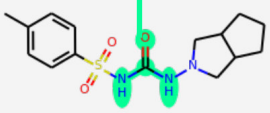
Screening of suceptible amines in the API and route of synthesis

Nitrogenous fragment susceptible to the formation of N-Nitrosamines

Functional group name

Description of the fragment and its connection with the formation of N-Nitrosamines

Screening for Susceptible Amines

Fragment	Group	Description
	Urea	Nitrosamines derived from ureas belong to the N-nitrosoamide group, which is highly unstable due to the union of two very electropositive functional groups (NNO and CO) and can decompose into the diazonium ion (Richard N. Loeppky 1994).

b

Risk assessment for N-Nitrosamines formation by FMEA (Failure Modes and Effects Analysis)

The objective of this analysis is to identify compounds that may create N-Nitrosamines and assess the associated risk using the **Risk Priority Number (RPN) parameter. A result of Y (yes) indicates the presence of risk, while N (no) denotes the absence of risk.**

Risk Assment for N-nitrosamines formation by FMEA

#	Potential sources of nitrosamine impurities (Cause)	Question Severity	Comments	Y/N	Severity	Occurence	RPN
1	Nitrosamines formation - Reactive conditions in production process (Reaction condition of nitrosamine formation)	10	Etapa de nitrosação (amina secundária, HCl e NaNO2), precisa da formação de nitrosamina como intermediário de	Y	10	6	60

The rating is done based on 4 parameters:

Criteria for evaluating RPN

Question Severity:

Potential precursors of N-Nitrosamines.

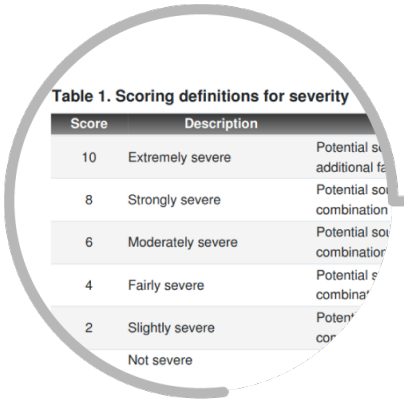


Table 1. Scoring definitions for severity

Score	Description	
10	Extremely severe	Potential s additional fa
8	Strongly severe	Potential so combination
6	Moderately severe	Potential so combinatio
4	Fairly severe	Potential s combina
2	Slightly severe	Poten cor
	Not severe	

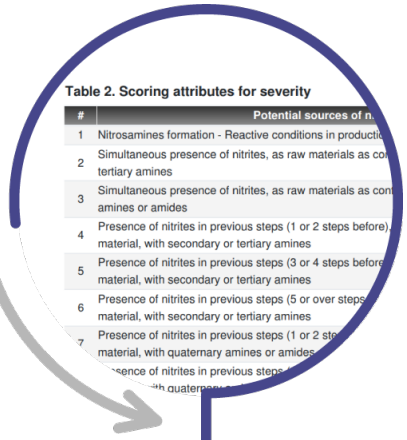


Table 2. Scoring attributes for severity

#	Potential sources of n
1	Nitrosamines formation - Reactive conditions in producti
2	Simultaneous presence of nitrites, as raw materials as co tertiary amines
3	Simultaneous presence of nitrites, as raw materials as con amines or amides
4	Presence of nitrites in previous steps (1 or 2 steps before) material, with secondary or tertiary amines
5	Presence of nitrites in previous steps (3 or 4 steps before material, with secondary or tertiary amines
6	Presence of nitrites in previous steps (5 or over steps material, with secondary or tertiary amines
7	Presence of nitrites in previous steps (1 or 2 ste material, with quaternary amines or amides
	ence of nitrites in previous steps

Severity:

Evaluation of the possible formation/contamination by N-Nitrosamines in the synthetic process.

Occurrence:

Indicates in which stage of synthesis a potential source of N-Nitrosamine formation occurred in relation to the final stage of synthesis.

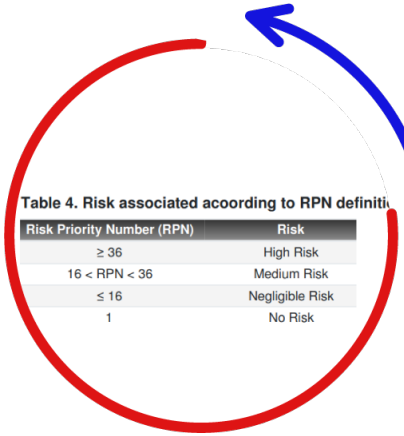
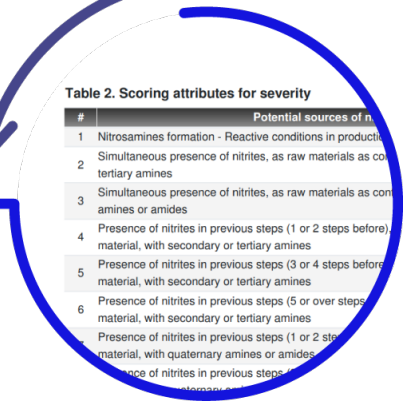


Table 4. Risk associated acoording to RPN definiti

Risk Priority Number (RPN)	Risk
≥ 36	High Risk
16 < RPN < 36	Medium Risk
≤ 16	Negligible Risk
1	No Risk



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6	Presence of nitrites in previous steps (5 or over steps material, with secondary or tertiary amines
7	Presence of nitrites in previous steps (1 or 2 ste material, with quaternary amines or amides
	ence of nitrites in previous steps

RPN:

Indicates the risk of formation/contamination of N-Nitrosamines.

c

Conclusion for calculating RPN:

The classification is made as follows: high risk, medium risk, negligible risk and no risk.
RPN > 16: It is recommended to perform a purge factor analysis.
RPN< 16: The risk is considered negligible or no risk.

Conclusion

#	Question	RPN	Risk
1	Nitrosamines formation - Reactive conditions in production process	60	0 20 40 60 80 100 High Risk
4	Presence of nitrites in previous steps (1 or 2 steps before), as raw materials as contained in starting material, with secondary or tertiary amines	36	0 20 40 60 80 100 High Risk
7	Presence of nitrites in previous steps (1 or 2 steps before), as raw materials as contained in starting material, with quaternary amines or amides	32	0 20 40 60 80 100 Medium Risk

d

Degradation products with susceptible amines and predicted nitrosamines

Prediction of degradation products containing secondary and tertiary amines/amides susceptible to the formation of N-Nitrosamines.

The following reactions are carried out to predict the structures resulting from forced degradation: Oxidation, acidic and basic hydrolysis, decomposition and photolysis.

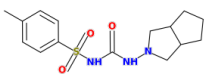
IDP-1-Y: Results for input molecules (e.g., API), where Y is the number for each nitrosamine derived from that molecule.

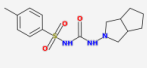
NDP-X-Y: Results for output degradation products (DPs), where X is the unique number for each DP, and Y is the number for each nitrosamine derived from that unique DP.

Degradation products with susceptible amines and predicted nitrosamines

Secondary amine:	Tertiary amine:
0	0
Secondary amide:	**Tertiary amide:**
4	0

Degradation Products

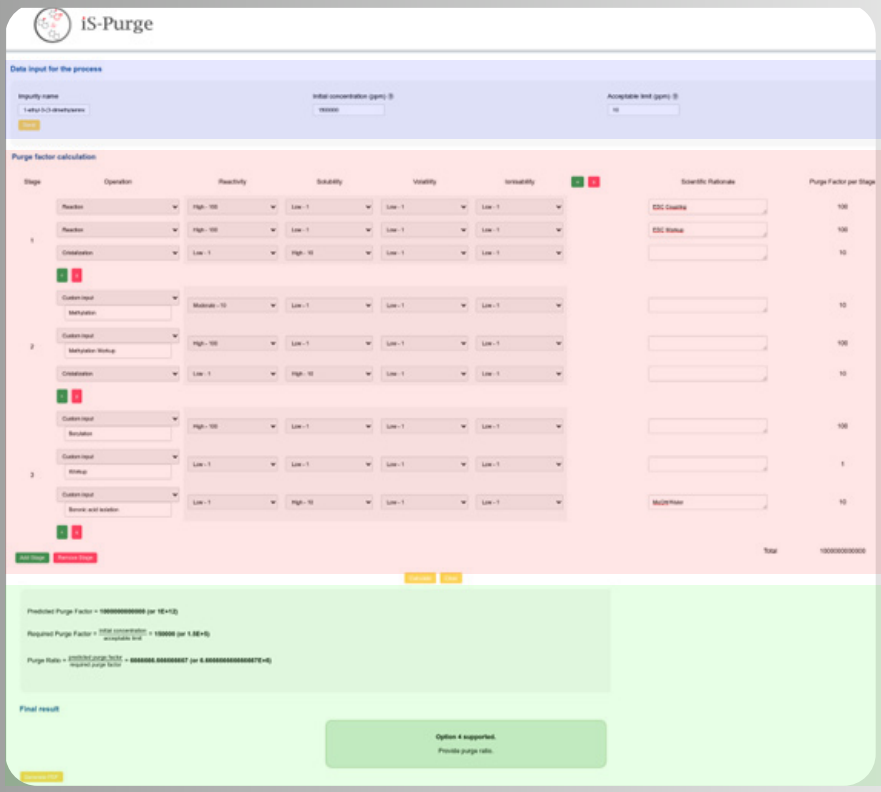


Product	N-Nitrosamine	Type	Label
 CC1C-CC(S(NC(NN2CC3C(CCC3)C2)=O)(=O)=O)=CC=1	 CC1=CC=C(S(=O)(=O)N(N=O)C(=O)NN2CC3CCCC3C2)C=C1	Secondary Amide	IDP1

3 iS-Purge™

Effective and simple calculation of purge factors (PFs) and purge rates (PRs) for N-Nitrosamines

If a potential risk of N-Nitrosamine formation has been identified in the synthetic API process, taking into account the formation steps and the reagents, solvents, catalysts, starting materials, formed intermediates, and other impurities, iS-Purge can be used to conduct a comprehensive evaluation of the entire drug manufacturing process in order to assess the possibilities of eliminating/purging the potentially present N-Nitrosamines. This process enables a more precise determination of the risk of N-nitrosamine presence in the final API.



- Compound name
- Initial Concentration
- Acceptable Limit

Choose the factors you want for evaluation.

Calculations:
- Predicted Purge Factor
- Required Purge Factor
- Purge Rate

a

Initial Data

Impurity:	Initial concentration:	Acceptable limit:
Thionyl chloride	1E+6 ppm	10 ppm

Initial concentration: concentration (in ppm) of the PMI or mutagenic impurity in its formation step in the synthetic route.

Acceptable limit: concentration (ppm) based on TTC or specific limit calculated for the PMI or mutagenic impurity (TTC or specific limit in micrograms/day *1000 divided by the maximum daily dose in mg/day.

b

Physical-chemical properties and processing conditions

A value is attributed to each parameter based on the physicochemical properties of the impurity in relation to the processing stage and conditions (unit operations).

Stage	Operation	Reactivity	Solubility	Volatility	Ionisability	Physical processing	Scientific Rationale	Purge Factor per Stage
1	Reaction	High - 100	Low - 1	Low - 1	Low - 1	1	SOCl2 is a very reactive intermediate (R = 100), and the high yields (89%) support a high reaction efficiency proposition.	100
	Washing	Low - 1	High - 10	Low - 1	Low - 1	1	Analyte has high solubility in DMF (S = 10), and intermediate 5 is isolated and washed under vacuum.	10
	Drying	Low - 1	Low - 1	High - 10	Low - 1	1	Dried under vacuum. Thionyl chloride boils at 79 °C (V = 10). There is no physical processing in this stage (PP = 1).	10
	Reaction	High - 100	Low - 1	Low - 1	Low - 1	1	SOCl2 will react with methylamine base and with aqueous brine (used to extract isolated intermediate) (R = 100).	100

c

Conclusion and Calculation

The iS-Purge software estimates the ability to purge (or remove) PMIs or MIs from the synthetic process, based on the theoretical method published by Teasdale et al. , evaluating 6 parameters:

Reactivity (Low – Value 1, Moderate – Value 10, or High - Value 100);
Solubility [Low (<10-33mg/L) – Value 1; Moderate (33-100 mg/L) – Value 3; or High (100 - >1000mg/L) – Value 10];
Volatility [Low (Boiling point >20°C above the process solvent) – Value 1; Moderate (Boiling point within ±10 °C of that of the process solvent) – Value 3; or High (Boiling point >20 °C below that of the process solvent– Value 10];
Ionisability: (Low – Value 1 or High - Value 10);
Custom input: if desired, custom values inputs could be added when guided by scientific rationale and/or expert review.
Stages and operations: One stage can be composed by one or more operations the synthetic process, defined by the user (Reaction, Extraction, Centrifugation, Filtration, Drying, Washing, Purification, and Crystallization). Custom operations could be added when guided by detailed scientific rationale.

Calculations

Predicted Purge Factor = **9000000000000 (or 9E+12)**

$$\text{Required Purge Factor} = \frac{\text{initial concentration}}{\text{acceptable limit}} = \textbf{100000 (or 1E+5)}$$

$$\text{Purge Ratio} = \frac{\text{predicted purge factor}}{\text{required purge factor}} = \textbf{90000000 (or 9E+7)}$$

d

Final Result

Option 4 supported
Provide purge ratio

Based on the purge ratios (PR) calculations, iS-Purge will advise if the Option 4 is supported based ICH M7 criteria and risk-based approach proposed by Burns et al.[4]:

If Purge Ratio > 1000: Option 4 is supported, and no additional data is necessary.
1000 > Purge Ratio > 100: Option 4 is supported, but additional evidence (literature or non-trace analytical testing) should be provided.
100 > Purge Ratio > 1: Option 4 is supported, but strong evidence (literature or trace analytical testing) should be provided.
Purge Ratio < 1: Option 4 is not supported, and options 1, 2, or 3 are recommended.

4

Nitro-Next™

Automatized Carcinogenicity Potency Scorer

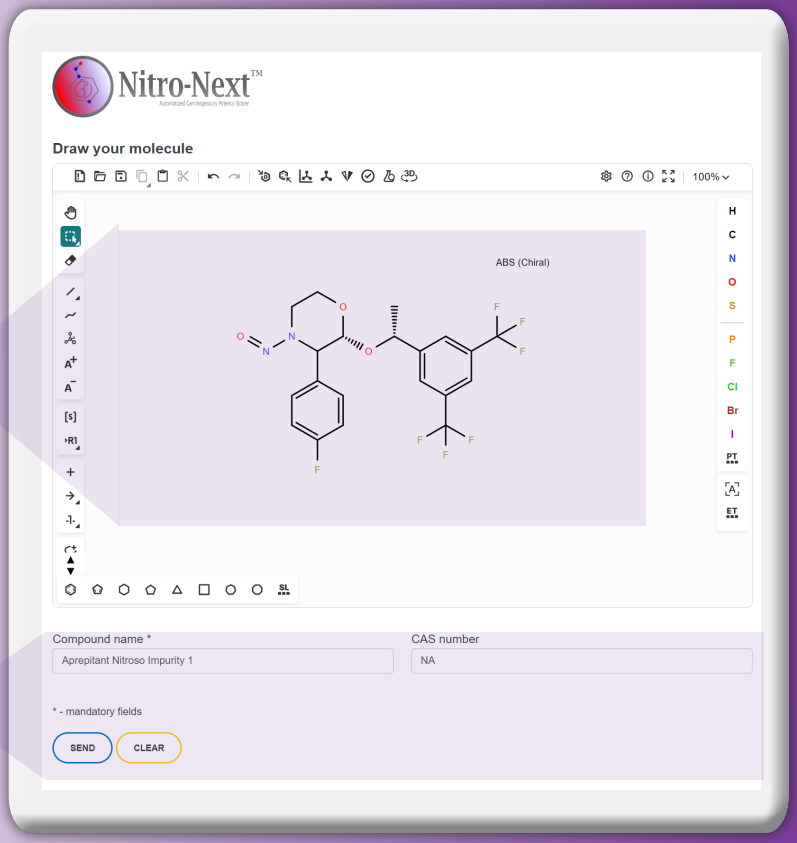
Automatized Carcinogenicity Potency Scorer

In compliance with EMA, FDA and Anvisa's RDC 677/2022 and Guide 50/2021, the software performs an *in silico* analysis to identify structural features related to the carcinogenic potency of N-Nitrosamines, enabling automated unambiguous safe limit setting for these molecules.

Draw your molecule or insert the structure using the SMILES code.

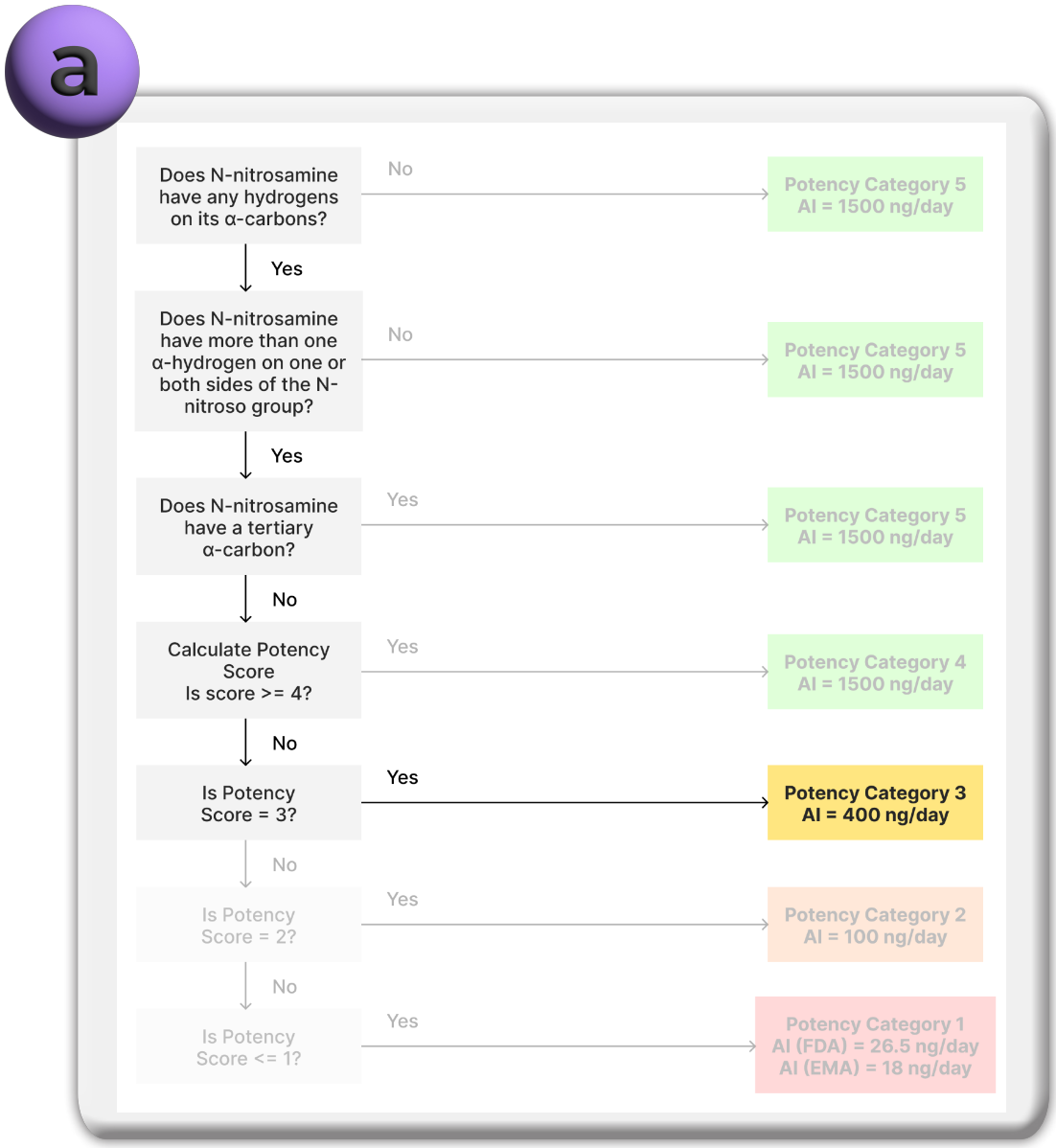
Insert the compoud name (mandatory) and CAS number (optional).

Then press the "SEND" button to start the analysis and generate the report.



4 Steps to a Complete AI Limit Calculation

Nitro-Next algorithm analyzes N-Nitrosamine structures following the EMA/FDA CPCA flowchart:



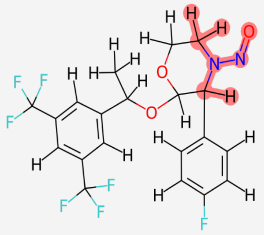
The software accurately determine both potency category and acceptable intake limit for structures within CPCA applicability domain. If a N-Nitrosamine is outside the CPCA scope, the software generates a user report presenting this information.

If potency score calculation is needed to determine potency category and derive the acceptable intake limit, Nitro-Next provides detailed information with visual representations for better comprehension of results.

b

Scoring according to α-hydrogen count

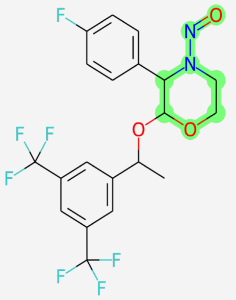
Nitro-Next identifies and counts a-hydrogens according to CPCA for appropriate score attribution. The resulting score is also presented:

Count of α-Hydrogens	Score	Feature
1,2	3	

c

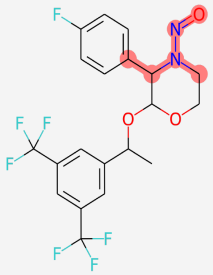
Scoring according to Deactivating Features

Deactivating features are identified, described, and attributed a score according to CPCA. The corresponding group is then highlighted green in the N-Nitrosamine structure:

Deactivating Feature	Score	Feature
N-nitroso group in a morpholine ring	1	

d Scoring according to Activating Features

The software additionally identifies and highlights activating features that may be present in N-Nitrosamines (red), assigning the appropriate score based on the groups identified:

Activating Feature	Score	Feature
Aryl group bonded to α-carbon (i.e., benzylic or pseudo-benzylic substituent on N-nitroso group)	-1	

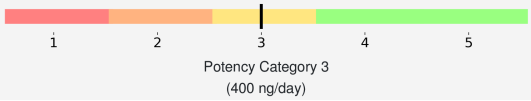
Final Result and Additional Information

The potency score calculation is demonstrated in detail and the resulting score defines the acceptable intake limit for the N-Nitrosamine under evaluation.

Potency Score = α-Hydrogen Score + Deactivating Feature Score + Activating Feature Score

Type	Score	Subtotal
α-Hydrogen Score	3	3
Deactivating Feature Score	1	1
Activating Feature Score	-1	-1

Potency Score = 3 + 1 – 1 = 3

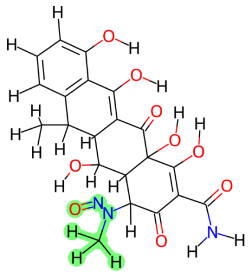
Potency Score	Potency Category (AI Limit)
3	

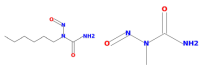
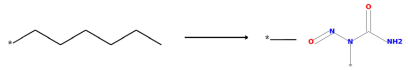
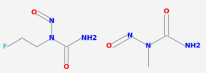
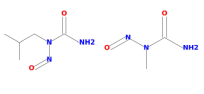
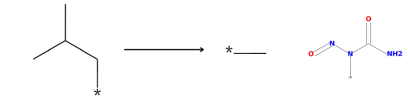
Potency Category 3
(400 ng/day)

If applicable, Nitro-Next performs the exclusive **Matched Molecular Pair (MMP)** analysis that can be used in Expert Review for attenuating or activating fragments.

The underlying principle of MMP analysis involves comparing two molecules (L and R) and simulating a transformation in the R portion. The associated property changes are expressed as “Ratio” and “Difference/Variation” of TD50 values or toxicological outcomes:

Fragment 1



Molecule L	Molecule R	Transformation	Context	Ratio	TD50 L	TD50 R	Difference / Variation
Most Similar							
				0.18	0.513	0.0927	-0.4203
				0.74	0.125	0.0927	-0.0323
				0.02	4.73	0.0927	-4.6373

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