Guide on the Control of Nitrosamines in Active Pharmaceutical Ingredients and Medicines

Guide No. 50/2021 - version 3

National Health Surveillance Agency - Anvisa

2023

Guide on the Control of Nitrosamines in Inputs
Active Pharmaceuticals and Medicines

IN FORCE AS OF 06/13/2023

This Guide expresses Anvisa's understanding of best practices with respect to procedures, routines and methods considered adequate for complying with technical or administrative requirements demanded by the Agency's legislative and regulatory frameworks.[1]

This is a non-normative regulatory instrument, of a recommendatory and non-binding nature, therefore, it is possible to use alternative approaches to the propositions set out here, provided they are compatible with the requirements related to the concrete case. Failure to comply with the content of this document does not characterize a health infraction, nor does it constitute grounds for rejecting petitions, provided that the requirements required by law are met.

The recommendations contained in this Guide take effect from the date of their publication on the Anvisa Portal.

[1] Ordinance No. 162, of March 12, 2021 , which deals with guidelines and procedures for improving regulatory quality at the National Health Surveillance Agency (Anvisa).

SUMMARY

1. SCOPE

This document presents recommendations regarding the control of nitrosamines in all Chemically synthesized Active Pharmaceutical Ingredients (APIs) and medicines for human use that contain them, as well as biological products, when applicable.

The recommendations are equally applicable to post-registration changes that may result in the formation of nitrosamines, such as changes related to the API, composition and packaging of the medication, not exclusively restricted to these.

2. INTRODUCTION

N-nitrosamines constitute a class of compounds characterized by the binding of a nitroso group (-N=O) to an amine functional group ($-NR_{two}$). Among the compounds in this class, there are some mutagenic, genotoxic and potentially carcinogenic agents in humans and, for this reason, they must be controlled at levels considered acceptable and safe. These compounds can be found in water, cured meat, processed fish, beer and other alcoholic and non-alcoholic beverages, cheese, soy sauce, oils, processed vegetables and human milk and their exposure within safe limits represents a low risk of harm to health. (EFSA CONTAM Panel, 2023) (EMA 2020). However, exposure above acceptable levels and for a long period may increase the risk of cancer (Horne et al., 2023). (

In 2018, regulatory agencies around the world became aware of the presence of nitrosamines above the levels allowed in drugs, after manufacturers of active pharmaceutical ingredients from the group of drugs commonly called “sartanas” – angiotensin II receptor antagonists, used to control blood pressure – issued warnings of their possible presence in this class of drugs .

Since then, these agencies have promoted actions in order to protect the health of patients. exposure to nitrosamines in medications above levels considered acceptable. In Brazil, the control actions promoted by Anvisa began with inspections carried out in 30 drug manufacturing companies, with 111 products having been inspected. As a result, 31 health actions were carried out, including interdictions, suspensions and recalls. The source of drug contamination was identified as coming mainly from the presence of solvents under chemical conditions that favor the formation of nitrosamines.

Faced with the case of “sartanas”, the main regulatory agencies in the world, together with drug companies began to investigate whether other drugs could also have nitrosamines above acceptable levels. In 2019, the presence of nitrosamines in other drug classes, such as nizatidine, ranitidine, and metformin, was reported. Additionally, the possible formation of nitrosamines from the primary packaging material containing nitrocellulose was evidenced. Since then, formation of nitrosamines has been observed in several other medicinal products, for example those containing varenicline, rifampicin, diosmin and sitagliptin.

As part of this investigation process, it was found, for example, that the presence of nitrosamines in ranitidine presented a different source than previously found in other products. For this drug, the formation of dimethylnitrosamine (NDMA) originates from an intermolecular degradation that occurs throughout the storage of the product, and which can be influenced by the crystal morphology of the molecule (King et al. 2020), in addition to being accelerated by the storage at temperatures above

room temperature. Such conditions may result in consumer exposure to unacceptable levels of this impurity (FDA 2020b).

In Europe, in the months following the discovery, several manufacturers of the raw material ranitidine had their Certificates of Suitability (*Certificate of Suitability*-CEP) revoked by the *European Directorate for the Quality of Medicines* (EDQM). It is also noteworthy that drugs containing ranitidine hydrochloride have been available to the world's population for over 30 years and have been used for the treatment of ulcers, esophagitis and gastric reflux, without reports of serious adverse events, which reinforces the need for evaluation and control of nitrosamines in all drug classes.

It is important to emphasize that although there is a very low risk of nitrosamines being present in biological products, these cannot be definitively discarded. In the light of current scientific knowledge, it is known that such risks are concentrated, for example, in products with chemically synthesized fragments, those packed in blisters containing nitrocellulose, biological products with excipients in their composition, or in which there is the intentional addition of agents nitrosants in the manufacturing process (EMA, 2020).

In this sense, although nitrosamines are not expected to be formed during the manufacture of most APIs and finished products (EMA 2020b), it is strongly recommended that manufacturers, distributors and fractionators of APIs and drug manufacturing and importing companies evaluate their products for the possible presence of these contaminants and take the necessary precautionary measures to minimize the risk and ensure the safety of these products.

This guide presents basic concepts about training, risk management related to presence, in addition to recommendations on control of nitrosamines in active pharmaceutical ingredients and medicines, as well as clarifies the responsibility of companies, presents strategies for calculating limits and addresses other concepts.

It should be noted that this is a document that can be changed as new studies and information is available regarding this area of knowledge, including acceptable limits of exposure to nitrosamines, in view of the lack of conclusive studies on long-term intake.

It is important to register international regulatory cooperation on this topic. In 2018, it was formed a strategic group composed of regulatory agencies from several countries to deal with the subject, the NISG, "*Nitrosamines International Strategic Group*". In 2020, a spin-off group of the NISG, the NITWG, or International Technical Working Group on Nitrosamines (*Nitrosamines International Technical Working Group*) with the attribution of addressing technical issues related to nitrosamines in more depth. Anvisa has been part of these groups since 2021.

3. LEGAL BASIS

Law No. 6,360, of September 23, 1976, which provides for the health surveillance to which subject to medicines, drugs, pharmaceutical and related inputs, cosmetics, sanitizing products and other products;

RDC Resolution No. 73, of April 7, 2016, which provides for post-registration changes, cancellation of registration of drugs with synthetic and semi-synthetic active ingredients;

RDC Resolution No. 76, of May 2, 2016, which provides for changes, inclusion and post-registration cancellation of specific drugs.

RDC Resolution No. 166, of July 24, 2017, which provides for the validation of methods analytics;

RDC Resolution No. 753, of September 28, 2022, which provides for the criteria for the granting the registration of drugs with synthetic and semi-synthetic active ingredients, classified as new, innovative, generic and similar;

RDC Resolution No. 359, of March 27, 2020, which establishes the Pharmaceutical Input Dossier (DIFA) and the Letter of Adequacy of Active Pharmaceutical Ingredient Dossier (CADIFA);

RDC Resolution No. 412, of August 20, 2020, which establishes the requirements and conditions for carrying out stability studies for the purposes of registration and post-registration changes of biological products;

Normative Instruction No. 65, of August 20, 2020, regulates the classification of changes post-registration and conditions and technical documents necessary to instruct requests for post-registration alteration and cancellation of registration of biological products;

RDC Resolution No. 511, of May 27, 2021, which provides for the admissibility of codes foreign pharmacists.

RDC Resolution No. 413, of August 20, 2020, which provides for post-registration changes and cancellation of registration of biological products;

RDC Resolution No. 576, of November 11, 2021, which provides for the notification of low-risk medications;

RDC Resolution No. 625, of March 9, 2022, which provides for the minimum requirements relating to the obligation, on the part of companies holding drug registrations, to communicate the implementation of the drug recall action to the competent health authorities and consumers, in the event of sufficient evidence or proof of quality deviation that represent a risk, injury or consequence health, as well as on the occasion of cancellation of registration related to safety and efficacy;

RDC Resolution No. 654, of March 24, 2022, which provides for Good Manufacturing Practices of Active Pharmaceutical Ingredients;

RDC Resolution No. 658, of March 30, 2022, which provides for the general guidelines of good drug manufacturing practices;

RDC Resolution No. 677, of April 28, 2022, which provides for risk assessment and risk control Potentially carcinogenic nitrosamines in Active Pharmaceutical Ingredients (API) and medicines for human use.

4. CHEMICAL AND REACTIONAL ASPECTS

N-nitrosamines have the general formula described below:

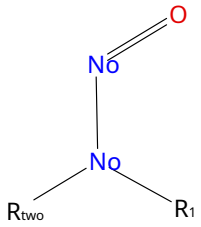


Figure 1- General structure of N-nitrosamines.

The chemistry of nitrosamines has been studied and documented since the last century, having this field of study was boosted in the 1950s by the observations of Magee and Barnes (1956) who related these substances to the onset of cancer. Data obtained in Norway, in the late 1950s and early 1960s, already indicated the presence of nitrosamines as a cause of hepatotoxicity in animals fed diet treated with nitrite (Ender et al. 1964).

Dimethylnitrosamine (NDMA) is currently classified by the IARC as probably carcinogenic to humans (group 2A) (IARC 1987), while two tobacco-specific nitrosamines (i.e., 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine (NNN)) are categorized as carcinogenic to humans (group 1) (IARC 2007).

The existence of these compounds is widespread. Fong and Chan (1973) observed that the ability of the *Staphylococcus aureus* reduce NO_3^- (nitrate) to NO_2^- (nitrite) from salts present in fish, could lead to the production of nitrosamines through the reaction of the nitrite formed with trimethylamine, a substance present in large quantities in decomposing fish. Water and smoked foods are also commonly cited as sources of nitrosamines.

The genotoxicity of nitrosamines is dependent on alpha-carbon hydroxylation, which occurs through enzymes of the microsomal P-450 system (CYP 450). Activation involves the production of diazonium ions, which decompose leading to the formation of carbocations, which are positively charged and electrophilic species capable of binding to DNA (Rath & Canaes 2009, Carlson et al. 2017). Currently, data from animal studies of 228 nitrosamines reveal that 82% are considered carcinogens. *in vivo* (Thresher et al. 2020); independent of the administered route (Li & Hecht 2022, Preussmann & Stewart 1984).

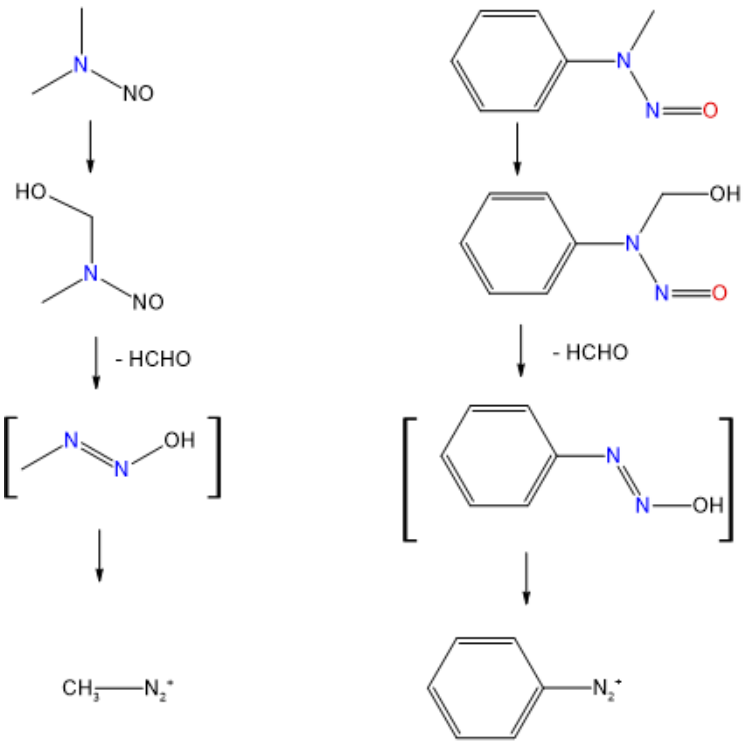


Figure 2- Hydroxylation of nitrosamines mediated by the P-450 system and subsequent generation of diazonium ions. Adapted from EMA (2020a) and (Rath & Canaes 2009).

Chemically, nitrosamines can be formed from amines and nitrosating agents. (usually oxidized nitrogen containing NyOx groups) under certain reaction conditions. The nitrosation of secondary amines (Fig. 3B), tertiary and quaternary ammonium compounds by nitrous acid is a general example of the formation of these compounds. Nitrous acid is an unstable compound obtained from sodium nitrite in an acid medium leading to the generation of nitrosonium ion (NO^+), which is responsible for the N-nitrosation of amines (Fig. 3A).

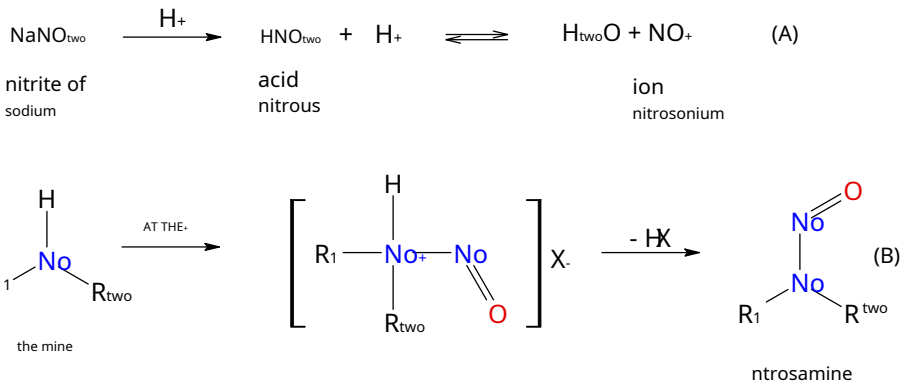


Figure 3 - Formation of nitrosamines by nitrosation with nitrous acid obtained from sodium nitrite in an acid medium.

In general, nitrosamines formed from primary amines are unstable and lead to direct formation of the corresponding diazonium ion; (Fig. 4C). That is, when one of the ligands R_1or $R_{two}$of the amine shown in Figure 3 is a hydrogen, tautomeric interconversion occurs in the primary nitrosamines formed (something analogous to keto-enol equilibrium) (Fig. 4A), which leads to the formation of a corresponding alkyldiazonium.

Diazoic acid tends to dehydrate in the presence of hydrogen ions and form diazonium ion (Fig. 4B). This ion is rapidly decomposed forming a carbocation and releasing nitrogen (N_{two}) (Roberts & Caserio 1977, Reusch 1999). In summary, there are no appreciable amounts of nitrosamines formed from primary amines by the decomposition tendency of the nitrosated product.

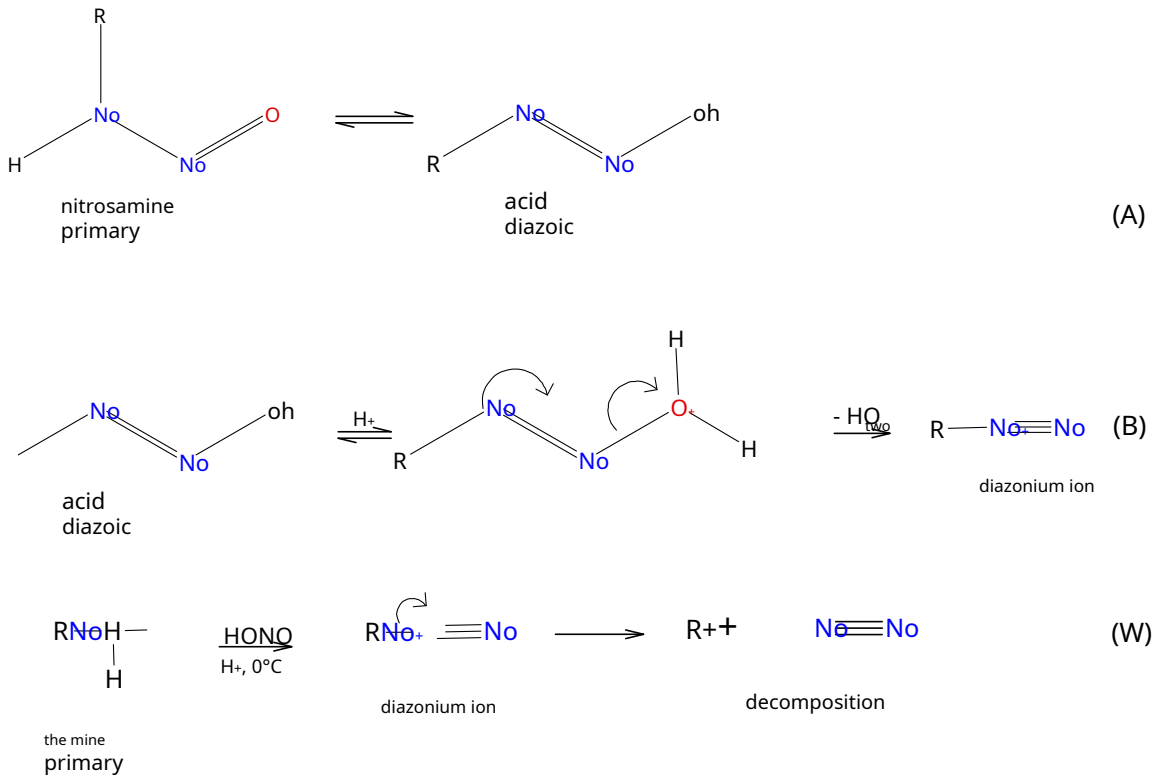


Figure 4- Carbocation formation from primary amines.

The structure of the nitrosamine obtained at the end of the nitrosation process will be determined primarily by the structure of the amine that has been nitrosated. As an example, the formation of dimethylnitrosamine (NDMA) from the nitrosation of dimethylamine (DMA) is illustrated as follows:

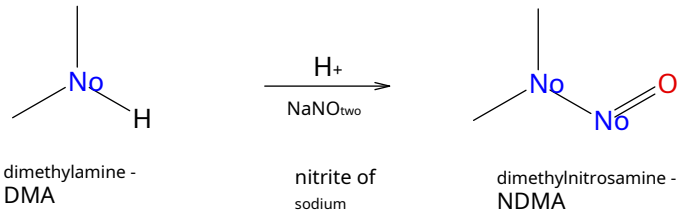


Figure 5 - Nitrosation of dimethylamine (DMA) and formation of dimethylnitrosamine (NDMA).

Likewise, acid nitrosation *No*-methyl-4-aminobutanoic acid (MBA) leads to the formation of carboxypropylmethylnitrosamine (NMBA):

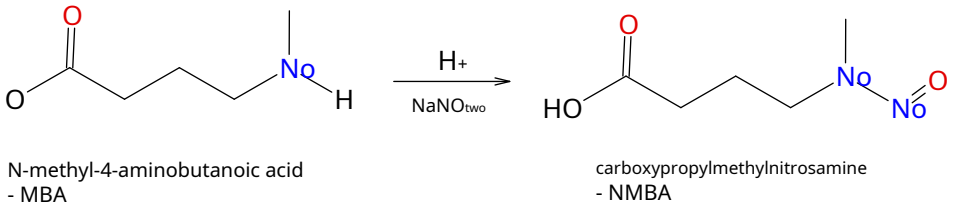


Figure 6 - Nitrosation of N-methyl-4-aminobutanoic acid (MBA) and formation of carboxypropylmethylnitrosamine (NMBA).

Both NDMA and NMBA were detected as impurities arising from the synthesis of APIs angiotensin II receptor antagonists (EMA 2020, FDA 2020). The reactions above describe what probably occurred to generate the NDMA and NMBA nitrosamines, i.e. acid formation

nitrous *in situ* from the presence of sodium nitrite, leading to the formation of the nitrosating agent (NO^+) and consequent nitrosation of the amines DMA and MBA, as shown in Figure 3A (EMA 2020).

It is known that other compounds containing NOx groups, such as nitrite salts and alkyl nitrites, nitrous anhydride (N_2O_3), dinitrogen tetroxide (N_2O_4), nitrosyl halides (NOCl), nitrosylthiocyanate and nitrosophenol, among others, are capable of nitrosating amines (Figs. 7 to 9). Processes such as curing meat, malting before fermentation or during chemical reactions can lead to the production of some of these compounds. Nitric oxide is capable of nitrosing in the presence of metals and organometallic compounds (EMA 2020a).

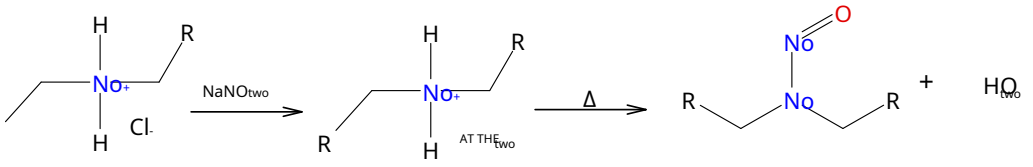


Figure 7 - Formation of secondary nitrosamine from ammonium salt and sodium nitrite.

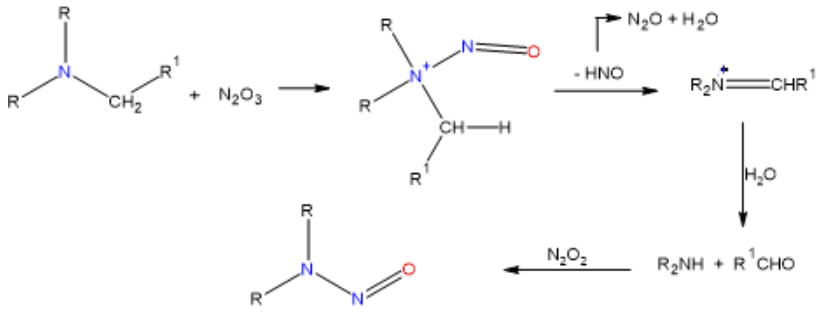


Figure 8- Formation of nitrosamine from tertiary amine and N_2O_3 as a nitrosating agent.

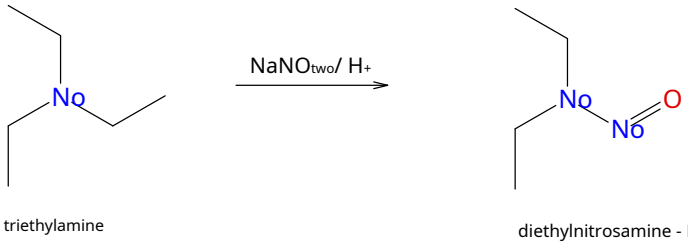


Figure 9 - Nitrosative dealkylation of triethylamine and formation of nitrosamine diethylnitrosamine (NDEA).

The formation of nitrosamines from tertiary amines is generally a slower process. than the process of nitrosation of secondary amines, since it involves a dealkylation step, followed by nitrosation of the dealkylated amine and, regarding the formation of simple nitrosamines, derived from trialkylamines, a much lower risk is expected when compared to the formation of nitrosamines derived from secondary amines, although this reduced reactivity may not occur with more complex tertiary amines (López-Rodríguez et al., 2020 and Ashworth et al., 2023).

a) Formation of nitrosamine as a contaminant in the process of obtaining Active Pharmaceutical Ingredients

Among the main causes of the formation of nitrosamines as contaminants in API, is the simultaneous use of secondary or tertiary amines and nitrosating agents, sources of NOx ($NaNO_2$, NO_2O_3 for example) in the synthesis or obtaining of intermediates or APIs. Not only can the deliberate use of these compounds in the same step lead to the formation of nitrosamines, but also the carriage of amines or sources of

NOx from previous steps to subsequent steps can generate a condition conducive to the formation of nitrosamines. Contaminated reagents, starting materials, solvents and catalysts can also lead to the formation of nitrosamines during synthetic steps.

Amines can also be generated *in situ*. For example, the solvent dimethylformamide (DMF) which can undergo acidic or basic hydrolysis under favorable conditions and generate dimethylamine (DMA), which is susceptible to nitrosation (Fig. 10).

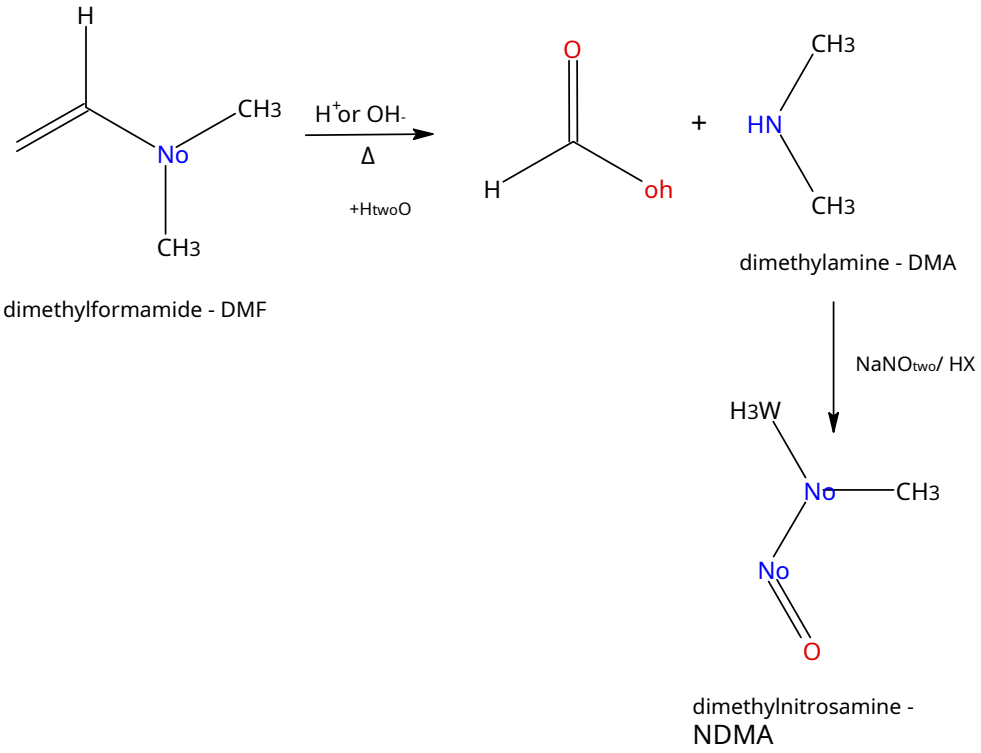


Figure 10 - Hydrolysis of dimethylformamide (DMF) and formation of NDMA.

Dimethylamine can also appear as an impurity in the solvent dimethylformamide (DMF), arising from the DMF synthesis process itself. Similarly, the solvent methylpyrrolidone (NMP) can undergo hydrolysis and generate substrate amenable to nitrosation (Fig. 11) (Klein 2017, EMA 2020).

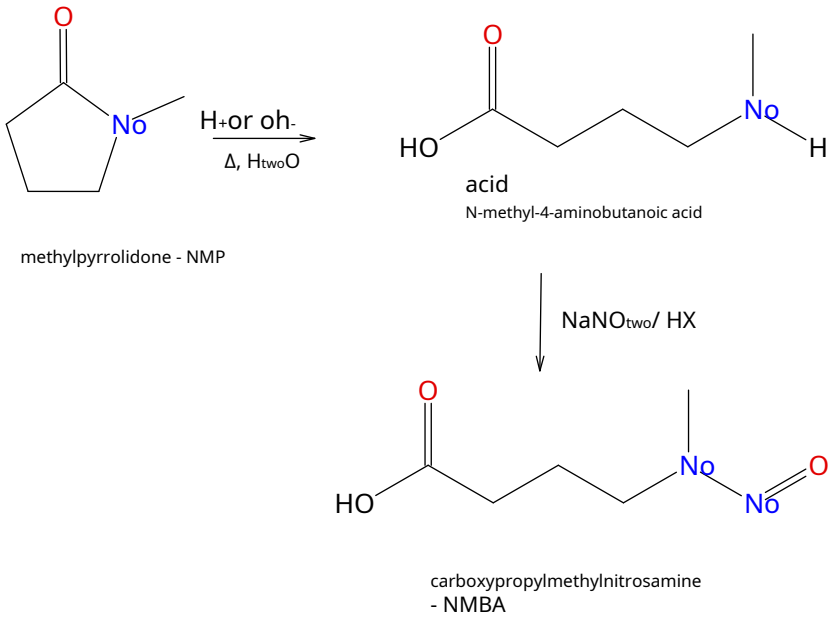


Figure 11 - Hydrolysis of methylpyrrolidone (NMP) and formation of NMBA.

The cases mentioned above are possible causes of the presence of NDMA nitrosamines and NMBA in some processes for obtaining APIs (EMA 2020), exemplifying how the process conditions should be evaluated regarding the potential for the formation of amines susceptible to nitrosation, especially when agents capable of generating nitrosating species are used, even if in different stages of the process, since the carrying of impurities from one stage to another can lead to the risk of nitrosamine formation. For example, the nitrite used in a given step can be carried to subsequent steps, going through processes of*work up*, crystallization or by other purification operations carried out (EMA 2020, FDA 2020).

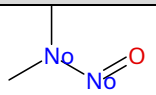
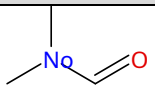
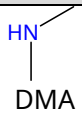
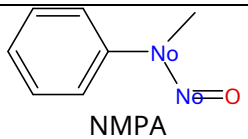
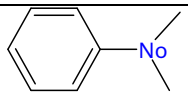
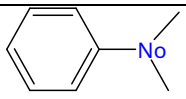
In addition to the transformations of inputs used in the process, exemplified with the cases of hydrolysis of DMF and NMP, direct reactions between ammonium salts and nitrosating agents can also occur. For example, tetrabutylammonium bromide (TBAB) or triethylamine hydrochloride (TEA.HCl) catalysts undergo nitrosative dealkylation, a reaction similar to that shown in Figure 9. However, the solvent dimethylacetamide and other amides can follow the same degradation pattern shown in Figure 9. Figures 10 and 11, generating amines capable of nitrosation. Worthy of note, in addition to the cases mentioned so far, is the possibility of oxidation of hydrazines, which can even happen through simple exposure to oxygen in the air (López-Rodríguez et al., 2020, Horne et al., 2023).

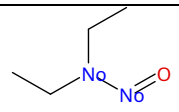
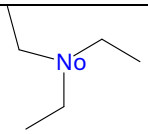
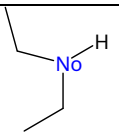
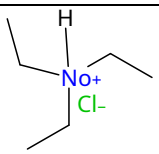
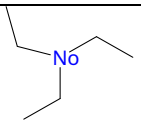
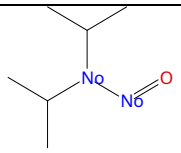
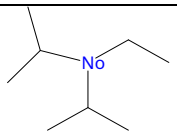
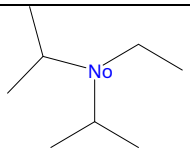
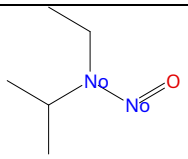
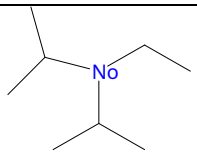
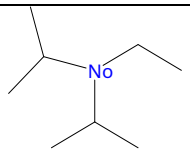
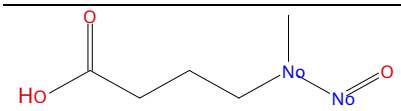
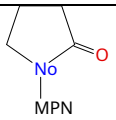
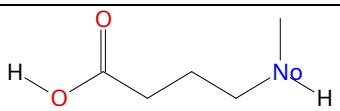
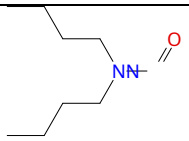
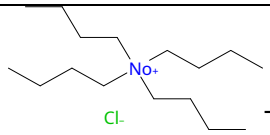
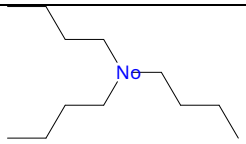
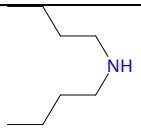
Due to the wide use of amines in several synthesis processes, it is possible that these are present as contaminants in various inputs. Additionally, the presence of less substituted amines in reagents such as tertiary amines or ammonium salts is possible. For example, diisopropylamine and isopropylethylamine are possible contaminants of diisopropylethylamine (DIPEA), as well as diethylamine is a likely impurity present in triethylamine (TEA). Furthermore, phase transfer catalysts such as triethylamine hydrochloride (TEA.HCl) have been identified as a potential source of triethylamine and diethylamine (EMA 2020).

It is of crucial importance to evaluate the process of obtaining an active pharmaceutical ingredient (IFA) in full, including assessment of procurement of key intermediates and starting materials. The possibility of carrying a certain substrate from one stage of the process to another, the use of reagents that have amines in their profile of contaminants or potential nitrosating agents and the process conditions, such as temperature and pH that may favor the degradation of reagents or solvents, are some important examples to be observed.

In this sense, it is important to know the degree of purity of the materials introduced and its potential impurities. Contamination within the same manufacturing unit or resulting from recovery/ reuse of solvents or materials conducted by third parties must always be considered in risk assessments of synthesis processes. Possible critical combinations that must be observed are situations in which the general conditions for the formation of nitrosamines are present as in the examples in Table 1.

Table 1 – Examples of critical combinations that can lead to the formation of nitrosamines.

Nitrosamine formed	Source in NOx	amine source	nitrosated amine
 NDMA	$NaNO_{two}$	 DMF	 DMA
 NMPA	$NaNO_{two}$	 N,N-DMA	 N,N-DMA

 NDEA	$NaNO_{two}$	 TEA	 DEA
		 TEA.HCl	 TEA
 DIPNA	$NaNO_{two}$	 DIPEA	 DIPEA
 EIPNA	$NaNO_{two}$	 DIPEA	 DIPEA
 NMBA	$NaNO_{two}$	 MPN	 MBA
 NDBA	$NaNO_{two}$	 TBAB	 TBA
			 DBA

In addition to nitrosation of amines, other factors must also be considered during the evaluation. less classic conditions that allow the formation of nitrosamines mentioned in the literature (López-Rodríguez et al. 2020), such as the reduction of nitramines, oxidation of hydrazines and formation from organometallic compounds.

b) Formation of nitrosamine as a contaminant in the drug manufacturing process

The formation of nitrosamines during drug production is less predictable in comparison with the API production. However, the considerations made in the previous section about the possible ways of formation of nitrosamines in the API may remain valid for the drug, as long as the reaction conditions are present.

The formation of nitrosamines in drugs can occur due to some factors linked to the production process of the drug and its unit operations, the interaction between the API and the excipient during the production process or during storage, the degradation of the API and other materials, the interaction of the formulation with the packaging material and, also, by cross-contamination between processes.

In the context of nitrosamine formation in the finished product, agent availability nitrosant can come from the raw materials used, it is known that excipients such as crospovidone, hypromellose, magnesium stearate, among others, may contain nitrite as impurities (Boetzel et al. 2022). The nitrite interaction of the excipients must be evaluated against the presence of amines, mainly secondary, arising from other excipients, API or API-related substances.

Some unit operations present potential risks for the formation of nitrosamines, such as processing in fluidized beds, due to the possibility of oxidation of disubstituted hydrazines or exposure of secondary amines to oxidized nitrogen species (López-Rodríguez et., 2020) (Horne et al., 2023). Wet granulation can facilitate contact between the API, or any substances related to amine function, and the nitrosating agents, especially if this process step occurs in an ideal pH range (Horne et al., 2023). Although the risks associated with water quality are generally considered to be low, it is essential to properly control the water quality and estimate the amount of nitrite, or the very presence of nitrosamines in the water. Depending on the conditions, the formation of nitrosamines can become significant at lower pH values (<

The degradation of ranitidine, which occurs in the finished product and which can be influenced by crystal morphology of the molecule, is an example of nitrosamine formation that involves API degradation and is influenced by factors other than those classically attributed to nitrosamine formation.

Packaging has already been associated with the presence of NDMA and NDEA, due to reactions between the amines present in printing inks and nitrocellulose present in packaging sealing sheets. During the high temperature sealing process, nitrosamines can evaporate and contaminate other pharmaceuticals in the package.

5. RISK MANAGEMENT

It is suggested that companies use the risk management principles described in the Guide ICH Q9 -*Quality Risk Management*(ICH 2005) as support in carrying out risk analyses. In this guide, we propose carrying out this analysis in three stages: Risk Assessment (1), Confirmatory Tests (2) and Nitrosamine Control for Regularized Products (3).

Step 1 called Risk Assessment consists of identifying and assessing the risk of formation and presence of nitrosamines. It is suggested that this step be initiated by analyzing the API synthesis route, which may be performed by the API manufacturer, distributor or fractionator, holder of the API registration (*holder*) or even by the drug manufacturer, if it holds the complete synthesis route of the active. The knowledge of the complete API synthesis encompasses the knowledge of the synthesis routes of the starting materials, the control of the materials and the knowledge of the synthesis route in detail. This information is commonly found in the restricted parts of the Active Pharmaceutical Ingredient Dossier (DIFA). After this evaluation, the company will proceed with the evaluation of the production process of the finished product and the potential for the formation of nitrosamines throughout the shelf life.

If a risk of the presence of nitrosamines is identified as a result of Step 1, the company must proceed to Step 2 and carry out the confirmatory tests in order to confirm or refute the presence of nitrosamines in the API or finished product, depending on the origin of the risk. At this stage, the company must verify that the identified and quantified nitrosamine has an acceptable intake limit defined and published by Anvisa as per Table 3 of this guide.

If the company disagrees with the limits of nitrosamines established in Table 3 (Limits of acceptance for nitrosamines) or the nitrosamine impurity identified does not yet have a harmonized and published acceptable intake limit, it must notify Anvisa and propose new limits based on scientific rationale through a protocol in the system Requests to GESEF (Security Assessment Management and Efficacy) using subject code 12194 - Nitrosamine Safety Limit Assessment – Company.

To carry out the tests, the company must use appropriate analytical procedures sensitive for the quantification of these impurities. The absence of nitrosamines is considered when they are below 10% of the acceptable intake limit, but other approaches may be justified, not exceeding the limit of 30%. Once the presence of nitrosamines has been confirmed based on the tests carried out in Step 2, the company must move on to Step 3, where the controls for nitrosamines in its products are defined.

In situations where there is identification of the presence of nitrosamines above acceptable limits, the company must adopt the necessary risk mitigation actions and notify Anvisa (Inspection and Surveillance Management - GGFIS) through the protocol in the Solicita system using the following subject codes: 70788 - ACTIVE PHARMACEUTICAL INGREDIENTS - Notification for Nitrosamine Assessment above of Acceptable Limits (manufacturers, distributors and fractionators) (company petition) and/or 70789 - MEDICINES - Notification for Evaluation of Nitrosamines above Acceptable Limits (manufacturers and importers).

It is important to highlight that in all cases the company is responsible for guaranteeing the quality and safety of its products with the assessment of the risk of ingestion by patients and adoption of appropriate actions to avoid or minimize the exposure of individuals to nitrosamines, including in situations of disagreement with the limits described in Table 3 and in the period until the limit is defined by Anvisa.

Risk control actions must include changes related to the root cause(s) identified in the investigation, as well as assessment of the need to adopt measures that restrict the availability of the product on the market (for example: suspension of distribution and marketing, recall) taking into account actions adopted for markets in other countries and aspects such as risk of market shortages and availability of therapeutic alternatives.

For known impurities above the limit stipulated in Table 3 - Acceptance Limits described in section 8 of this Guide, companies must immediately suspend the distribution and marketing of the drugs or APIs involved, in addition to assessing the need for collection, which must be treated as provided in Resolution RDC nº 677/2022 and nº 625/2022 and their updates .

Finally, if the presence of nitrosamines is confirmed and, as long as it is within the limits acceptable, the company must file with the Agency, if applicable, the respective post-registration petitions in accordance with the regulations applicable to each case. Test additions are immediate implementation or immediate notification and performed via subject-specific code. For petitions applicable to synthetic and semi-synthetic drugs and APIs, the following subject codes should be used:

- 12195 - GENERIC - Inclusion of the critical control test for nitrosamines in the medication - RDC 677/2022
- 12198 GENERIC - Inclusion of the control test for nitrosamines in the API with CADIFA - RDC 677/2022
- 12201 GENERIC - Inclusion of the control test for nitrosamines in the API without CADIFA - RDC 677/2022
- 12197 NEW - Inclusion of the critical test for control of nitrosamines in the drug - RDC 677/2022 NEW -
- 12200 Inclusion of the control test for nitrosamines in the API with CADIFA - RDC 677/2022 SIMILAR -
- 12196 Inclusion of the critical test for control of nitrosamines in the medication - RDC 677/2022 SIMILAR -
- 12199 Inclusion of the control test for nitrosamines in the API with CADIFA - RDC 677/2022 SIMILAR -
- 12202 Inclusion of the nitrosamine control test in the API without CADIFA - RDC 677/2022

It should be noted that in addition to the inclusion of tests, other post-registration changes may apply in order to mitigate the risk of the presence of nitrosamines. The cases below illustrate some examples of necessary post-registration alterations according to risk assessment notes:

Example 1: After identifying and assessing the risk of a certain product X, the company performed confirmatory tests and verified the presence of NDMA above the acceptable limit. According to the company's risk assessment, the root cause of the presence of nitrosamine was the reaction of nitrocellulose, a primary packaging component, with an amine present in the product's excipients during the process of closing the blister by heating. In order to adapt the product, the company proposes, as a post-registration change, a packaging change in order to remove nitrocellulose as a component of the blister. For this, a protocol of a type 7.c alteration (major change in the composition of the primary packaging) is carried out under the terms of RDC nº 73/2016, which requires an individual protocol and must await a manifestation from Anvisa. Considering the correct risk assessment carried out,

Example 2: After detecting and confirming the presence of more than one nitrosamine reaching unacceptable limits, the registration holder, through its investigation, detects that the root cause involves the API itself and probably the presence of nitrosamine from solvents that were used in the synthesis route. Assuming that the approved API manufacturer chose to discontinue the API in question, the applicant decides to change the API manufacturer to adapt the product. For this case, the condition of the presence or absence of CADIFA under the terms of RDC No. 359/2020 must be considered. If the proposed manufacturer already has CADIFA and the conditions described in amendment 1.g - Replacement of CADIFA holder (immediate implementation) of Annex I of RDC 361/2020 are also met, this change may be implemented immediately. If such conditions are not met or the proposed manufacturer does not have CADIFA, the change must await approval. In this case, considering that changing the manufacturer mitigates the risk of nitrosamines that existed due to the approved manufacturer, the previous risk assessment may justify the non-need for additional parallel post-registrations, and it may be sufficient to change the manufacturer of the IFA.

Example 3: As per the example above, the root cause was identified as a nitrosamine from the API manufacturing process, but in this case the company will make a change to the API process that will eliminate this risk. For this case, the condition of the presence or absence of CADIFA under the terms of RDC No. 359/2020 and whether the change involves an impact on the impurity profile must be considered. Considering the understanding of Questions and Answers of the RDC 73 Edition 4.2 (January 2021), that the removal of an impurity does not fit into the impact on the impurity profile, this can be considered of immediate implementation under the terms of RDC nº 361/2020 since that the change does not otherwise impact the impurity profile (eg generating another impurity) or the API specifications (eg changing its crystalline form). For example, in the case of changing DIFA without CADIFA, meeting the requirements of the standard, the change can be classified as a type 1.d change (Change from DIFA without CADIFA (immediate implementation)). In this case, as in the previous example, there would be no need for additional parallel post-registrations, it being sufficient to just change the IFA process.

Post-registration changes must be filed in accordance with current regulations, and containing all relevant documentation. Thus, for example, in cases where the nitrosamine control test is included in an API or synthetic medication, the petition will be for an individual protocol and immediate implementation, pursuant to RDC 73/2016. In cases where there is a change in the synthetic API synthesis route to mitigate the risk of nitrosamine formation, the change can be classified as a prior analysis (depending on the conditions in the specific case), and the fact that it has the objective of mitigation of the risk of nitrosamine formation does not mean that it can be implemented before analysis.

As for nitrosamines that do not yet have a defined acceptance limit, it is not expected that a post-registration test inclusion change is filed until the limits are approved by ANVISA. However, it is emphasized that the company holding the registration must adopt the necessary control measures and carry out the test as soon as possible after identifying its need.

It is important to mention that all documentation related to the risk management of nitrosamines must be filed at the company and at any time may be required by the Agency or verified during Good Manufacturing Practices Certification (CBPF) inspections, investigative inspections or registration audits.

Figure 12 presents a summary of the steps related to risk assessment and the necessary actions arising from the identified risk.

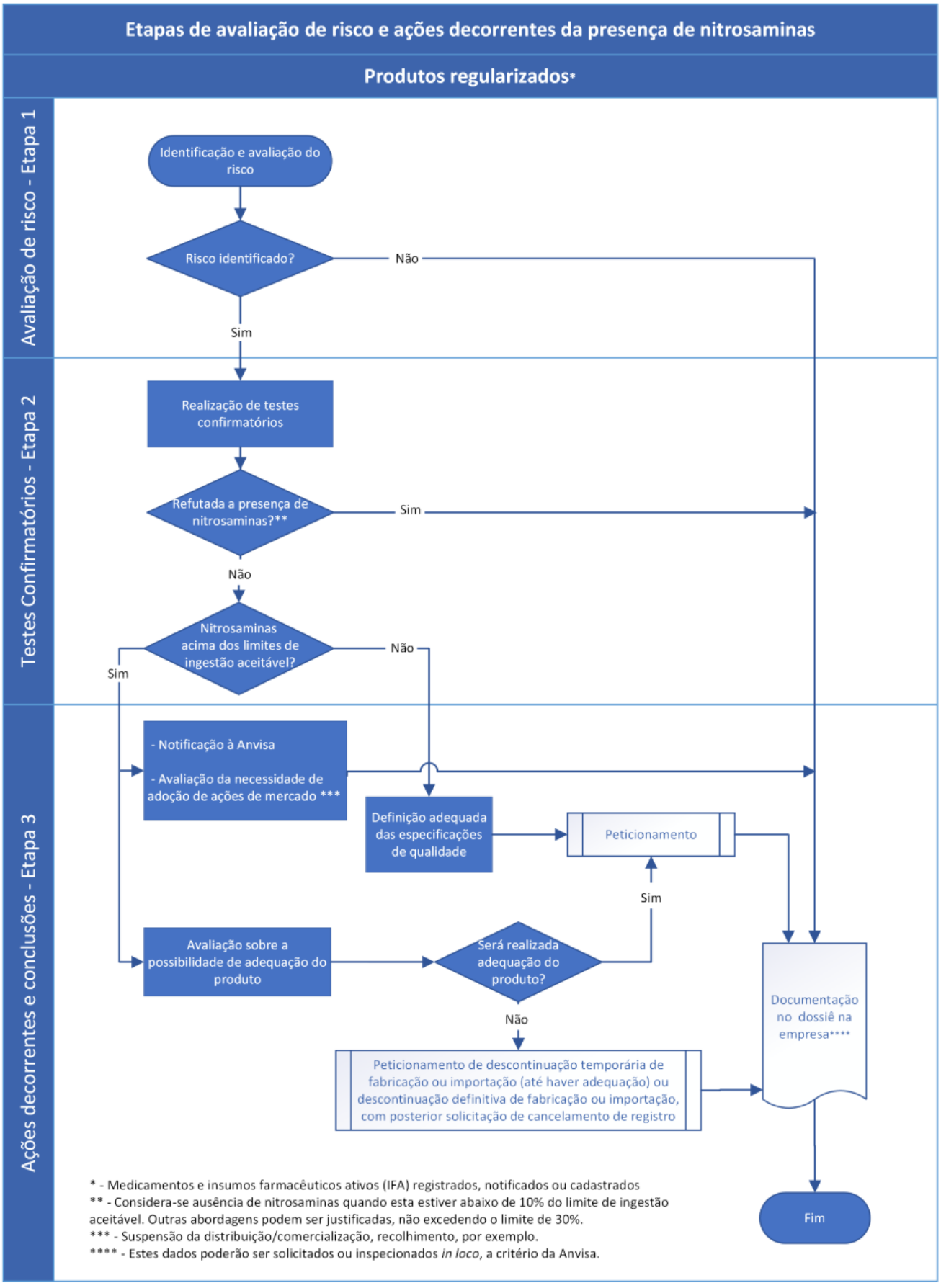


Figure 12- Steps related to risk assessment and necessary actions arising from the identified risk

6. PRIORITIZATION FACTORS FOR RISK ASSESSMENT (STEP 1)

It is known that the number of drugs registered by a company can be significant and, for this reason, it is suggested that the product evaluation sequence be established based on prior knowledge about them. Companies may consider factors such as recommended daily intake, duration of treatment, therapeutic indication, number of patients treated or others that they deem relevant according to the portfolio or the situation of the product. For example, the lack of commercialization of a certain product or condition that is approved (such as an alternative API manufacturer that is approved, but not used by the company) may justify this assessment not being prioritized at first. However, in cases where an approved condition has not been evaluated for the risk of containing nitrosamines because it was not being marketed,

Table 2 presents an example of prioritization, using the criteria "Duration of treatment versus Maximum Daily Dose", which was proposed by the entities representing the Brazilian Pharmaceutical Sector in response to the "Discussion Panel on the control of Nitrosamines in medicines", held on February 5, 2020 at the Anvisa Auditorium.

Table 2 - Prioritization by Duration of treatment X Maximum Daily Dose.

	duration of treatment		
Maximum Daily Dose	**> 1 year**	**1 to 12 months**	**≤ 1 month**
> 1000mg	Very high	High	Average
100mg to 1000mg	High	Average	Low
<100mg	Average	Low	Very low

To carry out the evaluation of drugs with identified risk, companies can also use tools such as Failure Mode and Effects Analysis (FMEA) and Failure Mode, Effects and Criticality Analysis (FMECA), as described in the ICH Guideline guideline Q9 -*Quality Risk Management*(ICH 2005).

a) Basic components

It is expected that manufacturers, distributors and fractionators of APIs and manufacturing companies and drug importers to work together and carry out risk assessments using quality management principles. In addition, the result of the work carried out must be based on scientific knowledge, always linking the protection of the patient and observing that the level of effort, formality and detail of the documentation is proportional to the level of risk.

In addition, the principles described in the ICH Guide M7(R1) -*Assessment and Control of Reactive DNA (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*(ICH 2017) regarding mutagenicity assessment, control strategies and changes in manufacturing processes of active substances can be applied.

It is relevant to point out that if the risk of nitrosamine formation has been assessed during the development phase of the API/finished product manufacturing processes, this information can be used to support the assessment referred to in this guide.

Finally, at the end of Step 1, companies should have sufficient arguments to answer the following questions:

- What is the risk of nitrosamine formation in the API synthetic process, taking into account the combination of reagents, solvents, catalysts and starting materials used, intermediates formed, impurities and degradants?
- What is the risk of nitrosamine contamination (eg from recovered materials such as solvents, reagents and catalysts, equipment, starting materials or intermediates)?
- What is the risk of nitrosamine formation during manufacture of the finished product or during storage throughout its shelf life (eg possible degradation or interaction with excipients and packaging material)?

b) Purge evaluation

If the presence of potential risk of formation of nitrosamines in the process is verified synthetic API, taking into account the reagents, solvents, catalysts, starting materials, formed intermediates and other impurities, a detailed evaluation of the entire process of obtaining the drug can be carried out with regard to the possibilities of elimination/purge of the(s)) potentially present nitrosamine(s).

In this analysis, if mastery of the process for obtaining the API, and its associated parameters, is such that it is possible to determine that the risk of the presence of nitrosamine(s) above the maximum permitted limit(s) is negligible, the control strategy may be based solely on process control , with no need for analytical tests. This strategy is analogous to option 4 of the ICH M7 Guide.

For such a control strategy to be acceptable, a formal risk analysis is required, considering the physicochemical properties of the nitrosamine(s) in question and the factors of the process for obtaining the API that impact on the fate and elimination/purge of the nitrosamine(s), including chemical reactivity, solubility, volatility, ionizability and processes specifically designed to remove the nitrosamine(s) in question. It is crucial that all factors considered by the company and which supported the elimination estimate at each stage of the process are clearly recorded and readily available to Anvisa. This type of analysis must necessarily be conducted by qualified personnel who have knowledge of the API obtaining process, in order to avoid erroneous purge estimates due to lack of knowledge of the specific process or erroneous application of elimination factors and other applicable concepts described in the literature. The outcome of the risk analysis should estimate an elimination/ purge factor for the contaminating nitrosamine(s) in question.

Contamination/elimination studies may be used as evidence of the ability of the process to eliminate/purge the nitrosamine(s) in question. When it is not possible to determine the absence, or the presence at negligible levels, from the risk analysis/estimated purge factor, the control strategy must include analytical tests on the API or on isolated intermediates.

Some scientific publications are available and can be consulted to guide how much to the best practices for calculating the purge factor, establishing necessary requirements to justify the elimination of the impurity, including for cases involving nitrosamines, and can be used as a reference in this step, for example: Teasdale et al. 2010, Teasdale et al. 2013, Barber et al. 2017, Burns et al. 2019, Burns et al. 2020.

7. CONFIRMATORY TESTS (STEP 2)

a) Analytical method

As with other impurities, laboratory evaluation of the presence of nitrosamines requires the application of sensitive and selective analytical procedures, often requiring the association of different analytical techniques such as chromatographic separation associated with identification and quantification by mass spectrometry, for example.

Although the Brazilian Pharmacopoeia still does not have a method for analyzing nitrosamines, several procedures have been developed and made public including by regulatory authorities such as the European Medicines Agency – EMA (EDQM 2020), American Food and Drug Agency - FDA (FDA 2019) and Canadian Medicines Agency - Health Canada (Health Canada 2019).

It is also necessary to consider the foreign pharmacopoeias admitted in Brazil, according to the RDC Resolution No. 511, of May 27, 2021 or its updates. These methods, if available, can be used by companies as a basis for analyzing their products.

Considering the aspects related to the formation of these contaminants and the catalog of company's products, a strategy to be considered is the company's development of a general and comprehensive method that may be suitable both for the analysis of active inputs, excipients, and finished products in their different stages (production, stability studies and others). However, considering the specificity of each product and the different types of matrix, a single method for all cases may not be possible.

It should be noted that, regardless of the strategy adopted and the adjustments made, the method of use must meet the criteria established in current legislation, Resolution RDC No. 166, of July 24, 2017, which provides for the validation of analytical methods, or their updates, or the ICH Guide Q2(R1) Validation of Analytical Procedures . In this sense, it is highlighted, but not restricted, the need to observe the limits of detection or quantification and selectivity, especially when this is applicable to different analysis matrices. It is important to highlight that the analytical validation must be planned considering the data available for the product. With this, it can be concluded that it is possible to use partial validation, limit testing, or even the need to perform additional tests, among other situations.

There are reports of the formation of nitrosamines even in the steps of sample preparation well and interference in the analysis by solvents commonly used in laboratory routine. Therefore, proper sample preparation is a critical step in the evaluation of these analytes, either due to the loss of impurities or their generation during this step (EMA 2020, King et al. 2020).

b) Batches to be tested

The step at which nitrosamine will be tested (intermediate, API or finished product) depends on the origin of the impurity. For example, when confirmatory testing is required for impurities from the API manufacturing process, these can be tested in the API or in a process intermediate, if their origin is prior to this intermediate. If the impurity is degradation, it is recommended that the tests be carried out on samples that represent the product during its validity, for example, batches submitted to stability studies, samples close to expiration, among others. In this case, the test is applicable both to the API and to the finished product.

The quantification assays of the API batches made by the API manufacturer can be used by the drug registration holder provided that there is a critical evaluation of the results, and that the

manufacturer has been qualified according to current Good Manufacturing Practices legislation. However, this does not exempt the need for risk assessment in the finished product, since the formation of the contaminant can occur in several stages of the production process.

Regarding the number of batches to be tested, this must be defined by evaluating company risk, as recommended below:

- For registered drugs, a minimum of 10% of annual batches, or 3 batches per year, whichever is greater, must be properly sampled and tested.
    - O If less than 3 batches are produced in the year, all manufactured batches must be tested.
    - O If more than one manufacturer, manufacturing process and/or sources of risk-related materials are used, more batches must be tested in order to cover all risk factors.
    - O When nitrosamine(s) are degradation impurity(s), at least 3 representative batches of the product throughout its shelf life must be tested.
    - OOther technically justified approaches may be accepted.
- For new registration or post-registration petition, the number of batches tested must be consistent with the quantity required by the current RDC.
    - O In addition, the number of lots required in the previous paragraph must also be observed. For example, for petitions requesting less than three batches, the implementation will be conditional on the company's commitment to test the implementation batches also later, in order to complete the required 3 batches. These data must be available for presentation to Anvisa, when requested or during inspection.
- When the drug manufacturer needs to perform the API analysis, the number of batches must be defined by the company's risk assessment.

8. CALCULATION STRATEGY FOR ASSIGNING ACCEPTANCE LIMITS

The compounds *No*-nitrosos are carcinogenic genotoxic agents of the group called “Group of concern” or *Cohort of Concern.* These are more potent impurities than most other mutagenic compounds, hence the use of the threshold based on the concept of “Threshold of Toxicological Concern” or TTC (*Threshold of Toxicological Concern)* established in the ICH Guide M7(R1) of 1.5µg/day is not applicable.

As described in the ICH Guide M7(R1), for such compounds it is expected that the acceptable intake is significantly lower than for the other potentially mutagenic impurities, so that the establishment of the limit should ideally be done on a case-by-case basis, using, for example, carcinogenicity data of structurally similar compounds.

The risk assessment approaches described in this Guide apply to all avenues of administration, and threshold corrections for different routes of administration are not applicable. Cases in which scientific data justify particularities of a specific route of administration must be evaluated individually.

For products intended for advanced cancer only as defined in the scope of ICH guideline S9, N-nitrosamine impurities should be controlled according to ICH guideline Q3A (R2) and ICH guideline Q3B (R2). If the active substance itself is mutagenic or clastogenic at concentrations

therapeutics, N-nitrosamine impurities should be controlled within limits for non-mutagenic impurities according to ICH M7 (R1).

a) Limits for single nitrosamine

The determination of acceptable limits or AI (*Acceptable Intake*) of the nitrosamines listed in this guide was based on the specific calculation guidance for each compound set out in the ICH Guide M7(R1) and harmonized with the limits already accepted by other regulatory authorities (EMA 2020b, EMA 2023, FDA 2020), which, in turn, were obtained from from carcinogenicity studies in animals or rationale supported by structural similarity. Limits for some known nitrosamines are described in Table 3.

Table 3 - Acceptance limits for nitrosamines

Nitrosamine (acronym)	Nomenclature	CAS	acceptable intake (ng/day)
NDMA[1]	dimethylnitrosamine	62-75-9	96.0
NDEA[1]	diethylnitrosamine	55-18-5	26.5
EIPNA[two]	ethylisopropylnitrosamine	16339-04-1	26.5
DIPNA[two]	diisopropylnitrosamine	601-77-4	26.5
NMBA[two]	carboxypropylmethylnitrosamine	61445-55-4	96.0
MeNP[two]	methylnitrosopiperazine	16339-07-4	26.5
NDBA[two]	dibutylnitrosamine	924-16-3	26.5
NMPA[1]	phenylmethylnitrosamine	614-00-6	34.3
NMOR[3]	nitrosomorpholine	59-89-2	127.0
NNV[4]	nitrosovarenicline	-	37.0
NDPA[two]	dipropylnitrosamine	621-64-7	26.5
NMPH[5]	methylphenidatonitrosamine	55557-03-4	1,300
-	nitrosamineparoxetine[5]	-	1,300
NPIP[1]	nitrosaminapiperidine	100-75-4	1,300
NDLX[6]	duloxetinanitrosamine	2680527-91-5	100
-	fluoxetinenitrosamine[6]	-	100
NTTP[4]	trifluoromethyltetrahydrotriazolnitrosopyrazine	-	37
NTHP[1]	nitrosaminetetrahydropyridine	55556-92-8	37
NMPEA[1]	methylphenylethylnitrosamine	13256-11-6	8
NNORT[7]	nitrosaminanortriptyline	55855-42-0	8
NNK[3]	butanonapyridinylmethylnitrosamine	64091-91-4	100
NDAB[8]	dabigatrananitrosamine	-	18
-	nitrosaminarasagiline[8]	2470278-90-9	18
-	nitrosaminatamsulosin[8]	-	18
NDELA[3]	diethanolaminenitrosamine	1116-54-7	1,900
NPYR[3]	nitrosaminepyrrolidine	930-55-2	1,700
NDPh[9]	diphenylaminenitrosamine	86-30-6	78,000
-	mephenamiconitrosamine acid[10]		78,000

[1]Limit calculated from the TD_{50} obtained by the harmonic mean of the carcinogenicity studies listed in the database *Carcinogenicity Potency Database* (CPDB) available at https://files.toxplanet.com/cpdb/index.html

[two]Threshold established based on structure-activity relationship (Q)SAR strategy with NMDA or NDEA.

[3]Limit based on TD value$_{50}$more sensitive derived from the TD study$_{50}$most robust available in the CPDB database

[4]Threshold derived using SAR and approach*read-across* having the value of TD$_{50}$of N-nitroso-1,2,3,6-tetrahydropyride as a starting point

[5]Threshold derived using SAR and approach*read-across* having the value of TD$_{50}$NPIP as a starting point

[6]Threshold derived using SAR and approach*read-across* having the value of TD$_{50}$of NNK as a starting point

[7]Threshold derived using SAR and approach*read-across* having the value of TD$_{50}$of NPEA as a starting point

[8]TTC-derived limit for nitrosamines of 18 ng/day

[9]Limit based on TD value$_{50}$more sensitive derived from the TD study$_{50}$most robust available in the CPDB database with application of the lower confidence interval (((% CI) of the estimated TD50 (TD50L01)

[10]Threshold derived using SAR and approach*read-across* having the value of TD$_{50}$of NDPh as a starting point

These acceptable intake values apply to a finished product containing only one nitrosamine. The limit determined for a specific product in ppm can be calculated through the ratio of the acceptable intake (in ng) to the Maximum Daily Intake (DMD) of the product (in mg). For example, considering the maximum daily dose of metformin of 2550 mg and the limit of 96 ng for NDMA, we have 0.038 ppm (96/2550) as an acceptable daily limit.

This calculation can also consider the factor referring to the duration of treatment, when the treatment lasts less than 10 years, according to the equation presented in item "*Less than Lifetime Approach*", when applicable.

The acceptable intake values predicted in this guide, especially those calculated based on structure-activity relationship, are interim limits, considering the best evidence available at the time of publication of this guide. These limits can be changed in case of availability of new scientific evidence for these compounds.

The company must notify Anvisa and present technical justification in cases of definition of limits higher than those presented in table 3, through a protocol in the Request system using subject code 12194 - Evaluation of safety limits for nitrosamines – Company.

b) More than one nitrosamine

Considering the case of detection of more than one nitrosamine in the same product, two alternatives can be used to define the limit of the sum of nitrosamines:

> **Option 1:**the sum of all nitrosamines present in the product must not exceed the acceptance limit for the most potent nitrosamine among those present.
> **Option 2:**individual limits for each nitrosamine are adjusted to ensure that the total risk from exposure to them does not exceed the negligible risk.

Option 2 considers a risk-based approach to accepting the presence of multiple nitrosamines, provided that it is demonstrated that the final risk does not exceed the ratio of 1:100,000, defined in the ICH Guide M7(R1) as an acceptable risk of cancer. Thus, if more than one nitrosamine needs to be controlled in the specification of the API or the finished product, a limit must be established for the sum of these nitrosamines, which must ensure that the risk remains negligible. A calculation example considering option 2 is described in Figure 14.

These approaches are only applicable if more than one nitrosamine is actually present. and needs to be controlled in the specification of the API or finished product. If there is a theoretical possibility of

presence of nitrosamines, but whose absence has been demonstrated and which do not need to be controlled in the specification, the limit for total nitrosamines must not consider such impurities.

Example:

For an API that contains only NDEA, the acceptable limit of 26.5 ng/day corresponds to a risk of 1:100,000. On the other hand, if it only contains NDMA, the acceptable limit of 96 ng/day corresponds to the same risk of 1:100,000.

In another scenario, for an API that contains both nitrosamines, if the original limits were maintained, the risk would correspond to the sum of both, therefore twice the initial risk - greater than the negligible risk. Thus, in order for the risk to be maintained at 1:100,000, the nitrosamine limits must be reduced in proportions that ensure a negligible total final risk, as described below:

If the limit of each one is reduced to 50% of the original value (13.25 ng/day for NDEA and 48 ng/day for NDMA), each one will represent 50% of the initial risk, and the sum of both will be equivalent to the negligible risk (1:100,000). Alternatively, variable proportions between the individual limits established for each nitrosamine can be used, for example, 30% of the NDEA limit (7.95ng/day) and 70% of the established limit for NDMA (67.2 ng/day), as represented in Figure 14.

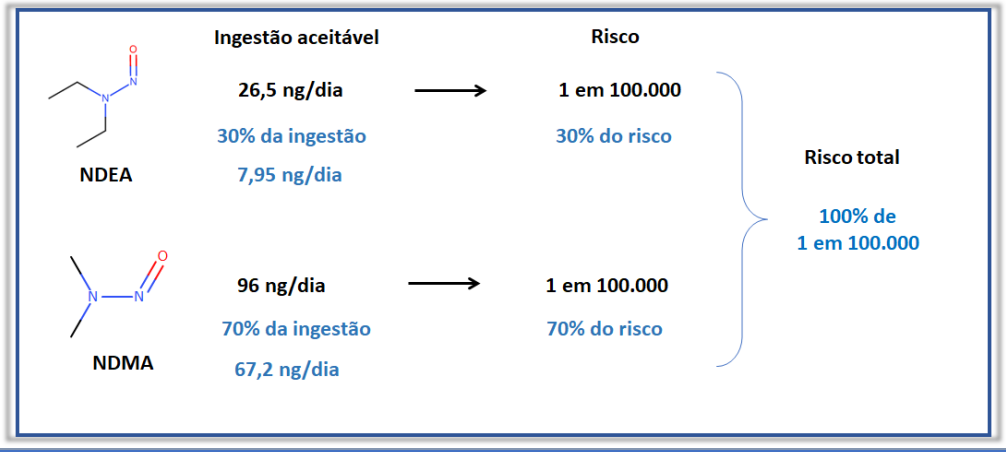


Figure 13 - Example of setting limits for more than one nitrosamine

c) New nitrosamines

During the risk assessment there is still the possibility of finding other nitrosamines, potential or real ones, in addition to those listed in Table 3. In view of the absence of specific limits, an approach similar to that previously performed for nitrosamines with limits listed in this guide is recommended, with the determination of a specific limit based on carcinogenicity studies, when available . In cases where carcinogenicity studies are not available, it is recommended to derive a threshold from structure-activity relationship comparison (*Structure Activity Relationship*-SAR) with known nitrosamines or the application of a specific TTC for the class of nitrosamines.

For limit determination based on TD_{50}carcinogenicity studies, these should meet criteria for quality and robustness as described in the ICH Guide M7(R1), eg studies with multiple doses (at least 3 groups) and 50 animals per dose per sex. The studies used for the purpose of

determination of the limit will be evaluated on a case-by-case basis, even if the harmonic mean of the DT_{50}be presented in *Carcinogenicity Potency Database (*CPDB).

In the absence of robust studies that can support this limit, the SAR approach is recommended, as long as it is used as a comparator to $TD_{50}$of a nitrosamine whose threshold has been derived from a robust carcinogenicity study with the most similar structure to the compound under test. The use of the SAR approach must be scientifically justified and adequately documented.

Finally, as an alternative to deriving a limit based on SAR, the specific TTC for the class of nitrosamines can be applied. Based on scientific knowledge and data available to date, the TTC for the nitrosamine class corresponds to 18 ng/day. This value corresponds to the 5th percentile of the TD values$_{50}$for 45 nitrosamines available in the LCDB Carcinogenicity Database.*Lhasa Limited Carcinogenicity Database*) (LhasaLimited 2020), whose methodology for deriving the $DT_{50}$was published by Thresher et al. (2019).

Although there are carcinogenicity data for a higher number of nitrosamines in the base of data*Carcinogenicity Potency Database*-CPDB, from which the data used for the determination of the general TTC for genotoxic carcinogens (1.5 µg/day) were extracted, the TD values$_{50}$of LCDB were calculated by selecting only studies that met additional quality criteria. Among these criteria are the removal of data for which there is no dose-response or the dose-response curves are not linear, exclusion of studies with a single group and TD values$_{50}$above 1,000,000 mg/kg (Thresher et al. 2019).

More recently, Thomas et al. (2021) corroborated the applicability of using the database LCBD data considering only more robust studies and statistically more conservative 5th percentile derivation. The authors argue that the limit of 18 ng/day allows considering a confidence limit that estimates the uncertainties and the different potencies of the nitrosamine class.

Options for deriving thresholds for new nitrosamines are illustrated in Figure 15.

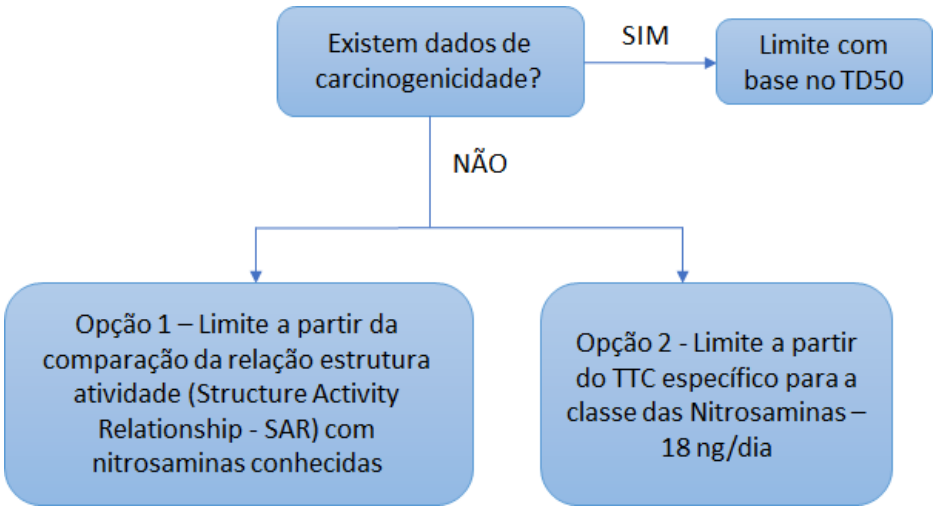


Figure 14- Decision tree for establishing limits for new nitrosamines

The company must notify Anvisa in cases of identification of a new nitrosamine (not contemplated in table 3) and present technical justification for defining its limit through a protocol in the Solicita system using subject code 12194 - Evaluation of safety limit for nitrosamines – Company.

d) Approach *Less Than Lifetime*

The term "*Less Than Lifetime*" is loosely translated to “less than a lifetime”. That This approach corresponds to the possibility of establishing higher limits for potentially mutagenic impurities when the duration of treatment is shorter than a lifetime.

This means that limits vary depending on the duration of treatment, based on a concept foundation of toxicology established by Haber's Law:

Concentration x Time = Constant

Therefore, the carcinogenic effect is based on both dose and duration of exposure. That concept is set out in Note 6 of ICH M7 (R1) (ICH 2017). Based on this concept, TTC values were established in ICH M7 (R1) (ICH 2017) that vary according to the duration of treatment, with limits greater than the limits defined for a lifetime (*lifetime*or over 10 years) safety factors were also incorporated to mitigate the risk of acute effects that could occur, such as a possible saturation of DNA repair enzymes.

The factors are presented in Table 4 and the rationale for their derivation is illustrated in Figure 16.

Table 4 - Factors for the duration of treatment in the calculation of the acceptable limit (Bercu et al, 2021).

duration of treatment	**< 1 month**	**1 to 12 months**	**1 to 10 years**	**> 10 years**
factor to be applied in limit calculation	80	13.3	6.7	1.0

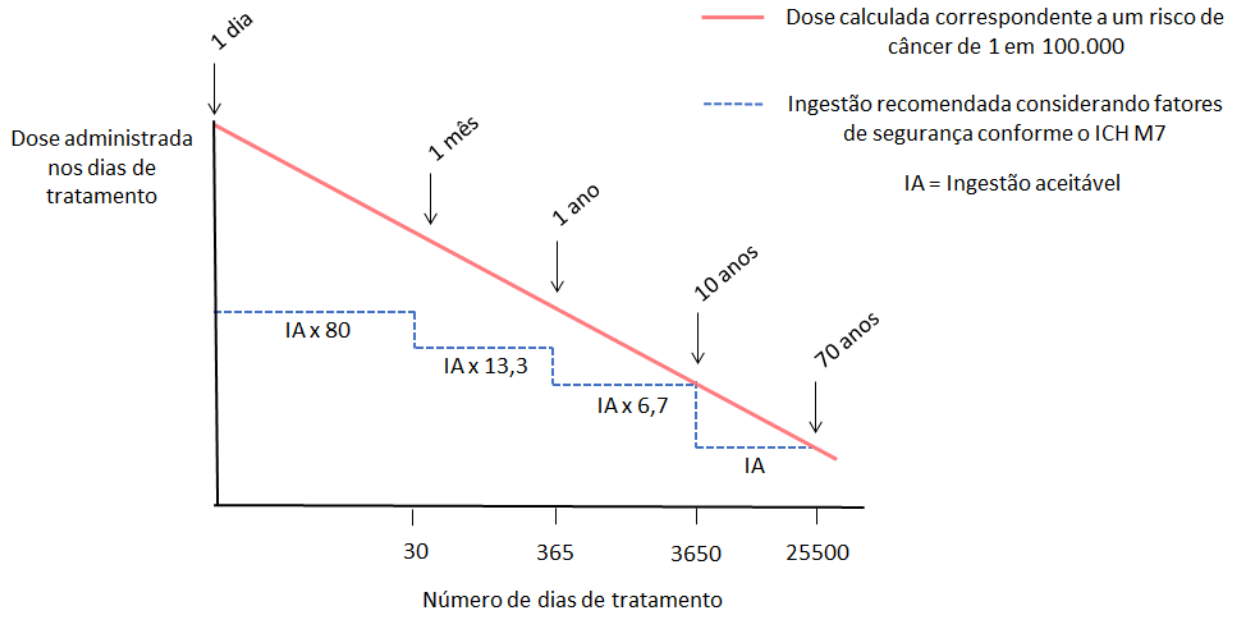


Figure 15- Correlation between duration of exposure and acceptable daily intake for mutagenic impurities and nitrosamines. Adapted from Guide ICH M7(R1)

In the case of nitrosamines, the TTC of 1.5 µg/day is not applicable due to their greater potency, placing them in the concern group (*cohort-of-concern*). However, the ICH Guide M7(R1) does not state that its concepts, such as the LTL approach, are not applied to compounds included in this group.

Considering the proposed factors described in Table 4, the acceptance limits for nitrosamines would result in higher limits than applied for lifetime exposure listed in Table 3. A case study for the NDEA was recently published suggesting its applicability (Bercu et al, 2021), considering that nitrosamines have similar toxicodynamics to other impurities potentially mutagenic, despite being more potent.

The applicability of the LTL concept for nitrosamines has been discussed internationally, however, there is still no consensus on the safety of its use as a starting point for calculating acceptable limits. Thus, analytical methods must be developed and validated so that their sensitivity (quantification limit) is adequate for the concentrations presented in situations (a), (b) and (c) of item 8 of this guide and the absence of nitrosamines during the Confirmatory tests, discussed in item 5 of this guide, should not be based on the limits obtained through the LTL approach. Given the lack of robust scientific evidence at this time, the possibility of overriding DNA repair mechanisms is questioned, especially considering other possible sources of nitrosamines such as polypharmacy, dietary and environmental exposure,

Thus, this Anvisa recommends that the LTL approach not be applied as a point of starting point for establishing the safety limit for nitrosamines. Anvisa recognizes that, when the safety limit for a lifetime is lower than the values of a single nitrosamine or more than one present in the product, considering the technical impossibility of adapting the product to the limit for a lifetime, the LTL approach may have its justified use, accompanied by a technical-scientific rationale, considering, for example, indication, dosage or risk of shortages, to be evaluated on a case-by-case basis by Anvisa. For such cases, the company must present the information through a protocol in the Solicita system using subject code 12194 - Evaluation of safety limit for nitrosamines – Company.

9. GLOSSARY

Risk analysis: process consisting of three components, namely (1) risk assessment, (2) risk management and (3) risk communication.

Approach *Less than Lifetime*: established assessments for cancer risk based on exposures when these are less than lifetime (70 years).

cohort-of-concern: group of highly potent mutagenic carcinogens comprising aflatoxin-like, N-nitroso- and alkyl-azolic compounds.

Acceptable Intake: An intake level that poses a negligible risk of cancer, or for serious/life-threatening indications, where risk and benefit are adequately balanced.

Threshold of toxicological concern, from English *Threshold of Toxicological Concern – TTC*: concept developed to define an acceptable intake for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects.

Acceptable limit: maximum acceptable concentration of an impurity in a drug substance or drug, derived from the acceptable intake and daily dose of the drug.

Material: term used to denote raw materials (starting materials, reagents, solvents), auxiliary materials, intermediates, active pharmaceutical ingredients, and packaging and labeling materials.

NDSRIs: From English *Nitrosamine Drug Substance Related Impurities*–refer to nitrosamines complex or structurally related to API.

Regularized products/products: drugs and active pharmaceutical ingredients (API) registered, notified or registered.

(Q)SAR and SAR: refers to the relationship between the molecular (sub)structure of a compound and its mutagenic activity using (Quantitative) Structure-Activity Relationships derived from experimental data.

Negligible risk: risk corresponding to a cancer incidence of 1 in 100,000.

TD_{50}: Chronic dose rate in mg/kg body weight/day that would cause tumors in half of the animals at the end of a standard lifespan for the species, taking into account the frequency of this type of tumor in the control animals.

10. REFERENCES

Ashworth IW et al. (2020). Potential for the Formation of N-Nitrosamines during the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water.Org. Process Res. Dev. **24**: 1629−1646.
Barber, C., et al. (2017). "A consortium-driven framework to guide the implementation of ICH M7 Option 4 control strategies."Regulatory Toxicology and Pharmacology **90**: 22-28.

Bercu, J., et al. (2021). "Use of less-than-lifetime (LTL) durational limits for nitrosamines: Case study of N-Nitrosodiethylamine (NDEA)"Regulatory Toxicology and Pharmacology **123**: 104926.

Boetzel, R., et al (2022). A Nitrite Excipient Database: A Useful Tool to Support N-Nitrosamine Risk Assessments for Drug Products.J Pharm Sci. June ;**112**:1615-1624. doi: 10.1016/j.xphs.2022.04.016. Epub 2022 Apr 29. PMID: 35500671.

Burns, MJ, et al. (2019). "New Semi-Automated Computer-Based System for Assessing the Purge of Mutagenic Impurities."Organic Process Research & Development **23**(11): 2470-2481.

Burns, MJ, et al. (2020). "Controlling a Cohort: Use of Mirabilis-Based Purge Calculations to Understand Nitrosamine-Related Risk and Control Strategy Options."Organic Process Research & Development **24**(8): 1531-1535.

Carlson, ES, et al. (2017). "A General Method for Detecting Nitrosamide Formation in the In Vitro Metabolism of Nitrosamines by Cytochrome P450s."Journal of visualized experiments: JoVE (127): 56312.

EDQM (2020). "Ad-hoc projects of the OMCL Network." 2021. Available at: https://www.edqm.eu/en/ad-hocprojects-omcl-network.

EMA (2020)a. Lessons learned from presence of N-nitrosamine impurities in sartan medicines - Overview and recommendations.EMA/526934/2019 , European Medicines Agency.

EMA (2020)b. Nitrosamine impurities in human medicinal products Procedure under Article 5(3) of Regulation EC (No) 726/2004.Procedure number: EMEA/H/A-5(3)/1490 , European Medicines Agency.

EMA (2023). “Questions and answers for marketing authorization 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” EMA/409815/2020 Rev.15. Available at: https://www.ema.europa.eu/en/documents/referral/nitrosaminesemea-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

Ender, F., et al. (1964). "Isolation and identification of a hepatotoxic factor in herring meal produced from sodium nitrite preserved herring."Naturwissenschaften **51**(24): 637-638.

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)(2023).“Risk assessment of N-nitrosamines in food.”EFSA Journal. **21**: 278.https://doi.org/10.2903/j.efsa.2023.7884

FDA (2019). "FDA Updates and Press Announcements on Angiotensin II Receptor Blocker (ARB) Recalls (Valsartan, Losartan, and Irbesartan)." 2021. Available at: https://www.fda.gov/drugs/drug-safety-andavailability/fda-updates-and-press-announcements-angiotensin-ii-receptor-blocker-arb-recalls-valsartanlosartan#5fdb8ed25acb8.

FDA (2020)a. "Information about Nitrosamine Impurities in Medications." 2020. Available at: https://www.fda.gov/drugs/drug-safety-and-availability/information-about-nitrosamine-impuritiesmedications.

FDA (2020)b. "FDA Requests Removal of All Ranitidine Products (Zantac) from the Market."FDA News Release . Available at: https://www.fda.gov/news-events/press-announcements/fda-requests-removal-all-ranitidineproducts-zantac-market.

Fong, YY and WC Chan (1973). "Dimethylnitrosamine in Chinese marine salt fish."Food and Cosmetics Toxicology **11**(4): 841-845.

Health Canada (2019). "Impurities found in certain angiotensin II receptor blocker (ARB) products, also known as sartans.", Available at: https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/angiotensin-receptor-blocker.html#a4.

Horne et al., (2023). Regulatory Experiences with Root Causes and Risk Factors for Nitrosamine Impurities in Pharmaceuticals.Journal of Pharmaceutical Sciences **112**: 1166−1182.

IARC (1987). Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42.**7**: 67. ISBN 978-92-832-1411-3

IARC (2007). Monographs on the Evaluation of Carcinogenic Risks to Humans. Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines.**89**: 553. ISBN 978-92-832-1589-9

ICH (2005). Q9 - Quality Risk Management. INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE.

ICH (2017). M7(R1) - Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE.

King, FJ, et al. (2020). "Ranitidine—Investigations into the Root Cause for the Presence of N-Nitroso-N,Ndimethylamine in Ranitidine Hydrochloride Drug Substances and Associated Drug Products."Organic Process Research & Development **24**(12): 2915-2926.

Klein, DR (2017). Preparation and Reactions of AmidesOrganic Chemistry **:**933.

Li, Y.; Hecht, SS (2022a). “Metabolic Activation and DNA Interactions of Carcinogenic N-Nitrosamines to Which Humans Are Commonly Exposed”.International Journal of Molecular Sciences 2022,**23**(9), 4559.

Lhasa Limited (2020). "Lhasa Carcinogenicity Database." Available at: https://carcdb.lhasalimited.org/carcdbfrontend/#.

López-Rodríguez, R., et al. (2020). "Pathways for N-Nitroso Compound Formation: Secondary Amines and Beyond."Organic Process Research & Development **24**(9): 1558-1585.

Magee, PN and JM Barnes (1956). "The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine."Br J Cancer **10**(1): 114-122.

Preussmann, R and Stewart, BW (1984). “N-Nitroso Carcinogens. In Chemical Carcinogens, 2nd ed.; ACS Monograph 182; Searle, EC, Ed.; American Chemical Society: Washington, DC, USA”**two**: 643–828.

Rath, S. and LS Canaes (2009). "Contamination of hygiene and cosmetic products by n-nitrosamines." New Chemistry **32**: 2159-2168.

Reusch, W. (1999). "Virtual Textbook of Organic Chemistry." Retrieved 9/23/2020 2020 from https://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/amine2.htm#amin7.

Roberts, JD and MC Caserio (1977). Organonitrogen compounds; I. Amines.Basic Principles of Organic Chemistry . Menlo Park, CA, WA Benjamin, Inc.**:**1095-1166.

Teasdale, A., et al. (2013). "Risk Assessment of Genotoxic Impurities in New Chemical Entities: Strategies To Demonstrate Control."Organic Process Research & Development **17**(2): 221-230.

Teasdale, A., et al. (2010). "A Tool for the Semiquantitative Assessment of Potentially Genotoxic Impurity (PGI) Carryover into API Using Physicochemical Parameters and Process Conditions."Organic Process Research & Development **14**(4): 943-945.

Thomas, R., et al. (2021). "Utilization of parametric methods to improve percentile-based estimates for the carcinogenic potency of nitrosamines."Regulatory Toxicology and Pharmacology : 104875.

Thresher, A., et al. (2019). "Generation of TD(50) values for carcinogenicity study data."Toxicol Res (Camb) **8**(5): 696-703.

Thresher, A. et al. (2020) Are all nitrosamines concerning? A review of mutagenicity and carcinogenicity data. Regul Toxicol Pharmacol**, 116**: 104749

Agência Nacional de Vigilância Sanitária – Anvisa

SIA Trecho 5, Área Especial 57, Lote 200

CEP: 71205-050

Brasília – DF

www.anvisa.gov.br

www.twitter.com/anvisa_oficial

Anvisa Atende: 0800-642-9782

ouvidoria@anvisa.gov.br