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Assessment of Regulatory Requirements for Nitrosamine Impurities on the Drug Development and Post Approval

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ABSTRACT

Nitrosamines describes a class of compounds having the chemical structure of a nitroso group bonded to an amine (R1N(-R2)-N=O). They are a group of carcinogens that are formed by the reaction of secondary and tertiary amines, amides, carbamates, and derivatives of urea with nitrite or other nitrogenous agents during the manufacturing of API and Finished products. Although they are also present in some foods and drinking water supplies, Nitrosamine-contaminated medicinal products have raised safety concerns towards the use of various drugs as they are probable human carcinogens when exposed above acceptable levels and over a long period of time. These can form at any stage from drug substance synthesis till product life cycle of the finished product and hence health authorities have issued public health alerts and tight guidance on nitrosamine contamination for all drug products in the market. This review emphasises, assessment of regulatory requirements on Nitrosamine impurity sources, the EMA and USFDA approach and recommendation to the applicant/Marketing authorisation holder on Risk evaluation, route cause, control strategy and mitigation plan for under progress and approved drug products.

Keywords
Drug Substance; Drug Product; Impurities; Nitrosamine; Regulatory Challenges

Introduction

Nitrosamines or N nitrosamines more appropriately refer to any molecule that has a nitroso functional group. A group with the chemical structure of a nitrosate group linked to an amine (R1N(-R2)-N=O) is described as nitrosamine as below.

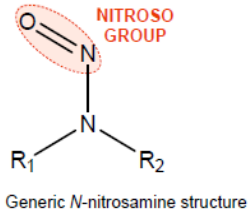

Figure 1: General structure of nitrosamine.

Nitrosamines are a group of carcinogens generated through reactions by nitrite or other nitrogenous nitrite secondary and tertiary amins, amides, carbamates and urea derivatives (including N2O3 and N2O4)[4].These chemicals are recognised as likely DNA reactive impurities in human carcinogenes (mutagenic carcinogens), which are also mentioned in ICH M7 (aflatoxin- like, N-nitroso-group and alkyl-azoxy compounds). The substance can arise through a nitrosating reaction from acidic nitrate salts (secondary, tertiary or quaternary amines) to nitrous acid (acidic salts).

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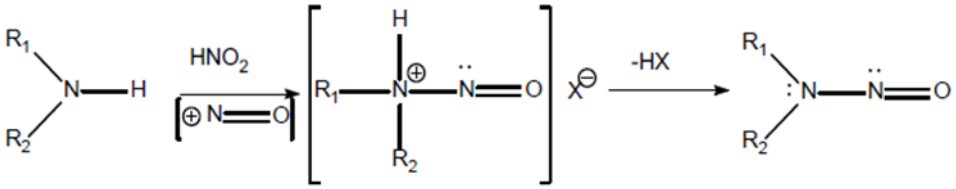

Figure 2: Nitrosating reaction of amines.

Though they are also found in certain foods and drinking water supplies, nitrosamine impurities are considered to be human carcinogenic. The general nature of precursors and the easy nature of acidic and neutral pH nitrosation[5]in consumer products, and medications has made nitrosamines a common and unpleasant guest. Nitrosamines are common substances and everyone in their everyday lives is exposed to some nitrosamines and does not cause any harm when taken at low quantities. However, the risk of cancer may grow with nitrosamine impurities if people are exposed to it over tolerable levels for any extended period of time. Nitrosamines are therefore deemed to be inacceptable in medicinal products. According to ICH M7(R1), Nitrosamines are regarded as a high-power mutagenic carcinogen, which is known by both the rodent carcinogenicity and mutagenicity data to be part of the "cohort of concern," classed as Class 1, impurities.[6]. The presence of nitrosamine in pharmaceuticals was shown to be a public health problem in 2018, following reports of dangerous concentrations of Nitrosamine Impurity (N-NDMA) in angiotensin II receptor blockers NDMA (ARBs) (Sartan products).

Although nitrosamine impurities were already recognized as environmental contaminants and have been found in water and food in small quantities, their presence in APIs was an unexpected and shocking discovery. In 2018, N-nitrosodimethylamine (NDMA) was identified in pharmaceutical products containing Valsartan API by the European Medicines Agency (EMA) and the Federal Drug Administration (FDA). Valsartan is an antihypertensive medication of the angiotensin II blockers class (ARB). A few more nitrosamine impurities were soon found, such as N-nitrosodiethylamine (NDEA) and Nitroso-N-methyl-4-aminobutyric acid (NMBA). The agencies published public health alarm reports and guidelines following these and other additional studies, which have restrictions, concerning nitrosamine contaminants in a number of medicinal items. This article proposes taking actions in detecting and preventing unacceptable amounts of nitrosamine (N-nitrosamine) contaminants in pharmaceutical products in the production of API and drug products. The article also discusses on criteria where impurities of nitrosamines can be formed. In recent unexpected findings of human-carcinogenic nitrosamine impurities in medicine such as ARBs (angiotensin 2), ranitidine and nizatidine, a potential nitrosamine risk assessment evaluation for a medicinal product at risk during its development and life cycle management was deemed necessary.

Methods

Web page content on the Internet: Numerous search engines have been used to collect literature, for example Science Direct, google scholar and many more. Online books have also been a helpful information source.

Collected documents and information on regulatory websites like:

a) USFDA: https://www.fda.gov
b) EMA: https://www.ema.europa.eu/en

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Sources of Nitrosamine impurity and their issue

The sources of nitrosamine impurities currently discovered are mentioned below:

- Use of sodium nitrite ($NaNO_2$) or another nitrosating chemical in the presence of secondary or tertiary amines at the same or different stage of manufacture.
- Use in combination with reagents, solvents (e.g., DMF, NMP, etc.) and catalysts which are prone for degradation of the secondary and tertiary amines for the same and other processes sodium nitrite ($NaNO_2$) or other nitrous compounds.
- In the API production process, use of the contaminated raw materials (e.g.solvents, reagents and catalysts).
- The use of contaminated reclaimed materials (e.g. solvents, reagents and catalysts) includes re-sourced materials for non-dedicated equipment for third parties not informed of their content and the processing of the reclaimed material.
- Use of contaminated starting and intermediate material from suppliers using techniques or raw materials with residual nitrosamines or nitrosating chemicals.
- The purposeful carry over of nitrosamines during the production process (e.g. as intermediates).
- Cross-contamination owing to various manufacturing or other processes on the same production line.
- Due to operator-related mistakes or inadequate information such as poor separating phases while operational activities, impurities are transferred across procedural processes.
- Degradation of the initial starting materials, intermediates and APIs, including the ones persuaded by inherent reactivity (e.g. presence of nitrogen, oxime, or other functionalities) or exogenous nitrogen and related compounds. This could also happen when the product is formulated and stored, and could also be affected by crystal structure, crystal habit and the conditions for storage (temperature, humidity etc.) For further details, refer to the ranitidine and published literature reference page 6 of Article 31 of Directive 2001/83/EC.
- Use of some materials for the packaging. In final products stored in blister packaging with nitrocellulose-containing lidding foil, nitrosamine contamination was discovered. Nitrocellulose degeneration products and low molecular amines present either in printing inks or in the FP have been demonstrated to develop and transfer to the product through the blister during the heat sealing procedure.
- Reaction of nitrosateable nitrogen group in APIs or their impurities in the formulation or storage[7].

THE EMA AND FDA APPROACH ON NITROSAMINE IMPURITY

The EMA approach on implementation of CHMP Opinion on the Nitrosamine Impurities in Human Medicines

On 10 September 2019, the EMA Executive Director (Ed) requested that CHMP carry out a scientific assessment in compliance with Article 5(3) of Regulation (EC) No 726/2004 on the presence of nitrosamine impurities in human medicines including the chemical-synthesized active inputs of pharmaceutics (APIs). A two-step approach was proposed to be followed [1]:

- First stage: It was emphasised that the Marketing Authorisation Holders (MAH) identify the possible presence of nitrosamine impurity for these products;

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- 2nd stage: to assess all available scientific knowledge on nitrosamine impurities and their impacts on the safe use of medications and to explore whether the current scope should be extended to other pharmaceutical products in consideration of the work that continues for the lessons learned in sartan examination. This assessment will operate as the basis for a coordinated approach and response throughout the EU and will inform regulatory authorities (RAs) on the steps to be taken after the detection of N-nitrosamine impurities in their drugs by MAHs.[1].

EMA Human Medicine Committee (CHMP) published an opinion mandating companies to take measures to restrict, the presence of nitrosamines in human medications to the maximum extend and to ensure levels of these impurities do not exceed defined limits. The steps will ensure that nitrosamines are either absent or below the established standards for the protection of public health. Companies will have to establish defined control strategy to prevent or minimise the occurrence of these, and enhance production processes if necessary. The risk of nitrosamines occurring in medicines will also need to be evaluated by companies and adequately tested when a risk is found.

Recommendations from the agency to be followed by Marketing Authorisation Holders/Applicants:

MAH/Applicants for all human medical products shall ensure that the presence of nitrosamines, whatever its market status or product type (i.e. generics, counter (OTC) medications and biological products), is managed and kept to a lower standard.[7]. The MAH/applicants recommended to ensure that their pharmaceutical products are manufactured and evaluated using the current state of technological and scientific advances processes and methods; MAH/applicant is therefore responsible to:

- Design their manufacture techniques and controls to minimise or mitigate the presence of nitrosamines in the APIs and FPs to the maximum feasible extent.
- Assess the risk of contamination by nitrosamine in the API(s) and FP(s) and make any modifications to the dossier as necessary (e.g. changes in procedure);
- Ensure that in accordance with Article 46(f) of Directive 2001/83/EC the active compounds and excipients used on its FPs are made in accordance with acceptable good manufacturing practice.

Compliance between MAHs / applicants with the foregoing duties is subject, in particular during the GMP inspections, to periodic inspections by competent regulatory agencies.[7]. In order to determine and, where appropriate, limit the risk of presence of nitrosamine contaminants, CHMP has started a "call for review" to request MAHs to assess their production methods and submit their results back to authorities.

The MAH/applicant to comply with the obligations outlined in the call for review to Marketing authorisation holders (MAHs), must conduct a risk evaluation/risk assessment of their manufacturing process for active pharmaceutical ingredients (APIs) and finished products for the presence of N-nitrosamines in their marketing authorisation applications (MAAs), as well as for authorised medicinal products for human medicines containing chemical synthesised drug substances and finished products with active substance of biological origin.[1].
There are three steps to the request for review:

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- Step 1: Risk assessment to be conducted by MAHs to identify whether APIs and/or FPs are susceptible to nitrosamine presence;
- Step 2: MAHs must perform a confirmatory testing to confirm or negate the existence of nitrosamines if the risk is found. MAHs should disclose their results at the earliest opportunity;
- Step 3: If the presence of nitrosamine(s) is confirmed, then the MAH/applicant shall file appropriate variation submissions to execute effective risk mitigation actions.

Moving future, a control strategy should be implemented by the MAH/applicant for nitrosamines on the active ingredients and FPs, including current, future strategies for minimising N-nitrosamine risk/contamination and controlling any future changes that may influence this risk.[1].

For the 'call for review', when and how should MAHs report steps 1 and 2 to Case:

Submission of step 1 outcome:

The Step 1 risk assessment should be concluded for products containing chemically synthesised APIs and reported by 31 March 2021 at the latest. In the case of a product with biological APIs, this should be completed and submitted by 01 July 2021 at the latest. For any product where possible risks have been identified in step 1, irrespective of the marketing status of the product, this risk assessment will have to be carried out. It is only permitted to provide a written confirmation of step 2 confirmational testing and the necessary variations within the framework of step 3 before the product is launched in situations where no batches of finished product are commercialized. All MAHs shall inform the competent authorities responsible of the results of their risk assessment (step 1)[7]

Submission of step 2 outcome:

Confirmative testing operations at Step 2 and submission of any necessary variations to marketing authorisations (Step 3) for products incorporating chemical synthesised drug substances should be planned to be concluded by September 26, 2022 at the latest. Confirmatory testing at step 2 and submission of any required variation to MAs(step 3) for the products using biological APIs are scheduled to be finalised by 1 July2023 at the latest.

In case testing confirms the presence of nitrosamine, MAHs should notify the competent authorities immediately regardless of the amounts observed. In the context of the following restrictions and the relevant measures advised to avoid or minimise nitrosamine patient exposure, the immediate danger of patients should be assessed accordingly.[7]

Limits applicable in medicinal products to nitrosamine:

The ICH M7 (R1) Guideline defines N-nitrosamines as a substance of "cohort of concern" being used to limit the drug products associated with the negligible risk of the so-called acceptable (AI) substances (toxicological risk of TTC of 1.5 ug/day) (theoretical excess of risk of cancer < 1 in 100 000 for life-time exposure). The above calculation is based on the assumption for a lifelong administration of the maximum daily dose of medicinal product as defined in ICH M7 (R1) guideline.[6] The review of the CHMP provides a limit based on ICH M7(R1) guidelines (AI limits corresponding to the theoretical excess cancer risk <1 by 100000) which is calculated based on lifetime daily exposure.[7].

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Calculation of the limit when a single known nitrosamine is identified [7]

For certain N-nitrosamines the following limitations have been defined and should be applied:

Table 1: Limit of specified Nitrosamine Impurities.

N-Nitrosamine (CAS number)	ng/day***
NDMA* (62-75-9)	96.0
NDEA*(55-18-5)	26.5
EIPNA**(16339-04-1)	26.5
DIPNA**(601-77-4)	26.5
NMBA**(61445-55-4)	96.0
MeNP**(16339-07-4)	26.5
NDBA**(924-16-3)	26.5
NMPA*(614-00-6)	34.3

These limits are applicable only if a FP contains a single N-nitrosamine.

*Limit calculated on the basis of harmonic mean TD50 derived from carcinogenic potency database (CPDB)
**Limit derived using structure-activity-relationship (SAR) /read-across approach
***The conversion to a specification limit in ppm for a particular medicinal product is calculated by dividing the respective above limit (ng) by the maximum daily dose (mg) of a given product as reflected in the SmPC.

Calculation of the limit when a new nitrosamine is identified[7]*:*

For detection of novel nitrosamines two situations are envisaged:

- TD50 calculations should be made for N-nitrosamines when identified on the individual material with appropriate specific data of animal carcinogenicity, the specific limit for lifetime exposure to the chemical should be computed and applied in line with the recommendation of ICH M7(R1).
- When N-nitrosamines are detected without sufficient information on a material to determine a specified lifetime exposure limit according to ICH M7(R1) guideline,
    1. The default option is to utilise a class-specific TTC for 18 ng/day nitrosamines (from the Lhasa Carcinogenic Potencies Database).
    2. If suitable, a technique based on SAR considerations is acceptable with proper justification in order to calculate an acceptable consumption limit.

The approach adopted by the MAH/applicant must be appropriately justified.

This is applicable to all rout of administration. Limit adjustments are generally unacceptable unless there are variances in different route of administration data. The MAH/applicant is advised to communicate with the respective agency to verify the suitability of the proposed approach.

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Calculation of limit when more than one nitrosamine is identified[7]

To define limits if there are more than one nitrosamine impurities two ways are regarded acceptable as mentioned in ICH M7 (R1):

1.The total daily intake of all N-nitrosamines that are recognised does not exceed the AI of the N-nitrosamines impurity which is known to be highly mutagenic. or

2.The computed overall risk level shall not exceed 1 in 100,000 for all identified N-nitrosamines.

The MAH/applicant must justify the approach selected.

The LT Limit (Less than life time) Calculation technique should be adopted only after consultation of the appropriate authorities, not for the calculation of the limit specified above, but only temporarily until further efforts to reduce the pollutant to or below the above-defined limits can be implemented. N-nitrosamine impurity shall be regulated according to the rules of ICH Q3A(R2) and ICH Q3B(R2) in goods designed for advanced cancer only as indicated in the ICH S9 guidance, as specified in the ICH S7 guideline Q&A document. If in case the drug substance is mutagenic or clastogenic at defined concentration, N-nitrosamine impurities should be assessed as non-mutagenic impurities in accordance with ICH M7 (R1).

All routes of administration will be subject to the same risk strategy. Limit correction is not normally acceptable unless statistics justify route-specific variances.

Discussion on analytical aspects

The CHM thinks it important to take careful note of analytical methodologies for N-nitrosamines:

- Possible presence of nitrite and secondary amines in the precursors sample.
- For any possible interferences, working methods should be validated
- Hydrophilicity and lipophilicity as well as the target analyte volatility/non-volatility
- Use an appropriate internal standard with high purity to control any loss when preparing the sample and ensure precise quantification.
- LOQ gives a minimum degree of accuracy and precision to quantify the sample and is therefore chosen over LoD for impurity testing, and decision-making
- LOQ should be at least or substantially below the permissible toxicological limit, considering the test objective (e.g. routine testing, justifying skip testing, justifying omission of specification)

Given the possibility of differing matrices and goal analytes, it cannot generally be suggested to employ either HPLC or GC in a universally applicable sample preparation procedure. However, for certain APIs and for finished dosage forms that use a sample suspension to produce a solution for sodium hydroxide and subsequently the extraction of the fluent-liquid using dichloromethane the sample preparation by the OMCL Swiss may apply. Specific validation in each scenario is nevertheless important.

Risk Evaluation:

The MAHs/applicants must complete risk assessments utilising the quality management standards described in ICH Q9, together with its API, FP manufacturers and their suppliers. The principles outlined in ICH M7 and the assessment report on the toxicological assessment of nitrosamine in human medical products, of Article 5(3) of the CHMP should

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be considered. The risk assessment should be performed taking in to consideration all possible sources of contamination and/or nitrosamine generation, including the root causes described in Nitrosamine Impurity Sources.[7]
If a risk is detected in the API and/or the FP following completion of a risk assessment, the MAHs shall report the risk they identified to the competent authorities, carry out confirmatory tests without further delay and enter into any necessary modifications in the submission. Even if it is noted that there is no risk, all MAHs should inform the competent authorities concerned of the outcome of their risk assessments (stage 1).

Control Strategy:

MAH/Applicant must derive a strategy to be followed with measures on present and future steps for the minimization of the risk of formation or contamination of nitrosamines, e.g. changes in the manufacturing process, implementation of specific test methods, and development of suitable techniques, premises and equipment measures. MAH/applicants must, to fulfil the requirements listed above,

- Perform risk evaluation to assess the manufacturing of drug substance (e.g on the raw materials, starting materials and route of synthesis) keeping in mind the available details on the existence of N-Nitrosamine in the drug substance.
- Perform risk evaluation for a completed dosage form in relation to sources of N- nitrosamines in the finished dosage form (API degradation, main packaging material, excipients, etc.).
- Ensure, in line with the latest state of research and technology, that their pharmaceutical products are formulated, manufactured and managed by relevant technology in accordance with Article 23 and Annex I of Directive 2001/ 83/EC and Article 16 of Regulation(EC) No 726/2004. Consequently, MAH/applicants must design their process and checks so that the presence of N-nitrosamines in the API and the finished product(s) is prevented or minimised, where possible, and any future adjustments in the production process shall be introduced as necessary.
- Ensure that drug substance and excipients in their finished dosage forms, are in line with GMP requirements as mentioned in Article 46(f) of Directive 2001/83/EC.
- MAH / applicants shall, will be subjected to frequent supervision by the authorities concerned to comply with the responsibilities indicated above.

Discussion on root causes and strategies to mitigate the presence of N-nitrosamines in human medicinal products

It is doubtful that NDMA and other water-based N-nitrosamines will represent a realistic source of API contamination. Nevertheless, procedures for degradation of medications resulting from the usage of water that is disinfected cannot be reneged. To date the deterioration of the API processes linked with impurities has not been recognise as contributing factors for N-nitrosamines in water or N-nitrosamines. Further, the contamination of secondary/tertiary alkylamine and alkyl ammonium quaternary salts in the low PPM region by N nitrosamines has been studied. Today, it is considered that the significance of these results in secondary and tertiary amines of unknown grade 40 years ago is uncertain. The solvent dimethyl acetamideICH Q3C (R7), in conjunction with nitrosating products, is a secondary source of NDMA, in addition to DMF and NMP.
ICH Q3C (R7) solvent nitromethane cannot be ruled out in combination with specific oxidants/catalysts and in combination with secondary and tertiary amines, to act as an agent of nitrosation. Concurrent occurrence of $NaNO_2$with

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solvents, reagents and catalysts might be directly associated with the secondary and tertiary amine sources, e.g. in N-nitrosamine impurities in sartans. This mixture of compounds is critical and very sensitive to N-nitrosamine generation and should be prevented or carefully monitored if all the production stages of the API have been justified. In addition, the MAHs/applicants should analyse the probable creation of the cohort of chemicals as N-nitro-sameness throughout the development of manufacturing processes. MAHs/applicants are reminded that all materials used in the manufacturing process, regardless of their intended use, should be specified in the dossier.

In general, the interactions between starting materials, intermediates, drug substance, solvents, reagents and catalysts should be investigated carefully during the development, production processes, in line with the relevant ICH Guidelines (Q3A, Q3C, Q3D, Q7, Q9, Q11, M7) and the EMA Active Substances Guideline.[2]

The following considerations should be taken in to account to minimise the presence of N-nitrosamines in human pharmaceutical products based on the above concerns and the feedback of QWP and the ad hoc expert group:

- Ensure that processes of production are designed / adjusted to prevent N-nitrosamine development or contamination
- Risk evaluation through synthetic pathways, starting materials, intermediate materials, raw materials (solvents, catalysts, etc) and finished manufacturing processes of product (raw materials, packaging, etc.) to identify possible and confirmed root causes in API synthesis and finished product for N-nitrosamines formation and contamination should be performed.
- A change of formulation, manufacturing process including the starting material, intermediate and drug substance or primary packing is warranted if presence of nitrosating agent is observed in risk evaluation.
- In case the combination of the nitrogen agents, solvents, reagents and catalysts is inevitable and justified, sufficient control measures should be taken. The API and the end product must, where necessary, reflect this in its control plan.
- The checkpoint should be selected to ensure that the impurity is present below the acceptable finished product limit for nitrosamines.
- ICH M7(R1) also mentions a skip test option for a single nitrosamine impurity of the API if it is consistently less than 30% of ICHM 7(R1) levels for the respective N-nitrosamines provided a well-known and well-controlled root cause of the detected nitrosamine as mentioned by QWP.
- The CHMP also accepted the 2nd QWP response to the effect that the amount of the respective single nitrosamine must be proved consistently at or under 10% of the ICH M7(R1) limit to warrant deletion of the specification. The LOQ must be established at least this level. (For the potential extra life time cancer risk of lower than 1:1 million is shown, below 10 per cent of the limit)

The FDA approach on Nitrosamine impurities

The existence of nitrosamine contaminants in certain medication products has being studied by the FDA since 2018. The FDA is concerned with exposure over long periods of time to nitrosamine impurity above permitted limits that can raise cancer risks. Seven theoretically existent nitrosamine impurities of NDMA, NDEa, NMBA, NIPEA, NDIPA, NDBA and NMPA were the concerns established by the FDA. Already five of those impurities (NDMA, NDEA, NMBA, NIPEA and NMPA) have been identified in the API and finished formulations[9].

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In order to address the concerns of the Agency about the risk of nitrosamine impurities, numerous applicants have contacted the FDA for the provided test data, research findings and answers to additional requests for information. In February2021, in order to advise applicant about risk evaluations and testing and other appropriate actions to reduce and mitigate nitrosamine impurities in active ingredients (APIs) and finished dosage forms, the FDA has implemented the "Control of Nitrosamine Impurities in Human Drugs". Since the problem of impurities of nitrosamine goes beyond the supply of drugs in the US, FDA and other regulators have partnered to exchange information, to coordinate inspection efforts, to share effective methods for analytical detection and identification of different nitrosamines and to find rapid safety and drug supply solutions.

Recommendations from the agency to be followed by Applicants:

Depending on the regulation status of the drug product, the FDA has recommended varied implementation time frames.

1.Approved or Marketed Drug Products

Manufacturers shall complete a risk assessment of authorised or marketed medicines within seven months after the release of the original advice in order to guarantee the safety of the United States drug supply.[9] Published on September 2020, with a suggested completion date on or before 31 March 2021. Confirmatory testing should commence as soon as nitrosamine risk is determined and start promptly on high-risk products. The confirmatory testing for pharmaceutical goods and filing of required amendments to applications for pharmaceutical products should be concluded within 3 years after the original guidelines are published, and the proposed completion date is October 1, 2023 or before.

The timetables also include activities undertaken by API manufacturers to support the drug products (i.e., risk assessment and testing). The Agency may ask for an accelerated risk assessment, a confirmatory test or other regulatory action based on the Agency's available information.

2. Applications under progress:

The risk assessment for nitrosamine impurities in APIs and pharmaceutical products and confirmatory testing is advised before submission of an initial application. However, if necessary, a risk assessment and confirmatory test submission might be provided as a variation (changes to DMF or application) if these are not available at the moment of the initial submission. Such variations should be provided as soon as feasible, to minimise negative impact on the application assessment schedule, after the original submission. Applications underway shall conduct a risk assessment immediately and report to the FDA if nitrosamine levels exceeding the AI threshold are found by a confirmatory test. If the impurities of nitrosamine are found over the LOQ but below the AI limitations, if necessary, the applicant shall inform the agency through a supplement. When it is considers important, the Agency shall coordinate with the applicant for consensus during or soon after the approval and prior to commercialization.

Assessment of Nitrosamine impurities

Nitrosamine molecules in various animal species are effective genotoxic agents and some are categorized as human carcinogens[10]by the International Research Agency for Cancer for Human Carcinogens (IARC)[10]. They are referred to in the ICH guidance for industry M7 as "cohort of concern" compounds (R1). The Guidance suggests the control at or below a level of any known mutagenic carcinogen, such as nitroso-compounds, so that there could be a minimal risk of human cancer from exposure to potentially mutagenic contaminants. FDA issued temporary accepted limits

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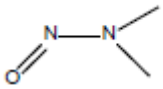
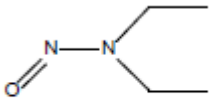
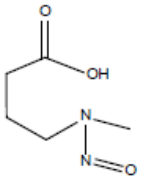
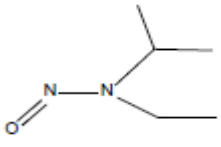
following the finding of nitrosamine contamination in ARBs[11]for such contaminants, it suggested that manufacturers take measures to assess and reduce or remove nitrosamine levels on their drugs when the interim level exceeds;

Limits that apply for Nitrosamine in medicinal products:

Positive known nitrogen impurities in drug products and APIs with published AI limits are presented below:

Table 2: Potential known nitrosamine impurities and published AI limits

Sr. No.	Name	Structure	FDA recommended Acceptable Intake Limit (ng/day)[1,2]
1	N-nitrosodimethylamine NDMA	 N-Nitrosodimethylamine (NDMA)	96
2	N-nitrosodiethylamine (NDEA)	 N-Nitrosodiethylamine (NDEA)	26.5
3	N-nitroso-N-methyl-4- aminobutanoic acid (NMBA)	 N-Nitroso-N-methyl-4-aminobutyric Acid (NMBA)	96
4	N-nitrosoisopropylethyl amine (NIPEA)	 N-Nitrosoisopropylethylamine (NIPEA)	26.5

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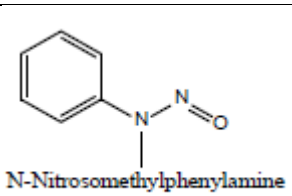
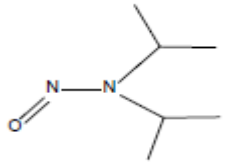
5	N-nitrosomethylphenylamine (NMPA)	 N-Nitrosomethylphenylamine (NMPA)	26.5
6	N-nitrosodiisopropylamine (NDIPA)	 N-Nitrosodiisopropylamine (NDIPA)	26.5

[1]The AI limit is a daily exposure to a compound such as NDMA, NDEA, NMBA, NMPA, NIPEA, or NDIPA that approximates a 1:100,000 cancer risk after 70 years of exposure.
[2]The conversion of AI limit into ppm varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug label (ppm = AI (ng)/MDD (mg)).

The above tabulated acceptance criteria is applicable only when a single nitrosamine impurity is in a drug product. In case there are additional impurity from the above table and that the total quantity of nitrosamine impurities in Table 1 is detected and higher than 26.5 ng/day (MDD) for the AI of the highest potent Nitrosamine), the applicant should contact the Agency on the basis of a maximum daily dosage (MDD) for assessment.

Acceptance limit of the impurities in the drug product based on the maximum daily dose:

$$\text{Limit in ppm} = \frac{\text{AI (ng)}}{\text{MDD (mg)}}$$

AI= Acceptable Intake

MDD= Maximum daily dose of the drug product

Table 3: Acceptable Nitrosamine limits based on MDD and without published AI limits

Sr. No.	Case	Acceptance criteria
1.	Acceptable limit for drug products with a MDD of less than 880 mg/day	Total nitrosamines should be not more than 0.03 PPM (Corresponds to not more than 26.5 ng/day)

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2.	Acceptable limit for drug products with a MDD of more than 880 mg/day	The limit for total nitrosamines should be adjusted so as not to exceed the recommended limit of 26.5 ng/day
3.	Nitrosamines without published AI limits (Manufacturers should use the approach outlined in ICH M7(R1))	Manufacturers Should use the approach outlined in ICH M7 (R1) to determine the risk associated with the nitrosamine and contact the agency about the acceptability of any proposed Limit. A compound-specific AI can be calculated based on rodent carcinogenicity potency data such as TD50 values (doses giving a 50% tumour incidence equivalent to a cancer risk probability level of 1:2) identified in the public literature. Linear extrapolation to a probability of 1 in 100,000 (theoretical cancer risk of 10^{-5} i.e., the accepted lifetime risk level used) is achieved by simply dividing the TD50 by 50,000. The AI (in mg/kg/day units) can be converted to mg/day by multiplying with the human body weight. (50 kg is the assumed body weight identified in the referenced guidance)

Methodology:

- In order to fulfil the low AIs for nitrosamines it is recommended to have sensitive techniques with quantification limits (LOQ) in parts per billion (ppb). The analytical procedures which can quantify at or below 0.03ppm should be used by the manufacturers of APIs and finished dosage form.
- manufacturing companies should establish analytical procedures where LOQ and detection limit (LOD) are as low as possible for the finished dosage form with high daily dose (e.g., greater than 1 g).
- In case multiple nitrosamines are evident then the test procedure must be validated for LOQs below 0.03ppm, to calculate maximum total nitrosamine level of 26.5ng/day. The LOQ should be below 0.02 ppm if the MDD is 1200 mg, for example.

Risk Evaluation:

General Root Causes for the Presence of Nitrosamine Impurities in APIs

There are many factors that can influence the formation or carry-over of Nitrosamine impurities, the possible reasons based on the current understanding are presented below.

- By the application of vulnerable procedures and nitrosamine-impurity materials.

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- The risk of nitrosamine creation is increased if nitrous acid is used in the presence of precursor amines to reduce residual azide (a reagent often utilised in tetrazole ring synthesis or induction into a molecule of functional azide group).

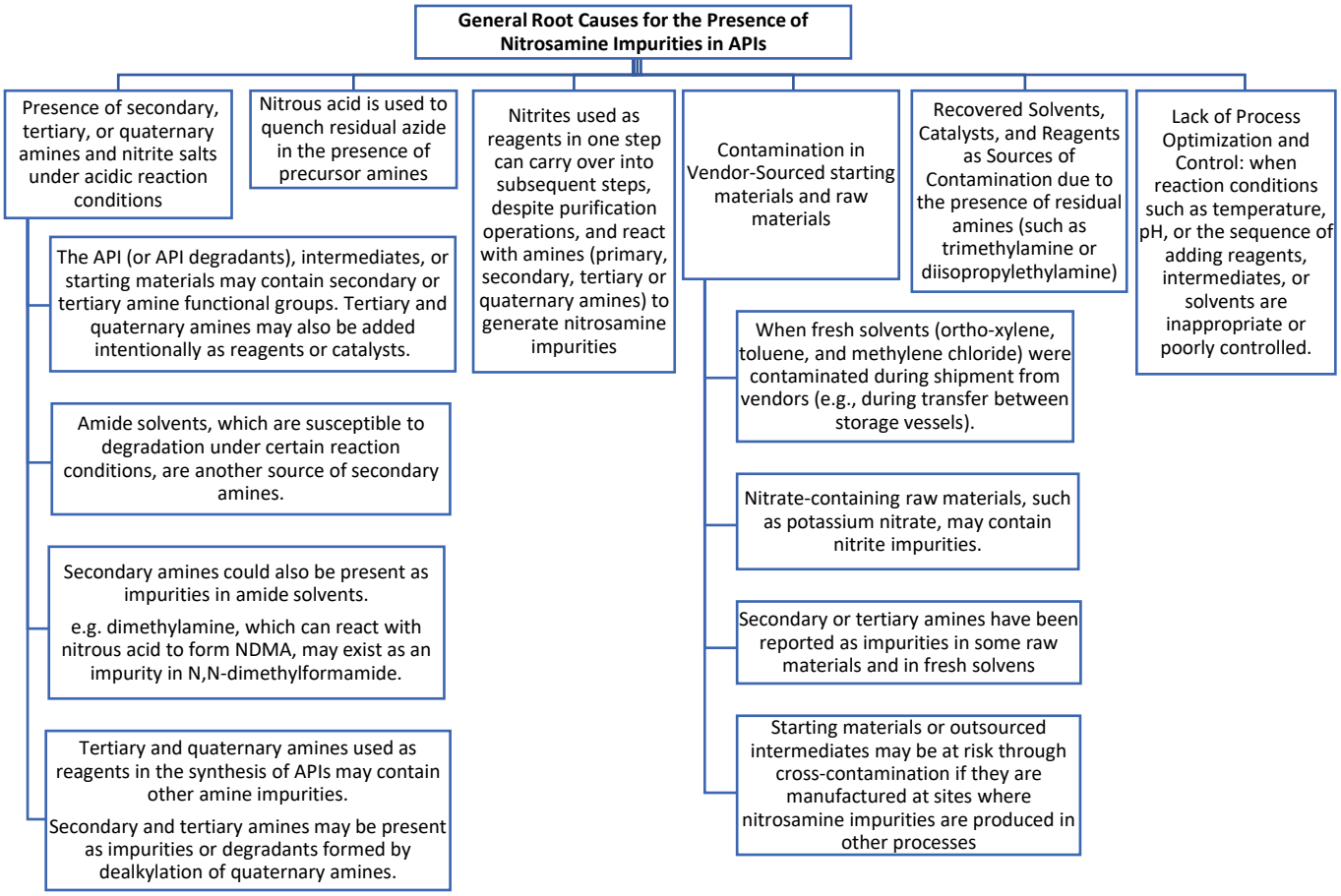

Figure 2: General root cause for the presence of Nitrosamine impurities in APIs

Within the same API process several reactions of the above-mentioned contamination of nitrosamines may develop. Multiple procedures can therefore be required to detect all potentially contaminating sources. The existence of nitrosamine impurities is unlikely to be detect in the typical procedure (e.g. HPLC) API pureness, identity and known impurities. Each failure mechanism could also result in various nitrosamines among batches of the same process and API producers in varying proportions, with contamination detected in certain batches but not all.

- Process-dependent level of amine impurity that may cause the API to contaminate with nitrosamine should be established by every drug substance supplier.

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- This list is not exhaustive as a range of synthetic transformations can be mediated by amine reagents. Manufacturers should assess the potential risk of nitrosamine production of additional chemicals having amine function groups.

Nitrosamine Impurities in Drug Products from Sources Other Than API Contamination:

Table 4: Nitrosamine Impurities in Drug Products from Sources Other Than API Contamination

Excipients	Nitrites are common nitrosating impurities that have been reported in many excipients at ppm levels. Nitrite impurities are found in a range of commonly used excipients, which may lead to nitrosamine impurities forming in drug products during the drug product manufacturing process and shelf-life storage period.
Utilities	The supplier qualification program[12] should take into account that nitrite impurities vary across excipient lots and may vary by supplier. Drug product manufacturers should also be aware that nitrite and nitrosamine impurities may be present in potable water. Potable water may contain low levels of nitrite and even nitrosamines from environmental contamination[13]. (e.g final rinsing, cleaning cycles). Purification process leading to pharmacopeial grade purified water, should remove organics including Nitrosamines.
Through degradation	Some drug products may undergo degradation pathways that form nitrosamine impurities; this could potentially occur during drug product storage

Discussion on root causes and strategies to mitigate the presence of N-nitrosamines in human medicinal products

Mitigating the Presence of Nitrosamine Impurities in APIs:

During synthesis route (ROS) development, the drug substance manufacturer can optimise the manufacturing process design for API's to reduce, or prevent generation of nitrosamine impurities. Manufacturers of API should refer to the recommendations made by ICH M7(R1) and the ICH Guidance for the Active Pharmaceutical Ingredients Industry Q7 (September 2016) and Q11 (November 2012) for the development and production of medicines. In this regard, the guidelines are to be followed by API manufacturer. During the process development, the following variables should be considered:

- The avoidance of reaction circumstances that might lead to the production of nitrosamines if not practicable, be shown to be well managed and consistently capable of decreasing nitrosamine impurities should be demonstrated through appropriate and robust fate and purge studies.

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- Use of bases other than secondary, tertiary, or quaternary amines (where possible) if ROS circumstances are likely to produce nitrosamines is recommended.
- Exercise caution while working with ROS that includes the use of amide solvents (e.g., N,N-dimethylformamide, N,N-dimethylacetamide, and N-methylpyrrolidone).
- In azide decomposition procedures, substituting alternative quenching agents for nitrites is an option.
- Optimizing and maintaining consistent control over reaction sequences, procedures, and reaction conditions (such as pH, temperature, and reaction time).
- Developing a manufacturing method that makes it easier to purge nitrosamine impurities by eliminating quenching stages from the primary reaction mixture in order to decrease the danger of nitrosamine production is being considered. The Active Pharmaceutical Ingredients (APIs) manufacturer shall audit their supply chain to monitor these raw materials, starting materials and intermediates and shall establish controls and consider additional specifications for risky materials in order to prevent contamination by nitrosamines.
- Recovery materials such as solvents, reagents, and catalysts should only be utilised in the same step or in a previous stage of the same manufacturing process when they are recovered from the manufacturing process. Before being put to use, recovered materials must fulfil all applicable criteria.
- API batch may be reprocessed or changed to limit the quantity of nitrosamine impurities in the end product in accordance with ICH Q7 to alter and regulate such operations. If a batch is discovered to include nitrosamine and later reprocessed or reworked in any form, such operations should be conducted under quality control monitoring.

If an impurity of nitrosamine is identified above the LOQ, the drug substance manufacturer should design a strategic approach to ensure nitrosamine levels remain under the AI limit. In order to ensure that nitrosamine levels remain reliably within the AI limit, manufacturers should design an adequate management strategy, including specification limits.

Any API batch discovered to contain nitrosamine impurity levels above the suggested AI shall not be distributed, unless the API is required for the prevention or mitigation of medication shortages with a previous FDA agreement.

Mitigating the Presence of Nitrosamine Impurities in Drug product:

The finished dosage form manufacturer should do risk assessments to establish the potential for the finished product to produce nitrosamine impurities. In order to assess the risk evaluation, the finished product manufacturer should include identification of API ROS or other process conditions, the risk evaluation shall also include the examination of any pathway(s) to introduce nitros-amines during manufacture or storage of drug products (including degradation). When there is an identified danger of nitrosamines in a medicinal product batches should be tested using sensitive and properly validated procedures. If impurity of nitrosamine is identified, producers should examine the root cause and adopt adjustments to the production process to minimise or reduce the amount of nitrosamine impurity.

When creating its control approach, finished dosage form manufacturer should assess if nitrites can be present during production procedures where at-risk APIs are used. They should also analyse if nitrosamines may occur in a finished medicament over the lifetime of the medicinal product. If nitrosamine is introduced into the drug product by means of

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the avoidable exogenous sources, manufacturers must remove contamination from the source. If impurity of a nitrosamine is identified above LOQ, a plan should be developed to ensure that the level of the nitrosamine continues in the limits of AI. The plan for controlling the nitros-amine shall include specifications limits. This control technique is also advisable in situations when nitrosamines are introduced due to the API structure, the API ROS, or the API or medicinal product manufacturing process. In view of existing uncertainty about nitrosamine contaminants and their existence in medications, tests should be carried out on each release batch. Sufficient process understanding and evidence of acceptable statistical control should support alternative techniques and be submitted to the FDA before implementation in a supplement.

If dosage forms are already distributed on batches with unacceptable nitrosamine impurities, manufacturers of medicines shall contact the FDA to assess the regulatory action of the medication products. The manufacturer should not release any drug product batch which may have levels of nitrosamine impurities above the recommended AI. If a recall is initiated, manufacturers should contact the Agency. According to Section 501 of the Food, Drug and Cosmetics Act, 51a drug not made, processed, packaged or kept in accordance with the CGMP is considered adulterated so as to assure compliance with specific quality and purity criteria. When required to avoid or minimise a shortage of a medicine, the FDA may exert its regulatory discretion.

Conclusion

Studies on nitrosamine presence have shown that a complete evaluation of potential nitrosamine contamination should be broader in the drug product, than the simultaneous usage of amines and nitrite sources in the API production system. Manufacturers of all final goods should assess and reduce these hazards under all circumstances that contribute to the nitrosamine content. A full summary of the elements to be taken into account is provided by the European Union and the FDA Agency for manufacturers. In cases of impurity below interim acceptable levels, the level of one nitrosamine is considered safe and those products can remain on the market. In the event of nitrosamine levels being determined above LOQ level but within the AI limit, it should be notified to the Agency and, the applicant shall work with the agency to solve these challenges during the course of the review or immediately before distribution after approval if deemed necessary by the authority. In circumstances when nitrosamine concentrations exceed permissible limits, or more than one nitrosamine is identified, those drugs should generally not be allowed on the market. However, every national authority, would evaluate the benefit risk ratio if the product is no longer available in the market, so that the impact on the market would also be balanced. Consequently, it can be decided that different brands or therapies would be available on the market and the clinical consequence of a halt or transfer to another therapy could be assessed. EMA and USFDA will continue to monitor the issue of nitrosamine contamination and provide updates as and when required. Attention should also be paid to the websites of the USFDA and EMA, where new information resulting from the ongoing investigations will be published.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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