Control of Nitrosamine Impurities in Human Drugs

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2024
Pharmaceutical Quality/Manufacturing Standards (CGMP)

Revision 2

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Preface

The Food Drug and Administration (FDA or Agency) is implementing this guidance without prior public comment because it has determined that prior public participation is not feasible or appropriate (see section 701(h)(1)(C)(i) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 371(h)(1)(C)(i)) and 21 CFR 10.115(g)(2) and (g)(3)). FDA made this determination because of the importance of providing timely information to manufacturers and applicants regarding the detection of nitrosamine impurities, including nitrosamine drug substance-related impurities (NDSRIs), and the resulting recommendations to conduct risk assessments, testing, and other appropriate actions to reduce and mitigate the risk of nitrosamine impurities in active pharmaceutical ingredients (APIs) and drug products.

Comments may be submitted at any time for Agency consideration. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to https://www.regulations.gov. All comments should be identified with the docket number FDA-2020-D-1530 and complete title of the guidance in the request.

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Contains Nonbinding Recommendations

Control of Nitrosamine Impurities in Human Drugs Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance recommends steps manufacturers and applicants of active pharmaceutical ingredients (APIs)[2] and drug products should take to detect and prevent unacceptable levels of nitrosamine[3] impurities in drug products.[4] The guidance also describes conditions that may introduce nitrosamine impurities. The unexpected finding of nitrosamine impurities, which are probable or possible human carcinogens, in certain drug products has made clear the need for a risk assessment strategy for the potential presence of nitrosamines in any drug product.

The discovery of nitrosamines in some drug products led FDA and other international regulators to conduct a detailed analysis of these impurities in affected APIs and drug products.[5] Based on the Agency's current understanding, this guidance discusses potential root causes of nitrosamine formation and advises API and drug product manufacturers and applicants that they should use the

[1] This guidance has been prepared by the Office of Pharmaceutical Quality in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

[2] For the purpose of this guidance, the term *active pharmaceutical ingredient* should be interpreted to mean active ingredient in a drug product (see 21 CFR 210.3(b)(7) and 21 CFR 314.3(b) defining *active ingredient*). The terms active pharmaceutical ingredient and drug substance are also used interchangeably in this guidance.

[3] The term *nitrosamine* as used in this guidance means *N-nitrosamine*.

[4] This guidance is applicable to applicants of new drug applications (NDAs) and abbreviated new drug applications (ANDAs). This guidance is also applicable to applicants of biologics license applications (BLAs) submitted to CDER for products containing chemically synthesized fragments, sponsors of proposed drug products, drug master file (DMF) holders, and manufacturers of marketed products that are not the subject of approved applications (such as drug products compounded by outsourcing facilities pursuant to section 503B of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 353b) and drug products subject to section 505G of the FD&C Act (21 U.S.C. 355h) (i.e., over-the-counter (OTC) monograph drugs)). This guidance also applies to contract manufacturers working with API or drug product manufacturers. This guidance applies to drug products currently available on the U.S. market as well as those that are the subject of pending applications.

[5] FDA has been collaborating with other agencies to share scientific knowledge and current thinking on technical safety and quality topics relating to nitrosamines and to promote technical convergence among member jurisdictions when possible. Such agencies include the European Medicines Agency; European Directorate for the Quality of Medicines & Healthcare; Health Canada; Therapeutic Goods Administration (Australia); Brazilian Health Regulatory Agency (Anvisa); Ministry of Health, Labour and Welfare/Pharmaceuticals and Medical Devices Agency (Japan); Health Sciences Authority (Singapore); Swissmedic (Switzerland); World Health Organization; Nitrosamine International Strategic Group; and Nitrosamines International Technical Working Group.

three-step mitigation strategy described in this guidance to (1) conduct risk assessments of their approved or marketed products and products with pending applications, (2) perform confirmatory testing if a risk is identified, and (3) report changes to prevent or reduce nitrosamine impurities to FDA in accordance with applicable requirements.[6] Most importantly, API and drug product manufacturers should take appropriate actions to reduce or prevent the presence of nitrosamine impurities in APIs and drug products.[7]

Although nitrosamine impurities have been found in some drug products, and there have been some recalls of batches of those products when there were unacceptable levels of these impurities,[8] nitrosamine impurities might exist in other APIs and drug products due to the use of vulnerable processes and materials that may produce nitrosamine impurities. Therefore, the recommendations made in this guidance apply to the following:

- All chemically synthesized APIs
- Drug products containing chemically synthesized APIs or fragments (including biological products containing synthesized fragments)[9]
- Drug products at risk due to other factors described in this guidance (see section III.B., C., and D.)
- Semisynthetic and fermentation products that are at risk due to their structures, similar to chemically synthesized APIs

This guidance revises the guidance of the same title issued in February 2021. Among other changes, this revision includes a new section that describes nitrosamine drug substance-related impurities (NDSRIs), potential root causes of NDSRIs, and mitigation strategies to prevent or reduce the presence of NDSRIs. With this revision, this guidance describes two general structural classes of nitrosamine impurities: small-molecule nitrosamine impurities (nitrosamine impurities that do not share structural similarity to the API and are found in many different drug products) and NDSRIs that share structural similarity to the API and are generally unique to each API (see section III.A.).

NDSRIs are also addressed in the guidance for industry *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)* (August 2023) (RAIL

[6] For the applicable requirements regarding reporting changes, see 21 CFR 314.70, 21 CFR 314.97, and 21 CFR 601.12 regarding changes to approved applications; 21 CFR 314.60 and 21 CFR 314.96 regarding amendments to pending applications; and 21 CFR 314.420(c) regarding changes to DMFs.

[7] Records regarding actions taken should be readily available. Changes must be made in compliance with current good manufacturing practice. See, for example, sections 501(a)(2)(B) (21 U.S.C. 351(a)(2)(B)) and 704 (21 U.S.C. 374) of the FD&C Act, 21 CFR 211.180, and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry *Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients* (September 2016).

[8] See section IV.A. of this guidance.

[9] As noted earlier, this guidance applies to biological products that fall under CDER's regulatory oversight.

guidance).[10] In the RAIL guidance, FDA explains that to reflect the evolving and highly technical nature of the relevant information, FDA intends to provide certain updated information in connection with the RAIL guidance at an FDA web page (nitrosamine guidance web page).[11] This includes updated information about recommended acceptable intake (AI) limits for nitrosamine impurities, recommended safety testing methods for nitrosamine impurities, and recommended analytical testing methods for nitrosamine impurities. As described in more detail below, FDA also intends to provide updated information in connection with this guidance at the same FDA web page, including recommendations under Other Emerging Scientific and Technical Issues and Recommended Timelines.[12] This associated information will be updated periodically.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

FDA has been investigating the presence of nitrosamine impurities in certain drug products since 2018 (see Appendix A for a complete timeline of FDA's involvement in investigating nitrosamine impurities). Nitrosamine impurities have been found in drugs such as angiotensin II receptor

[10] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[11] See the FDA/CDER Nitrosamine Impurity Acceptable Intake Limits web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related.

[12] As FDA becomes aware of new and emerging information on nitrosamine impurities, it may communicate information on the identification of new nitrosamine impurities and FDA's understanding of the root cause of such impurities and their formations. It may also communicate new recommended AI limits, recommendations for prevention or mitigation to address such nitrosamine impurities, and recommended timelines for implementing the mitigation recommendations. When recommending timelines for implementation, FDA may consider factors such as the potential risk to the public health, the state of scientific knowledge, the scope of the problem, the feasibility and complexity of implementing effective prevention or mitigation strategies, and the risk of drug shortages. For example, to address a new nitrosamine impurity, the most effective mitigation may be reformulation, which could require substantial time to complete, and thus FDA may recommend a longer timeline. A different nitrosamine impurity may be best addressed through replacement of packaging, for which FDA may recommend a shorter timeline. If the need for the change is urgent due to a significant public health concern posed by the nitrosamine impurity, immediate implementation may be recommended. Recommended interim AI limits and their associated estimated durations may be posted, if warranted, to prevent a disruption in the U.S. drug supply. In some cases, FDA may also consider international harmonization in establishing timelines. This information may be found on the nitrosamine guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits. This associated information will be updated periodically.

blockers,[13] histamine-2 blockers (ranitidine and nizatidine),[14] antidiabetic medications (metformin[15] and sitagliptin[16]), antibiotics (rifampin and rifapentine),[17] and smoking cessation medication (varenicline[18]). FDA continues to learn of the existence of nitrosamine impurities in drug products in various drug classes and is working with manufacturers and applicants to assess their products and determine appropriate actions.

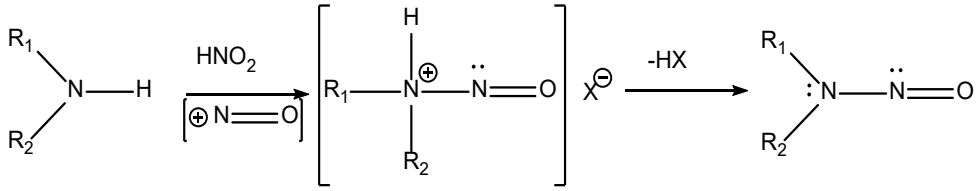
Because the nitrosamine impurity issue extends beyond the U.S. drug supply, FDA and other regulatory authorities have partnered to share certain information, coordinate inspection efforts, communicate effective analytical methods to detect and identify various nitrosamine impurities, and develop rapid solutions to ensure the safety and quality of the drug supply.

III. NITROSAMINE IMPURITIES AND ROOT CAUSES OF FORMATION

A. Nitrosamine Impurities

The term *nitrosamine* describes a class of compounds having the chemical structure of a nitroso group bonded to an amine ($R^1N(-R^2)-N=O$), as shown in Figure 1. The compounds can form by a nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions). A different class of precursor is 1,1-disubstituted hydrazine, which can be oxidized to form a nitrosamine. The compounds 1-cyclopentyl-4-nitrosopiperazine and 1-methyl-4-nitrosopiperazine are formed via this hydrazine oxidation process (Horne et al. 2023).

Figure 1. Representative Reaction to Form Nitrosamines



Nitrosamine compounds are potent genotoxic agents in several animal species and some are classified as probable or possible human carcinogens by the International Agency for Research

[13] The first nitrosamine detected in angiotensin II receptor blockers was *N*-nitrosodimethylamine (NDMA), which is a genotoxic and carcinogenic agent in animals and is classified as probably carcinogenic to humans (Class 2A carcinogen) by the World Health Organization's International Agency for Research on Cancer. Other nitrosamines, including *N*-nitrosodiethylamine and *N*-Nitroso-*N*-methyl-4-aminobutyric acid, have also been detected in various angiotensin II receptor blocker products.

[14] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine

[15] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-metformin

[16] https://www.fda.gov/drugs/drug-safety-and-availability/fda-works-avoid-shortage-sitagliptin-following-detection-nitrosamine-impurity

[17] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamines-rifampin-and-rifapentine

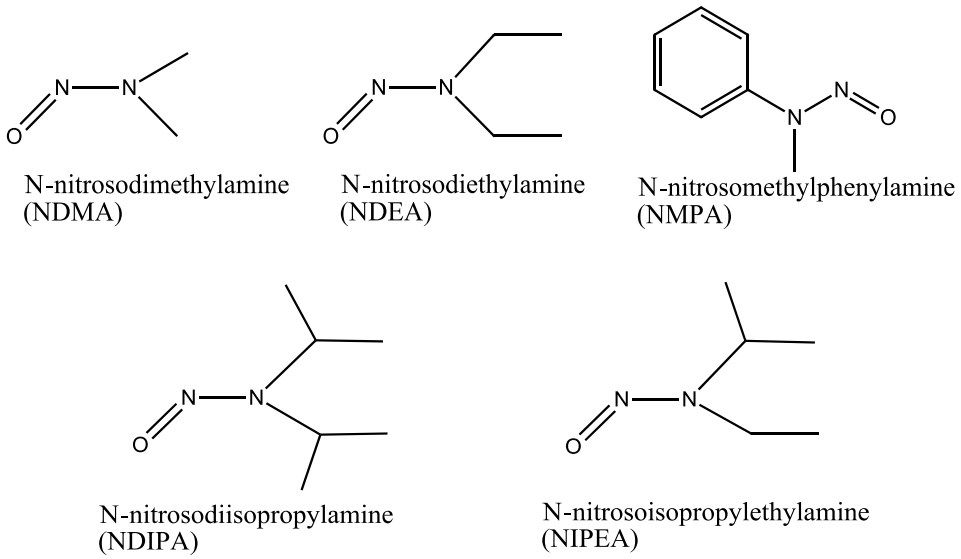
[18] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix

on Cancer.[19] They are referred to as *cohort of concern* compounds in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (July 2023). ICH M7(R2) recommends control of any known mutagenic carcinogen, such as nitroso-compounds, at or below a level such that there would be a negligible human cancer risk associated with the exposure to the compound.

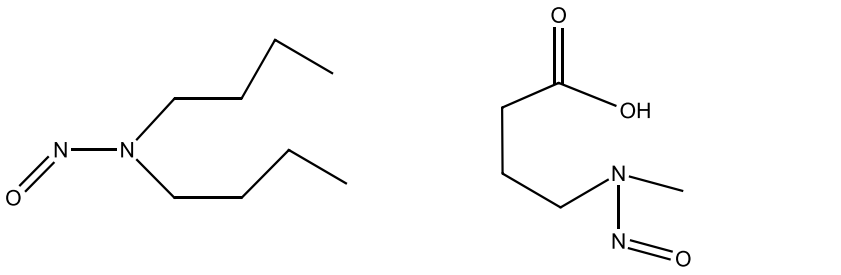
1. Small-Molecule Nitrosamines

Several small-molecule nitrosamine impurities could be present in APIs and/or drug products, including *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosomethylphenylamine (NMPA), *N*-nitrosodiisopropylamine (NDIPA), *N*-nitrosoisophenylethylamine (NIPEA), *N*-nitrosodibutylamine (NDBA), and *N*-nitroso-*N*-methyl-4-aminobutyric acid (NMBA) (see Figure 2).

Figure 2. Chemical Structures of Potential Small-Molecule Nitrosamine Impurities in APIs and Drug Products



[19] See the International Agency for Research on Cancer Monographs on the Identification of Carcinogenic Hazards to Humans web page at https://monographs.iarc.who.int/list-of-classifications.



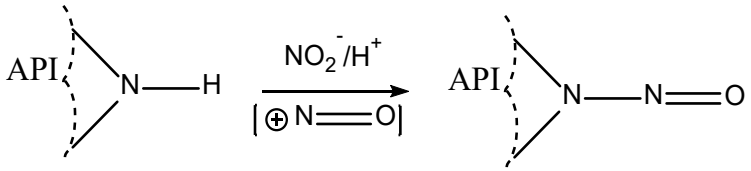
N-nitrosodibutylamine (NDBA)

N-nitroso-N-methyl-4-aminobutyric acid (NMBA)

2. NDSRIs

NDSRIs are a class of nitrosamines sharing structural similarity to the API (having the API or an API fragment in the chemical structure) and are generally unique to each API. NDSRIs form through nitrosation of APIs (or API fragments) that have secondary, tertiary, or quaternary amines when exposed to nitrosating compounds such as nitrite impurities in excipients. Figure 3 shows the representative reaction of an API containing a secondary amine functional group in its structure with nitrite under acidic conditions.

Figure 3. Representative Reaction of NDSRI Formation



B. Root Causes of the Presence of Small-Molecule Nitrosamine Impurities in APIs

Information gathered by FDA suggests several general root causes of the presence of nitrosamine impurities in APIs.

1. General Conditions That Lead to Nitrosamine Formation

Formation of nitrosamines is possible in the presence of secondary, tertiary, or quaternary amines[20] and nitrite salts[21] under acidic reaction conditions. Under these conditions, nitrite salts may form nitrous acid, which can react with an amine to form a nitrosamine (see Figure 1). There is a greater risk of nitrosamine formation if nitrous acid is used to quench residual azide (a reagent commonly used in tetrazole ring formation or introduction of azide functional group into a molecule) in the presence of precursor amines.

[20] Secondary and tertiary amines may be present as impurities or degradants of quaternary ammonium salts.
[21] Secondary, tertiary, and quaternary amines and nitrite also can be called nitrosamine precursors.

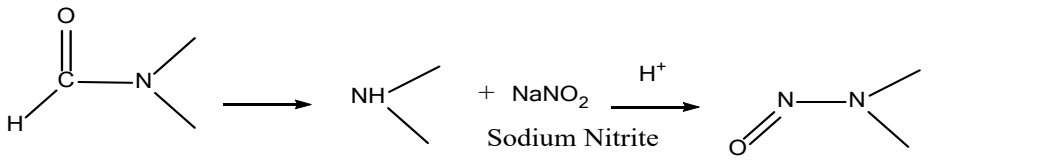
Nitrites used as reagents in one step can carry over into subsequent steps, despite purification operations, and react with amines to generate nitrosamine impurities. Therefore, whenever nitrite salts are present, carryover into subsequent steps cannot be ruled out. In general, processes that use nitrites in the presence of secondary, tertiary, or quaternary amines are at risk of generating nitrosamine impurities.

2. Sources of Secondary, Tertiary, and Quaternary Amines That Can Form Nitrosamines

Amines may be present in a manufacturing process for a variety of reasons. The API (or API degradants), intermediates, or API raw materials[22] may contain secondary or tertiary amine functional groups. Tertiary and quaternary amines may also be added intentionally as reagents or catalysts. All of these types of amines can react with nitrous acid or other nitrosating agents to form nitrosamines (Smith and Loeppky 1967; Fiddler et al. 1972; Gillatt et al. 1984).

Amide solvents, which are susceptible to degradation under certain reaction conditions, are another source of secondary amines. For example, under high reaction temperatures for an extended reaction period, *N*,*N*-dimethylformamide can degrade into dimethylamine, which can react with nitrous acid to form NDMA (see Figure 4). *N*-methylpyrrolidone, *N*,*N*-dimethylacetamide, and *N*,*N*-diethylacetamide also have similar degradation pathways to form secondary amines that can react with nitrous acid to form nitrosamine impurities. Secondary amines could also be present as impurities in amide solvents. For example, dimethylamine, which can react with nitrous acid to form NDMA, may exist as an impurity in *N*,*N*-dimethylformamide.

Figure 4. Formation of NDMA From *N*,*N*-Dimethylformamide



Tertiary and quaternary amines used as reagents in the synthesis of APIs may contain other amine impurities. Tertiary amines, such as triethylamine, have been shown to contain low levels of other secondary amines (such as dipropylamine and isopropylethylamine). Secondary and tertiary amines may be present as impurities or degradants formed by dealkylation of quaternary amines. For example, a common phase-transfer catalyst, tetrabutylammonium bromide, may contain tributyl- and dibutylamine impurities. The amine impurity level that may lead to nitrosamine impurities in the API is process-dependent and should be determined by each API manufacturer.

[22] For the purpose of this guidance, *raw material* is a general term used to mean starting materials, reagents, and solvents intended for use in the production of intermediates or APIs.

This list of the aforementioned sources is not exhaustive, as amine reagents can be used to mediate a wide range of synthetic transformations. Manufacturers should evaluate other reagents containing amine functional groups for potential risk of nitrosamine formation.

3. Vendor-Sourced Raw Materials Containing Nitrosamine Impurities

Nitrosamine impurities can be introduced when vendor-sourced raw materials contain nitrosamines or precursors. The Agency has observed the following root causes of nitrosamine impurities in vendor-sourced materials:

- Nitrosamines have been found in fresh solvents (*ortho*-xylene, toluene, and methylene chloride) when impurities were carried over during transfer between storage vessels used in the shipment of solvents.

- Sodium nitrite is a known impurity in some starting materials (such as sodium azide) and may be present and react with amines under acidic conditions to form nitrosamines. Nitrate-containing raw materials, such as potassium nitrate, may contain nitrite impurities. The amount of nitrite impurity that can be tolerated is process-dependent and should be determined by each API manufacturer.

- Secondary or tertiary amines have been reported as impurities in some raw materials (see details in section III.B.2.) and in fresh solvents such as toluene.

- API starting materials and API intermediates may be at risk through cross-contamination at the manufacturing site if these materials are manufactured where nitrosamine impurities can carry over from other processes.

Awareness of the supply chain of API raw materials is an important factor in preventing nitrosamine impurities and cross-contamination of APIs. For example, without supplier oversight, API manufacturers may not be aware of nitrosamine impurities or precursors in API starting materials they have sourced from vendors; a manufacturer whose process is not normally susceptible to nitrosamine formation may not realize that vendor-sourced material may have had impurities introduced during production or transport.

4. Recovered Solvents, Reagents, and Catalysts as Sources of Nitrosamine Impurities

Recovered materials such as solvents, reagents, and catalysts may pose a risk of nitrosamine impurities due to the presence of residual amines (such as trimethylamine or diisopropylethylamine). If the recovery process involves a quenching step (i.e., nitrous acid used to decompose residual azide), nitrosamines could form during solvent recovery. These nitrosamines may be entrained if they have boiling points or solubility properties similar to the recovered materials, depending on how recovery and subsequent purification takes place (e.g., aqueous washes or distillation). This further increases the risk of nitrosamines in material recovery. For

these reasons, some drug products using APIs manufactured by certain "low" risk processes[23] were found to contain nitrosamine impurities. The Agency has observed the following due to this root cause:

- A manufacturing site may produce the same API by more than one synthetic process that uses common solvents. If any of those synthetic processes produces nitrosamines or contains precursor amines, the solvents sent for recovery are at risk. The use of recovered solvents that are commingled from different processes or across manufacturing lines without control and monitoring can introduce nitrosamine impurities. If a recovered solvent with nitrosamine impurities is used to manufacture an API, the API will contain the impurities even if the synthetic route is not normally susceptible to nitrosamine formation.

- Recovery of raw materials (e.g., solvents, reagents, and catalysts) is often outsourced to third-party contractors. Process outsourcing can pose a risk if the third-party recovery facility does not receive enough specific information on the contents of the materials it is processing and relies solely on routine recovery processes.

- Raw materials can contain nitrosamine impurities if adequate cleaning of equipment between customers, or between different materials, is not carried out or is not validated as capable of removing each impurity of concern. It was reported that nitrosamine impurities were introduced into recycled *ortho*-xylene and toluene due to inadequate cleaning and use of shared storage equipment between different customers. Inadequate and unvalidated cleaning procedures can also lead to cross-contamination if precautions to avoid nitrosamines are not in place before materials from different customers are combined for recovery. For example, nitrosamine impurities were introduced into lots of the catalyst tri-*N*-butyltin chloride (used as a source of tri-*N*-butyltin azide) at a third-party contractor facility due to the combining of catalyst lots from multiple customers.

5. Quenching Process as a Source of Nitrosamine Impurities

There is a risk of nitrosamine formation when a quenching step is conducted directly in the main reaction mixture (i.e., when nitrous acid is added to the reaction mixture to decompose residual azide). This allows nitrous acid to come into direct contact with residual amines in the raw materials used in the manufacturing process. The nitrosamine impurities could be carried to the subsequent steps if adequate removal or purification operations are not in place, or if the operations are not optimized for removing specific impurities of concern. These impurities can enter the downstream process once they are introduced. Even if the quenching process is conducted outside of the main reaction mixture (see section III.B.4.), there is a risk if recovered materials containing nitrosamine impurities are introduced into the main process.

6. Lack of Process Optimization and Control

Another potential source of formation of nitrosamine impurities is lack of optimization of the manufacturing process for APIs when reaction conditions such as temperature, pH, or the sequence

[23] "Low" risk processes are those deemed not normally susceptible to nitrosamine formation.

of adding reagents, intermediates, or solvents are inappropriate or poorly controlled. FDA has seen instances in which reaction conditions varied widely between batches and even between different processing equipment in the same facility for the same API. Additionally, certain manufacturing processes using forced air, such as fluid bed drying at elevated temperature and jet milling, can create favorable conditions for nitrosamine formation in at-risk APIs when nitrogen oxides in the air react with the APIs.[24]

The multiple root causes of nitrosamine impurities listed above can occur within the same API process. Therefore, multiple strategies may be necessary to identify all potential sources of nitrosamine formation. Typical routine tests (e.g., high performance liquid chromatography) for API purity, identity, and known impurities are unlikely to detect the presence of nitrosamine impurities. Further, each failure mode could result in different nitrosamines in different amounts across batches from the same process and the same API manufacturer, with nitrosamine impurities detected in some batches but not all.

C. Nitrosamine Impurities in Drug Products From Sources Other Than APIs

Nitrites are common nitrosating impurities that have been reported in many excipients at parts per million (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. A manufacturer's supplier qualification program[25] should take into account that nitrite impurities vary across excipient lots and may vary by supplier. Drug product manufacturers and applicants should also be aware that nitrite and nitrosamine impurities may be present in potable water. Additionally, when nitrosamine precursors such as secondary, tertiary, and quaternary amines, including API fragments, exist as impurities in a drug substance, these precursors can react with nitrites in excipients or nitrites from other sources used in the manufacturing process and form small-molecule nitrosamines or NDSRIs in drug products.[26]

Some container closure systems, including secondary packaging components, and manufacturing equipment could be a source of nitrite or nitrosamine impurities. These impurities may leach into the drug products during manufacturing or storage resulting in small molecule nitrosamine impurities or NDSRIs. The risk for such impurities should be assessed during extractable and leachable studies.

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[24] In general, at-risk APIs include APIs with secondary, tertiary, and quaternary amine functional groups. They also include any API with a route of synthesis that uses at-risk materials (see section III.B.2.).

[25] In accordance with the ICH guidance for industry *Q10 Pharmaceutical Quality System* (April 2009), the manufacturer's and applicant's pharmaceutical quality system extends to control of the quality of purchased materials. A supplier qualification program is a manufacturer's system (e.g., audits, material evaluations, qualification) for selecting material suppliers who can provide materials from approved sources using a defined and agreed-upon supply chain.

[26] An example of this scenario is dimethylamine, which may exist as an impurity in a drug substance. When dimethylamine is carried over into a drug product manufacturing process, it can react with nitrite impurities in the excipients to form NDMA. The nitrosamine formation could be prevented by controlling the nitrosamine precursor, in this case dimethylamine, in the drug substance. Other secondary amine impurities in APIs also have similar risks. See section III.B.2.

D. Root Causes of the Presence of NDSRIs in Drug Products

NDSRIs can be generated during the manufacturing process or during storage of a finished drug product over its shelf life. The known root causes of the presence of NDSRIs in drug products are (1) nitrosating impurities such as residual nitrite in excipients or other sources of nitrite impurities present in drug products (see section III.C.), which lead to nitrosation of the active ingredient to yield NDSRIs under certain conditions, and (2) NDSRIs carried over from APIs (see section III.B.2. and 3.). Generally, the presence of high levels of NDSRIs has been associated with drug products rather than APIs because NDSRI formation most often results from a reaction between the API or an API fragment and nitrite impurities present in the drug product.[27] Consequently, NDSRI levels may increase during storage. Drug products containing APIs with secondary, tertiary, or quaternary amine[28] groups are considered at risk for NDSRI formation if exposed to a nitrosating agent.

IV. RECOMMENDATIONS FOR CONTROL AND MITIGATION OF NITROSAMINES IN APIs AND DRUG PRODUCTS

Because nitrosamines are probable or possible human carcinogens, FDA recommends that manufacturers and applicants consider the potential causes of nitrosamine formation described in this guidance as well as any other pathways observed and evaluate the risk for nitrosamine formation in their APIs and drug products. Manufacturers and applicants should prioritize the evaluation of at-risk APIs and drug products (as described above) based on factors such as maximum daily dose (MDD), duration of treatment, therapeutic indication, and number of patients treated.[29] As new information becomes available and FDA's understanding of nitrosamines in drugs evolves, FDA may recommend that certain drug products become higher priorities for risk assessments.

Manufacturers and applicants should refer to the ICH guidance for industry *Q9(R1) Quality Risk Management* (May 2023) for details related to hazard identification, analysis, and management. Manufacturers and applicants of APIs and drug products should take appropriate measures to prevent unacceptable levels of nitrosamine impurities in their products throughout the product life cycle.

A. Acceptable Intake Limits

[27] APIs may contain low levels of an NDSRI resulting from reactions with reagents or nitrosating species produced during some manufacturing processes.

[28] APIs with tertiary amine functional groups inherently have lower risk for nitrosamine formation than those with secondary amines because tertiary amines are generally less reactive. Risks from tertiary amines include the existence of secondary amine precursors or degradation to generate secondary amines that later react with nitrosating species such as nitrites in the excipients or other nitrite sources in the drug product. APIs containing functional groups such as amides that may undergo hydrolytic degradation during manufacture and storage to generate secondary amines can also be considered at risk for NDSRI formation. Quaternary amines have even lower risk for nitrosamine formation than tertiary amines.

[29] For example, a drug product with an MDD of 2,000 milligrams (mg) in general would pose a greater risk than a drug product with an MDD of 200 mg with the same level of the same type of nitrosamine. Similarly, a drug product intended for only short-term use (e.g., a 7-day course of an antibiotic) poses less risk than a drug product intended for chronic use, if the two products have comparable levels of nitrosamines.

An AI limit as defined in ICH M7(R2) is a level that approximates an increased cancer risk of one additional case in 100,000 subjects based on a conservative assumption of daily exposure to a mutagenic impurity in drug substances and drug products over a lifetime (70 years).[30] As described in ICH M7(R2),[31] a threshold of toxicological concern concept was developed to define an *acceptable intake* of 1.5 micrograms per day for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects. Highly potent mutagenic carcinogens, referred to as the cohort of concern and including *N*-nitroso compounds, are theoretically associated with a potential for significant carcinogenic risk at intakes below the threshold of toxicological concern.

FDA recommends that an AI limit be determined by using the predicted Carcinogenic Potency Categorization Approach when robust carcinogenicity data and additional safety information, including bacterial mutagenicity data, on specific nitrosamine compounds are not available (see the RAIL guidance for an explanation of the Carcinogenic Potency Categorization Approach). Other approaches for determining an AI limit for a specific nitrosamine could be based on database and literature searches for available carcinogenicity and bacterial mutagenicity data or in vivo and/or in vitro testing using the specific compound (see Appendix B). A read-across analysis from a structurally similar surrogate with robust carcinogenicity data can also be used, if scientifically justified. These approaches are described in the RAIL guidance. These approaches can be applied in determining the AI for both NDSRIs and small-molecule nitrosamine impurities.

FDA published recommended AI limits for certain NDSRIs on the nitrosamine guidance web page.[32] However, unlike small-molecule nitrosamine impurities, most NDSRIs lack mutagenicity and carcinogenicity data, making risk assessments challenging. Because of that lack of data, manufacturers and applicants should refer to the RAIL guidance and the updated information on the nitrosamine guidance web page[33] for determining the predicted carcinogenic potency categorization and corresponding recommended AI limit for an NDSRI at hypothetical risk of forming from an API. FDA may not have recommended an AI limit for all possible NDSRIs. In that situation, manufacturers and applicants can determine an AI limit by using the Carcinogenic Potency Categorization Approach methodology and contact the Agency to determine the acceptability of the proposed AI limit.[34]

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[30] FDA has sometimes used the term *acceptable daily intake.* This guidance uses the term *AI limit*.

[31] ICH M7(R2) does not apply to APIs and drug products intended to treat patients with advanced cancer indications, as defined in the ICH guidance for industry *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals* (March 2010). ICH guidances for industry *Q3A Impurities in New Drug Substances* (June 2008) and *Q3B(R2) Impurities in New Drug Products* (August 2006) apply to nitrosamine impurities that are present in drug products indicated for treatment of advanced cancer.

[32] To reflect the evolving and highly technical nature of the relevant information, FDA intends to provide certain updated information on safety testing methods for nitrosamine impurities, in connection with this guidance and the RAIL guidance, at the nitrosamine guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits. This associated information will be updated periodically. For the most recent version, check the web page.

[33] Ibid.

[34] Manufacturers and applicants should refer to the nitrosamine guidance web page for FDA's recommended AI limits. If an applicant is considering an AI limit where there is no FDA-recommended AI limit or that differs from what FDA has recommended, the applicant should consult FDA's regulations and guidance to determine the appropriate submission (see §§ 314.70(b)(2), 314.97, and 601.12 and the guidance for industry *Changes to an*

FDA's recommended AI limits for nitrosamines, including NDSRIs, are communicated directly to an applicant or manufacturer or through FDA guidance (e.g., this guidance; the RAIL guidance). FDA recommends that manufacturers and applicants refer to FDA's recommended AI limits when developing control strategies and in determining AI limits for nitrosamine impurities in their APIs and drug products.[35] Manufacturers or applicants may provide a scientifically justified rationale to pursue an AI limit different than the FDA-recommended limit.

Recommended AI limits reflect a permissible level of exposure to the impurity and are typically determined in units of nanograms per day (ng/day). For the purpose of establishing controls such as specification limits, conversion of an AI limit into parts per million will vary by product and is calculated based on a drug's MDD (parts per million = AI (ng/day)/MDD (mg)). MDD for purposes of calculating a control limit for nitrosamines is typically the MDD reflected in the drug product labeling. If the AI limit cannot be determined using the approaches described in this section, FDA recommends that 26.5 ng/day be used as the AI limit.[36]

FDA-recommended AI limits correspond to individual nitrosamine impurities and are applicable only if a drug product contains a single nitrosamine. FDA recommends that when more than one nitrosamine is identified, the total limit of nitrosamines should not exceed the recommended AI limit for the most potent nitrosamine in the drug product. FDA also recognizes that if the recommended AI limits for the individual nitrosamines vary greatly, it might not be practical to base the total nitrosamine limit on the most potent individual nitrosamine limit and an alternative approach might be appropriate.

An alternative flexible AI limit approach can be used to establish specifications when multiple nitrosamine impurities (e.g., both small-molecule nitrosamines and NDSRIs) are present in drug products.[37] Using the approach described in Appendix C, a manufacturer or applicant can ensure that the levels of the nitrosamine impurities when totaled result in an exposure level that does not exceed the acceptable cancer risk of 1:100,000 as outlined in ICH M7(R2).[38]

Generally, sensitive analytical methods with appropriate limits of quantitation (LOQs) are needed to test whether drug products can meet the AI limits recommended for nitrosamine impurities. In accordance with the ICH guidance for industry *Q2(R1) Validation of Analytical Procedures: Text and Methodology* (November 2005), the detection and quantitation limit should be commensurate with the level at which the impurities must be controlled. The LOQ should be scientifically justified based on these principles. FDA's nitrosamine guidance web page associated with this guidance includes examples of validated analytical test methods recommended for detecting

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Approved NDA or ANDA (April 2004)). FDA will evaluate the proposed AI limit as part of the technical assessment. For OTC monograph drugs, manufacturers should contact FDA at CDER-OPQ-inquiries@fda.hhs.gov if an AI limit that has not been recommended in guidance is being considered.

[35] API manufacturers should control nitrosamine impurities to ensure that the drug products in which the APIs are used will meet the recommended AI limits.

[36] If the total nitrosamine level is below 26.5 ng/day, no additional safety data beyond the data needed to establish that the nitrosamine level in a drug product is below 26.5 ng/day are needed.

[37] Such products could include fixed-combination drug products with more than one API.

[38] As explained earlier, in accordance with ICH M7(R2), the level that approximates an increased cancer risk of one additional case in 100,000 people is based on a conservative assumption of daily exposure to the impurity over a lifetime (70 years).

nitrosamine impurities in several different APIs and drug products.[39,40] Validation is needed to determine if a method developed and validated for detecting nitrosamine impurities in certain types of drug products or formulations is appropriate for detection of the same nitrosamine impurity in another drug product or formulation.

B. Recommended Three-Step Mitigation Strategy

API and drug product manufacturers and applicants should take the following three steps to mitigate nitrosamine impurities in their products:

1. Assess the risk of nitrosamine impurities in APIs, marketed products, and products under approved and pending applications. Risk assessments should be conducted in a timely manner based on the prioritization of drugs.[41] Manufacturers and applicants do not need to submit risk assessment documents to the Agency, but they should retain these documents so that they are available if requested.

2. Perform confirmatory testing when there is any risk for the presence of nitrosamine impurities.[42] Due to nitrosamines' physiochemical properties (low molecular weights, some volatility, and high toxicity), the analytical test methods for nitrosamines need to have specificity, excellent chromatographic separation, and highly sensitive detection capability.

3. Report changes implemented to prevent or reduce nitrosamine impurities in APIs and drug products to FDA. This includes submission of any drug master file (DMF) amendments in accordance with 21 CFR 314.420(c) and changes to approved applications as required under 21 CFR 314.70, 21 CFR 314.97, and 21 CFR 601.12 and pending applications under 21 CFR 314.60 and 21 CFR 314.96.

Manufacturers and applicants should ascertain and address (as applicable) the presence of nitrosamine impurities, including NDSRIs, using the three-step mitigation strategy described in this section. Recently, FDA has learned that some manufacturers or applicants have conducted risk assessments for small molecule nitrosamine impurities for some drug products and have not included NDSRIs in the assessment. FDA recommends that manufacturers and applicants reevaluate their risk assessments if NDSRIs were not previously considered; in general, risk assessments should be revisited periodically as part of risk management.[43] If NDSRIs are

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[39] To reflect the evolving and highly technical nature of the relevant information, FDA may provide certain updated information on testing methods for nitrosamine impurities in connection with this guidance at the FDA nitrosamine guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits. This associated information will be updated periodically.

[40] Manufacturers, applicants, and laboratories are encouraged to make validated test methods publicly available (e.g., by posting on the method developer's website) to facilitate faster testing of other similar drug products.

[41] In accordance with quality management principles, manufacturers and applicants should consider manufacturing changes and shifts over the product life cycle that may affect the potential for nitrosamine impurities, including new sources of raw materials or excipients. Risks should be reassessed periodically (see ICH Q9(R1)).

[42] Testing using appropriately validated methods can be performed by the API manufacturer or by a qualified laboratory.

[43] See ICH Q9(R1).

detected at levels that are higher than the recommended AI limits, FDA recommends that manufacturers and applicants develop control strategies and/or design approaches to control NDSRIs within acceptable levels.

C. Recommendations for API Manufacturers

Although nitrosamines are not expected to form during the manufacture of the majority of APIs, all manufacturers of chemically synthesized APIs should take appropriate actions to mitigate the risk of nitrosamine impurities in APIs where there is a potential for nitrosamine formation.

API manufacturers should review their API manufacturing processes and conduct risk assessments to identify the potential for nitrosamine impurities. If a risk of nitrosamine impurities is identified, confirmatory testing of batches should be performed using sensitive and appropriately validated methods. If an appropriate risk assessment determines that there is no potential for nitrosamine impurities, or a risk is identified but no nitrosamine is detected, there is no need to take further action. If a nitrosamine impurity is detected, API manufacturers should investigate the root cause. They should implement appropriate follow-up actions, including changes in the manufacturing process to reduce or prevent nitrosamine impurities from forming (see section V.).[44] The Agency encourages API manufacturers to notify through the field alert reporting system if initial assessments and testing show nitrosamine formation in APIs that have been sold for use in the U.S. market and to inform drug product manufacturers that have been supplied the API lots so that they can determine if a recall is warranted and contact the Agency, as appropriate, to prevent a drug shortage.[45]

1. Mitigating the Presence of Nitrosamine Impurities in APIs

FDA recommends that API manufacturers take the following actions:

- API manufacturers should optimize the design of the manufacturing process for APIs during route of synthesis (ROS) development to minimize or prevent the formation of nitrosamine impurities. API manufacturers should refer to the recommendations in ICH M7(R2) and the ICH guidances for industry *Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients* (September 2016) and *Q11 Development and Manufacture of Drug Substances* (November 2012) in this respect. The following factors should be considered during process development:

  - Avoiding reaction conditions that may produce nitrosamines whenever possible; when not possible, demonstrating that the process is adequately controlled and is capable of consistently reducing nitrosamine impurities within the recommended AI limits through appropriate and robust fate and purge studies

  - Using bases other than secondary, tertiary, or quaternary amines (when possible) if ROS conditions may form nitrosamines

[44] See section V.C. for reporting changes to approved applications and DMFs.
[45] See the guidance for industry *Field Alert Report Submission: Questions and Answers* (July 2021). The Center for Drug Evaluation and Research's Drug Shortage Staff can be contacted at drugshortages@fda.hhs.gov.

  - If possible, avoiding the use of amide solvents (e.g., *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, and *N*-methylpyrrolidone), and when their use is unavoidable, exercising caution including assessing whether nitrosamines can form

  - Replacing nitrites with other quenching agents for azide decomposition processes

  - Optimizing and consistently controlling the sequences of reactions, processes, and reaction conditions (such as pH, temperature, and reaction time)

  - Designing a manufacturing process that facilitates the purge of nitrosamine impurities in the subsequent processing steps

- API manufacturers should remove quenching steps (when there is a risk of nitrosamine formation (e.g., using nitrous acid to decompose residual azide)) from the main reaction mixture to reduce the risk of nitrosamine formation. The API, or an intermediate formed through a reaction using an azide salt, can be separated from the mother liquor in the organic phase. The aqueous waste phase separated from the organic phase should then be quenched with nitrous acid without contacting the API, its intermediate, or solvents intended for recovery.

- API manufacturers should audit their supply chains and monitor them for any at-risk API raw materials and intermediates.[46] API manufacturers should maintain records including the name(s) of the raw material or intermediate supplier(s) and the raw material or intermediate manufacturer(s) (if not the supplier),[47] and any repackers and distributors who handle the materials before API manufacture. When appropriate, API manufacturers should establish controls and consider additional specifications for at-risk materials to prevent formation of nitrosamine impurities.

- To avoid cross-contamination when recovered materials such as solvents, reagents, and catalysts are used in the manufacturing process, API manufacturers should use recovered material only in the same step or in an earlier step (if there is sufficient purification) of the same process from which it was collected. The recovered materials should meet appropriate standards before reuse. If the recovery of materials is outsourced to third-party contractors, the API manufacturer should audit the contractors' validation of cleaning procedures and other controls. API manufacturers should follow recommendations in ICH Q7 to prevent cross-contamination with nitrosamines or nitrosamine precursors. API manufacturers should also verify with their suppliers whether the purchased materials used in their processes are recovered.

[46] See section III.B. for a description of *at-risk materials*.
[47] See ICH Q7 for recommendations on records that should be maintained.

- API manufacturers should be aware that potable water used in API manufacture may contain low levels of nitrites and even nitrosamines from environmental sources.[48] The existence of nitrites in processing water may lead to nitrosamine formation in API manufacture. Therefore, to avoid unacceptable levels of nitrosamine impurities in APIs, API manufacturers should analyze nitrite and nitrosamine levels in water and use water that has been purified to remove unacceptable impurities.

  If a nitrosamine is introduced to the API through exogenous sources[49] that can be avoided, manufacturers should eliminate the source of nitrosamine impurities.

- If API manufacturers reprocess or rework API batches to control nitrosamine impurity levels, the quality unit should oversee any reprocess or rework of such batches. Reprocessing or reworking operations should follow recommendations found in ICH Q7.

2. Control of Nitrosamine Impurities in APIs

If a nitrosamine impurity is detected above the LOQ, the API manufacturer should develop a strategy to ensure that the nitrosamine level remains at or below the recommended AI limit. Manufacturers should develop an appropriate control strategy to ensure that the nitrosamine level reliably remains below the recommended AI limit in the API, taking into consideration batch-to-batch variations.[50] Given existing uncertainties regarding nitrosamine impurities and their presence in drugs, for at-risk APIs with an impurity detected above 10 percent of the recommended AI limit, manufacturers should test each batch on release and the stability samples on the retest dates for the presence of nitrosamine impurities. A control strategy is also recommended when the risk of formation of nitrosamines is inherent due to the API structure, the API ROS, or the manufacturing process of the API. Alternate approaches (e.g., upstream test of an intermediate) should be supported by sufficient process understanding and evidence of adequate statistical control and should be submitted to FDA in a supplement prior to implementation, as appropriate.[51]

Any API batch found to contain levels of nitrosamine impurities above the recommended AI limit should not be released by the API manufacturer for distribution. As described above, the API manufacturer should contact the Agency if the API may be needed to prevent a disruption in the drug supply.

D. Recommendations for Drug Product Manufacturers and Applicants

1. Risk Assessment and Confirmatory Testing

[48] See the latest version of the World Health Organization's *Guidelines for Drinking-Water Quality* at https://www.who.int/water_sanitation_health/water-quality/guidelines/en/.
[49] For the purpose of this guidance, *exogenous sources* refer to materials such as solvents and raw materials used in synthesis or processing that may introduce impurities.
[50] The control strategy should be updated as new knowledge is gained.
[51] All changes in specifications from those in the approved application must be submitted in a prior approval supplement unless exempted by regulation or guidance (see §§ 314.70(b)(2), 314.97, and 601.12 and the guidance for industry *Changes to an Approved NDA or ANDA*).

Drug product manufacturers and applicants should conduct risk assessments to determine the potential for small-molecule nitrosamine impurities and NDSRIs in drug products. A risk assessment should involve collaboration with the API manufacturer to aid in the identification of the API ROS or other process conditions of the API's manufacture that put the drug product at risk for nitrosamine impurities. The risk assessment should also include evaluation of any pathway (e.g., degradation and nitrosamine precursor impurities such as dimethylamine or other secondary amine precursors) that may introduce the risk of nitrosamine formation during drug product manufacture or storage. If an appropriate risk assessment determines that there is no potential for nitrosamine impurities, there is no need to take further action.

If a risk of nitrosamines in a drug product is identified (see section V.A.), confirmatory testing of batches should be performed using sensitive and appropriately validated methods. If a nitrosamine impurity is detected, manufacturers and applicants should investigate the root cause and implement changes in the manufacturing process to prevent or reduce nitrosamine impurities such that they ensure that nitrosamine levels remain within their corresponding recommended AI limits.

2. Control of Nitrosamine Impurities in Drug Products

a. Establishing reliability of API suppliers

Drug product manufacturers and applicants must test representative samples of all incoming components, including lots of at-risk API, before use, as required under 21 CFR 211.84. To meet the current good manufacturing practice (CGMP) regulations in 21 CFR part 211, subpart E, and be consistent with the ICH guidance for industry *Q10 Pharmaceutical Quality System* (April 2009), drug product manufacturers and applicants should continue testing each API lot for nitrosamine impurities until they have verified that the API supplier can consistently manufacture API that meets the recommended AI limits throughout its expiry or retest date. Testing should continue if there are variations or an upward trend of nitrosamine impurities within the API expiry period that may lead to levels exceeding AI limits. Additionally, when identified nitrosamine impurities are included in the API specification and the manufacturer relies on the supplier's certificate of analysis for nitrosamine test results, the reliability of the supplier's analyses must be validated at appropriate intervals.[52]

Drug product manufacturers and applicants, when designing their control strategy, should evaluate whether nitrites could be present during manufacturing processes where at-risk APIs are used. Manufacturers and applicants should also determine whether nitrosamine precursors are present as impurities in the drug substance because they may form nitrosamine impurities during the drug product manufacturing. Manufacturers and applicants should also evaluate whether nitrosamines could form in a finished drug product over the drug product's shelf life. If a nitrosamine is introduced to the drug product through exogenous sources that can be avoided, manufacturers and applicants should eliminate the source of nitrosamine impurities.

[52] See § 211.84(d)(2).

b. Establishing specifications in drug products

If a nitrosamine impurity is detected above the LOQ, the manufacturer or applicant should develop a strategy to ensure that the nitrosamine level remains within the recommended AI limit. The control strategy should include specification limits for the identified nitrosamine impurities in the drug product if the confirmatory testing finds levels of the nitrosamine impurity above 10 percent of the recommended AI limit,[53] including in stability samples at expiry. A control strategy is also recommended when there is a significant risk of formation of nitrosamine due to the API structure, the API ROS, or the manufacturing process of the API. Additionally, given existing uncertainties regarding nitrosamine impurities and their presence in drugs, even if initial test results are below 10 percent of the recommended AI limit, testing is recommended of regulatory (primary or exhibit) and validation batches, and when changes are introduced in the manufacturing process, excipients, API, or other critical elements that may result in nitrosamine formation during the product life cycle.[54] Alternate approaches should be supported by sufficient process understanding and evidence of adequate statistical control and, in the case of approved products, must be submitted to FDA in accordance with §§ 314.70, 314.97, and 601.12, as appropriate.[55]

Generally, any drug product batch found to contain levels of a nitrosamine impurity above the FDA recommended AI limit[56] should not be released by the drug product manufacturer for distribution[57] and may warrant removal from the market, because such drug products may be considered adulterated under section 501 of the Federal Food, Drug, and Cosmetic Act (FD&C Act),[58] for example if they are not manufactured, processed, packed, or held in conformity with CGMP. As discussed further below, FDA may exercise enforcement discretion when warranted to prevent or mitigate a shortage of a drug.[59]

Manufacturers and applicants should contact the Agency if drug product batches with levels of nitrosamine impurities above the recommended AI limit are already in distribution.[60] Additionally, applicants holding new drug applications (NDAs) and abbreviated new drug applications (ANDAs) are required to submit field alert reports as described in 21 CFR 314.81(b)(1) if testing shows that any batch of distributed drug product fails to meet the specifications established in the

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[53] See section V.A. for discussion of FDA's recommendations when confirmatory testing finds that the level of a nitrosamine impurity is not more than 10 percent of the recommended AI limit.

[54] See the guidance for industry *Process Validation: General Principles and Practices* (January 2011). Testing of validation batches should include both initial process performance qualification batches and subsequent validation batches, as appropriate.

[55] See section V.C.

[56] In certain circumstances, consistent with those described in section V.C., manufacturers or applicants may provide a scientifically justified rationale to pursue an AI limit different than the FDA-recommended limit.

[57] See 21 CFR 211.22(a), § 211.84, 21 CFR 211.165(a), and 21 CFR 211.192 regarding examples of requirements for testing and release for distribution.

[58] See 21 U.S.C. 351.

[59] See MAPP 4190.1 Rev. 4 *Drug Shortage Management*. MAPPs can be found on the Manual of Policies and Procedures web page at https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/cder-manual-policies-procedures-mapp. See also section 506D(b) and (c) of the FD&C Act.

[60] Manufacturers and applicants can contact the Drug Shortage Staff in CDER at drugshortages@fda.hhs.gov. To discuss issues involving recalls, manufacturers and applicants can contact the recall coordinator assigned to the product type and location. Contact information for recall coordinators is available at https://www.fda.gov/safety/industry-guidance-recalls/ora-recall-coordinators.

relevant application, including specifications on nitrosamine impurities. Similarly, applicants that hold a biologics license application are required to report biological product deviations as described in 21 CFR 600.14.

Investigational new drug application sponsors should be aware of potential nitrosamine impurities in APIs and drug products so that they can be addressed before submission of an NDA or biologics license application, and consider throughout product development the recommendations described in this section.[61] Investigational new drug application sponsors should communicate identified nitrosamine risks to the Agency to facilitate discussion of considerations for a proposed or ongoing clinical trial.

3. Recommendations for Drug Product Manufacturers and Applicants on Reduction or Prevention of NDSRI Formation in Drug Products

Manufacturers and applicants should consider the following mitigation strategies when NDSRIs are detected:

- Screen excipients using a supplier qualification program that accounts for potential nitrite impurities across excipient suppliers and excipient lots to reduce the risk of NDSRI formation in drug products. Alternatively, reformulate the drug product with an alternate excipient that has lower levels of nitrites.

- Design drug products incorporating an antioxidant (such as ascorbic acid (vitamin C), ascorbate salt (such as sodium ascorbate), alpha-tocopherol, or propyl gallate), which may inhibit NDSRI formation in drug products. Recent research has demonstrated that the addition of a small amount of antioxidants to formulations may significantly inhibit the formation of NDSRIs in drug products (Shakleya et al. 2023).

- Modify the microenvironment to neutral or basic pH in the drug product formulation. The formation of NDSRIs typically occurs under acidic conditions; in a neutral or basic environment, the kinetics of these reactions are significantly reduced. Thus, formulation designs that incorporate excipients, such as sodium carbonate, that modify the microenvironment to neutral or basic pH could inhibit NDSRI formation (Shakleya et al. 2023).[62]

FDA encourages manufacturers and applicants to consider other innovative strategies to prevent or reduce the formation of NDSRIs to acceptable levels in drug products. Each manufacturer or applicant should determine the potential benefit and demonstrate the suitability of any formulation approach.

V. IMPLEMENTATION OF RECOMMENDED AI LIMITS

[61] See 21 CFR 312.21 and 21 CFR 312.23(a)(7)(i).

[62] Addition of a pH modifier should not change the salt form of the API in an ANDA. The salt or ester form of the active ingredient in the proposed generic drug product must be the same as that in its reference listed drug. See section 505(j)(2)(A)(ii) of the FD&C Act.

A. Assessment of Test Results

Manufacturers and applicants should follow the three-step mitigation strategy described in section IV. This strategy includes conducting a risk assessment followed by confirmatory testing if a risk is identified. Generally, if manufacturers and applicants conduct a risk assessment and determine a drug product is at risk of nitrosamine formation, they should perform confirmatory testing to determine if their drug product contains nitrosamine impurities.

Typically, the confirmatory testing involves sampling the drug product for the specific nitrosamine, and the test either confirms the presence of the impurity or shows that it is not present. Alternatively, a manufacturer or applicant may subject its drug product to testing to demonstrate that based on the chemical structure, a nitrosamine will not form in its drug product. For example, in instances where formation of nitrosamine impurities may occur through nitrosation of the API or API fragments, such as the NDSRIs that are identified by FDA in the nitrosamine guidance web page, a manufacturer or applicant may demonstrate in the risk assessment that subjecting the drug to nitrosating conditions (i.e., targeted forced degradation) will not form nitrosamine impurities in the drug product. In those instances, omission of confirmatory testing may be justified by the risk assessment.[63]

In the absence of such a data-driven justification, confirmatory testing should be performed if a risk is identified by the manufacturer or applicant or by FDA (e.g., if the risk for a specific nitrosamine impurity is identified on FDA's nitrosamine guidance web page). Confirmatory testing should be performed on at least three representative batches.[64] Based on the results, manufacturers and applicants should use the following factors to determine whether changes should be implemented to prevent or reduce the presence of nitrosamine impurities:

1. If the confirmatory testing indicates that nitrosamine levels are not more than 10 percent of the recommended AI limit,[65] then a specification (method and acceptance criterion) is not needed provided the root cause is well understood and manufacturing process controls are established and validated. Nitrosamine levels, and the need for controls, should be reevaluated during the process validation studies and throughout the product life cycle if changes are introduced in the manufacturing process, excipients, API, or other materials used in the manufacturing process (see section IV.C.2.). However, if

[63] In cases where FDA has identified the NDSRI, risk assessment information should be provided upon request by FDA (e.g., in response to an information request). Applicants can provide this information in an annual report. Manufacturers, including manufacturers of OTC monograph drugs and other marketed products that are not the subject of approved applications should retain this information at the facility.

[64] Manufacturers should test three representative batches currently marketed in the United States representing various time points within the labeled expiry, including the end of expiry, and all available API sources should be included in the confirmatory testing. The strength(s) that may be at greatest risk should be selected.

[65] FDA has taken an approach adopted by international partners that recognizes the level of 10 percent of the recommended AI as posing a negligible toxicological risk. The European Medicines Agency and Health Canada have recognized levels at or below 10 percent of the AI limit in a drug product as justification for omission of a specification. See "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.16 7 (July 2023) and Health Canada's "Guidance on nitrosamine impurities in Medications" (March 2024).

during any of these points in the life cycle results are above 10 percent of the recommended AI limit, a specification should be established. Provided that results remain within 10 percent of the recommended AI limit, for approved drug products, confirmatory test results can be included in the annual report to inform the Agency of the test results.

2. If the confirmatory testing indicates nitrosamine levels exceed 10 percent of the recommended AI limit but are within the recommended AI limit, a control for nitrosamines should be established in the release and stability specifications. For approved drug products, this information should be submitted in a supplement as changes being effected in 30 days.[66]

3. If the confirmatory testing indicates nitrosamine levels exceed the recommended AI limit and changes in formulation, manufacturing process, or packaging are warranted, manufacturers and applicants should implement such changes that are demonstrated to ensure that nitrosamine levels remain within the recommended AI limit. For approved drug products, when these changes meet the criteria described in § 314.70(b) or § 601.12(b) as major changes, they must be submitted to the Agency in a prior approval supplement.

   If a manufacturer or applicant proposes an alternative AI limit for nitrosamine impurities or identifies an NDSRI that is not included on the nitrosamine guidance web page, a proposed AI limit or predicted carcinogenic potency category associated with an AI limit should be submitted to FDA for evaluation. For approved drug products, this information must be submitted in accordance with § 314.70(b) or § 601.12(b).

As stated above, manufacturers of over-the-counter (OTC) monograph drugs and other marketed products that are not the subject of approved applications should follow the applicable recommendations described in this guidance, including the recommendations to conduct risk assessments, perform confirmatory testing, and implement changes as needed to mitigate nitrosamine impurities in their drug products. Results of confirmatory testing, when conducted, should be maintained at the facility. In complying with CGMP, manufacturers and applicants must confirm that, following a change (e.g., revision of a formulation or manufacturing process), drug products continue to meet specifications[67] at the time of release and at the time of use as defined by the expiry date.[68]

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[66] See §§ 314.70(c)(1) and 601.12(c). A supplement must be submitted at least 30 days before distribution of the drug product made using the change for any change in a drug product quality control that has a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product. Although these controls are expected to increase assurance that the drug substance or drug product will have the characteristics of identity, strength, quality, purity, or potency that it purports or is represented to possess, in the case of new controls for nitrosamine impurities, the Agency has not designated this change as one that can be implemented upon receipt by the Agency of a supplement (i.e., a changes being effected in 0 days supplement).

[67] See 21 CFR 211.160(b)(3).

[68] See § 211.165(a) and 21 CFR 211.137(a).

FDA is providing these recommendations to help manufacturers and applicants mitigate the risk that their products contain unsafe levels of nitrosamine impurities. Drug products that contain or fail to adequately address the risk of nitrosamine impurities may not meet applicable requirements of the FD&C Act, including sections 501 and 505. For example, such products would violate the Act if they are not manufactured, processed, packed, or held in conformity with CGMP in accordance with section 501(a)(2)(B).

Relevant records and other information that demonstrate compliance with CGMP requirements must be made available for FDA review during an inspection conducted under section 704(a)(1) of the FD&C Act or when requested by FDA in advance or in lieu of an inspection as described in section 704(a)(4) of the FD&C Act. Manufacturers should contact FDA at CDER-OPQ-inquiries@fda.hhs.gov when testing reveals that nitrosamine impurities in a drug product exceed FDA's recommended AI limits. Manufacturers should also be prepared to provide FDA with test results and root cause analyses in such cases. Additionally, manufacturers of OTC monograph drugs and other marketed products that are not the subject of approved applications should contact FDA at the mailbox above if considering adopting an AI limit that exceeds the AI limit recommended by FDA.

B. Recommended Timeline for Implementing Risk Assessments, Confirmatory Testing, and Submission of Required Changes for Nitrosamines

FDA recommends different implementation timelines depending on the regulatory status of the drug product and the type of nitrosamine impurity at issue.[69]

1. Approved or Marketed Drug Products

For small molecule nitrosamine impurities, as previously communicated, FDA recommended that manufacturers and applicants conclude a risk assessment of approved or marketed products by March 31, 2021. To ensure the safety of the U.S. drug supply, FDA recommended that manufacturers and applicants conclude confirmatory testing of drug products and submission of required changes in drug applications for small molecule nitrosamines by October 1, 2023.[70]

As discussed in the RAIL guidance, FDA recognized that some manufacturers and applicants did not consider NDSRIs in their original risk assessments. Therefore, FDA recommended that if NDSRIs were not considered in previous risk assessments, manufacturers and applicants complete a risk assessment for NDSRIs by November 1, 2023, as part of overall risk management. FDA recommends conclusion of NDSRI confirmatory testing of drug products and submission of required changes in drug applications by August 1, 2025.[71,72]

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[69] This applies to NDAs, ANDAs, and BLAs submitted to CDER for products containing chemically synthesized fragments, sponsors of proposed drug products, DMF holders, and manufacturers of marketed products that are not the subject of approved applications (such as drug products compounded by outsourcing facilities pursuant to section 503B of the FD&C Act (21 U.S.C. 353b) or drug products subject to section 505G of the FD&C Act (21 U.S.C. 355h) (i.e., OTC monograph drugs)).

[70] Note that in addition to the recommended date for manufacturers and applicants to make changes, manufacturers and applicants must retain records regarding such changes in accordance with § 211.180.

[71] See section V. in the RAIL guidance.

[72] See footnote 70.

FDA acknowledges that the implementation timelines include investigating the root cause of an impurity and its formation, identifying changes that will eliminate the root cause (e.g., change in manufacturing process, change in supplier), and confirming that any proposed changes will minimize the risk of nitrosamine formation without otherwise adversely affecting product quality, bioequivalence (BE)/bioavailability (BA), and safety. The timelines also include activities conducted by API manufacturers (i.e., risk assessment and testing) to support the drug products in which they are used. FDA may request an expedited risk assessment, confirmatory testing, or other actions based on available information that shows a drug product may have an increased risk.

Once risk assessments and confirmatory testing have been completed for both small molecule nitrosamines and NDSRIs, manufacturers and applicants should continue to take appropriate actions to ensure that their drug products are safe. As FDA's and manufacturers/applicants' understanding of nitrosamine impurities evolve with advances in the science and generation of data, FDA recommends that manufacturers and applicants continue to conduct risk assessments expeditiously and inform FDA if confirmatory testing finds new or previously identified nitrosamine levels above the recommended AI limit.

2. Drug Products in Development and Under FDA Review

a. Presubmission stage

Prospective applicants should ascertain the risk of nitrosamine impurities as early as possible in the development process and, if feasible, before performing in vivo tests such as BE studies. FDA recommends that applicants conduct a scientifically based risk assessment for nitrosamine impurities in APIs and proposed drug products and perform confirmatory testing as appropriate before submission of an original application. However, the risk assessment and submission of confirmatory testing, if appropriate, and changes to the DMF or application can be submitted in an amendment if they are not available at the time of the original submission filing. Such an amendment should be submitted as quickly as possible after the original submission filing to minimize any potential adverse effect on the application assessment timeline.[73]

b. After submission

Applicants with pending applications should conduct the risk assessment expeditiously and inform FDA if confirmatory testing finds nitrosamine levels above the recommended AI limit.[74] Under section 505(d)(3) of the FD&C Act, if FDA finds that "the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of such drug are inadequate to preserve its . . . quality and purity," it "shall issue an order refusing to approve the application." Accordingly, under FDA's regulations, an NDA "is required to contain" a chemistry, manufacturing, and controls section that includes, among other things, a description

[73] For NDA submissions and BLAs submitted to CDER, applicants should discuss the need for an amendment with the Agency at the pre-NDA or pre-BLA stage.

[74] For NDA submissions and BLAs submitted to CDER, the applicant should contact the specific drug product's review division. For ANDAs, the applicant should contact the project manager specified for the ANDA.

of the manufacturing and packaging procedures and in-process controls for the drug product, and the specifications necessary to ensure the quality and purity of the drug.[75] The Agency will work with applicants in an effort to resolve issues during the review cycle.

FDA generally adheres to review goals established as part of the current Prescription Drug User Fee Act and the Generic Drug User Fee Amendments.

C. Reporting Changes to Mitigate Nitrosamine Impurities

Applicants must report changes implemented to prevent or reduce nitrosamine impurities in accordance with FDA regulations (§§ 314.60, 314.70, 314.96, 314.97, and 601.12). Holders of pending applications must update their applications through submission of an amendment according to §§ 314.60 and 314.96.

1. Reporting Changes by API Manufacturers

If an API DMF holder makes process changes in the ROS as a result of the risk assessment and confirmatory testing, the DMF holder must submit an amendment to the DMF and inform each drug product manufacturer or applicant that references the DMF (including those with pending and approved applications), in accordance with § 314.420(c). The applicant must also report such changes in the application, as appropriate, in accordance with §§ 314.60, 314.70, 314.96, 314.97, and 601.12. If the API is manufactured by the applicant and not covered by a DMF, the manufacturer must report such ROS changes in the application in accordance with §§ 314.60, 314.70, 314.96, 314.97, and 601.12, as applicable. If a batch of API is found to contain a nitrosamine and is reprocessed or reworked in any way, these operations should be reported in the DMF or application (as applicable).

Although each DMF may contain only a single synthetic route, if a change in synthetic process is needed to avoid nitrosamine formation and it is not possible to immediately stop using the original manufacturing process, the API manufacturer should submit both processes in the DMF and provide an estimate for the earliest feasible time frame for the removal of the original process. The different synthetic processes should be identified by separate codes to designate batches manufactured through each process. If the original DMF is not updated within a reasonable time frame, the new or revised process should be submitted in a separate DMF.

2. Reporting Changes for Drug Products Reformulated to Mitigate Nitrosamines

As described above in section IV.D.3., one mitigation strategy to reduce or prevent the presence of nitrosamine impurities in drug products is the addition of antioxidants or pH modifiers to the drug formula. This strategy has been shown to be effective by FDA laboratories (Shakleya et al. 2023). Accordingly, if confirmatory testing finds nitrosamine impurities in a drug product, applicants and manufacturers may choose to reformulate their drug products by adding antioxidants or pH modifiers to reduce or prevent nitrosamine impurity formation, as discussed below.

[75] See 21 CFR 314.50(d)(1) and 21 CFR 314.94(a)(9)(i).

Applicants and manufacturers must implement any formulation changes in accordance with applicable requirements.[76] In addition, following a change in formulation, applicants and manufacturers, in complying with CGMP, must confirm that products continue to meet specifications, at the time of release and at the time of use as defined by the expiry date.[77] Applicants and manufacturers should consider the recommendations regarding stability testing provided below. If manufacturing changes are made in conjunction with a reformulation, such changes must be reviewed and approved by the quality control unit.[78]

As provided in §§ 314.70(b)(1) and 314.97(a), changes to an approved NDA or ANDA that have a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product (i.e., a "major change") require submission and approval of a supplement before distribution. Reformulation of an approved drug product is generally considered a major change requiring a prior approval supplement.[79]

The FDA postapproval change guidance considers a change in components or composition that has the effect of adding a new excipient to be a Level 3 change.[80] Level 3 changes are those that are likely to have a significant effect on formulation quality and performance, and typically, for such changes, an in vivo BE study comparing the relative BA of the modified product to the previously approved formulation (NDAs) or to the reference product (ANDAs) would be recommended, in addition to chemistry and dissolution documentation. Similarly, if an applicant pursues reformulation approaches for a product that is the subject of an application pending with the Agency, the Agency recommends the applicant submit an amendment containing the information noted above (see also section V.C.).[81]

When a product is reformulated, applicants should provide data to confirm that the product continues to meet established specifications over its shelf life and that the requirements regarding BA and BE under 21 CFR 320.21 are met.

Applicants can use meeting requests to discuss with FDA formulation changes before submission of a supplement or amendment.[82] Such submissions should generally contain stability and BE studies to support the change. As described below, stability recommendations follow basic principles outlined in the ICH guidance for industry *Q1A(R2) Stability Testing of New Drug Substances and Products* (November 2003) and the guidance for industry *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (November 1995).

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[76] See, for example, §§ 314.70(b)(2), 314.97, 601.12, and 21 CFR parts 211 and 330.
[77] See 21 CFR 211.160(b)(3) and § 211.137(a).
[78] See 21 CFR 211.100(a), § 211.160(a), and § 211.165.
[79] See §§ 314.70(b) and 314.97.
[80] See the guidance for industry *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (November 1995).
[81] See §§ 314.60 and 314.96.
[82] Generally, a Type C meeting or pre-ANDA meeting can be requested for this purpose.

a. Stability data to support reformulated drug products

FDA has developed recommendations in this guidance for drug products reformulated (changed in composition) to reduce or eliminate nitrosamine impurities. The amount of stability data recommended to support reformulated drug products varies depending on whether the nitrosamine levels in the product have been demonstrated to be stable at early time points. Applicants must follow the requirements in § 314.70(a)(2) or § 601.12(a)(2) to assess the effects of the change. All manufacturers and applicants, including those producing OTC monograph drugs, must assess stability of the reformulated drug product within the context of their stability program established to comply with 21 CFR 211.166, ensure that reformulated drug products comply with appropriate specifications under 21 CFR 211.160(b)(3), and document and maintain records of all stability testing performed in compliance with 21 CFR 211.194(e). Manufacturers and applicants should verify that changes have been effective in achieving the desired outcome with no unintended consequences for product quality.

Due to the lack of historical information on NDSRIs and limited data at this time on the effectiveness of individual mitigation strategies, a significant body of data on reduction or elimination of NDSRIs under different conditions is not available. Because of this uncertainty, FDA recommends that such changes be supported by manufacture and testing of samples from three batches of drug products. For approved drug products, at the time of supplement submission, applicants should provide stability data for 3 months at accelerated (time points 0, 1, 2, and 3 months) and long-term (0 and 3 months) stability conditions for these three batches. Applicants with pending applications should submit this information in an amendment. For application products, FDA may also request 6 months of accelerated stability data before approval if analysis shows the potential for an upward trend in NDSRI levels that may lead to out-of-specification results. Long-term stability data through the proposed shelf life from the first three production batches should be reported in the annual report.[83] Manufacturers of drugs that are not approved under section 505 or 351 of the Public Health Service Act, including OTC monograph drugs and drugs compounded under section 503B of the FD&C Act for which such a change is made, should retain similar supporting information at the facility, and must document and maintain records of all stability testing performed in compliance with § 211.194(e).

b. Bioequivalence studies for reformulated products

As noted above, reformulations of an approved or pending NDA or ANDA generally require the submission of a supplement or amendment containing an in vivo BA/BE study comparing the relative BA of the modified product to the previous formulation (NDAs) or to the reference listed drug (ANDAs), in addition to chemistry and dissolution documentation.[84]

The regulations at § 320.21(a) and (b) require applicants who submit NDAs or ANDAs to include evidence measuring the in vivo BA for NDAs or demonstrating BE to the reference listed drug for ANDAs, or information to permit FDA to waive in vivo BA or BE studies for

[83] The expiry period of the reformulated drug product can be the same as the expiry period of the original drug product if the stability profile of the reformulated drug product is comparable to that of the previously approved drug product (except the nitrosamine impurities).
[84] See §§ 314.60, 314.70, 314.96, and 314.97.

NDAs or ANDAs, respectively. Similarly, applicants with pending applications who pursue reformulation must provide such evidence for a reformulated product in an amendment to support approval of the NDA or ANDA.[85] Additionally, § 320.21(c)(1) requires any person submitting a supplemental application to include the submission of evidence or information described in § 320.21(a) and (b) if there is a "change in the manufacturing process, including a change in product formulation . . . beyond the variations provided for in the approved application." Under § 320.21(c), application holders must provide "evidence or information set forth in paragraphs (a) and (b)" to demonstrate BA or BE, as applicable, if they reformulate their product. The regulations at 21 CFR 320.24 set out the types of evidence and available approaches to measure BA or establish BE, listing acceptable in vivo and in vitro approaches, including "[a]ny other approach deemed adequate by FDA" (§ 320.24(b)(6)).

Generally, changes in components or composition that have the effect of adding a new excipient, such as a reformulation adding antioxidants or pH modifiers to control nitrosamines within applicable FDA-recommended AI limits, would be considered a Level 3 change.[86] Typically, for such changes, an in vivo BA/BE study comparing the modified product to the previous formulation (NDAs) or to the reference listed drug (ANDAs) would be recommended, in addition to chemistry and dissolution documentation. However, there may be circumstances where demonstrating BA or BE without an in vivo study may be adequate and acceptable.

As noted above, the regulations provide for the acceptable types of evidence and available approaches to measure BA or establish BE, including "any other approach deemed adequate by FDA" under § 320.24(b)(6). FDA initiated research and testing to evaluate the effect of adding a new excipient to a product to control for nitrosamine formation. This effort began when FDA became aware that APIs containing secondary amine and/or dimethyl tertiary amine functional groups were at risk for nitrosamine formation, which affected a substantial number of FDA-approved drug products and OTC monograph drugs. This effort was further prompted by FDA's consideration of the number of drugs potentially implicated (pending, approved, and OTC monograph), the potential need for applicants and manufacturers to reformulate multiple products, and the resources needed to demonstrate BA or BE while maintaining drug supply and minimizing market disruption. FDA believes that applying the approach described in more detail below may be adequate and acceptable to measure BA or demonstrate BE pursuant to § 320.24(b)(6) in certain cases.

Based on the research (Shakleya et al. 2023) that (1) demonstrated that the addition of small amounts of antioxidants to formulations may significantly inhibit the formation of NDSRIs in drug products, and (2) indicated that incorporating excipients that modify the microenvironment to neutral or basic pH (i.e., pH modifiers) could also inhibit NDSRI formation, FDA initiated additional studies to investigate the effects of certain antioxidants on the stability of drug formulations, in vitro permeability of model drugs, and on intestinal drug transporters.

[85] See §§ 314.60 and 314.96.

[86] See the guidance for industry *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*.

Results from these studies indicate that the addition of small amounts of certain antioxidants (i.e., ascorbic acid, α-tocopherol, propyl gallate, or cysteine hydrochloride) to biopharmaceutics classification system (BCS) III drugs[87] does not affect the permeability of the drug substance or intestinal transporter activities, and therefore would not be expected to change drug absorption in vivo or affect BA or BE (Kulkarni et al. 2024; Lu et al. 2024).

These studies were conducted on BCS III drugs that are characterized as having low permeability and are considered to be more susceptible to the effects of excipients.[88] Because FDA-supported research shows that the addition of certain antioxidants does not affect permeability of drugs that already have low permeability (BCS III drugs), it is reasonable to infer that addition of such antioxidants will have no (or minimal) effect on permeability of drugs that have high permeability (i.e., BCS I and II drugs). Thus, the addition of an antioxidant would not be expected to affect their associated drug absorption in vivo. Therefore, we conclude that the results from the studies conducted on BCS III drugs are appropriate to extrapolate to BCS I and II drugs.

FDA did not specifically study the effect of reformulating products using a pH modifier (e.g., an excipient such as sodium carbonate that modifies the microenvironment by increasing the pH) on product performance. However, scientifically, it is not expected that pH modifiers would have a significant effect on the permeability of an API with amine functional groups when added to a formulation to inhibit nitrosamine formation.[89]

Based on the research outcomes and information above, FDA believes that immediate-release (IR) solid oral and oral suspension products (which are expected to behave similarly to solid oral IR products in terms of rapid dissolution and absorption through the gastrointestinal tract) incorporating an API that is BCS I, II, or III and reformulated to include one of the antioxidants evaluated by FDA or a pH modifier would not be expected to change in quality or clinical performance.

Consistent with this research and information, FDA believes there may be circumstances where applying an approach pursuant to § 320.24(b)(6) to demonstrate BA or BE may be adequate and acceptable. More specifically, consistent with § 320.24(b)(6), BA or BE may be demonstrated without an in vivo study where a product reformulation is supported by appropriate chemistry data and information on the added antioxidant or pH modifier, and comparative dissolution data confirm the lack of any adverse effect of such an excipient on drug performance.

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[87] The BCS is a scientific approach based on the aqueous solubility and intestinal permeability characteristics of the API. The BCS categorizes drug substances into one of four BCS classes: class I: high solubility, high permeability; class II: low solubility, high permeability; class III: high solubility, low permeability; class IV: low solubility, low permeability. The assessment of permeability should preferentially be based on the extent of absorption derived from human pharmacokinetic studies (e.g., absolute BA or mass balance (see the ICH guidance for industry *M9 Biopharmaceutics Classification System-Based Biowaivers* (May 2021))). ICH M9 recommends permeability studies but also allows for human in vivo data on permeability derived from published literature.

[88] See ICH M9.

[89] In general, pH modifiers are not expected to significantly affect the permeability of APIs since small amounts of pH modifiers would be neutralized by stomach acid once the drug product is administered. Dissolution testing can detect differences in solubility and release of the APIs, if they exist, as a result of the addition of a pH modifier.

The following circumstances describe instances where the approach described above may be adequate and acceptable to demonstrate BA or BE and support approval of a product reformulated to address risk of nitrosamine formation.[90] In all cases below, it is assumed that the manufacturing process remains generally the same with the only exception being the addition of the new excipient.

- **IR solid oral or IR oral suspension drug products for which the reformulation strategy is described below.**

  If the product contains an API that is classified as BCS I, II, or III,[91] and the reformulation incorporates a pH modifier,[92] or one of the specific antioxidants studied in FDA-supported research (ascorbic acid, α-tocopherol, propyl gallate, or cysteine hydrochloride), in an amount no more than 10 mg per dose or maximum daily exposure (whichever is lower),[93] submitting comparative dissolution testing data on the pre- and post-change products (in quality control dissolution medium and multimedia of pH 1.2, 4.5 and 6.8) may be sufficient to demonstrate BA or BE, consistent with § 320.24(b)(6), and support the formulation change for the product. The Agency may request in vivo BE studies if the submitted information is considered to be inadequate to support BE.

- **IR solid oral or IR oral suspension drug products for which the reformulation strategy is other than those described above.**

  If the product contains an API that is classified as BCS I, II, or III,[94] and incorporates one of the specific antioxidants included in FDA-supported research (ascorbic acid, α-tocopherol, propyl gallate, or cysteine hydrochloride) but in an amount higher than 10 mg per dose or the maximum daily exposure, whichever is lower,[95] or an antioxidant other than the four antioxidants described in FDA-supported research (Kulkarni et al. 2024; Lu et al. 2024), submission of appropriate studies and dissolution testing as specified below for BCS I, II, and III, respectively, may be sufficient to demonstrate BA or BE, consistent with § 320.24(b)(6), and support the formulation change for the product.

  – For products containing BCS I and II APIs, because of their high permeability that diminishes the effect of transporter activities (if the API is a transporter substrate), comparative dissolution testing of the pre- and post-change products (in quality

[90] This approach does not apply to narrow therapeutic index drug products.
[91] As noted in footnote 87, published literature may be appropriate to support the determination of the BCS class of the API in a drug product.
[92] For ANDAs, the level of pH modifier should not exceed the maximum daily exposure levels of FDA-approved products. Applicants can calculate the maximum daily exposure based on product labeling for approved products or by searching FDA's Inactive Ingredient Database.
[93] Propyl gallate has a maximum daily exposure of 7 mg in the FDA Inactive Ingredient Database.
[94] As noted in footnote 87, published literature may be appropriate to support the determination of the BCS class of the API in a drug product.
[95] For ANDAs, the level of antioxidant should not exceed the maximum daily exposure levels of FDA-approved products. Applicants can calculate the maximum daily exposure based on product labeling for approved products or by searching FDA's Inactive Ingredient Database. For NDAs, the levels of added antioxidants should be justified to support the safety of the excipient and its level.

control dissolution medium and multimedia of pH 1.2, 4.5, and 6.8) may be sufficient to demonstrate BA or BE and support a formulation change. In limited circumstances, FDA may request additional studies, such as in vitro bridging studies (permeability and transporter activity) as described in this subsection, or in vivo BE studies, to support a formulation change for BCS I and II products if the submitted information is considered to be inadequate to support BE.

– For products containing BCS III APIs, supportive studies to demonstrate that the antioxidant does not affect passive intestinal permeability and transporter activity if the API is a transporter substrate and comparative dissolution testing of the pre- and post-change products (in quality control dissolution medium and multimedia of pH 1.2, 4.5, and 6.8), may be sufficient to demonstrate BA or BE. The Agency may request in vivo BA/BE studies if the submitted information is considered to be inadequate to support BA/BE.

FDA will consider meeting requests, as appropriate, to discuss approaches to establish BA or BE.[96]

For IR solid oral or IR oral suspension products containing BCS IV APIs, due to their poor solubility and permeability, and modified-release products for all BCS classes, due to the complexity of the release mechanism, FDA does not believe the alternative approach to establishing BE or BA described above would be appropriate. Instead, studies such as a validated in vitro-in vivo correlation, physiologically based pharmacokinetic modeling, or in vivo BE studies are appropriate to demonstrate BA or BE and support a formulation change.

Determining whether applying the approaches discussed here for establishing BA or BE pursuant to § 320.24(b)(6) may be adequate and acceptable is a review issue and a fact-specific assessment within the context of a specific submission for a particular drug.

Applicants are encouraged to communicate with FDA if proposing novel BA/BE approaches. Requests for FDA to consider other alternative approaches to demonstrate BA or BE for these products will be evaluated on a case-by-case basis. FDA will consider meeting requests, as appropriate, to discuss BA/BE approaches with applicants and application holders.

VI. MAINTAINING THE DRUG SUPPLY

FDA can work with manufacturers and applicants to mitigate the risk of nitrosamine impurities in APIs and drug products to avoid interruptions in the drug supply. If drug product batches already in distribution contain levels of nitrosamines above the FDA-recommended AI limit,[97] and manufacturing changes or recalls are likely to lead to a disruption in the drug supply, then manufacturers and applicants should immediately contact the Center for Drug Evaluation and

[96] BE proposals can be discussed with FDA through Type C meeting requests, supplements and amendments, controlled correspondences, and pre-ANDA meetings.

[97] In certain circumstances, consistent with those described in section IV.A., manufacturers or applicants may provide a scientifically justified rationale to pursue an AI limit different than the FDA-recommended limit.

Research's Drug Shortage Staff at drugshortages@fda.hhs.gov. Drug Shortage Staff can assist manufacturers and applicants in meeting any obligations to report discontinuances or interruptions in their drug manufacture under section 506C of the FD&C Act[98] and regulations under § 314.81(b)(3)(iii). Timely contact with the Drug Shortage Staff also enables FDA to consider, as soon as possible, what actions, if any, may be needed to avoid shortages and protect the health of patients who depend on the affected drug products.[99]

When contacted about a potential disruption in the drug supply, FDA intends to evaluate each circumstance on a case-by-case basis. FDA may work directly with a specific manufacturer or applicant of the marketed drug and intends to consider whether it is appropriate to recommend an interim AI limit for a temporary period. If FDA recommends an interim AI limit, it generally does not intend to object, for example based on applicable underlying CGMP violations, to the distribution of such drug product batches that contain nitrosamine impurity levels at or below the recommended interim AI limit during the specified period under certain circumstances, which will be determined on a case-by-case basis. FDA intends to post such recommended interim AI limit on the nitrosamine guidance web page in connection with this guidance.[100]

[98] See 21 U.S.C. 356c.

[99] FDA may exercise enforcement discretion when warranted to prevent or mitigate a shortage of a drug.

[100] To reflect the evolving and highly technical nature of the relevant information, FDA intends to provide updated information on these recommended interim AI limits, in connection with this guidance, at the FDA nitrosamine guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cder-nitrosamine-impurity-acceptable-intake-limits. This associated information will be updated periodically. For the most recent version, check the web page.

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Guidances for Industry[1]

- Draft guidance for industry *Postapproval Changes to Drug Substances* (September 2018)[2]
- Guidance for industry *Bioavailability Studies Submitted in NDAs or INDs — General Considerations* (April 2022)
- Guidance for industry *Changes to an Approved NDA or ANDA* (April 2004)

[1] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.
[2] When final, this guidance will represent FDA's current thinking on this topic.

- Guidance for industry *CMC Postapproval Manufacturing Changes To Be Documented in Annual Reports* (March 2014)

- Guidance for industry *Field Alert Report Submission: Questions and Answers* (July 2021)

- Guidance for industry *Process Validation: General Principles and Practices* (January 2011).

- Guidance for industry *Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)* (August 2023)

- Guidance for industry *SUPAC-IR: Immediate Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation* (November 1995)

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- ICH guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (July 2023)

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- ICH guidance for industry *Q9(R1) Quality Risk Management* (May 2023)

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- ICH guidance for industry *Q11 Development and Manufacture of Drug Substances* (November 2012)

- ICH guidance for industry *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals* (March 2010)

APPENDIX A:
TIMELINE OF FDA INVESTIGATIONS REGARDING NITROSAMINE IMPURITIES

The following timeline details FDA's investigations and actions regarding nitrosamine impurities:

- In June 2018, FDA was informed of the presence of an impurity identified as *N*-nitrosodimethylamine (NDMA) in the angiotensin II receptor blocker valsartan.[1] Through investigation, the Agency determined that numerous lots of valsartan and several other angiotensin II receptor blocker drug products from different manufacturers and applicants contained unacceptable levels of nitrosamines. The manufacturers and applicants voluntarily recalled the affected batches of these drug products,[2] which led to a drug shortage in some of the affected products.[3] In addition, FDA evaluated processes that use amines that are common in API synthesis and learned that common synthetic pathways could introduce other types of nitrosamine impurities besides NDMA.

- In September 2019, FDA learned that some common heartburn products (ranitidine, commonly known as Zantac, and nizatidine, commonly known as Axid) contained unacceptable levels of NDMA.[4] FDA recommended that manufacturers and applicants voluntarily recall ranitidine and nizatidine products with NDMA levels above those FDA calculated using the methods in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (July 2023).[5,6,7] Preliminary findings from FDA stability testing raised concerns that NDMA levels in some ranitidine products stored at room temperature can increase with time to unacceptable levels. FDA's preliminary results using accelerated stability testing demonstrated that elevated levels of NDMA were measured in products after 2 weeks. On April 1, 2020, FDA requested that all ranitidine products be withdrawn from the U.S. market.

- In December 2019, FDA became aware that some metformin diabetes medicines in other countries were reported to have NDMA. In light of this information, FDA acquired samples of metformin to test for NDMA. By February 2020, the Agency had identified NDMA in

[1] https://www.fda.gov/news-events/press-announcements/fda-statement-fdas-ongoing-investigation-valsartan-impurities-and-recalls-and-update-fdas-current
[2] For a list of recalled products, see FDA's Recalls, Market Withdrawals, & Safety Alerts web page at https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts.
[3] See FDA's web page on drug shortages at https://www.fda.gov/drugs/drug-safety-and-availability/drug-shortages and FDA's list of recalled angiotensin II receptor blocker products at https://www.fda.gov/drugs/drug-safety-and-availability/search-list-recalled-angiotensin-ii-receptor-blockers-arbs-including-valsartan-losartan-and.
[4] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-ndma-zantac-ranitidine
[5] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.
[6] https://www.fda.gov/news-events/press-announcements/statement-new-testing-results-including-low-levels-impurities-ranitidine-drugs
[7] https://www.fda.gov/news-events/press-announcements/fda-statement-fdas-ongoing-investigation-valsartan-impurities-and-recalls-and-update-fdas-current

some samples but did not find levels exceeding the recommended acceptable intake (AI) limit. In May 2020, further FDA testing revealed that certain lots of metformin extended-release formulation contained NDMA above the Agency's recommended AI limit. Based on that testing, FDA requested that identified applicants voluntarily recall these lots of the extended-release metformin.

- In August 2020, FDA learned that two antibiotic drugs used to treat tuberculosis — rifampin and rifapentine — contained 1-methyl-4-nitrosopiperazine (MNP) and 1-cyclopentyl-4-nitrosopiperazine (CPNP), respectively. To avoid shortages and ensure continued patient access to these necessary drugs, FDA did not object to manufacturers and applicants distributing MNP-containing rifampin and CPNP-containing rifapentine until they could reduce or eliminate these impurities.

- In July 2021, FDA was informed that applicants were voluntarily recalling some batches of the smoking cessation drug varenicline (commonly known as Chantix) because of the potential presence of *N*-nitroso-varenicline, an NDSRI, above the recommended AI limit. FDA did not object to manufacturers and applicants distributing varenicline tablets containing *N*-nitroso-varenicline above FDA's recommended AI limit, but below the interim recommended AI limit, until they could eliminate the impurity or reduce it to acceptable levels.[8] By August 2021, manufacturers were able to reduce levels in marketed products to below FDA's recommended AI limit.

- In August 2022, FDA became aware of the NDSRI impurity 7-*N*-nitroso-3-(trifluoromethyl)-5,6,7,8-tetrahydro[1,2,4]triazolo-[4,3-a]pyrazine (NTTP) in certain samples of the antidiabetic drug sitagliptin. FDA encouraged manufacturers and applicants to contact them if a marketed sitagliptin product had NTTP levels exceeding the recommended AI limit. FDA determined on a case-by-case basis whether those drugs should be released for distribution.[9]

[8] https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-and-press-announcements-nitrosamine-varenicline-chantix

[9] https://www.fda.gov/drugs/drug-safety-and-availability/fda-works-avoid-shortage-sitagliptin-following-detection-nitrosamine-impurity

APPENDIX B:
FDA DETERMINATION OF RECOMMENDED ACCEPTABLE INTAKE LIMITS

FDA's identification of recommended acceptable intake (AI) limit values follows the procedures recommended in the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance for industry *M7(R2) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (July 2023).[1] A compound-specific AI limit can be calculated based on rodent carcinogenicity potency data such as the dose resulting in a 50-percent tumor incidence (TD_{50} value) identified in the public literature. Doses resulting in a 50-percent tumor incidence are equivalent to a cancer risk probability level of 1:2. Linear extrapolation to a probability of 1 in 100,000 (i.e., the accepted lifetime risk level used) is achieved by simply dividing the TD_{50} by 50,000. The AI limit (in milligram (mg)/kilogram (kg)/day units) can then be converted to mg/day by multiplying by the human body weight (50 kg is the assumed body weight identified in the referenced guidance).

$$\text{AI (mg/day)} = \frac{TD_{50}\text{ (mg/kg/day) x 50 kg}}{50{,}000}$$

Linear extrapolation from a TD_{50} value is considered appropriate to derive an AI limit for M7 Class 1 impurities (known mutagenic carcinogens) with no established threshold mechanism. In many cases, the carcinogenicity data are available from the Carcinogenicity Potency Database or the Lhasa Carcinogenicity Database.[2] When these databases contain a precalculated TD_{50} value for a selected chemical, this value generally may be used to calculate the AI limit if it is based on robust carcinogenicity data.

A summary of the AI limit derivation for *N*-nitrosodimethylamine (NDMA) is provided as an example. NDMA was identified as a mutagenic carcinogen in several species and is listed as a probable or possible human carcinogen by the Environmental Protection Agency's Integrated Risk Information System program. TD_{50} values for NDMA are 0.0959 mg/kg/day (rat)[3] and 0.189 mg/kg/day (mouse) according to the Carcinogenic Potency Database.[4] For the AI limit calculation, the lower (more conservative) value of the rat is used. The resulting AI limit associated with a 1 in 100,000 cancer risk over 70 years of exposure is calculated by dividing the TD_{50} by 50,000 and then multiplying by 50 to account for a patient with a 50-kg body weight, resulting in 0.0000959 mg/day NDMA, or approximately 96 ng/day NDMA.

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[1] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[2] The Carcinogenicity Potency Database is available at https://files.toxplanet.com/cpdb/index.html. The Lhasa Carcinogenicity Database is available at https://carcdb.lhasalimited.org. As indicated in the Field Descriptions from the Lhasa Carcinogenicity Database, it includes data from the Carcinogenic Potency Database.

[3] Peto, R, R Gray, P Brantom, and P Grasso, 1991, Dose and Time Relationships for Tumor Induction in the Liver and Esophagus of 4080 Inbred Rats By Chronic Ingestion of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine, Cancer Research, 51:6452–6469.

[4] Carcinogenicity Potency Database entry for *N*-nitrosodimethylamine (CAS 62-75-9) (NDMA) accessed at https://carcdb.lhasalimited.org/study-information/44605816

Hence, a daily lifelong intake of 96 ng/day NDMA corresponds to a theoretical cancer risk of 10^5 and therefore represents an AI limit when present as an impurity.

When carcinogenicity study data for an impurity are unavailable or of lesser quality as described in ICH M7(R2) addendum,[5] the predicted Carcinogenic Potency Categorization Approach is recommended by FDA for determining an AI limit (see the RAIL guidance).

[5] See the guidance for industry *M7(R2) Addendum: Application of the Principles of the ICH M7 Guidance to Calculation of Compound-Specific Acceptable Intakes* (July 2023).

APPENDIX C: EXAMPLE OF CONTROL AND SPECIFICATION FOR THE RECOMMENDED ACCEPTABLE INTAKE LIMIT FOR MULTIPLE NITROSAMINES IN ONE DRUG PRODUCT

The following approach can be used to establish specification limits for small-molecule nitrosamines and nitrosamine drug substance-related impurities.

The flexible approach can be used to control the total nitrosamine level when multiple nitrosamines may be present in the same drug product to meet the recommended acceptable intake (AI) limit based on the increased cancer risk of 1:100,000.[1]

> Each individual nitrosamine should be calculated as a percentage of its AI limit, such that the sum of all the nitrosamines does not exceed 100 percent. The calculation for total nitrosamines is described as below:
>
> $$\sum_{i=2}^{n} \frac{Xi}{AIi} * 100\% \leq 100\%$$
>
> Where Xi is the amount of each single nitrosamine i in parts per million (ppm), AIi is the AI limit of each nitrosamine in ppm, and n is not more than 3.

An example of a flexible approach for total recommended AI limits for multiple nitrosamine impurities in one drug product is shown below.

Drug A has maximum daily dose 80 milligrams/day

		Acceptance Criteria	Stability Results			
			0 month		3 months	
Nitrosamine	AI Limit (ng*/day)	Concentration Limit (ppm)[2]	Nitrosamine level (ppm)	% of AI Limit	Nitrosamine level (ppm)	% of AI Limit
Nitrosamine 1	26.5	NMT* 0.33	0.10	30.30	0.15	45.45
Nitrosamine 2	37	NMT 0.46	0.05	10.87	0.20	43.48
Nitrosamine 3	1,500	NMT 18.75	1.00	5.33	3.00	16.00
Total nitrosamine[1]		Sum of all nitrosamines NMT 100%		46.50 (pass)		104.93 (fail)

* ng = nanogram; NMT = no more than

[1] Total nitrosamine is the sum of the percentage of AI limit for each individual nitrosamine. No more than 100 percent means the theoretical cancer risk is no more than 1:100,000.

[2] The concentration limit here is the AI limit for the nitrosamine in ppm which is calculated based on a drug's maximum daily dose (ppm = AI (ng/day)/maximum daily dose (milligrams/day)).

[1] Overall cancer risk estimate should be 1:100,000 considering all potential sources of nitrosamines, from drug substance and drug product, including those leaching into the drug product from container closure systems.

If an approach is proposed for total recommended nitrosamine limits other than that described above, or there are more than three different nitrosamines detected in one drug product, manufacturers and applicants are encouraged to contact the Agency.[2]

[2] Applicants can discuss the proposed limit of total nitrosamine impurities with FDA via (1) Type C meeting requests for NDAs, supplements, and amendments, (2) pre-ANDA meetings, and (3) controlled correspondences.