Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs)

Guidance for Industry

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(2). Submit one set of either electronic or written comments on this guidance at any time. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this document, contact (CDER) Jason Bunting (301) 796-1292.

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

August 2023
Pharmacology/Toxicology

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Preface

The Food and Drug Administration (FDA or Agency) is implementing this guidance without prior public comment because the Agency 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 additional timely information to manufacturers and applicants regarding recommended acceptable intake limits of nitrosamine drug substance-related impurities (NDSRIs), a class of nitrosamine impurities that have been identified in many drug products and also could be present in active pharmaceutical ingredients (APIs). Since the publication of the guidance for industry *Control of Nitrosamine Impurities in Human Drugs*, FDA has received reports of NDSRIs, which share structural similarity to the API and are unique to each API. Based on the chemical structure of the API, there is a risk of NDSRIs forming in a substantial number of drug products. This guidance is being implemented immediately, but it remains subject to comment in accordance with the Agency's good guidance practices.

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.

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities 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 provides manufacturers and applicants of drugs, including prescription and over-the-counter (OTC) drug products,[2,3] with a recommended framework for predicting the mutagenic and carcinogenic potential of nitrosamine drug substance-related impurities (NDSRIs) that could be present in drug products and recommends acceptable intake (AI) limits for NDSRIs. NDSRIs are a class of nitrosamine impurities that have been identified in many drug products[4] and also could be present in active pharmaceutical ingredients (APIs).[5] These impurities share structural similarity to the API (having the API or API fragment in the chemical structure) and are therefore unique to each API. NDSRIs generally form in the drug product

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

[2] For purposes of this guidance, we use the term *drug* or *drug product* to refer to human drug and biological products, including drug-led and biologic-led combination products, regulated by CDER, unless otherwise specified. See section II (Scope) for full description of the applicability of this guidance document.

[3] For purposes of this guidance, we use the term *manufacturers and applicants* to refer collectively to new drug application, abbreviated new drug application, and biologics license application applicants and application holders, sponsors of proposed drug products, drug master file (DMF) holders, owners, manufacturers, or compounders of marketed drug 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) or drug products subject to section 505G of the FD&C Act (i.e., OTC monograph drugs)), unless otherwise specified, and active pharmaceutical ingredient (API) and drug product manufacturers.

[4] Generally, the presence of high levels of NDSRIs has been associated with drug products rather than APIs because NDSRI formation usually results from a reaction between the API or API fragment and nitrosating agents in the drug formulation. However, NDSRIs can potentially form in APIs when nitrosating agents are present in the API manufacturing process or when APIs undergo processing steps that can potentially induce their formation such as fluid bed drying at an elevated temperature and jet milling because these can create favorable conditions in which nitrogen oxides can react with at-risk APIs. Because the presence of NDSRIs is predominantly associated with drug products rather than APIs, for simplicity, we use the term *drug product* or *drug products* throughout this guidance. However, the recommendations in this guidance also apply to APIs to the extent that NDSRIs are associated with APIs.

[5] The term *API* used throughout this guidance refers to the active ingredient in a drug product. See 21 CFR 210.3(b)(7) and 314.3(b) (defining *active ingredient* and *drug substance*). The terms *API* and *drug substance* are also used interchangeably in this guidance.

through nitrosation of APIs (or API fragments) that have secondary or tertiary amines when exposed to nitrosating agents such as residual nitrites in excipients used to formulate the drug product. NDSRIs often lack carcinogenicity and mutagenicity study data (typically from animal studies) from which an AI limit can be determined. This guidance provides a recommended methodology for AI limit determination that uses structural features of NDSRIs to generate a predicted carcinogenic potency categorization and corresponding recommended AI limit that manufacturers and applicants can apply, in the absence of other FDA recommended AI limits, in their evaluations of approved and marketed drug products as well as products in development or under review by FDA.

Uncertainty about the presence and acceptability of the level of an NDSRI in drug products raises regulatory challenges and has led to some applicants conducting unnecessary studies or, in some cases, discontinuing drug products from the market. Because nitrosamine impurities have been identified in many drug products, disruptions in supply and access have increased, sometimes resulting in drug shortages. These challenges can impact patient access to medications, particularly with respect to drug products that are considered medically necessary.

This guidance recommends a risk-based safety assessment of NDSRIs and can be used by manufacturers and applicants to identify AI limits for NDSRIs in their drug products and APIs in conjunction with the recommendations in the guidance for industry *Control of Nitrosamine Impurities in Human Drugs* (February 2021) (Nitrosamine Guidance).[6] FDA recognizes that the AI limits recommended by the Agency and those generated by manufacturers and applicants may evolve with advances in the science and generation of data for nitrosamines. As industry groups and consortia perform investigations that provide insights into mutagenicity and carcinogenicity risks of nitrosamine impurities, FDA encourages sharing such information to help expand the available knowledge base.

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. SCOPE

This guidance applies to drugs, including prescription and OTC drug products that are the subject of an approved or pending new drug application (NDA) or abbreviated new drug application (ANDA), as well as products[7] not marketed under a drug application, including nonprescription drugs subject to section 505G of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (i.e., OTC monograph drugs), or otherwise subject to current good manufacturing practice (CGMP). This guidance also applies to prescription and OTC drug products in clinical development. In

[6] FDA published the Nitrosamine Guidance on September 3, 2020 (85 FR 55017) and updated it on February 24, 2021. We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.
[7] See footnote 2.

addition, this guidance applies to biological products[8] that contain chemically synthesized fragments or biologic-led combination products that contain a drug constituent part whether such products are in development or the subject of an approved or pending biologics license application (BLA). The recommendations in this guidance apply to both drug product and drug substance manufacturers.[9]

The recommendations in this guidance do not apply to NDSRIs that are detected in products indicated for use in patients with advanced cancers (i.e., drug products falling under the scope of International Council for Harmonisation (ICH) guidance for industry *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals* (March 2010) (ICH S9)). Principles of ICH S9, ICH guidance for industry *Q3A(R2) Impurities in New Drug Substances* (June 2008), and ICH guidance for industry *Q3B(R2) Impurities in New Drug Products* (August 2006) may be applied to set impurity specifications for these products.

III. BACKGROUND

FDA has been addressing the evaluation and management of nitrosamine impurities in certain drug products since 2018 when FDA was informed of the presence of an impurity identified as *N*-nitrosodimethylamine (NDMA) in the angiotensin receptor blocker valsartan.[10] The initial reports of nitrosamine impurities in drug products were of small molecule nitrosamines such as NDMA, compounds that form by a nitrosating reaction between amines (secondary, tertiary, or quaternary amines) and nitrous acid (nitrite salts under acidic conditions), generally due to API synthesis conditions that introduce nitrous acid, nitrite and amide solvents. In 2020, FDA issued guidance to industry, the Nitrosamine Guidance, recommending that manufacturers of APIs and drug products take steps to detect and prevent unacceptable levels of nitrosamine impurities in drug products, or to avoid their presence when feasible. The Nitrosamine Guidance introduced a three-step process that manufacturers and applicants should take to mitigate nitrosamine impurities in their drug products: (1) conduct risk assessments for nitrosamines in their APIs and drug products; (2) conduct confirmatory testing if risks are identified; and (3) report changes implemented to prevent or reduce the presence of nitrosamine impurities in APIs and drug products in approved and pending NDAs and ANDAs.

[8] As explained in footnote 2, we use the term *biological products* in this guidance to refer only to biological products regulated by CDER.
[9] See footnote 5.
[10] See the FDA Statement on FDA's Ongoing Investigation Into Valsartan Impurities and Recalls and an Update on FDA's Current Findings, available at https://www.fda.gov/news-events/press-announcements/fda-statement-fdas-ongoing-investigation-valsartan-impurities-and-recalls-and-update-fdas-current.

As explained in the Nitrosamine Guidance, manufacturers of APIs and drug products should take appropriate measures to prevent unacceptable levels of nitrosamines impurities in their drug products.[11] Because nitrosamine compounds have the potential to be potent genotoxic agents in several animal species and some are classified as probable or possible human carcinogens,[12] they are included in a group of high potency mutagenic carcinogens referred to as *cohort of concern* compounds in the 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)). The ICH M7(R2) guidance provides recommendations on control of any known or potential mutagenic carcinogens, such as nitroso-compounds at or below a level associated with negligible human cancer risk associated with exposure to these impurities.[13]

An AI limit is a level that approximates an increased cancer risk of one additional case in 100,000 people based on a conservative assumption of daily exposure to the impurity over a lifetime (70 years). To phrase it another way, an AI limit is a daily exposure to a compound such as NDMA that approximates a 1:100,000 cancer risk after 70 years of exposure.[14] This risk level represents a small theoretical increase in risk when compared to human overall lifetime incidence of developing any type of cancer, which is greater than 1 in 3. If a potential risk for a nitrosamine impurity has been identified, and appropriate testing has confirmed the nitrosamine level, then an appropriate control strategy leveraging process understanding and analytical controls should be developed to ensure that the nitrosamine impurity is at or below the AI limit.[15] As recommended in ICH M7(R2), an AI limit is generally determined based on database and literature searches for carcinogenicity and bacterial mutagenicity data.

As FDA's understanding of NDRSIs is evolving, this guidance recommends a risk-based safety assessment of NDSRIs. The predicted carcinogenic potency categorization approach recommended in this guidance complements the approach recommended in ICH M7(R2). It is based on principles of ICH M7(R2) that recommend the use of structure-activity relationship (SAR) concepts to assess and classify the mutagenic and carcinogenic risk of impurities to limit potential carcinogenic risk in drug products. The science on structure-activity relationships of *N*-nitroso compounds has progressed, so that at this time, FDA recommends that this predictive methodology be used to assess the carcinogenic potency of NDSRIs and more accurately predict their mutagenic potential. This is important because it is currently unknown if all or some NDSRIs are in fact high potency mutagenic carcinogens. This guidance considers that not all NDSRIs have the same carcinogenic potency, as reflected by the different recommended AI limits associated with each potency category.

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[11] Nitrosamine Guidance at 9.

[12] See the International Agency for Research on Cancer (IARC) Monographs on the Identification of Carcinogenic Hazards to Humans web page at https://monographs.iarc.who.int/list-of-classifications. See, e.g., NDMA, *N*-nitroso piperidine (NPIP), 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK), *N*-nitroso pyrrolidine (NPYR), and *N*-nitroso morpholine (NMOR).

[13] See ICH M7(R2), available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[14] See Nitrosamine Guidance at 10 and Appendix B (FDA Determination of Acceptable Intake Limits).

[15] An AI limit can be converted into a parts per million (ppm) control limit for a manufacturer or applicant. The conversion varies by product and is calculated based on a drug's maximum daily dose (MDD) as reflected in the drug labeling (ppm = AI (nanograms (ng))/MDD (milligrams)). Nitrosamine Guidance at 10.

A. Challenges in Determining AI Limits for NDSRIs

Determining an AI limit for NDSRIs is often more challenging than determining AI limits for small molecule nitrosamines, primarily because NDSRIs are unique to each API and, therefore, there are usually limited or no existing safety data (e.g., rodent carcinogenicity data) on NDSRIs.[16] FDA has communicated recommended AI limits for a limited number of NDSRIs, but unlike more commonly known nitrosamines (such as those identified in the Nitrosamine Guidance), a recommended AI limit has not previously been determined for most NDSRIs.

A recommended AI limit is based on a safety assessment that includes evaluation of the mutagenic and carcinogenic potential of the impurity and represents the level at or below which FDA has determined that the impurity or impurities would not pose a safety concern for patients taking the drug product. A compound-specific AI can be calculated based on rodent carcinogenic potency data such as TD_{50} values (doses giving a 50 percent tumor incidence equivalent to a cancer risk probability level of 1:2) identified in the published scientific literature. Carcinogenicity data may be available from the Carcinogenic Potency Database (CPDB) or Lhasa Carcinogenicity Database (LCDB).[17] TD_{50} values for approximately 140 nitrosamines, mostly small molecule nitrosamines, were originally reported in the CPDB and have been incorporated into the LCDB. The carcinogenic potency values range over four orders of magnitude, and some nitrosamines have been found to be non-carcinogenic.[18] However, many studies are not robust and cannot solely be relied on for calculating AI limits.

When the mutagenic potential of an NDSRI is not adequately characterized, FDA and applicants have used (quantitative) SAR methods to support the identification of a robustly-tested surrogate that is similar in structure and reactivity to the NDSRI to generate an estimate of carcinogenic potency from which an AI limit can be scientifically determined. In this scenario, surrogates are compounds containing an *N*-nitroso structural alert in the same chemical environment as an NDSRI and for which robust carcinogenicity data are available.[19] The rationale for the choice of surrogate (similar in structure and reactivity) is significant because test data from the identified surrogate are then used to generate an estimate, either quantitatively or qualitatively, for the data-poor compound (commonly referred to as a *read-across analysis*).[20]

The structural environment surrounding the *N*-nitroso group of the NDSRI is an important factor when selecting appropriate reference compounds for a read-across analysis. It may include

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[16] See ICH M7(R2) at 9 to 11 and Note 4 on calculating a compound-specific AI limit.

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

[18] For example, see Thresher A, Gosling JP, and Williams R, 2019, Generation of TD50 Values for Carcinogenicity Study Data, Toxicol Res, 8:696-703; Thresher A, Foster R, Ponting DJ, et al., 2020, Are All Nitrosamines Concerning? A Review of Mutagenicity and Carcinogenicity Data, Regul Toxicol Pharmacol, 116:104749.

[19] For example, see Cross KP and Ponting DJ, 2021, Developing Structure-Activity Relationships for *N*-Nitrosamine Activity, Comput Toxicol, 20:100186; Thomas R, Tennant RE, Oliveira AAF, and Ponting DJ, 2022, What Makes a Potent Nitrosamine? Statistical Validation of Expert-Derived Structure-Activity Relationships, Chem Res Toxicol, 35:1997–2013; and Ponting DJ, Dobo KL, Kenyon MO, and Kalgutkar AS, 2022, Strategies for Assessing Acceptable Intakes for Novel *N*-Nitrosamines Derived From Active Pharmaceutical Ingredients, J Med Chem, 65:15584–15607.

[20] See section IV.

consideration of the degree of substitution, steric bulk, electronic influences, potential for metabolic activation, stability/reactivity of the resulting metabolites, and overall molecular weight.

IV. RECOMMENDATIONS FOR AI LIMITS BASED ON PREDICTED CARCINOGENIC POTENCY CATEGORIZATION

A. Introduction to Predicted Carcinogenic Potency Categorization Approach

This section describes a recommended methodology that uses predicted carcinogenic potency categorization to assign a recommended AI limit to an NDSRI based on the NDSRI's activating and deactivating structural features. In the context of this guidance, activating or deactivating features are defined as molecular substructures that are associated with an increase or decrease, respectively, in carcinogenic potency. The predicted carcinogenic potency categorization approach to determine a recommended AI limit incorporates SAR concepts described in recent scientific publications for nitrosamine compounds.[21] The approach assumes that the α-hydroxylation mechanism of metabolic activation[22] is responsible for the mutagenic and highly potent carcinogenic response observed for many nitrosamines. Structural features that directly increase or decrease the favorability of the activation mechanism or that increase the clearance of the nitrosamine by other biological pathways will have a corresponding effect on carcinogenic potency. Therefore, a prediction of the mutagenic potential and carcinogenic potency of an NDSRI can be generated based on its structural features.

The predicted carcinogenic potency categorization approach described in this guidance applies to NDSRIs bearing a carbon atom on both sides of the *N*-nitroso group, where the carbon is not directly double bonded to a heteroatom (i.e., *N*-nitrosamides, *N*-nitrosoureas, *N*-nitrosoguanidines and other related structures are excluded). Additionally, the predicted carcinogenic potency categorization approach does not apply to NDSRIs where the *N*-nitroso group is within an aromatic ring (e.g., nitrosated indoles).

The predicted carcinogenic potency categorization approach enables manufacturers and applicants to identify the appropriate potency category and associated recommended AI limits for NDSRIs in APIs and drug products and to facilitate development of methods for confirmatory testing of impurity levels in drug batches. Potency categorization offers a science-based predictive solution to recommending AI limits for NDSRIs. FDA scientists, in collaboration with other international drug regulatory authorities, developed an approach to evaluate the predicted carcinogenic potency of NDSRIs based on the structural features of these compounds. The approach is based on information from published data on small molecule nitrosamines that are available through CPDB and LCDB, combined with chemical and biological mechanistic considerations described in recent nitrosamine scientific publications.[23] The goal is to set recommended AI limits by predicting the potency of the substances using activating and deactivating structural features present in

[21] See footnote 18.
[22] Li Y and Hecht SS, 2022, Metabolic Activation and DNA Interactions of Carcinogenic N-Nitrosamines to Which Humans Are Commonly Exposed, Int J Mol Sci, 23:4559.
[23] See footnote 18.

nitrosamines. In developing the model, relative weights were assigned to activating and deactivating features based on SAR trends reported in the published literature, SAR trends observed in the CPDB and LCDB, and a general scientific understanding of the chemical and biological mechanistic pathways of the compounds. The approach based on structural features can then be applied to an NDSRI to predict the relative carcinogenic potency of the NDSRI. This approach considers the effects of multiple features in the molecule and leverages a larger body of carcinogenicity data in the published scientific literature than is available for establishing an AI based on a surrogate nitrosamine molecule. FDA anticipates that the analysis of these structural features, and the weighting that has been assigned to these features, may change over time as the science advances and additional data are generated.

The predicted carcinogenic potency categorization and resulting recommended AI limit approach described in this guidance should not be applied to NDSRIs in circumstances in which FDA otherwise recommends an AI limit (e.g., based on compound-specific assessments or read-across analysis from a surrogate).[24] Generally, FDA has communicated recommended AI limits directly to an applicant or manufacturer or through an FDA guidance (the Nitrosamine Guidance). FDA may continue to do so, including in connection with this guidance.[25] When FDA communicates a recommended AI limit based on compound-specific assessments or read-across analysis from a surrogate, manufacturers and applicants should apply that recommended AI limit rather than using the predicted carcinogenic potency categorization approach in this guidance to identify a recommended AI limit.

FDA continues to work with international regulators in the development and use of this modeling approach to identify recommended AI limits for NDSRIs to minimize the potential impact of nitrosamine impurities on the drug supply.

The Agency recognizes that the science is evolving in the prediction of mutagenic potential and carcinogenic potency based on SAR concepts. Therefore, the predicted carcinogenic potency categorization method described in section IV.B is a conservative approach that represents the best available science at this time and is expected to be further refined and expanded as new data become available. Additionally, as with all guidance documents, manufacturers and applicants can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations.

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[24] In corresponding with certain manufacturers and applicants, FDA previously recommended that certain NDSRIs, for which there is no compound-specific data and no suitable surrogate for a read-across analysis, be controlled at levels that do not exceed a conservative AI limit of 26.5 ng/day. For these NDSRIs, we now recommend that manufacturers and applicants use the framework recommended in this guidance to determine an appropriate predicted carcinogenic potency category and corresponding recommended AI limit.

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

B. Specific Application of the Predicted Carcinogenic Potency Categorization Approach

FDA recommends the following AI limits based on the predicted carcinogenic potency categorization approach for NDSRIs.

Table 1 shows the predicted carcinogenic potency categories and associated recommended AI limits. Notably, the associated recommended AI limits generally represent the conservative lower limit for the range of potencies falling within each category.

Table 1. The Five Predicted Carcinogenic Potency Categories and Associated Recommended AI Limits for NDSRIs

Potency Category	Recommended AI (ng/day)	Comments
1	26.5	The recommended AI limit of 26.5 ng/day[*] is equal to the class-specific limit for nitrosamine impurities based on the most potent, robustly tested nitrosamine, *N*-nitrosodiethylamine (NDEA).[**] NDSRIs assigned to Category 1 are predicted to have carcinogenic potency no higher than the class-specific limit for nitrosamine impurities.
2	100	The recommended AI limit of 100 ng/day is representative of two potent, robustly tested nitrosamines, *N*-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(butanone) (NNK), which have recommended AI limits of 96 ng/day and 100 ng/day, respectively. NDSRIs assigned to Category 2 are predicted to have carcinogenic potency no higher than NDMA and NNK.
3	400	Compared to Potency Category 2, NDSRIs in this category have lower carcinogenic potency due to, for example, the presence of a weakly deactivating structural feature. The recommended AI limit was set to reflect a 4-fold decrease in carcinogenic potency from Category 2.
4	1500	NDSRIs assigned to Category 4 may be metabolically activated through an alpha-hydroxylation pathway but are predicted to be of low carcinogenic potency, for example, because the pathway is disfavored due to steric or electronic influences, or because clearance pathways are favored. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7(R2).[***]
5	1500	NDSRIs assigned to Category 5 are not predicted to be metabolically activated via an α-hydroxylation pathway due to steric hindrance or the absence of α-hydrogens, or are predicted to form unstable species that will not react with DNA. The recommended AI limit of 1500 ng/day is set at the TTC per ICH M7(R2).[***]

AI = acceptable intake; ng = nanogram; NDSRI = nitrosamine drug substance-related impurities; TTC = threshold of toxicological concern.

[*] For products intended for marketing in the United States, FDA recommends an AI limit of 26.5 ng/day for Category 1, even if a different limit is recommended in other regulatory regions.

[**] See the guidance for industry *Control of Nitrosamine Impurities in Human Drugs* (February 2021).

[***] See the International Council for Harmonisation 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)). The threshold of toxicological concern (TTC) of 1.5 micrograms/day (1500 ng/day) as explained in ICH M7(R2), represents an AI for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effect.

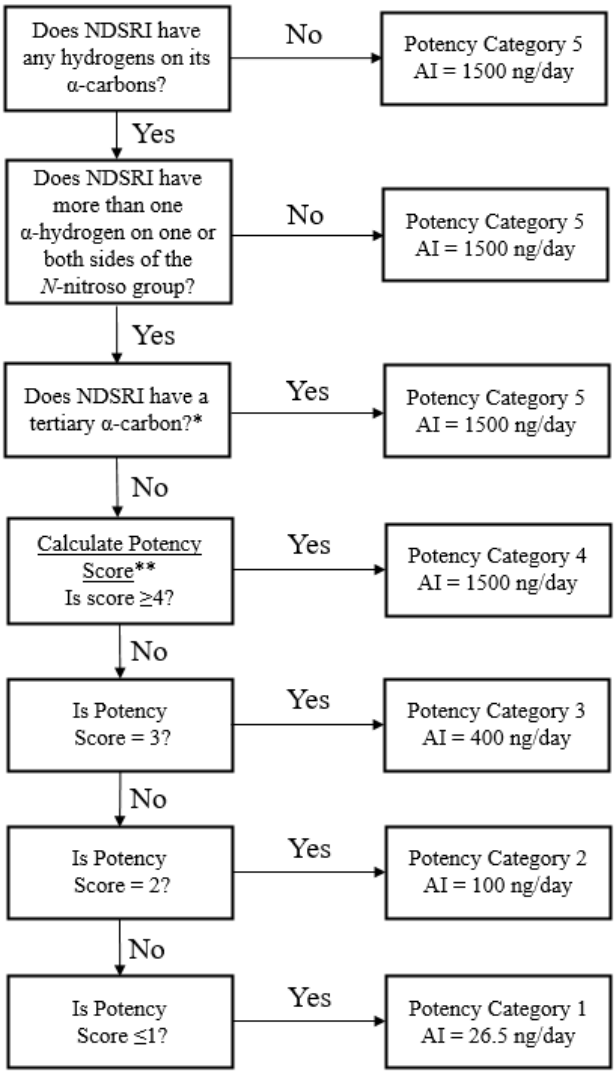
C. Determining the Predicted Carcinogenic Potency Categorization and Associated Recommended AI Limit

The flowchart shown in Figure 1 describes the recommended process of assigning an NDSRI to a predicted carcinogenic potency category, with a corresponding recommended AI limit, based on an assessment of activating and deactivating structural features present in the NDSRI. Two example calculations for determining the Potency Score and corresponding potency category are provided in Appendix A. The α- and β-carbons in the NDSRI referenced in the flowchart are defined relative to the *N*-nitroso group, as illustrated in Figure 2.

As noted above, the predicted carcinogenic potency categorization and recommended AI limit approach described in this guidance applies to NDSRIs bearing a carbon atom on both sides of the *N*-nitroso group, where the carbon is not directly double bonded to a heteroatom (i.e., *N*-nitrosamides, *N*-nitrosoureas, *N*-nitrosoguanidines and other related structures are excluded). Additionally, the potency categorization approach does not apply to NDSRIs where the *N*-nitroso group is within an aromatic ring (e.g., nitrosated indoles). Figure 1 illustrates how to predict the potency categorization of an NDSRI, and crosswalk to the recommended AI limit. For NDSRIs containing two *N*-nitroso groups, the group with the highest predicted carcinogenic potency category should be used to determine the recommended AI limit for the entire molecule.[26]

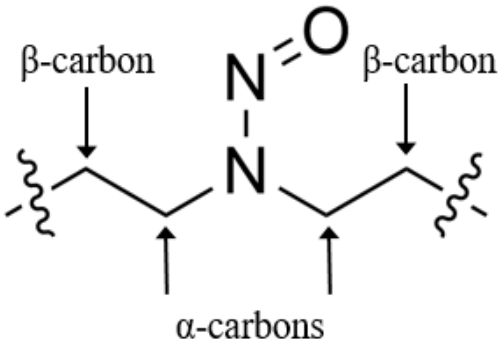
[26] For NDSRIs containing more than two *N*-nitroso groups, the applicant or manufacturer should contact the Agency for further guidance.

Figure 1. Flowchart to Predict the Carcinogenic Potency Category of an NDSRI and Identify an Associated Recommended AI Limit



NDSRI = nitrosamine drug substance-related impurities; AI = acceptable intake; ng = nanogram.
* A tertiary α-carbon is defined as an α-carbon atom in an sp^3 hybridization state, bonded to three other carbon atoms.
**To calculate Potency Score, see Appendix A.

Figure 2. Structural Representation of α- and β-carbons on an NDSRI.



D. Recommendations for Manufacturers and Applicants Whose Drug Products Contain Multiple Nitrosamines

As discussed above, the recommended AI limits for NDSRIs correspond to a single NDSRI in a drug product. If more than one nitrosamine impurity (NDSRI(s) and/or small molecule nitrosamine(s)) is identified and the total level of nitrosamines exceeds the recommended AI limit for the most potent nitrosamine in the drug product based on the maximum daily dose, the manufacturer or applicant should contact the Agency. FDA also recognizes that if the recommended AI limits for the individual nitrosamines vary greatly, basing the total nitrosamine limit on the most potent individual nitrosamine limit might not be practical. In such cases, FDA encourages manufacturers to contact the Agency if proposing a nitrosamine limit other than the recommended AI of the most potent nitrosamine impurity detected.[27]

E. Recommended AI Limits for NDSRIs at Risk of Formation in Certain APIs

FDA has identified a number of APIs that have secondary or tertiary amines and are therefore at risk for forming NDSRIs. Hypothetically, under certain conditions related to the formulation and manufacturing process for the drug product, such as residual nitrites in excipients used to formulate the drug product, these APIs could form NDSRIs. FDA applied the predicted carcinogenic potency categorization to these APIs. More specifically, based on scientific analysis of chemical structures, we have concluded that when an API contains hypothetical nitrosated forms of secondary amine- and dimethyl tertiary amine- groups, these recommended

[27] Applicants can discuss with FDA or propose an AI limit of total nitrosamine impurities through the following mechanism, as appropriate: Type C meeting requests for NDAs and 351(a) BLAs, pre-ANDA meetings, and pre-submission meetings for ANDAs as well as supplements, amendments, controlled correspondences for NDAs, ANDAs, and BLAs.

AI limits based on predicted carcinogenic potency should be applied to the NDSRIs to the extent that they form in these APIs.[28]

V. IMPLEMENTATION OF RECOMMENDED AI LIMITS FOR NDSRIs, INCLUDING THOSE BASED ON THE PREDICTED CARCINOGENIC POTENCY CATEGORIZATION

A. Recommended Timeline for Implementing Risk Assessments, Confirmatory Testing, and Submission of Required Changes for NDSRIs

FDA expects manufacturers and applicants to ascertain the presence of nitrosamine impurities, including NDSRIs, using the three-step mitigation strategy described in sections III and V of the Nitrosamine Guidance, but is clarifying here that the recommended timelines in that guidance do not apply to risk assessments, confirmatory testing, and submission of required changes related to NDSRIs.[29] For NDSRIs, FDA recommends different implementation timelines for manufacturers and applicants depending on the regulatory status of the drug product.

1. Recommended Timeline for Approved or Marketed Drug Products

FDA has learned that some manufacturers and applicants have considered the nitrosamine impurities originally identified in the Nitrosamine Guidance but have not considered NDSRIs in their risk assessments. FDA recommends that if NDSRIs were not considered in previous risk assessments, manufacturers and applicants reevaluate the risk *within 3 months* of publication of this guidance, with a recommended completion date by November 1, 2023, as part of overall risk management. Confirmatory testing using sensitive and appropriately validated methods should start as soon as the risk of an NDSRI is identified and should begin immediately for drug products considered to be at high risk. If NDSRIs are detected in the drug product at levels that exceed the recommended AI limit (e.g., associated with their predicted carcinogenic potency category), then FDA recommends that manufacturers and applicants develop control strategies and/or design approaches to control NDSRIs within acceptable levels.[30]

To enable manufacturers and applicants to complete the three-step mitigation strategy based on increased awareness of NDSRIs, FDA recommends conclusion of NDSRI confirmatory testing

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[28] To reflect the evolving and highly technical nature of the relevant information, FDA intends to provide a list of APIs that hypothetically could be at risk of forming NDSRIs due to the presence of secondary or tertiary amines. This list will include associated recommended AI limits for these hypothetical NDSRIs based on predicted carcinogenic potency categorization, in connection with this guidance, at the CDER web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related. This is not an all-inclusive list of APIs that have the potential to form NDSRIs. In addition, the recommended AI limits for the APIs on this website do not necessarily correspond to the presence or level of any NDSRI(s) found in any particular drug product. This associated information will be updated periodically. For the most recent version, check the web page.

[29] See, e.g., 21 CFR 314.60, 314.70, 314.96, 314.97, and 601.12.

[30] See section V.B.1. FDA intends to issue additional guidance on approaches to reduce or prevent NDSRI formation in drug products.

of drug products and submission of required changes in drug applications by August 1, 2025.[31] By August 1, 2025, manufacturers and applicants should ensure that any NDSRIs in their drug products meet the FDA-recommended AI limit (e.g., associated with their predicted carcinogenic potency category).[32]

FDA acknowledges that the implementation timeline includes investigating the root causes of NDSRI impurities, identifying effective changes as appropriate (e.g., changes in manufacturing process, component supplier, or product formulation),[33] and confirming that any proposed changes will minimize the presence of NDSRIs without otherwise adversely affecting drug product quality. FDA may request an expedited risk assessment, confirmatory testing, or other regulatory action based on information available to the Agency.

If a risk of NDSRIs in a drug product is identified, confirmatory testing of batches should be conducted using sensitive and appropriately validated methods.[34] If a nitrosamine impurity is detected, manufacturers should investigate the root cause and implement changes in the manufacturing process or product formulation to eliminate, mitigate, or reduce nitrosamine impurities consistent with the recommendations in the Nitrosamine Guidance.[35] Manufacturers must implement any changes in accordance with appropriate requirements.[36] In addition, applicants must submit proposed formulation changes through supplements or amendments to their applications.[37] Generally, FDA considers reformulation of an approved drug product a major change requiring a prior approval supplement (PAS).[38]

If an NDSRI falls at or below the FDA-recommended AI limit (e.g., associated with its predicted carcinogenic potency category),[39] the manufacturer or applicant should still develop an appropriate control strategy to ensure that the nitrosamine level is reliably at or below that AI

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[31] See, e.g., 21 CFR 314.60, 314.70, 314.96, 314.97, and 601.12.

[32] 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. In those cases, we recommend that manufacturers complete this process in time to submit any relevant changes to applications by August 1, 2025.

[33] See, e.g., FDA, Updates on Possible Mitigation Strategies To Reduce the Risk of Nitrosamine Drug Substance-Related Impurities in Drug Products, available at https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities. Last accessed June 4, 2023.

[34] To reflect the evolving and highly technical nature of the relevant information, FDA may provide certain updated information on testing methods for NDSRIs, in connection with this guidance, at the CDER web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related. This associated information will be updated periodically. For the most recent version, check the web page.

[35] See Nitrosamine Guidance at 14.

[36] See, e.g., requirements related to manufacturing changes in section 506A of the FD&C Act (21 U.S.C. 356a). See also 21 CFR 314.70, 21 CFR 314.97, and 21 CFR 601.12. Regardless of the change, the methods used in, and the facilities and controls used for, the manufacture, processing, packing, or holding of a product, including packaging and labeling operations, testing, and quality control of products, must comply with CGMP (see sections 501 of the FD&C Act (21 U.S.C. 351) and 21 CFR parts 210, 211, 212, and 600 through 680.) All manufacturing changes must be reviewed and approved by the quality control unit (see 21 CFR 211.100(a) and 211.160(a)).

[37] 21 CFR 314.70, 314.97, and 601.12

[38] See 21 CFR 314.70(b); see also 21 CFR 314.97(a) and 601.12(b).

[39] 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.

limit. Where applicable, applicants should report changes to an approved application.[40] If an NDSRI level is detected above the FDA-recommended AI limit (e.g., associated with its predicted carcinogenic potency category),[41] see the recommendations in section V.B.

2. Recommended Timeline for Drug Products in Development and Under FDA Review

a. Pre-submission stage

FDA recommends that an applicant conduct a risk assessment for NDSRIs and conduct confirmatory testing as appropriate prior to submission of an original application. However, the risk assessment and submission of confirmatory testing, if appropriate, and changes to the drug master file or application may be submitted in an amendment if these are not available at the time of the original application submission. Such an amendment should be submitted as quickly as possible after the original application submission to minimize any potential adverse impact on the application assessment timeline.[42]

b. Applications pending with the Agency

Applicants with pending applications should conduct the risk assessment expeditiously and inform FDA if confirmatory testing finds NDSRI levels above the AI limits recommended in this guidance.[43] If an NDSRI is detected above the recommended AI limit, the applicant should amend the application as appropriate. The Agency will work with the applicant in an effort to resolve issues during the review cycle.

FDA intends to review applications consistent with the performance goals and procedures agreed to by FDA and industry in the respective *commitment letters* that accompany the Prescription Drug User Fee Act reauthorization for fiscal years 2023 to 2027 (PDUFA VII) and the Generic Drug User Fee Amendments reauthorization for fiscal years 2023 to 2027 (GDUFA III).

B. Recommendations for Manufacturers and Applicants That Have Marketed Products With Levels of NDSRIs Above the FDA Recommended AI Limits

Generally, any drug product batch found to contain levels of an NDSRI above the FDA-recommended AI limit (e.g., associated with its predicted carcinogenic potency category),[44] should not be released by the drug product manufacturer for distribution and may warrant removal from the market, because such drug products may be considered adulterated under

[40] See 21 CFR 314.70(b)(2) and the guidance for industry *Changes to an Approved NDA or ANDA* (April 2004); see also 21 CFR 314.97 and 21 CFR 601.12.
[41] 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.
[42] For NDA submissions, applicants should discuss the need for an amendment with the Agency at the pre-NDA stage.
[43] For NDAs, the applicant should contact the review regulatory project manager for the specific product. For ANDAs, the applicant should contact the project manager specified for the ANDA.
[44] 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.

section 501 of the FD&C Act,[45] 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.[46] Manufacturers should contact the Agency if a recall is initiated.[47]

1. Additional Considerations for Maintaining the Drug Supply for Marketed Products

If drug product batches already in distribution contain levels of NDSRIs above the FDA-recommended AI limit (e.g., associated with their predicted carcinogenic potency category),[48] 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 Research's Drug Shortage Staff at drugshortages@fda.hhs.gov. Contacting the Drug Shortage Staff can assist manufacturers in meeting any obligations to report discontinuances or interruptions in their drug manufacture under section 506C of the FD&C Act and implementing regulations under 21 CFR 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 products.[49]

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 or not 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 distribution of such drug product batches that contain NDSRI levels at or below the recommended interim AI limit during the specified period under certain circumstances on a case-by-case basis.

In certain cases where FDA does not intend to object to the distribution of drug products from multiple drug manufacturers that contain NDSRI levels at or below the recommended interim AI limit, FDA intends to post such recommended interim AI limit on the FDA website in connection with this guidance.[50]

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

[46] FDA, Drug Shortages Management, Manual of Policies and Procedures 4190.1 Rev.3 (Nov. 30, 2018). 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-manual-policies-procedures-mapp. See also section 506D(b) and (c) of the FD&C Act.

[47] Manufacturers 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.

[48] 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.

[49] See footnote 46.

[50] 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 CDER web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/updated-information-recommended-acceptable-intake-limits-nitrosamine-drug-substance-related. This associated information will be updated periodically. For the most recent version, check the web page.

A decision to release lots remains solely the firm's responsibility. Firms are responsible for ensuring that their drugs are manufactured in compliance with all applicable requirements, including CGMP, and FDA expects them to vigilantly monitor and promptly report to FDA any adverse drug experiences or other findings that may affect product quality or safety.[51]

C. Approaches to Justify or Qualify a Proposed Alternative AI Limit

If the observed level of an NDSRI in a drug product exceeds the FDA-recommended AI limit (e.g., associated with the predicted carcinogenic potency category for that NDSRI), the Agency recommends that manufacturers and applicants pursue mitigation efforts to reduce or remove the NDSRI.[52] A manufacturer or applicant should submit a scientifically justified rationale to pursue an AI limit higher than the FDA-recommended limit associated with the predicted carcinogenic potency category for that NDSRI. Alternative approaches using safety data, such as obtaining compound-specific data or using read-across assessment to a suitable surrogate, could be used to support a higher AI limit. Importantly, manufacturers and applicants should note that the Agency may request additional safety data, beyond what is described here, to support alternative AI limits. If compound-specific data or a read-across approach is pursued, we recommend the following:

a. Mutagenicity assessment:[53,54] When testing the NDSRI using a bacterial mutagenicity assay (Ames assay), FDA recommends use of the full complement of testing strains described in Organisation for Economic Co-operation and Development (OECD) 471,[55] utilizing the preincubation method and using both rat and hamster S9 at concentrations of 30 percent. FDA recommends using S9 fractions typically prepared from animals treated with an inducer of CYP450s, like a combination of phenobarbital and beta naphthyl flavone. Manufacturers and applicants should use the recommended pre-incubation time of 30 minutes as part of the optimal conditions for detecting a signal for mutagenicity.

b. Read-across assessment based on surrogate: An NDSRI may have a well-justified AI limit based on a surrogate that maintains a 1 in 100,000 cancer risk estimate and is higher than the recommended AI limit associated with the predicted carcinogenic

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[51] See, e.g., 21 CFR 314.80(a) and (c), and 314.81(b)(1).

[52] FDA, Updates on Possible Mitigation Strategies To Reduce the Risk of Nitrosamine Drug Substance-Related Impurities in Drug Products, available at https://www.fda.gov/drugs/drug-safety-and-availability/updates-possible-mitigation-strategies-reduce-risk-nitrosamine-drug-substance-related-impurities. Last accessed June 4, 2023.

[53] For nitrosamines, enhanced testing conditions for the Ames assay are recommended due to the reported reduced sensitivity of the assay under standard conditions for some nitrosamines. FDA is investigating experimental conditions that improve Ames assay sensitivity for nitrosamines and NDSRIs. See also Li X et al., 2023, Revisiting the Mutagenicity and Genotoxicity of N-nitroso Propranolol in Bacterial and Human In Vitro Assays, Regul Toxicol Pharmacol, 141:105410.

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

[55] See OECD (2020), Test No. 471 Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, available at https://doi.org/10.1787/9789264071247-en .

potency category for that NDSRI in certain cases. When conducting a read-across assessment based on a surrogate, manufacturers and applicants can perform a (Q)SAR analysis to predict mutagenic potential by following recommendations described in ICH M7(R2). To calculate an AI limit, the Agency recommends selection of surrogates with robust carcinogenicity data.[56] The Agency recognizes that a robustly tested surrogate nitrosamine that adequately represents the structural and mechanistic characteristics of the NDSRI is frequently unavailable. Therefore, when appropriately justified, the following compounds should be considered for use as surrogates due to the availability of robust mutagenicity and carcinogenicity data: NDMA, *N*-nitroso piperidine (NPIP), 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone (NNK), *N*-nitroso pyrrolidine (NPYR), and *N*-nitroso morpholine (NMOR).[57]

Approaches to justify or qualify a proposed alternative AI limit, as described in this section, should be submitted to FDA in a supplement or amendment, as appropriate, before implementation.[58]

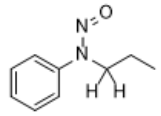
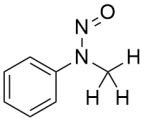
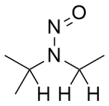
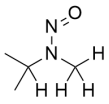
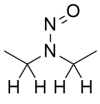
[56] See also section III.
[57] See footnote 12.
[58] Generally, changes in specifications from those in the approved application must be submitted in a prior approval supplement unless otherwise exempted by regulation or guidance (see 21 CFR 314.70(b)(2) and 314.97(a) and the guidance for industry *Changes to an Approved NDA or ANDA*; see also 21 CFR 601.12(b)(2)).

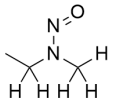
APPENDIX A: DETERMINATION OF POTENCY SCORE

For nitrosamine drug substance-related impurities (NDSRIs) not assigned to Potency Category 5, the Potency Score is calculated as the sum of the α-Hydrogen Score (Table A), Deactivating Feature Score (Table B), and Activating Feature Score (Table C) based on selected structural features present in the NDSRI. An NDSRI structure is expected to match exactly one of the α-hydrogen definitions in Table A, but it may contain multiple or no structural features identified in Tables B and C. In cases where one or more features from Tables B and C are contained in the NDSRI, the Potency Score should be calculated as outlined in the box below. In cases where the NDSRI contains no features from Tables B and C, the Potency Score will be equal to the α-Hydrogen Score.

Potency Score = **α-Hydrogen Score** + **Deactivating Feature Score** (sum all scores for features present in NDSRI) + **Activating Feature Score** (sum all scores for features present in NDSRI)

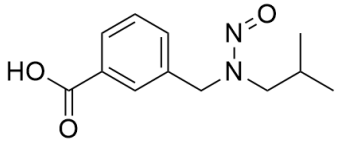
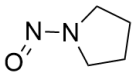
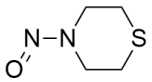
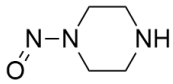
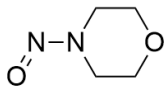
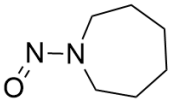
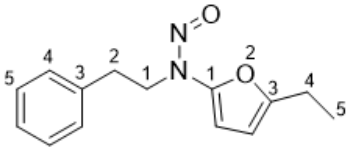
Table A. Count of hydrogen atoms on each α-carbon (lowest count first) and corresponding α-Hydrogen Score. Examples are intended to be illustrative only and are not intended to be exhaustive.

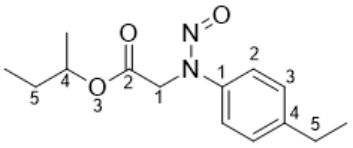
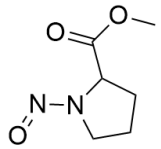
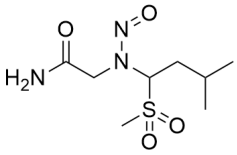
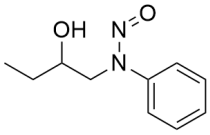
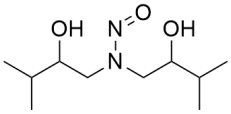
Count of Hydrogen Atoms on Each α-Carbon, Lowest First	Example	α-Hydrogen Score
0,2		3[$]
0,3		2
1,2		3
1,3		3
2,2		1

2,3		1

[$]A score of 3 applies when the methylene α-carbon is not part of an ethyl group. If the methylene α-carbon is part of an ethyl group, a score of 2 should be applied.

Table B. List of deactivating features and associated scores. To calculate Deactivating Feature Score, sum the individual scores for all listed features present in the NDSRI structure. Each deactivating feature row in the table may only be counted once. For NDSRIs where the *N*-nitroso group is within more than one ring, the feature score for only the smallest matching ring should be applied. Examples are intended to be illustrative only and are not intended to be exhaustive.

Deactivating Feature	Example	Individual Deactivating Feature Score
Carboxylic acid group anywhere on molecule		+3
N-nitroso group in a pyrrolidine ring		+3
N-nitroso group in a 6-membered ring containing at least one sulfur atom		+3
N-nitroso group in a 5- or 6-membered ring[*]		+2
N-nitroso group in a morpholine ring		+1
N-nitroso group in a 7-membered ring		+1
Chains of ≥5 consecutive non-hydrogen atoms (cyclic or acyclic) on both sides of acyclic *N*-nitroso group. Not more than 4 atoms in each chain may be in the same ring.		+1

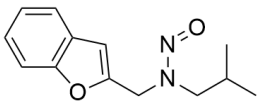
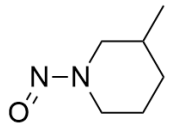
		
Electron-withdrawing group** bonded to α-carbon on <u>only one</u> side of *N*-nitroso group (cyclic or acyclic)		+1
Electron-withdrawing groups** bonded to α-carbons on <u>both</u> sides of *N*-nitroso group (cyclic or acyclic)		+2
Hydroxyl group bonded to β-carbon*** on <u>only one</u> side of *N*-nitroso group (cyclic or acyclic)		+1
Hydroxyl group bonded to β-carbons*** on <u>both</u> sides of *N*-nitroso group (cyclic or acyclic)		+2

* Excludes examples where *N*-nitroso group is in a pyrrolidine ring, a 6-membered ring containing at least one sulfur atom, or a morpholine ring (all counted separately).

**Excludes carboxylic acid and aryl (counted separately), and ketone (conflicting data). Additional electron withdrawing group examples are limited to those described in Cross KP and Ponting DJ, 2021, Developing Structure-Activity Relationships for *N*-Nitrosamine Activity, Comput Toxicol, 20:100186, where they are referred to as "β-carbon electron withdrawing groups."

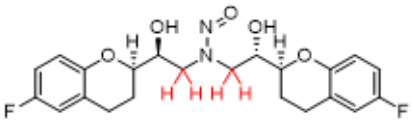
***β-Carbon must be in an sp^3 hybridization state for this feature to apply.

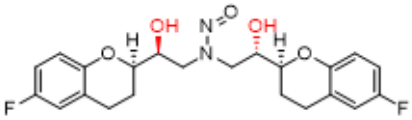
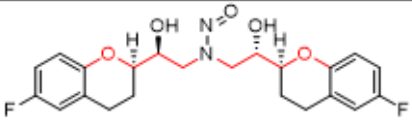
Table C. List of activating features and associated scores. To calculate Activating Feature Score, sum the individual scores for all listed features present in the NDSRI structure. Each activating feature row in the table may only be counted once. Examples are intended to be illustrative only and are not intended to be exhaustive.

Activating Feature	Example	Individual Activating Feature Score
Aryl group bonded to α-carbon (i.e., benzylic or pseudo-benzylic substituent on *N*-nitroso group)		-1
Methyl group bonded to β-carbon (cyclic or acyclic)		-1

Example 1 below shows how the predicted carcinogenic potency categorization approach flow chart (Figure 1) can be applied to the hypothetical NDSRI, *N*-nitroso-l-nebivolol. A Potency Score of 4 is calculated for *N*-nitroso-l-nebivolol, resulting in its placement in Potency Category 4 with an associated recommended acceptable intake limit of 1500 nanograms (ng)/day.

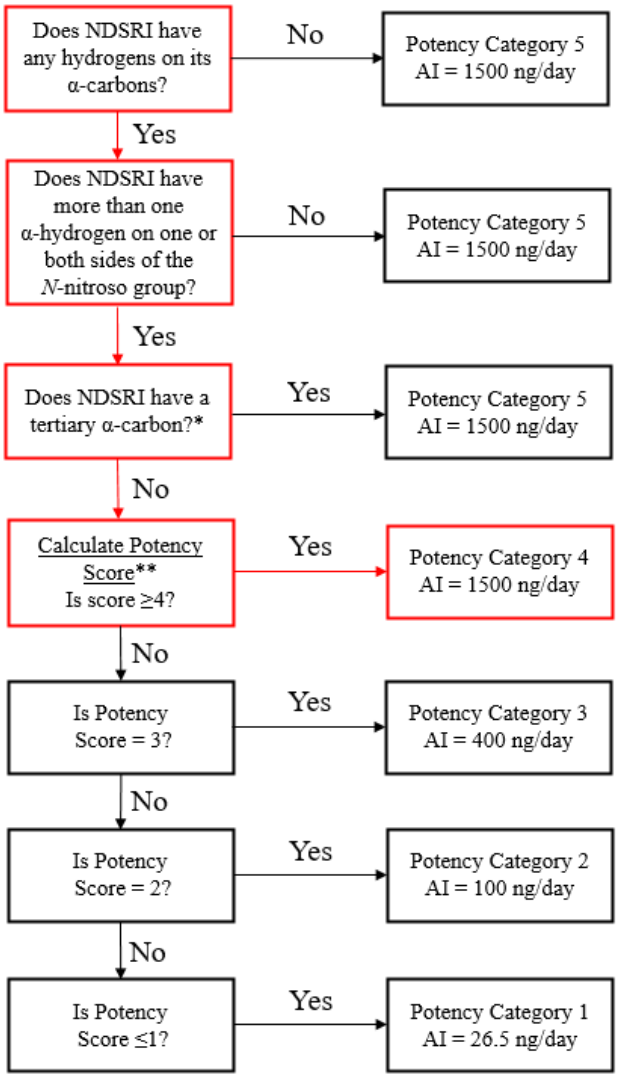
Example 1 – *N*-Nitroso-l-nebivolol

Count of α-Hydrogens	Score	Feature Highlighted in Red
2,2	1	

Deactivating Features	Score	Feature Highlighted in Red
Hydroxyl group bonded to β-carbons*** on both sides of *N*-nitroso group (cyclic or acyclic)	+2	
Chains of ≥5 consecutive non-hydrogen atoms (cyclic or acyclic) on both side of acyclic *N*-nitroso group. Not more than 4 atoms in each chain may be in the same ring.	+1	
No Activating Features Present		

Potency Score = 1 + 2 + 1 = 4	Potency Category 4	AI = 1500 ng/day

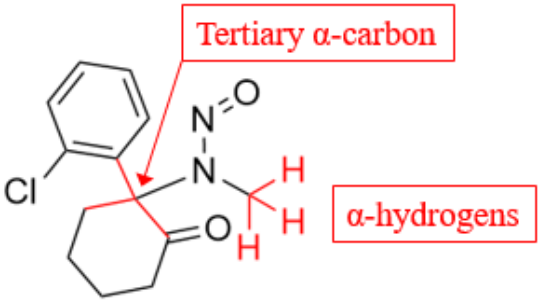
***β-Carbon must be in an sp^3 hybridization state for this feature to apply.



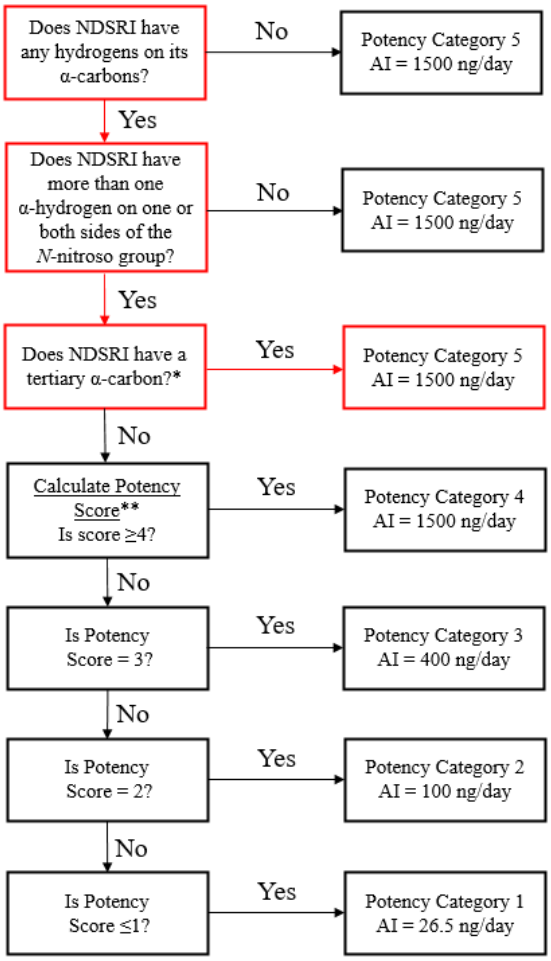
NDSRIs = nitrosamine drug substance-related impurities; AI = acceptable intake; ng = nanogram.
* A tertiary α-carbon is defined as an α-carbon atom in an sp^3 hybridization state, bonded to three other carbon atoms.
**To calculate Potency Score, see Appendix A.

Example 2 below shows how the predicted carcinogenic potency categorization approach flow chart (Figure 1) can be applied to the hypothetical NDSRI, *N*-nitroso-ketamine. *N*-nitroso-ketamine is placed in Potency Category 5 with an associated recommended acceptable intake limit of 1,500 ng/day.

Example 2 – *N*-Nitroso-ketamine



Potency Category 5	**AI = 1500 ng/day**



NDSRI = nitrosamine drug substance-related impurities; AI = acceptable intake; ng = nanogram.
* A tertiary α-carbon is defined as an α-carbon atom in an sp^3 hybridization state, bonded to three other carbon atoms.
**To calculate Potency Score, see Appendix A.