

New Dietary Ingredient (NDI) Notifications and Related Issues; Identity and Safety Information About the NDI: Guidance for Industry

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For questions regarding this document, contact the Human Foods Program at 240-402-1200.

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**U.S. Department of Health and Human Services
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Human Foods Program**

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New Dietary Ingredient (NDI) Notifications and Related Issues; Identity and Safety Information About the NDI: Guidance for Industry¹

This guidance represents the current thinking of the Food and Drug Administration (FDA or we) 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 the approach 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

Under section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 350b(a)(2)), the manufacturer or distributor of a new dietary ingredient (NDI) that has not been present in the food supply as an article used for food, or of a dietary supplement that contains the NDI, must submit a new dietary ingredient notification (NDIN) to FDA at least 75 days before introducing the product into interstate commerce. The notification must contain the information that provides the basis on which the manufacturer or distributor of the NDI or dietary supplement containing the NDI has concluded that product will reasonably be expected to be safe under the intended conditions for use. (*Id.*; see also 21 CFR 190.6.)

This guidance is intended to help provide recommendations to manufacturers and distributors of NDIs and dietary supplements containing an NDI regarding the information that should be included in an NDIN. This guidance finalizes the recommendations in Sections VI.A, Identity Information about the NDI and the Dietary Supplement, VI.B, History of Use or Other Evidence of Safety, and VI.C, Summary of the Basis for Your Conclusion of Safety, of FDA's revised draft guidance, *Dietary Supplements: New Dietary Ingredient Notifications and Related Issues*. In this guidance, the terms "you" or "your" refer to the manufacturer or distributor of an NDI or dietary supplement containing an NDI.

In July 2011, we published a draft guidance entitled "Guidance for Industry: Dietary Supplements: New Dietary Ingredient Notifications and Related Issues," in compliance with our statutory requirements under section 113(b) of the FDA Food Safety Modernization Act (Pub. L. 111-353). This draft guidance discussed FDA's views and recommendations on when an ingredient intended for use in a dietary supplement is an NDI, when the requirement to submit an NDIN to FDA applies, the types of data and information that manufacturers and distributors

¹ This guidance has been prepared by the Office of Food Chemical Safety, Dietary Supplements & Innovation (OFCSDSI) and the Office of Policy, Regulations and Information & International Engagement (OPIE) in the Human Foods Program at the U.S. Food and Drug Administration.

should consider when they evaluate the safety of a dietary supplement containing an NDI, what to include in an NDIN (including recommendations about identity and safety information), and the procedures for submitting an NDIN. In 2016, we replaced the 2011 draft guidance with a revised draft guidance, a portion of which is being finalized in this document. Since issuing this revised draft guidance in 2016, FDA has finalized other portions of the guidance through subsequent guidance documents. We have also updated the 2016 revised draft guidance to indicate which language remains in “draft” status. We understand the importance of finalizing other parts of the 2016 revised draft guidance, and we plan to finalize other individual sections as we complete our review and analysis of those sections.

FDA’s guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA’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 FDA’s guidances means that something is suggested or recommended, but not required.

II. Background

The term “dietary supplement” is defined in section 201(ff) of the FD&C Act (21 U.S.C. 321(ff)), and the term “new dietary ingredient” is defined in section 413 of the FD&C Act. Under section 413(a)(2) of the FD&C Act, the manufacturer or distributor of an NDI, or of the dietary supplement that contains the NDI, must submit a premarket notification to FDA at least 75 days before introducing the product into interstate commerce or delivering it for introduction into interstate commerce, unless the NDI and any other dietary ingredients in the dietary supplement “have been present in the food supply as an article used for food in a form in which the food has not been chemically altered,” as set forth in section 413(a)(1) of the FD&C Act.

As defined in section 201(ff)(1) of the FD&C Act, a “dietary ingredient” is any one of the following:

- A vitamin;
- A mineral;
- An herb or other botanical;
- An amino acid;
- A dietary substance for use by man to supplement the diet by increasing the total dietary intake; or
- A concentrate, metabolite, constituent, extract, or combination of any dietary ingredient from the preceding categories.

An NDI is defined in section 413(d) of the FD&C Act as a dietary ingredient that was not marketed in the United States before October 15, 1994. Thus, a substance cannot be an NDI unless it is also a dietary ingredient. Dietary ingredients marketed in the United States before October 15, 1994, are not NDIs and, therefore, do not require an NDIN.

Under section 413(a)(2) of the FD&C Act, an NDIN must contain the information, including any citation to published articles, that provides the basis on which the manufacturer or distributor of the NDI or dietary supplement (the notifier) has concluded that the dietary supplement containing the NDI will reasonably be expected to be safe. If the required premarket notification is not submitted to FDA, section 413(a) of the FD&C Act provides that the dietary supplement containing the NDI is deemed to be adulterated under section 402(f) of the FD&C Act (21 U.S.C. 342(f)). Even if the notification is submitted as required, under section 413(a) of the FD&C Act, the dietary supplement containing the NDI is adulterated under section 402(f) of the FD&C Act unless there is a history of use or other evidence of safety establishing that the NDI, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. For clarification, FDA does not approve NDI notifications; no FDA approval is required or issued at any point in this process.

To help industry comply with the FD&C Act's premarket notification requirements for dietary supplements that contain an NDI, FDA issued an NDIN regulation (21 CFR 190.6 and 62 FR 49886, September 23, 1997). The NDIN regulation specifies the information the manufacturer or distributor must include in its premarket NDIN (see 21 CFR 190.6(b)):

- The name and complete address of the manufacturer or distributor that is submitting the notification (21 CFR 190.6(b)(1));
- The name of the NDI that is the subject of the premarket notification. For botanicals, the Latin binomial name must be given, including the author citation (i.e., the name of the scientist who gave the botanical its Latin binomial name) (21 CFR 190.6(b)(2));
- A description of the dietary supplement (or dietary supplements) that contains the NDI, including:
 - the level of the NDI in the dietary supplement (21 CFR 190.6(b)(3)(i)); and
 - the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the supplement's labeling, the ordinary conditions of use of the supplement (21 CFR 190.6(b)(3)(ii));
- The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe (21 CFR 190.6(b)(4));² and
- The signature of a person authorized by the manufacturer or distributor to sign the notification on its behalf (21 CFR 190.6(b)(5)).

This guidance focuses on the NDIN requirements found at 21 CFR 190.6(a) and 190.6(b)(2), (3), and (4) and provides FDA's recommendations about what you should submit in your NDIN to satisfy these requirements.

² For purposes of this guidance, FDA is using the phrase "proposed conditions of use" as shorthand for the language in 21 CFR 190.6(b)(4) stating "conditions recommended or suggested in the labeling of the dietary supplement."

III. Definitions

The following definitions represent FDA's current thinking on the meaning of the terms below in the context of the new dietary ingredient provisions of the FD&C Act and regulations. The definitions are intended for use only in that context and may not be appropriate in other contexts.

Acceptable Daily Intake (ADI): The maximum amount of the ingredient that can be ingested daily over a lifetime without an appreciable health risk.

Acceptance criteria: Numerical limits, ranges, or other criteria for the tests described. They are used to determine whether to accept or reject the ingredient or product being analyzed.

Alternative methods (also referred to as new alternative methods or new approach methods (NAMs)): Innovative testing strategies that reduce, refine, or replace traditional animal testing.

Amino acid: An alpha-amino carboxylic acid used as a constituent of proteins or peptides.³

Botanical or herbal: A plant, alga, or fungus; a part of a plant, alga, or fungus (e.g., bark, leaves, stems, roots, flowers, fruits, seeds, berries, or parts thereof); or an exudate (secretion) of a plant, alga, or fungus.

Component: A substance that is part of a mixture, including substances that cannot be isolated from the whole, as well as those that can. Once isolated, a component of a mixture is also a constituent.

Concentrate: An article in which constituents are more concentrated than in the original. An herbal concentrate is an extract from which all or most of the extraction solvent has been removed, reducing the product to a solid, semi-solid, or syrupy form. The solvent and the process by which the concentrate is made are part of the definition of the concentrate.

Configurational isomer: See **Stereoisomers**.

Constituent: An article that is a physical part of the whole and can be isolated from the whole.

Enantiomers: Mirror-image isomers (optical isomers) that generally have similar chemical and physical properties, but different biological properties in a chiral environment.

Estimated Daily Intake (EDI): The highest total daily intake level of the ingredient under the proposed conditions of use.

EDI/ADI Ratio: The quantitative comparison of the estimated versus the acceptable daily intake.

³ Letter from Michael M. Landa, Acting Director, Center for Food Safety and Applied Nutrition, FDA, to Marc Ullman, Ullman, Shapiro & Ullman, LLP, responding to Citizen Petition FDA-2009-P-0298 from OVOS Natural Health Inc. (Feb. 23, 2011). Docket No. FDA-2009-P-0298 [Document ID: FDA-2009-P-0298-0008]. Available at: <https://www.regulations.gov/document/FDA-2009-P-0298-0008> accessed December 17, 2025).

Extract: A product consisting of a solvent combined with a dietary substance or botanical biomass by a process that physically separates constituents from the dietary substance or botanical and dissolves them into the solvent. The extract can be further concentrated through drying to a dry powder or semi-solid form.

Formulation: A formula that: (1) lists the identity and quantity of each dietary ingredient and other ingredients (formulation aids) of a dietary supplement; and (2) describes the administered form (e.g., powder, liquid, capsule).

Geometric isomers: Compounds that have the same molecular formula but differ from each other in the way that the atoms are oriented in space, and therefore have different chemical, physical, and biological properties (unless interconverted in the gut).

Ingestion: Taking an article, such as a dietary supplement or other food, into the stomach and gastrointestinal tract by swallowing.

In-process controls: Checks, tests, or other measures performed at any point, step, or stage during the manufacturing process to monitor the process and, as appropriate, adjust operations to ensure conformance with established specifications. Examples of in-process controls include blend uniformity testing, weight checks, temperature control, and pH monitoring.

Live microbial dietary ingredient: A single-celled prokaryotic or eukaryotic microorganism that is intended to be viable at the point of ingestion.

Margin of safety: A calculation derived from the NOAEL (No-Observed-Adverse-Effect Level) in a single study and the highest total daily intake level determined from the proposed conditions of use (the EDI). A margin of safety is a measure of how close the EDI is to the level that has been shown to have no adverse effect in animal or human studies (the NOAEL).

Maximum tolerated dose (MTD): The highest dose that causes no more than a 10 percent reduction in body weight and does not produce mortality, clinical signs of toxicity, or pathologic lesions that would be predicted to shorten the natural life span of an experimental animal for any reason other than the induction of neoplasms.⁴

Metabolite: In the dietary supplement context, a metabolite of a dietary ingredient is a molecular intermediate that incorporates structural elements of the ingested dietary ingredient and whose flux or net production in the human body increases on ingestion of the dietary ingredient. A metabolite can be part of (or an intermediate of) the catabolic or metabolic pathway of a dietary ingredient. FDA considers X to be a metabolite of Y if ingestion of Y by humans results in net production of/increased flux of X, incorporating structural elements of Y.⁵

⁴ Hayes, A.W., & Kruger, C.L. (Eds.). (2014). Hayes' Principles and Methods of Toxicology (6th ed.). CRC Press. Available at: <https://doi.org/10.1201/b17359> (accessed December 17, 2025).

⁵ See Hardy, Constance J. (Executive Secretary, Dietary Supplements Subcommittee of the FDA Food Advisory Committee). Summary Minutes of March 25, 2003, Meeting of the Dietary Supplements Subcommittee; College

Mineral: A substance of defined chemical composition which provides a form or source of inorganic elements to the diet. An element is one of a class of substances that cannot be separated into simpler substances by chemical means. Examples: calcium, iodine, and zinc.

No-Observable-Effect Level (NOEL): The highest dose or total daily intake level at which no effects of any kind (beneficial, neutral, or adverse) are observed in a properly designed and executed toxicological study.

No-Observed-Adverse-Effect Level (NOAEL): The highest dose or total daily intake level that did not elicit an adverse effect in a properly designed and executed toxicological study.⁶

Quality control: A planned and systematic operation or procedure for ensuring that a dietary supplement consistently meets established specifications for the dietary supplement.

Salt of a dietary ingredient: A neutral compound that is formed by the union of an acid or a base with a counter ion and that dissociates to the starting ingredients after ingestion.

Safety factor or uncertainty factor: A multiplier used to account for uncertainty about the extent to which data gathered in one context can be used to predict the safety of a substance in other contexts. For example, safety factors attempt to account for differences between animals and humans (uncertainty factor of interspecies variation), differences in sensitivity among humans (uncertainty factor of intraspecies variation), and extrapolation of subchronic to chronic data (uncertainty factor of extrapolated data from subchronic to chronic). Safety factors can be combined multiplicatively to account for multiple sources of uncertainty. Safety factors are used in calculating an acceptable daily intake (ADI) for various FDA-regulated products, including color additives, food additives, and new animal drugs.

Stereoisomers: Molecules that are identical in atomic composition and bonding but differ in the three-dimensional arrangement of the atoms.

Subchronic: Refers to toxicological studies that are 1 to 3 months in duration.

Target population: The population group or groups (e.g., sex, age, health status) that a manufacturer or distributor identifies (e.g., in product labeling, promotional materials, an NDIN) as those for whom the product is appropriate or recommended. Examples of target populations include adults, children 14 and over, and women going through menopause.

Traditional medicine: The sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not,

Park, MD; dated June 3, 2003. Available at: <https://www.fda.gov/media/177658/download?attachment> (accessed December 17, 2025).

⁶ See Hayes, A. Wallace, editor. Principles and Methods of Toxicology. 5th ed. Boca Raton: CRC Press; 2023.

used in the maintenance of health, as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illnesses.⁷

Uncertainty factor: See **Safety factor**.

Vitamin: An organic substance that is a minor component of foods, is essential for normal physiological functions (e.g., maintenance, growth, or development), is normally not produced endogenously (within the body) in amounts adequate to meet normal physiologic needs, and which causes, by its absence or underutilization, a clinically defined deficiency syndrome.

IV. Identity Information About the NDI and the Dietary Supplement Containing the NDI

1. What is the purpose of including information about the identity of the NDI and the dietary supplement containing the NDI in the NDIN?

The purpose of including identity information in an NDIN is:

- to establish what the NDI is, including that it is a dietary ingredient as described in section 201(ff)(1) of the FD&C Act;
- to identify the other ingredients and components of the dietary supplement; and
- to explain (we suggest through a brief narrative) the qualitative and quantitative relationship between the NDI and the substances that are the subject of the history of use or other evidence of safety in your notification (see question IV.5).

A clear and concise presentation of the information that you rely on to establish the identity of your NDI and, as applicable, your dietary supplement containing the NDI will facilitate the review of your NDIN. Without complete and accurate identity information, FDA cannot evaluate whether there is a history of use or other evidence establishing that the NDI or the dietary supplement containing the NDI will reasonably be expected to be safe under your proposed conditions of use.

2. What types of identity information should you include in the NDIN?

Under 21 CFR 190.6(b)(2) and (3), you must provide the following information regarding identity:

- the name(s) of the NDI(s) that is (are) the subject of the NDIN, including the Latin binomial name (which includes the author of the Latin binomial) of any herb or other botanical; and
- a description of the dietary supplement or dietary supplements that contain the NDI, including: (i) the level of the NDI in the dietary supplement; and (ii) the conditions of

⁷ G. Bodeker et. al. (2005). World Health Organization Global Atlas of Traditional, Complementary, and Alternative Medicine, page 13. Available at: <https://www.who.int/publications/i/item/9241562862> (accessed December 17, 2025).

use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the supplement's labeling, the ordinary conditions of use of the supplement.

In addition to the information required under 21 CFR 190.6, we recommend that you include the following information in the identity section of your NDIN to facilitate our review of the identity of your NDI and the basis for the safety of the dietary supplement:

- the trade name of the NDI (if different from the name of the NDI) and any other names by which the NDI is known;
- a description of the NDI, which should include the physical properties of the NDI, a description of the chemical or molecular composition or structure of the NDI, or both;
- verification of the NDI identity, which may be accomplished by providing analytical methods, data, and calculations;
- controls and/or acceptable ranges for batch-to-batch variability, where applicable;
- the identity and level of any impurities and contaminants that may adulterate or that may lead to adulteration of the NDI or the finished batch of the dietary supplement;
- additional information specific to the type of dietary ingredient, as recommended in question IV.6 (discrete chemical entity), IV.7 (configurational isomers), IV.8 (salts), IV.9 (covalently modified derivatives of a dietary ingredient), IV.10 and IV.11 (botanicals), IV.13 (extracts and concentrates), IV.14 (metabolites), IV.15 (enzymes), IV.16 (ingredients produced using fermentation), IV.17 (live microbial dietary ingredients), and IV.20 (similar substance or product);
- the identity and level of the other dietary ingredients and all other ingredients or components in the dietary supplement, such as those used for a technical or functional effect in the product (e.g., binders, fillers, and color additives⁸); and
- evidence that the other ingredients in the dietary supplement are of food grade purity.

For the purpose of an NDIN, FDA recommends that you establish identity specifications for the NDI and for those components of the NDI and dietary supplement that are relevant to establishing the basis for the safety of the dietary supplement. Your NDIN should provide a detailed description of the identity specifications in table form, as recommended in question IV.5. If you establish such specifications, FDA also recommends that you have data to support that such specifications are met in your NDIN.

In addition, for a manufactured NDI or dietary supplement containing the NDI, you should describe the manufacturing process, which helps FDA understand the identity of the NDI and the dietary supplement containing the NDI. You should provide detailed information about the

⁸ FDA also recommends that your NDIN list the applicable color additive regulation. See Color Additives Listed for Use in Food: Exempt from Certification (21 CFR 73, Subpart A) at <https://www.ecfr.gov/cgi-bin/text-idx?SID=79a76b1d7e7a98ae9459d88005ab7058&mc=true&node=pt21.1.73&rgn=div5>.

Color Additives Listed for Use in Food: Subject to Certification (21 CFR 74, Subpart A) at https://www.ecfr.gov/cgi-bin/text-idx?SID=79a76b1d7e7a98ae9459d88005ab7058&mc=true&node=pt21.1.74&rgn=div5#_top; Listing of Certified Provisionally Listed Colors and Specifications (21 CFR 82) at <https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-82>.

aspects of the manufacturing process that are relevant to safety and identity, as recommended in question IV.3 below.

3. How much detail should the description of the manufacturing process contain?

The description should have sufficient detail to enable FDA to understand the overall process used to make the NDI and the dietary supplement containing the NDI. You should identify any points in the process that you know to be relevant to the safety of the dietary supplement and describe the quality controls used in the manufacturing process (such as evaluating the performance of the manufacturing process to ensure conformance to proper specifications). In addition, you should provide any in-process controls and measurements used in the manufacturing process of your NDI and dietary supplement containing the NDI. Examples include critical parameter monitoring (e.g., gas flow rate, temperature, pH, mixing time), analytical testing at key stages (e.g., concentration, purity, blend uniformity), process verification (e.g., ensuring reactions, extractions, separations proceed correctly), and quality attribute testing (e.g., moisture, particle size, bulk density, appearance).

Detailed descriptions of manufacturing can be limited to those portions relevant to safety and identity, if they can be identified. For example, you might establish a specification to limit mold contamination of a component used to make your NDI (e.g., aflatoxin in corn). You might also use a specification for the temperature of a key extraction step to prevent formation of a toxic byproduct and/or a specification for that byproduct in an analysis of an interim material or of the final product. You may describe the entire process and all specifications or select only those that are relevant to the identity and safety information that provides the basis for the safety of your NDI and dietary supplement containing the NDI. Examples of manufacturing process information relevant to identity include extraction methods and solvents used (e.g., water extraction, ethanol extraction); process parameters such as temperature, pressure, and duration; concentration, drying, or other processing steps; and the physical form of the final NDI (e.g., powder, liquid, extract). Examples of manufacturing process information relevant to safety include standardization procedures to ensure consistent potency and composition across batches and filtration or purification steps to remove undesirable components.

4. What is a specification?

A specification is a set of standards developed by the manufacturer or distributor of a material (e.g., an NDI or a dietary supplement). The specification includes standards for each of the components of the material and for the entire material. For the purpose of an NDIN, FDA recommends that the specifications discussion include critical safety attributes. You may omit attributes not relevant to safety or identity. FDA also recommends that the specifications include a list of tests, the acceptance criteria for each test, and the analytical methods used to support the acceptance criteria.

FDA believes it is important for acceptance criteria to be specific. For example, it is not informative to state that a chromatogram or a spectrum “matches the reference sample” unless every peak matches (both height and location) or there is a description of which peak or peaks match and how they match (e.g., description of the acceptable variation in peak retention time

and peak height or area under curve). The use of “fingerprint” analysis of complex spectra or chromatography of mixtures containing many ingredients does not require knowledge of the identity of all or even any of the peaks, but FDA expects that it would involve matching sufficient numbers of peaks across the entire spectrum or chromatogram to ensure the validity of the test result. In addition, it is appropriate to identify components that are known to be toxic by a single acceptance criterion (e.g., “less than”). Acceptance criteria for other components (e.g., marker compounds in botanical extracts, vitamins, minerals, preservatives, antioxidants, or functional excipients) should be expressed as a range, as appropriate. The source and authenticity of analytical standards (or reference standards) should also be documented.

Analytical methods are an essential part of any specification and should contain a detailed set of directions that must be followed exactly for the results to be accepted for the stated purpose. The directions should cover all steps, from preparing the test sample to reporting the results of the analysis. A complete description of the method may be provided, whether it is in-house or included as a publication. For example, a description of a high-performance liquid chromatography (HPLC) method should include a description of the chromatographic column, column temperature, standards preparation, solvent elution conditions, flow rate, injection volume, run time, concentration calculations for analytes, and the source and authenticity of any reference standards that are integral to understanding how a method is used to identify the analyte.

5. What component specifications for your ingredients should be included in the notification?

Manufacturers and distributors of dietary supplements must establish specifications for the components of their products (see 21 CFR 111.70(b)). The required types of component specifications are:

- an identity specification for each component;
- component specifications necessary to ensure that specifications for the purity, strength, and composition of dietary supplements manufactured using the components are met; and
- limits on the types of contaminants that may adulterate or may lead to adulteration of the dietary supplement.⁹

Your notification should list and explain the role of specifications that are relevant to the identity of the NDI and to the safe consumption of the dietary supplement containing the NDI, including how you arrived at the criteria for acceptance or rejection based on the results of each test, check, or measurement in the specification. This can include specifications for starting materials used to make your NDI, process controls during manufacturing, and/or interim or final product specifications for the NDI or the dietary supplement containing the NDI. For ease of reference, we recommend listing the specifications in table form (see examples in Tables 1 and

⁹ This includes setting and measuring quantitative limits for contaminants that may be present in the source material or may result from the manufacturing process, which must be controlled to ensure that the dietary supplement consistently meets the established specifications.

2). You should describe the controls in place to maintain the strength, composition, and purity of the NDI or dietary supplement containing the NDI throughout the shelf life of the product.

If your notification relies on history of use or other evidence of safety for materials other than your NDI, you should explain, based on the manufacturing method and specifications of your NDI, the qualitative and quantitative relationship between your NDI and the materials used to demonstrate safety. For example, if your NDI is a mixture of polyphenolic compounds extracted from grapes, you might use information such as quantitative HPLC analysis to relate the quantity of those compounds in a serving of your ingredient to the quantity in a serving of unprocessed grapes or grape juice.

Table 1. Example: Dietary Ingredient Specification Sheet or Table

Test	Acceptance Criteria	Analytical Method (Referenced Method or In-House Method Name)
Appearance: Color/physical state	White to off white/powder	Visual, R-01545 ¹
Dietary ingredient #1 identity	The principal spot of the sample solution has an R _f value within $\pm$ units of that of the reference standard solution under identical chromatographic conditions. The principal spot of the sample solution corresponds in position, color, and intensity to that of the reference standard solution.	High-Performance Thin-Layer Chromatography (HPTLC), R-03140 ¹
Dietary ingredient #1 assay	$a \pm b$ mg	HPLC, R-02030 ¹
Related substances: Total related substances	No more than (NMT) specified limit in % of total peak area of the dietary ingredient	HPLC, R-02030 ¹
Component ² #1 identity	The retention time of the principal peak of the sample solution corresponds to that of the reference standard solution ($\pm$ % relative retention time) under identical chromatographic conditions.	HPLC, R-02030 ¹
Component #1 assay	$a \pm b$ mg	HPLC, R-02030 ¹
Microbial limits, if applicable: Total Aerobic Plate Count <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	NMT specified limit in CFU/g Not detected in g or less than the detectable limit of the test Not Detected in g or less than the detectable limit of the test	USP <61>

Test	Acceptance Criteria	Analytical Method (Referenced Method or In-House Method Name)
Apparent pH, 25 °C (if applicable)	Specified limit or range	USP <791> or in-house method
Residual solvent, e.g., ethanol, acetone, hexane (if applicable) ³	NMT specified limit in ppm	Gas Chromatography (GC), R-01901 ¹
Total heavy metals or toxic elements (if applicable)	NMT specified limit in ppm	USP <232>
Individual heavy metals or toxic elements, e.g., Hg, Pb, As, Cd (if applicable)	NMT specified limit in ppm	USP <232>
Pesticides (if applicable) ⁴	NMT specified limit in ppm	USP <561>

¹ In-house analytical methods, which should be described in sufficient detail in the NDIN for FDA to evaluate them. Use of a method published by an authoritative source (such as AOAC International or the United States Pharmacopeia (USP)) or described in a peer-reviewed journal (such as *Journal of Chromatography*) is also appropriate, as long as a reprint or copy of the publication is provided.

² Non-dietary ingredients (e.g., excipients, binders, and fillers) in the article.

³ Solvents that were used in the manufacturing process.

⁴ For agriculturally-derived products that are normally treated with pesticides or may contain residual pesticides due to proximate exposure in neighboring fields or roads.

Table 2. Example: Dietary Supplement Specification Sheet or Table

Test	Acceptance Criteria	Analytical Method (In-House Method # or Reference #)
Appearance: Color/physical state	White to off white/powder	Visual, R-01545 ¹
Dietary ingredient #1 identity	The retention time of the principal peak of the sample solution corresponds to that of the reference standard solution ($\pm$ % relative retention time) under identical chromatographic conditions.	HPLC, R-02030 ¹
Dietary ingredient #1 assay	$a \pm b$ mg/g	HPLC, R-02030 ¹
Dietary ingredient #2 identity	The retention time of the principal peak of the sample solution corresponds to that of the reference standard solution ($\pm$ % relative retention time) under identical chromatographic conditions.	HPLC, R-02030 ¹
Dietary ingredient #2 assay	$a \pm b$ mg/g	HPLC, R-02030 ¹
Related substances: Total related substances	No more than (NMT) specified limit in % of total peak area of dietary supplement	HPLC, R-02030 ¹
Component #1 (non-dietary ingredient)	Identity and assay tests as above in Table 1.	
Component #2 (non-dietary ingredient)	Identity and assay tests as above in Table 1.	
Microbial limits, if applicable: Total Aerobic Plate Count <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	NMT specified limit in CFU/g Not detected in g or less than	USP <61>

Test	Acceptance Criteria	Analytical Method (In-House Method # or Reference #)
	the detectable limit of the test Not detected in g or less than the detectable limit of the test	
Apparent pH, 25 °C (if applicable)	Specified limit or range	USP <791> or in-house method
Residual solvent, e.g., ethanol, acetone, hexane ²	Specified limit in ppm	GC, R01901 ¹
Total heavy metals or toxic elements (if applicable)	NMT specified limit in ppm	USP <232>
Individual heavy metals or toxic elements, e.g., Hg, Pb, As, Cd (if applicable)	NMT specified limit in ppm	USP <232>
Pesticide #1 (if applicable) ³ Pesticide #2 (if applicable) ³	NMT specified limit in ppm NMT specified limit in ppm	AOAC method # EPA method #

¹ In-house analytical methods, which should be described in sufficient detail in the NDIN for FDA to evaluate them. Use of a method published by an authoritative source (such as the AOAC International or the USP) or described in a peer-reviewed journal (such as *Journal of Chromatography*) is also appropriate, as long as a reprint or copy of the publication is provided. If using a published method, the number of the method should be included.

² Only if solvents were used in the manufacturing process.

³ For agriculturally-derived products that are normally treated with pesticides or may contain residual pesticides due to proximate exposure in neighboring fields or roads.

6. What additional information should you submit if your NDI is a discrete chemical entity?

FDA recommends that you provide sufficient information to uniquely characterize your NDI as a discrete molecular entity (or mixture of discrete molecular entities). Information that uniquely characterizes a single molecular entity should include the common or usual name of the molecular entity, the molecular formula and formula weight, the structural formula (as noted, for example, in PubChem or International Union of Pure and Applied Chemistry (IUPAC)), and, if available, the Chemical Abstracts Service (CAS) registry number and the Unique Ingredient Identifier (UNII) code generated by FDA's Global Substance Registration System.

Other relevant information might include:

- specifications for your raw materials, and evidence that your raw materials conform to the specifications and are food grade chemicals (e.g., safe for human consumption);
- a detailed description of each step in the production process, including the controls implemented and parameters monitored at each step to ensure conformance to established specifications, including:
 - reaction conditions (e.g., pressure, catalysts, solvent) in the synthesis and purification process;
 - the in-process and quality controls used in the manufacturing process (e.g., temperature, time, pH, shielding gas);
 - flow diagrams of the manufacturing process;

- if a mixture, the composition of your NDI, including the identity and quantity (including units and any ranges) for each component; and
- a description of how undesirable byproducts of manufacturing (e.g., unreacted chemical reagents, reaction byproducts, and solvents like methanol or hexane) are removed or prevented.

7. What additional information should you submit if your NDI is a configurational isomer?

If the substance exists as a configurational isomer (stereoisomer), such as an enantiomer or a geometric isomer, the isomer in question should be specified and characterized. For an enantiomer, the notification should include the correct stereoisomeric structure and the correct chemical name with the appropriate R or S designations. For a geometric isomer, the notification should include the correct configurational isomeric structure and the correct chemical name with the appropriate cis (Z) or trans (E) designations.

Other systems of nomenclature (such as D or L for amino acids) are also appropriate as long as the name unambiguously identifies which isomer(s) are present.

8. What additional information should you submit if your NDI is a salt?

FDA recommends that you describe the extent to which the salt will dissociate following ingestion, particularly if the history of use or other evidence of safety describes forms of the ingredient other than the salt that is the subject of the notification. FDA also recommends that, in your NDIN, you have data or documentation to support that your NDI will dissociate following ingestion. For example, you can provide a dissociation study performed with your NDI. In addition, you should include a discussion of whether different salt forms have different properties, and how this relates to the safety of your NDI.

9. What additional information should you submit if your NDI is a covalently modified derivative of a dietary ingredient?

Covalent modification chemically alters the ingredient and changes its identity. Examples include covalent bonding of one dietary ingredient to another or exchanging a functional group (e.g., an alcohol) for another (e.g., an acid or an ester). The chemical structure of the new ingredient should be described explicitly and clearly. FDA recommends that you provide data or documentation to support that your NDI will hydrolyze following ingestion, particularly if the history of use or other evidence of safety describes the hydrolyzed form of the NDI. For example, you can provide a hydrolysis study performed with your NDI.

10. What additional information should you submit if your NDI is a botanical or is derived from a botanical?

You must provide the Latin binomial name, including the author citation, for any ingredient that is a botanical or derived from a botanical (21 CFR 190.6(b)(2); see also 21 CFR 101.4(h)). The Latin binomial name should be in accordance with internationally accepted rules on

nomenclature, such as those found in the International Code of Nomenclature for algae, fungi, and plants (ICN) (formerly known as the International Code of Botanical Nomenclature). FDA recommends using the most recent edition of ICN.¹⁰ We recommend that you also specify the part of the plant from which the ingredient is derived because different plant parts may contain different constituents or different levels of the same constituents. You may, in addition, provide a common or usual name for your botanical ingredient.

We also recommend providing the following to help us evaluate the identity of your botanical NDI:

- description of the specific tests or examinations you used to ensure correct taxonomic identity, including identification of any authenticated botanical reference materials or authoritative botanical descriptions used;
- conditions of propagation, particularly if they involve deliberate manipulation of propagation in a manner that is significantly different than common plant propagation and breeding practices;
- conditions of cultivation (e.g., wild harvest, field, hydroponics, or greenhouse) and geographical origin of plant material, particularly if necessary to accurately identify the NDI or if relevant to your conclusion that the ingredient is reasonably expected to be safe;
- periods during which the botanical is cultivated and harvested (season or month(s) and year, age of plant, or both) and the stage of maturity of the harvested plant part;
- post-harvest processing and packing and whether the botanical is used in a raw or dehydrated state;
- the form in which the botanical is used (e.g., whole, chopped, cut-and-sifted, or powdered);
- documentation of a properly prepared and curated voucher of the botanical source material and the location where it is stored; and
- the full Latin binomial name (with author) of any known adulterant species that must be excluded from use in production of the NDI, and a description of how the potential adulterant is excluded from use.

11. How much detail should the description of the production methods for your botanical NDI contain?

FDA recommends that you describe the production methods for your botanical NDI in sufficient detail to demonstrate that the NDI is the same as or similar to the botanical materials that are the subject of the safety information. Thus, cultivation of plants, algae, or fungi in wild or standard conditions might not require extensive explanation. However, non-traditional production conditions should be explained. For instance, if you culture *Saccharomyces cerevisiae* in a medium with unusually large amounts of selenium, you should describe the fermentation

¹⁰ Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J. & Smith, G. F. (eds.) 2018: International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. Available at: <https://doi.org/10.12705/Code.2018> (accessed December 17, 2025).

process, as well as the levels and types of selenium compounds in your final product. If you use traditional or molecular methods to produce a variety with novel properties, you should describe the variety and production method in sufficient detail to establish the identity of the NDI derived from it.

12. How should the identity section of your NDIN deal with toxins in related plants or microorganisms?

FDA recommends that you identify the toxins or classes of toxins or other deleterious constituents or properties (e.g., antibiotic resistance genes in microorganisms or toxigenic properties for which the toxin is unidentified) known to be present in the same species or in a family or genus that is phylogenetically related to the NDI. FDA also recommends that you document the absence (or the amount, if present) of those toxins or other deleterious constituents or properties in the NDI. Documentation should include analytical procedures, data, and calculations. Identification below the species level (e.g., plant variety or strain designation) can be relevant to the safety determination when some varieties or strains of a species are known to contain toxins.

13. How should you describe an extract or concentrate?

FDA recommends that you include the following in the description of your extract or concentrate:

- overview of the manufacturing process, including a general description of each step (e.g., a flowchart including process controls), followed by a description of the method of manufacturing in sufficient detail to make clear the identity of the final product (the finished extract or concentrate) and how it is similar to and different from the starting material;
- description and the amount, expressed as a percentage or range of percentages, of the original starting materials used in the manufacturing process, along with specifications;
- description and the amount, expressed as a percentage or range of percentages, of all added ingredients, including all solvents used, along with specifications for residual solvents other than water in the finished NDI or dietary supplement containing the NDI;
- concentration or dilution ratio, or range of concentration or dilution ratios, of the finished extract or concentrate relative to the original starting material. If the concentration or dilution ratio is based on the weight of fresh herb, rather than dried, this fact should be disclosed;
- content, minimum content, or range of content of any marker substances, expressed as a percentage of the finished extract or concentrate (excluding added substances), accompanied by (1) a description of whether the marker is a marker of toxicity or a surrogate marker, and (2) a calculation or estimate of the relative level of each marker in the NDI compared to the original starting material;
- the names and specifications for any marker substances deemed relevant to the identity of the NDI (e.g., markers whose presence or absence is relevant to the identity of the botanical or that must occur in a particular ratio to each other to confirm identity);
- how the extract or concentrate is standardized from batch to batch;

- measures taken to identify and remove byproducts or contaminants (e.g., nonfood solvents) that may be present due to production methods or to reduce such contaminants to within acceptable limits;
- measures taken to control contaminants (e.g., pesticides, heavy metals, and filth) that may be present in raw materials from which the NDI is derived; and
- quantitative limits on contaminants that must be controlled to ensure the NDI's safety, if any are present in the source material or may result from the manufacturing process.

14. What additional information should you submit if your NDI is a metabolite?

If the notification asserts that the NDI is a metabolite, you should document the basis for this assertion. For example, the notification should cite evidence (e.g., published scientific literature or data from studies performed using the NDI) showing that the level of the NDI in the human body increases with intake of the precursor dietary ingredient. (See definition of “metabolite” in section III.)

15. What additional information should you submit if your NDI is an enzyme?

If your ingredient is an enzyme, you should describe the post-fermentation harvest and processing, including filtration, washing, and preservation methods. In addition, the specifications portion of the identity section of your NDIN should describe the following:

- the analytical method used to determine enzyme activity;
- the specifications for enzyme activity in the NDI; and
- the acceptance criteria for enzyme activity and for the number of units of activity per serving of the NDI in the dietary supplement containing the NDI.

16. What additional information should you include if your NDI is produced using fermentation?

The NDIN should include information about the organism(s) and fermentation process used to culture the microorganism that produces the NDI. The safety of the fermenting organism for use in food production should be discussed. Poorly defined microbiological mixtures are acceptable if there is a long history of use in production of food (e.g., mixtures used to make dairy products like kefir or cheese), and the fermentation substrate is consistent with that history of use. The NDIN should describe the history of use of the fermenting organism(s) to produce food or, in the absence of such history, should explain in detail how the manufacturing process excludes toxins and other undesirable byproducts of fermentation from the finished NDI.

The information about the fermentation process should describe the complete media formulation (i.e., the raw materials used to produce the fermentation media should be identified in the notification), the fermentation vessel(s), the fermentation conditions, the methods used to harvest the NDI from the fermentation mixture, and any specifications for the production organism in the finished NDI, particularly if the production organism is not inactivated or removed.

The NDIN should also address methods used to ensure the integrity of the production organism, such as how you guard against contamination and genetic drift or change. FDA is particularly concerned about contamination when fermentation occurs outside of a sterile production vessel (e.g., production of algae in ponds).

Note that the use of a major food allergen in the fermentation medium may require a separate notification or petition to FDA if the presence of the allergen is not declared on the resulting NDI or finished product label of the dietary supplement containing the NDI (see section 403(w) of the FD&C Act).

17. What additional information should you include if your NDI is a live microbial dietary ingredient?

FDA recommends that you include a complete description of the organism, including:

- origin of the strain;¹¹
- description of method used to isolate the strain;
- methods used to establish the identity of the strain, such as whole genome sequencing (WGS) or identification by internationally recognized third-party repositories (e.g., the American Type Culture Collection), including determination of inter-strain differences compared to a type-strain or well-characterized strain within the same species;
- location where the reference strain is deposited that provides a permanent record of the strain identity; and
- the relationship of the strain to the strain(s) of the same species used to establish the history of use or other evidence of safety for the NDI.

The use of scientific names (Latin binomial name with author citation) is required for botanical ingredients (21 CFR 190.6(b)(2)) and is recommended for bacteria. For bacteria, FDA recommends using the Bacteriological Code (1990 Revision),¹² validated lists of names in the International Journal of Systematic and Evolutionary Microbiology, and public lists of prokaryotic nomenclature (e.g., Prokaryotic Nomenclature Up to Date¹³ or the List of Prokaryotic Names with Standing in Nomenclature¹⁴).

FDA will pay particularly close attention to the proper identification of organisms from genera or species that do not have a long history of food use and to those from genera, like *Bacillus* and *Streptococcus*, which contain both species with long histories of food use and species that include human pathogens. FDA regards all members of a species that include human pathogens

¹¹ FDA considers each strain of a bacterial or yeast species to be a separate ingredient.

¹² Lapage, S. P.; Sneath, P. H. A.; Lessel, E. F.; Skerman, V. B. D.; Seeliger, H. P. R.; Clark, W. A., editors. International Code of Nomenclature of Bacteria (Bacteriological Code), 1990 Revision. Washington (DC): American Society for Microbiology Press; 1992.

¹³ Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Prokaryotic Nomenclature Up-to-Date. Available at: <https://lpsn.dsmz.de>. [Note that content on this website is updated frequently. Use the search function in the embedded link to retrieve the current validated name of a bacterial organism.]

¹⁴ Parte, A.C., editor. List of Prokaryotic Names with Standing in Nomenclature (LPSN) Database. Available at: <http://www.bacterio.net/>. [Note that content on this website is updated frequently. Use the search function in the embedded link to retrieve the current validated name of a bacterial organism.]

as potentially harmful to human health and, therefore, inappropriate for use as dietary ingredients, because of the risk for horizontal transfer of genes containing pathogenic traits between members of the same bacterial species. Examples of species that should not be used in dietary supplements include *Escherichia coli*, *Enterococcus faecalis*, and *Enterococcus faecium*.

You should explain how your strain was obtained and how it varies from other member strains of the same species. If your strain was genetically modified using either random mutagenesis or bioengineering, you should describe the process used and how you characterized the properties of the new strain.

FDA also considers the manufacturing process, including information on fermentation process and the fermentation media used, as an intrinsic part of the identity of an ingredient that is viable at the time of ingestion. We recommend that the fermentation and other parts of the manufacturing process relevant to safety and identity be described in detail in your NDIN, as recommended in questions IV.3 and 16.

FDA will pay particular attention to the viability of microorganisms in the NDI for a live microbial product. The per-serving level of a viable microorganism depends on both the mass (in grams) and the viability (e.g., number of colony-forming units) of the organism in the final product. The composition of the growth medium and the fermentation conditions of the organism are also relevant to the safety of the product, particularly when they alter the form of the organism (e.g., spore vs. vegetative) or the composition of the ingredient (e.g., when the ingredient includes both the organism and the growth medium).

More details about what safety information your NDIN should include when your NDI is a live microbial are included in question VIII.1.

18. What information should you provide in your NDIN if the NDI or dietary supplement containing the NDI will include an expiration date or “use by” date?

The expiration or “use by” date should be based on appropriate supportive stability data showing that (1) no new degradants will form during the recommended shelf life of the product under the conditions of storage specified in the NDIN, or under normal storage conditions, or, if degradants form, include the identity and levels of expected degradants as well as data on the safety of the degradants; and (2) the NDI or dietary supplement containing the NDI will continue to meet the critical safety attributes of identity, strength, and purity through its recommended expiration or “use by” date. You should provide these supportive data, including the test procedures and calculations, in the NDIN.

19. What information should you submit to describe the conditions of use that you intend to recommend for your dietary supplement?

Your NDIN must describe the conditions of use that will be recommended or suggested in the labeling of your dietary supplement or, if no conditions of use will be recommended or suggested in the labeling, the ordinary conditions of use of the supplement (21 CFR 190.6(b)(3)(ii)). Conditions of use include the serving size, serving form (e.g., capsule or powder), frequency of use (e.g., number of servings per day and interval between servings), duration of use, maximum

total daily intake level, instructions for use, target population, and any restrictions on use, such as excluded populations. The maximum total daily intake level of the dietary supplement is calculated by multiplying the serving size and the number of servings per day. In addition, you should include the maximum level or amount of the new dietary ingredient in each serving of the dietary supplement (include units).

For purposes of review, daily lifetime use by all age groups and other populations at the highest recommended serving size will be assumed unless the NDIN specifies restrictions on conditions of use (e.g., excluded populations or frequency and duration of intake). Population restrictions could include exclusion of children, pregnant or lactating women, or sensitive individuals who should not consume the product. Allergen warnings are an example of a population restriction on conditions of use. The conditions of use to be recommended or suggested in the labeling of the dietary supplement(s) containing the NDI should be described prominently in the administrative section near the beginning of the NDIN.

20. What identity information should you submit if your notification relies on history of use or other evidence of safety for a substance or product that is similar to, but not exactly the same as, your NDI and dietary supplement containing the NDI?

FDA recommends that you explain how the substance or product that is the subject of the safety information is similar to your NDI and dietary supplement containing the NDI, and provide a rationale for how the safety information that is presented for the similar substance or product is relevant to the safety of your NDI and dietary supplement. FDA recommends that you use chemical, microbiological, and botanical characterizations to support your rationale, as appropriate. Note that developing such a rationale requires knowledge of the identity (e.g., composition, preparation/manufacturing conditions) of the related substances that were studied or that have a history of safe use. One approach could be to first use this identity information to explain how your NDI and dietary supplement containing the NDI is qualitatively and quantitatively similar to the substances which you are citing. We recommend you specify similar characteristics that support extrapolating data from the similar substance to the NDI (e.g., if both contain the same skeleton moieties). Then, you could discuss the scientific rationale that supports extrapolating conclusions from a safety evaluation of the related substance or product to your NDI and dietary supplement containing the NDI. We recommend you support your assertions with published scientific literature.

V. Summary of the Basis for Your Conclusion of Safety

1. What safety information is required to support an NDIN?

Your NDIN must contain information that is the basis on which you have concluded that the NDI or dietary supplement containing the NDI will reasonably be expected to be safe under the supplement's labeled conditions of use. (Section 413(a)(2) of the FD&C Act; 21 CFR 190.6(a).)

FDA's regulation at 21 CFR 190.6(b)(4) requires that your NDIN include the history of use or other evidence of safety that establishes that the dietary ingredient, when used under the

conditions recommended or suggested in the dietary supplement's labeling, will reasonably be expected to be safe.¹⁵ The NDIN must include citations to published articles or other evidence that are the basis on which you have concluded that the NDI or dietary supplement containing the NDI will reasonably be expected to be safe. (*Id.*)

Your NDIN should provide evidence of a history of use or other evidence of safety (in vitro/in silico study data, other alternative methods, animal testing, clinical testing, or a combination of these, as discussed in Section VII below). In some cases, it may be appropriate to provide a combination of history of safe use and other evidence of safety. For example, if you are recommending a serving size, duration of use, or frequency of use that exceeds the historically documented safe use evidence, you should submit other evidence of safety to fill the data gap between the historical use level and proposed conditions of use. However, submitting other evidence of safety is generally not recommended if the proposed serving size, duration of use, and frequency of use for the NDI or dietary supplement containing the NDI are the same as or less than the levels for which there is a documented history of safe use.

For dietary supplements that contain dietary ingredients or other components in addition to the NDI, the NDIN should include safety information for the finished product as well as for the NDI because it is not possible to conclude that the dietary supplement containing the NDI will reasonably be expected to be safe without considering the safety of these other components.

2. What should you include in the safety narrative for the NDI?

Your NDIN should include a “safety narrative.”¹⁶ Your safety narrative should contain your objective evaluation of the history of safe use or other evidence of safety, along with an explanation of how the evidence of safety provides a basis to conclude that the NDI or dietary supplement containing the NDI, when used under the conditions described in the NDIN, will reasonably be expected to be safe. In general, this information should include an adequate history of safe use, safety studies, or a combination. The safety narrative should present a logical, scientific interpretation of all safety data to support an expectation of safety for this NDI or dietary supplement containing the NDI.

The safety narrative should estimate the total daily human intake of the NDI or dietary supplement containing the NDI and describe any potential toxicity or health concerns associated with human consumption, particularly if there are both toxicity and health concerns that may result from the proposed use of the dietary supplement by a vulnerable population. The

¹⁵ Throughout this guidance, FDA uses the term “history of safe use” to refer to history of use in humans that establishes that the dietary ingredient, when used under the conditions recommended or suggested in the dietary supplement's labeling, will reasonably be expected to be safe. We use the term “other evidence of safety” to refer to other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe.

¹⁶ This guidance uses the term “safety narrative” to refer to the single, comprehensive narrative document (similar to the Safety Information template) provided in the NDIN. In the Centralized Online Submission Module (COSM), New Dietary Ingredient Notification Step-by-Step Submission Guide, the “safety narrative” is referred to as the “safety information attachment” and references the Safety Information template. Available at: <https://www.fda.gov/food/registration-food-facilities-and-other-submissions/cfsan-online-submission-module-cosm> (accessed December 17, 2025).

description of toxicity and health concerns should include the effects of binders, fillers, formulation aids, and other non-dietary ingredients present in the dietary supplement, particularly if they alter the safety profile of one or more ingredients, such as by increasing bioavailability of an ingredient after ingestion.

The safety narrative should also describe the function of each non-dietary ingredient, including the technical effect and the quantity needed to achieve that technical effect. References to the applicable food additive regulation, color additive regulation, generally recognized as safe (GRAS) regulation, or GRAS notification are also recommended.

You may choose to provide other evidence of safety based on studies of related dietary ingredients that are different from your NDI as a way to address FDA's requirements without initiating new studies. For example:

- If your notification relies on other evidence of safety (21 CFR 190.6(b)(4)), and the NDI differs from the ingredient that is the subject of the safety evidence, the safety narrative should qualitatively and quantitatively¹⁷ compare the ingredients tested in each of the studies cited with the NDI.
- If you cite a study on the feeding of a whole herb to a test subject and the NDI is an extract of that herb, the safety narrative should qualitatively and quantitatively compare the serving and total daily intake of the herb in the study to the proposed dose and total daily intake of the NDI. Whenever possible, the safety narrative should identify the doses/intake level with effect and without effect in each *in vivo* study, and the relationships between observed adverse effects and other related observed effects should be described.
- In the event of a data gap, the NDI safety narrative should identify the NOAEL and describe the toxicity data or adverse events that were the basis for determining it. When using a related test article, additional justification should be provided as to how this safety conclusion is relevant to the NDI.
- The safety narrative should also describe the ADI for the NDI (where applicable) and explain how it was calculated. Additional calculations (e.g., molecular weight comparison, adjustment based on concentration) should be completed if the test article used to calculate the ADI is a related but not the same article as the NDI.
- The safety narrative should state the basis for the margin of safety for the NDI and how the margin of safety was calculated, with additional justification if the margin of safety was calculated using a related test article.

3. If your product contains more than one NDI, how should your safety narrative address that information?

The safety narrative should describe your approach to safety and reference all relevant data. When your NDIN describes a dietary supplement containing more than one NDI, you can

¹⁷ Several factors can be used to evaluate an NDI qualitatively and quantitatively. Qualitative analysis could encompass the type of species, part of the plant used, and/or processing of the NDI or dietary supplement containing the NDI. Quantitative analysis generally would involve the serving size of the NDI or the dietary supplement containing the NDI.

demonstrate the safety of the NDIs by providing either: (1) safety data on the combination of NDIs and a description of the individual constituents; or (2) safety data on the individual NDIs and an explanation of how the combination of NDIs does not present any new safety concerns.

When most or all of the history of safe use or other evidence of safety data are for the combination of NDIs, FDA recommends presenting the data on the combination of NDIs and a description of the individual NDIs. However, when the combination of NDIs represents several single-constituent NDIs with robust individual data sets, it may be more appropriate to submit safety data on individual NDIs and describe how the combination of NDIs does not present new safety concerns.

VI. History of Use

1. What does FDA consider an “adequate” history of safe use in an NDIN?

An “adequate” history of safe use should demonstrate that the NDI, or a substance or product that is related to the NDI, has been consumed, for example, as food, in large and genetically diverse populations with limited or no adverse events associated with consumption. The history of safe use should then be compared to the proposed conditions of use for the NDI or dietary supplement containing the NDI. Such comparison should help you to determine what, if any, additional data are necessary to demonstrate a reasonable expectation of safety. If you determine that history of safe use evidence alone demonstrates that the NDI or dietary supplement containing the NDI can reasonably be expected to be safe under the proposed conditions of use, FDA does not recommend submitting additional data in your NDIN.

If the evidence supporting your history of safe use contains gaps, or if the proposed conditions of use of the NDI differ from the historical conditions of use, we recommend that you submit animal studies, clinical studies, alternative methods as discussed in question VII.3, or a combination of these, to help demonstrate that there is a reasonable expectation of safety under the proposed conditions of use of the NDI or dietary supplement containing the NDI. More information about the information recommended to address data gaps can be found in question VIII and in Appendix A.

2. What data and information should be submitted to demonstrate an NDI’s history of safe use?

There are different ways that your NDIN can demonstrate a history of safe use that establishes that the NDI or dietary supplement containing the NDI is reasonably expected to be safe under the proposed conditions of use, as required by 21 CFR 190.6(b)(4). In general, your NDIN should provide evidence that the substance was safely consumed as a food or dietary supplement or as a component of a more complex mixture (e.g., calcium in milk, beta-glucan in oatmeal) at levels equal to or higher than those that would be consumed by someone taking the NDI-containing supplement under the proposed conditions of use.

This history of safe use data and information provided in your NDIN may be from the United States or another country. It is important that the data and information demonstrate that the

substance that historically was consumed as a food or dietary supplement is comparable to the proposed NDI.

Composition and other identifying characteristics

To demonstrate a history of safe use, FDA recommends that your NDIN include:

- a description and comparison of the identity of the NDI and the historically consumed article; and
- an explanation of how the compositions of the two are related.

The composition and other identifying characteristics of the NDI and the historically consumed article should be described in sufficient detail to demonstrate that safe use of the historically consumed article is relevant to the safety of the NDI and to provide a basis to conclude that the NDI or dietary supplement containing the NDI will reasonably be expected to be safe under the proposed conditions of use. For example, if the NDI has historically been used as a component of a more complex mixture, you should demonstrate how the NDI is qualitatively and quantitatively related to the historically consumed component. Likewise, if the NDI is itself a combination of dietary ingredients, your NDIN should demonstrate how the component dietary ingredients in the NDI are related to historically consumed ingredients or components.

Historical intake data and information

The safety narrative for your NDIN should include historical intake data, which include:

- the dose (per-serving intake) and total daily intake;
- duration of use;
- frequency of intake (e.g., daily chronic, intermittent);
- product labels; and
- any additional information that describes the conditions of use of the historically consumed material.

If consumption is not uniform within the population, you should provide information about the mean and high (e.g., 90th percentile) exposure levels. You should also discuss the size and relevant characteristics of the consuming population (e.g., everyone vs. limitations based on age, sex, or health status). It is important for the history of safe use information to reflect the size and population of the expected consumer population.

For these data to demonstrate a history of safe use, the intake level for the historically consumed article should be the same as or higher than the anticipated intake level of the NDI in the dietary supplement, based on the conditions of use described in the NDIN. For example:

- Information showing that a steroid hormone is present in nanogram amounts in a serving of milk or beef—foods that have a long history of safe use—would not support the safety of a highly concentrated bovine extract that contains the steroid hormone in milligram amounts. In contrast, consumption of cow's milk could be used to support the safety of a specific protein purified from milk at a serving level equal to or lower than the amount of the protein found in an 8-ounce serving of milk.

- If your NDI is an oil made from a plant or fish and you can show that the oil consists only of a mixture of fatty acids, each of which you can identify and demonstrate to be widely consumed at higher levels in conventional foods, you may be able to conclude that the dietary supplement containing the NDI will reasonably be expected to be safe based on compositional information alone.

3. What attributes of history of safe use data are important to include in your NDIN?

The length of an ingredient's history of use is important to demonstrating reliability. A description of the population consuming the ingredient and the ways in which they use it are also important. In addition, the number of consumers who used the ingredient and the frequency of intake are at least as important as the number of years over which the ingredient has been used.

If you are relying on history of safe use data alone, FDA generally recommends 25 years of widespread use to be the minimum to establish a history of safe use.¹⁸ If your NDIN contains data with fewer years of widespread use, we recommend providing additional information to help demonstrate that such timeframe provides sufficient, reliable data that are appropriate in the dietary supplement context.

4. What documentation should an NDIN include to demonstrate a history of safe use?

FDA's regulation at 21 CFR 190.6(b)(4) requires that your NDIN include "citation to published articles or other evidence that is the basis" on which you have concluded that the NDI or dietary supplement containing the NDI will reasonably be expected to be safe under the proposed conditions of use. Where you reference published information, you must provide copies of those references in your NDIN, including complete and accurate English translations if such references are in a foreign language. (*Id.*)

Examples of documentation of an NDI's history of use in food may include:

- published data and information, such as peer-reviewed scientific literature;
- reports from an authoritative body or source;¹⁹
- survey data on food or nutrient composition and consumption (e.g., the National Health and Nutrition Examination Survey (NHANES); FDA Total Diet Study; or other federal agency-sponsored studies);
- advertisements or other published promotional material describing the composition of products;

¹⁸ This approach is consistent with the approach followed by the European Union. See, e.g., the European Union's definition of "history of safe food use in a third country," which "means that the safety of the food in question is confirmed with compositional data and from experience of use and continued use for at least 25 years in the customary diet of a large part of the population of a country." Council Regulation No. 2015/2283, 2015 O.J. (L 327) 1, 8, available at <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R2283> (accessed December 17, 2025).

¹⁹ For purposes of this guidance, we consider an "authoritative body or source" to mean an internationally recognized entity that regularly reviews protocols, seeks comments from stakeholders, and makes updates.

- published agricultural or food production data; and
- cookbooks or other published recipes documenting the use of an ingredient to prepare conventional foods.

Other examples of documentation of history of safe use may include:

- survey or consumption data from non-Federal agency sources;
- product sales or production data, such as sales records, commercial invoices, and amounts of production;
- product labels; and
- compositional analyses, such as liquid or gas chromatography, mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy.

5. Should you cite the history of safe use of an NDI in traditional medicine?

The history of safe use of an NDI in traditional medicine can help to establish a reasonable expectation of safety for the NDI's use in a dietary supplement. However, because differences in composition, conditions of use, and target population often limit the relevance of a history of safe use in traditional medicine to the safety of an NDI in a dietary supplement, FDA recommends providing other evidence of safety to explain any data gaps that might exist between the traditional medicine history of safe use and proposed conditions of use.

Often, traditional medicinal products are chemically and compositionally different from the NDI that is the subject of the NDIN. Therefore, it is important to document and explain how any information about a substance's history of safe use in traditional medicine is qualitatively and quantitatively related to the NDI and its proposed conditions of use.

It also is important to document safety-related restrictions on use in accordance with the tradition. For example, if the written or oral tradition for a specific ingredient indicates that it should be used for no more than one week, that information should be addressed as part of your justification of safety.

6. How should your NDIN explain the differences from the historical use with respect to the NDI or dietary supplement containing the NDI?

The safety narrative should describe and discuss situations in which the proposed conditions of use and composition of the NDI differ from the documented conditions of use and composition of the historically consumed substance (e.g., when the NDI is derived from a plant variety bred to produce an additional constituent or to remove a toxic constituent).

If the historical usage differs meaningfully from the proposed use of the NDI, we recommend that you submit "other evidence of safety" (21 CFR 190.6(b)(4)) to help demonstrate that there is a reasonable expectation of safety under the proposed conditions of use. This other evidence of

safety may take the form of additional animal studies, human studies,²⁰ or both. Such studies should be designed to address gaps in the history of safe use evidence.

Below we provide examples of historical differences where we recommend that you submit additional supportive data in your NDIN. These examples are based on the general principle that the risk of a substance is likely to increase as intake increases above levels safely consumed in the past.

- the NDI has a higher serving or higher amount ingested than the historically consumed substance;
- the NDI and the historically consumed substance have a different route of administration (e.g., an article that has a history of safe use in sublingual form and is now intended for ingestion as an NDI);
- the NDI has a longer duration of use than the historically consumed substance;
- there are other changes in the NDI that could increase exposure to potential toxic effects;
- there are changes in preparation of the NDI compared to the form historically used; and
- there are other differences in the proposed use of the NDI, such as a change in target population, that could raise new safety issues.

If you are unsure about whether to submit other evidence of safety, you may request a pre-NDIN meeting with FDA to obtain more information.²¹

7. Are you required to submit a comprehensive survey of every historical use of the NDI?

FDA's regulations require that your NDIN contain data and information on which you base your reasonable expectation of safety (21 CFR 190.6(b)(4)). FDA does not expect history of safe use data in your NDIN to contain every known historical use of the NDI. Rather, your NDIN should contain the information that was important to your determination, demonstrating that it was safely used by a large-enough population to show that the NDI would be reasonably expected to be safe under the conditions of proposed use.

For example, if you have an NDI that is a constituent of soybeans, and you have documentation that soybeans have a history of safe use in a large population in Asia, you should submit that data. However, if there is also data describing safe *lower* historical consumption of soybeans in the United States or Europe, then we do not recommend you submit those data to support the safety of an NDI, because those data would be superfluous.

²⁰ We note that clinical studies must comply with FDA's regulations for the protection of human subjects (21 CFR parts 50 and 56). A clinical study intended to evaluate the safety of a dietary supplement or a dietary ingredient generally does not require an IND if the only endpoints measured are safety endpoints.

²¹ Contact information for the Office of Dietary Supplement Programs can be found on our webpage at <https://www.fda.gov/food/dietary-supplements>. Meeting requesters should be aware that meeting materials, like other FDA records, may be requested under the Freedom of Information Act (FOIA) (5 U.S.C. 552), and, unless the information being requested is trade secret, confidential commercial information (CCI), or is otherwise exempt from disclosure under FOIA and our regulations, it will be released to requesters.

By contrast, if there is also data describing historical consumption of the substance that calls into question the safe historical consumption of the substance, then FDA would expect the safety narrative to include such historical use of the substance and an explanation of how such data relates to your determination that the NDI would be reasonably expected to be safe under the proposed conditions of use.

8. Should your NDIN include an estimate of the intake of historically consumed materials related to the NDI if those related materials were relied on to establish history of use?

Yes, if your conclusion that the NDI or dietary supplement containing the NDI will reasonably be expected to be safe is based on a history of safe use of materials other than the NDI itself, FDA recommends that you estimate the historical intake of the materials that you determine to be relevant and include this information in your NDIN.

In developing these estimates, you should consider the complete pattern of intake, including dose, duration, and frequency of intake, as well as the size of the population known to have consumed the substance. The distribution of intake within the population (e.g., the mean and 90th percentile amounts consumed) is also important.

There are several sources where you can find references and information on methods of estimating consumer intake of food ingredients, including dietary ingredients in dietary supplements. FDA recommends guidance documents available on FDA's website that provide our current thinking regarding estimation of dietary intake.²² In addition, we recommend consulting risk assessment publications provided by the World Health Organization.

9. How do you determine whether the frequency of historical use was “daily chronic” or “intermittent”?

FDA recommends that you include frequency of use data as part of your NDIN to help explain whether the NDI or dietary supplement containing the NDI can reasonably be expected to be safe under the proposed conditions of use.

For purposes of this guidance:

- Daily chronic use of the historically consumed material means ingestion at least once a day, every day, for at least three months in a row and can include use for multiple months or years at a time.
- Intermittent use means less than daily chronic use. Intermittent use can be either daily and finite in duration, or non-daily and a lifetime in duration. An example of intermittent use could be consumption of digestive enzymes (e.g., lactase, alpha galactosidase) to help digestion of specific foods.

²² See, e.g., Guidance for Industry: Estimating Dietary Intake of Substance in Food (Aug. 2006), available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-estimating-dietary-intake-substances-food>, and resources on FDA's webpage on Dietary Supplements, available at <https://www.fda.gov/food/dietary-supplements> (accessed December 17, 2025).

Your NDIN should include other evidence of safety where there are gaps between your history of safe use data and the proposed conditions of use (21 CFR 190.6(b)(4)). For example, if the history of safe use data are based on intermittent use and the proposed conditions of use are daily chronic use, there is a safety gap that should be addressed in your NDIN.

VII. Other Evidence of Safety

1. What information does FDA recommend including in an NDIN when “other evidence of safety” is needed?

If there is a data gap between the history of safe use and the proposed conditions of use for the NDI or the dietary supplement containing the NDI, FDA recommends that you provide other evidence of safety. The safety study or studies you include in your NDIN will depend on the type of data gap you are seeking to address. More information about these studies and how they can be helpful is provided below.

Genetic toxicity tests

These tests include:

- a two-study battery (bacterial mutagenesis and *in vitro* cytogenetics) that includes a test for gene mutations in bacteria and either an *in vitro* mouse lymphoma thymidine kinase+/- gene mutation assay (preferred) or another suitable *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells; or
- a three-study toxicity battery (bacterial mutagenesis, *in vitro* cytogenetics, and *in vivo* mammalian test) that includes a test for gene mutations in bacteria; either an *in vitro* mouse lymphoma thymidine kinase+/- gene mutation assay (preferred) or another suitable *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells; and an *in vivo* test for chromosomal damage using mammalian hematopoietic cells.

Selection between the two- or three-study battery should be determined by the extent of documentation on a history of safe use or available published safety data on the NDI or dietary supplement containing the NDI; the less historical or safety data there are, the more studies are recommended to characterize the safety of the new ingredient.

Alternative methods: In vitro biochemical or cell-based assays / In silico modeling

These tests can include:

- *In vitro* biochemical assays such as receptor binding assays;
- *In vitro* cell-based assays such as transwell intestinal absorption assays, cytotoxicity assays, and mechanistic assays; and
- *In silico* modeling such as PB/PK modeling and QSAR modeling.

In vitro and *in silico* alternative methods are unlikely to be sufficient to demonstrate safety on their own; however, data from alternative methods (also referred to as new alternative methods or new approach methods (NAMs)), could be helpful to fill data gaps or support safety

conclusions from history of safe use or other *in vivo* studies. As most alternative methods have not gone through formal validation, it is important to define how the method is fit-for-purpose in the context of demonstrating safety for dietary supplements and to provide detailed information on the materials, methods, and models, including the scope and limitations of the assay. See questions VII.3 and VII.8 for more information.

FDA supports the use of NAMs that produce scientifically valid data that are appropriate within a context of use to meet a specific regulatory need. Some *in vitro* methods, such as those listed in the genetic toxicity battery (described above), have undergone formal validation (i.e., Organisation for Economic Co-operation and Development (OECD)) and are commonly leveraged to support safety and regulatory decision-making. Other alternative methods have not been formally validated but may still inform safety conclusions.

Preclinical animal studies

These studies can include:

- single-dose acute study and/or an absorption, distribution, metabolism, and excretion (ADME) study to determine an LD50 or use the highest safe dose to categorize your ingredients (Globally Harmonized System Classification of Chemicals; Hazard Categories);
- 14-day repeat-dose study in animals to establish an MTD;
- 90-day subchronic oral study in animals to determine a NOAEL and complete safety calculations, which would include a margin of safety and/or EDI/ADI ratio (Estimated Daily Intake/Acceptable Daily Intake); and
- one-generation rodent reproductive study to assess safety in sensitive populations, such as pregnant women and the developing embryo (appropriate when an NDI is labeled for use in pregnant, lactating women, or young children).

The selection of preclinical animal studies should also be based on the available history of safe use or other published safety data on the NDI or dietary supplement containing the NDI. However, the preclinical animal studies described above are intended to build on each other for the development of safety data. For example, a single-dose study likely would not be sufficient to demonstrate safety without the support of a 90-day subchronic study. If acute toxicity or tolerated doses are documented in the published literature, conducting a single-dose or 14-day study of your own may not be recommended. Not all studies listed above are always recommended to be included in your NDIN. The safety study or studies you include in your NDIN will depend on the type of data gap you are seeking to address. Any decision about what studies you selected to complete or not complete should be described in the safety narrative.

Clinical human studies

These studies can include:

- single-dose tolerability and/or ADME study in humans to demonstrate human exposure, at a serving similar to or greater than the proposed serving size. This could also be used as a comparison to ADME study conducted in preclinical animals with shared characteristics used to support an expectation of safety; and

- repeat-dose tolerability and/or ADME study in humans to demonstrate exposure in a human population comparable to preclinical studies and provide data on the consumer experience with servings at or above those proposed for the NDI or dietary supplement containing the NDI.

The decision to conduct a clinical human study and what design to follow will largely be informed by the existing data on your NDI and the remaining data gaps that need to be addressed. Any decision about what studies you selected to complete or not complete should be described in the safety narrative. Single-dose studies will provide different data than repeat-dose studies; the studies described above should be leveraged as appropriate. The clinical human studies listed above are not required to establish a reasonable expectation of safety, but you may find that conducting a human study could be useful to demonstrate the safety of your NDI (e.g., where available animal models are not ideal to represent human exposure).

Appendix A contains a Standard Safety Testing Table, which is intended to help illustrate the traditional collection of safety studies that could be used to help fill data gaps between historical use and proposed conditions of use.

2. Does FDA recommend submitting additional studies as other evidence of safety to supplement a history of safe use?

FDA's NDIN regulation at 21 CFR 190.6(b)(4) requires that your NDIN include the history of safe use or other evidence of safety that establishes that the dietary supplement containing the NDI, when used under the proposed conditions of use, will reasonably be expected to be safe. We recommend that you first consider data on history of safe use in evaluating the safety of an NDI, as you may determine that the history of safe use is sufficient to demonstrate that the NDI or the dietary supplement containing the NDI is reasonably expected to be safe under the conditions of proposed use.

There are circumstances where additional studies may not be helpful. For example, additional safety data are *not* recommended:

- If the NDI has been previously consumed by humans and:
 - the proposed use level is similar to or less than the levels safely consumed by humans in the past; and
 - the population expected to consume the NDI is the same as, or a subset of, the population that safely consumed the substance in the past.
- If the NDI is reasonably expected to be safe based on:
 - a large margin of safety between the level shown to cause no observed adverse effects in animals (found in published literature) and the intake level that would result from the proposed conditions of use of the NDI; or
 - longstanding and widespread use of the ingredient as a constituent of a conventional food or dietary supplement product at or below the intake level that would result from the proposed maximum serving level of the NDI.

If the proposed use of the NDI leads to intake levels that are the same as or less than the levels for which there is a documented history of safe use, FDA does not recommend submitting additional safety data in your NDIN in the following circumstances:

The dietary supplement containing the NDI is intended for:	The documented historical use data support:
Daily chronic use	Safe daily chronic use in the same population or a broader population
Intermittent use	Safe intermittent use or safe chronic use in the same population or a broader population

3. How can alternative methods be used to demonstrate other evidence of safety?

Alternative methods or NAMs are testing strategies that reduce, replace, or refine the use of animal testing to support risk or safety assessment for FDA-regulated products. In general, considerably more research is needed for tools that might replace, reduce, or refine the large battery of animal studies that support a multitude of toxicity and safety assessments.²³ Most current alternative methods cannot predict effects that occur in highly complex, interacting systems.²⁴ However, FDA supports the development of NAMs that more accurately identify, predict, evaluate, and reduce the degree and likelihood of risks, with the aim to provide enhanced prediction of the risk and/or safety outcomes.²⁵

FDA supports the use of NAMs when evaluating NDIs or dietary supplements containing an NDI. If you choose to include NAMs in your NDIN to demonstrate other evidence of safety, the submission should provide information demonstrating that the NAM is scientifically valid, fit-for-purpose, reliable, and appropriate for use in the dietary supplement context.

4. Should you submit safety studies if the test articles are similar but not identical to the NDI or the dietary supplement containing the NDI?

FDA generally recommends that the test article in safety studies described in your NDIN be identical to the NDI or the dietary supplement containing the NDI that is the subject of your NDIN. However, in the absence of safety data on the NDI or supplement itself, it may be useful to provide data on the safety of a related substance or product. Whether the test article is the same as the NDI or is a related substance or product, FDA recommends that you include a qualitative and quantitative comparison of the test article(s) and the NDI in the safety narrative.

If the NDI is a component of another substance for which safety studies are available, it may be helpful to submit data from those studies, accompanied by an explanation of why the data on the related substance support the safety of your NDI. For example, data from a study involving the

²³ See About Alternative Methods, available at <https://www.fda.gov/science-research/advancing-alternative-methods-fda/about-alternative-methods> (accessed December 17, 2025).

²⁴ See Advancing New Alternative Methodologies at FDA, available at <https://www.fda.gov/media/144891/download>, 2021 (accessed December 17, 2025).

²⁵ See *id.*

oral administration of the dried ground root of a plant could be relevant to the safety of an NDI that is an isopropanol extract of the same root if you document that the components of the isopropanol extract are present in the NDI at the same or lower levels than in the ground root fed to the study subjects. Likewise, the safety of an ester ingredient can be inferred if you can provide data to demonstrate that the ingredient is rapidly hydrolyzed in the stomach or intestine into an acid and an alcohol, and that the acid and the alcohol each have well-documented safety studies.

You should keep in mind that the more different the composition of the test article in a study is from that of the NDI, the more difficult it will be to demonstrate that the study provides relevant safety evidence.

5. Are you required to use specific published protocols in your NDIN?

You do not have to use specific published protocols in your NDIN. As applicable, your safety narrative should contain citations to any protocols used as part of the testing to demonstrate the reasonable expectation of safety of the NDI or dietary supplement containing the NDI (see 21 CFR 190.6(a)).

If your NDIN includes unpublished studies, you should cite an authoritative body or source for the protocol and explain how information generated by the study using the protocol supports the safety of the NDI or the dietary supplement in which the NDI will be used. We recommend that your safety narrative explains why you determined that the protocol or the battery of protocols you chose is appropriate for the safety endpoints that are being investigated. If you decide to deviate from a standard or published protocol, you should explain why you altered the protocol and how the alteration affects the relevance of the study results to the safety of your product.

There are many internationally recognized safety protocols and testing batteries developed for other types of products.²⁶ The following sources contain guidelines for safety protocols, including genotoxicity, cytotoxicity, repeated dose oral toxicity, reproductive toxicity, and teratology studies:

- OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, published by the Organisation for Economic Co-operation and Development (published July 4, 2023);²⁷
- Harmonized Test Guidelines, published by the Office of Chemical Safety and Pollution Prevention of the U.S. Environmental Protection Agency (EPA);²⁸ and
- Principles and Methods for the Risk Assessment of Chemicals in Food, Chapter 4: Hazard Identification and Characterization; Toxicological and Human Studies, published

²⁶ The NDI safety standard is different than the standard for food additives, drugs, and other FDA-regulated products. Recommendations in guidance documents that are tailored to the safety assessment needs of other products may not always be appropriate for dietary ingredients or dietary supplements.

²⁷ (OECD. OECD Test Guidelines for Chemicals: Health Effects. Available at: <https://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm> (accessed December 17, 2025).

²⁸ EPA, Office of Chemical Safety and Pollution Prevention (OCSPP). Test Guidelines for Pesticides and Toxic Substances. OCSPP Harmonized Test Guidelines. Updated June 29, 2022. Available at: <https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances> (accessed December 17, 2025).

jointly by the Food and Agriculture Organization of the United Nations and the World Health Organization.²⁹

6. How do you identify a potential hazard using a standard genetic toxicity test, and what should you do after identifying a potential genetic toxicity hazard?

A positive finding in one or more of the standard genetic toxicity tests constitutes a clear but non-quantitative identification of a potential hazard. If you receive positive results in genetic toxicity tests, additional safety testing, such as an evaluation of carcinogenicity from two-year or lifetime chronic toxicity assays, is recommended. Your safety narrative should include an explanation of the genetic toxicity results and your evaluation of their relevance.

7. What are the characteristics of a quality study using alternative methods?

When developing or using NAMs, you should consider factors including, but not limited to, the context of use, applicability domain, scope, and biological relevance of the method. While the breadth and scope of NAMs are vast and complex, there are shared quality characteristics that should be taken into account.³⁰

For example, with *in vitro* biochemical and cell-based assays, key characteristics include the use of quality materials from reputable sources, such as reagents that have undergone quality control (QC) testing and authenticated, well-characterized cell lines with documented sources and passage history. Methods should be clearly defined and documented through established standard operating procedures (SOPs) that are followed consistently to ensure reproducibility. Positive and negative controls should be included in each experiment to monitor assay performance and detect potential drift or variability. Additionally, proper maintenance and calibration of instrumentation is critical, with equipment regularly serviced, calibrated, and maintained with complete records to ensure data integrity and reliability. Finally, transparent and consistent record keeping is important, with all experimental procedures, observations, and data thoroughly documented.

As another example, with *in silico* models and computational approaches, many of the same quality characteristics listed for *in vitro* methods apply, including transparent model development

²⁹ Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. 2009, updated in 2020. Available at: https://iris.who.int/bitstream/handle/10665/44065/WHO_EHC_240_7_eng_Chapter4.pdf?sequence=7 (accessed December 17, 2025).

³⁰ See footnotes 23 and 24; Additionally, see e.g., ICCVAM Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies Washington (DC): U.S. Environmental Protection Agency; 2024 Mar. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK614924/> (noting detailed descriptions of best practices, quality tools, and analytical method assessment in Section 3.2.2, Tables 5, and Table 6.); see generally CDER 2026 - General Considerations for the Use of New Approach Methodologies in Drug Development, <https://www.regulations.gov/docket/FDA-2025-D-6131> (noting validation considerations such as context of uses, human biological relevance, technical characterization, and fit-for-purpose are relevant factors to evaluate quality NAMs).

and validation or use of known quality models.³¹ The underlying algorithms, training datasets, and validation procedures should be clearly documented or provided in software information. As with *in vitro* models, the applicability domain should be defined, explicitly stating the chemical space and biological endpoints for which the model is appropriate. Performance metrics such as sensitivity, specificity, and accuracy should be reported to demonstrate model reliability. Any variable (e.g. thresholds) should be stated in final reports. Finally, version control and reproducibility are important, with software versions, input parameters, and computational workflows documented to enable independent verification of results.

8. Should you include repeat-dose toxicity testing in your NDIN?

As dietary supplements are often used at regular intervals over an extended period of time (e.g., daily for a number of years), it is important that studies conducted help evaluate the cumulative effects of the use of the NDI or dietary supplement containing the NDI. As such, in the absence of sufficient history of use data or other data of current use to demonstrate the reasonable expectation of safety of the NDI or dietary supplement containing the NDI, a repeat-dose toxicity study is recommended to help demonstrate the safety of the NDI or dietary supplement containing the NDI.

In general, the purpose of repeat-dose toxicity testing is to define toxic effects on body systems and target organs based on repeated and/or cumulative exposure to the test substance, or to constituents and/or metabolites of the test substance, in animals. Repeat-dose testing defines the nature of the tissue or organ damage, particularly in relation to dose and duration of exposure. Repeat-dose testing is also used to identify doses associated with toxic and biological responses and to define an NOAEL.

Your safety narrative should include the design, results, and conclusion of the study or refer to the study report that includes that information. Your safety narrative should discuss a range of doses at or above the proposed serving for the NDI or dietary supplement containing the NDI described in the conditions of use, and ideally, a test article that has the same composition and form as the notified NDI or dietary supplement containing the NDI. If your test article does not have the same composition as the NDI or dietary supplement containing the NDI, the NDIN should provide a description of the qualitative and quantitative differences between the two articles and an explanation of how one supports an expectation of safety for the other.

9. What route of administration should be used for repeat-dose toxicity studies?

FDA recommends that the route of administration of your test article in repeat-dose toxicity studies be oral gavage because it is the method that most resembles human oral (ingestion) consumption of dietary supplements and provides the least amount of uncertainty between animal dose and human serving when translating animal data to a reasonable expectation of human safety. However, not all ingredients or dose levels are amenable to or achievable through oral gavage.

³¹ See footnote 30.

Feeding or water drinking studies are acceptable oral administration studies. However, feeding or drinking water administration is considered significantly different from oral gavage administration and may introduce additional toxicity concerns. Additional documentation to prove test article intake and/or waste should be included with feeding/drinking administration studies to support an effective review of the study. Without these additional data, your feeding/drinking study may be found insufficient to support an expectation of safety for your test article, NDI, or dietary supplement containing the NDI.

You may submit studies using an alternate route of exposure (e.g., spray formulation, inhalation), but for these data to be relevant to human oral (ingestion) consumption, an additional justification (and potentially additional data) should accompany the study to explain how the alternative route of exposure supports an expectation of safety for oral (ingestion) consumption of the NDI or dietary supplement containing the NDI.

10. How do you select the MTD for use in animal safety studies?

To maximize the chance that toxicity associated with the test article can be detected, the highest dose (commonly referred to as the “top dose”) in animal studies should be the MTD.³² FDA recommends that you use shorter-term studies to estimate the MTD for longer studies; for example, the results of a 14-day study should be known before the dose for a 90-day study can be determined.

Considering a broad range of biological information is essential to determining the correct MTD. For example, data concerning changes in body and organ weight and clinically significant alterations in hematological, urinary, neurological, and clinical chemistry parameters, in combination with more definitive toxic, gross, or histopathologic endpoints, can be used to estimate the MTD.

The safety narrative should include a description of the process used to select the MTD for the study if it is not readily apparent. FDA recognizes that there may be limitations on using a top dose, such as animal handling considerations (i.e., the amount that can be safely administered by gavage or the amount in feed that still permits proper nutrition). We also recommend noting such limitations in your safety narrative.

11. What is the difference between an NOEL and an NOAEL, and which should you use?

The NOAEL is a number signifying the highest dose or total daily intake level that did not elicit an adverse effect in humans or animals in a properly designed and executed toxicological study.³³ The purpose of the NOAEL is to identify a safe level of a substance (that is, the level at which no adverse effects are observed). The NOEL is the highest dose or total daily intake level at which no effects of any kind (beneficial, neutral, or adverse) are observed. Therefore, the

³² Lower doses should be used to establish the dose-response relationship and the no-effect dose.

³³ Hayes, A.W., & Kruger, C.L. (Eds.). (2014). *Hayes' Principles and Methods of Toxicology* (6th ed.). CRC Press. Available at: <https://doi.org/10.1201/b17359> (accessed December 17, 2025).

NOEL value provides little to no information on toxicity levels, which are relevant for safety assessments.

The NOAEL communicates valuable information relevant to the adverse effects that the NOEL does not, and use of the NOAEL is consistent with other risk assessment procedures. Therefore, we recommend using the NOAEL to calculate the margin of safety in the safety narrative. A comparative discussion of the effects observed at different doses of an NDI should appear in the safety narrative for the NDI, as it is central to the overall safety evaluation.

FDA anticipates that many dietary ingredients, because they are intended to have beneficial nutritional effects or other effects on the structure or function of the body, will cause changes in parameters that are measured in animal and clinical safety studies. FDA also anticipates that, as dose and total intake increase, effects that are neutral or beneficial at lower exposures may become adverse effects or be supplanted by adverse effects. Thus, it is important that the NDIN contain a discussion of the nature of the effects that are observed in safety studies. This discussion should distinguish between adverse effects and other effects (i.e., neutral or beneficial effects).

12. Should you include a subchronic oral toxicity study in your NDIN?

In the absence of sufficient data that would demonstrate the reasonable expectation of safety of the NDI (e.g., a history of safe use, data on current safe use, safety data of related ingredients, and/or data from alternative methods), a subchronic oral toxicity study is a common way to demonstrate the safety of an ingredient. Subchronic oral toxicity studies can be used to identify the MTD of a substance (e.g., with doses selected based on shorter-term, repeat-dose studies), as well as the substance's NOAEL, if conducted pursuant to an internationally recognized protocol from an authoritative source.

Subchronic oral toxicity studies are generally conducted for 90 days (3 months); FDA considers protocols described as lasting 12 or 13 weeks to be an equivalent timeframe. The appropriate animal species and study design may vary depending on the safety questions associated with the NDI being studied.

Toxicity data and the NOAEL identified by the subchronic oral study may be used to:

- predict the organ toxicity or other types of toxicity that are likely to be associated with human or animal consumption of unsafe quantities of the test article;
- determine the need for and design of additional animal studies, such as specialized toxicity studies and chronic toxicity studies; and
- assess the safety of short-term, repeat-dose exposure to the test article, either for consumers or for participants in clinical trials.

Your safety narrative should include the design, results, and conclusion of the study or refer to the study report that includes that information.

13. Should you include reproductive toxicity, teratology, and multi-generation reproductive studies in your NDIN?

If the NDI or the dietary supplement containing the NDI is recommended for use by pregnant or lactating women, we recommend including data to demonstrate safety in this sensitive population. These data could include a sufficient history of safe use, *in vitro/in silico*/NAMs to address a specific toxicity mechanism or targeted animal studies (described below). Your safety narrative should include the design, results, conclusion, and limitations of the study or refer to the study report that includes that information about the study or studies conducted. The study or studies you include in your NDIN will depend on your ingredient, its safety profile, its mechanism of toxicity and the proposed conditions of use. More information about these studies and how they can be helpful is provided below.

The *in vitro* / *in silico* / NAMs studies designed to assess developmental and reproductive toxicity (DART) endpoints serve the following purposes:

- *In vitro* studies may provide information on mode of action in specific vulnerable populations (developing conceptus or female reproductive system).
- *In silico* predictions may provide information on reactivity or bioactivity of the NDI.
- NAMs studies may provide support for mechanistic basis for effects and inform the use of safety factors to measure uncertainty.

The targeted animal studies serve the following purposes:

- **Reproductive toxicity studies** provide information regarding the effects of a dietary ingredient on all aspects of reproduction, including sexual behavior, spermatogenic and estrus cycles, gonadal function, fertility, parturition (giving birth), lactation, and prenatal development.
- **Teratology studies** provide information on whether the test article causes developmental effects in the offspring of a test animal, including congenital malformations and effects on neurodevelopment.
- **Multi-generation reproductive studies** provide growth and reproductive function data regarding the effects of the test article on male and female offspring of test animals and on the growth and reproductive function of their offspring in the subsequent generation(s); provide the basis for excluding women of child-bearing age or reproductive capacity of men from exposure to the NDI.

If pregnant or lactating women are specifically recommended not to use the NDI or dietary supplement containing the NDI, you may not find it useful to conduct these studies.

14. Should you identify the NOAEL for all test substance-related changes in reproductive and teratology studies?

Yes, you should identify the NOAEL for parental animals and their offspring in each generation in safety studies, including reproductive and teratology studies. In addition to information about reproductive success, data from the study should be used to provide information on development (i.e., growth and function of the offspring) and teratogenesis (i.e., birth defects, both structural and functional). You should also identify the NOAEL for all test substance-related changes in genotoxicity, cytotoxicity, and repeated dose oral toxicity studies. Your safety narrative should

include the design, results, and conclusion of the study or refer to the study report that includes that information.

15. Should your NDIN account for known risks or toxicities that may be specific to the NDI or dietary supplement containing the NDI?

For ingredients or constituents that may have recognized or documented toxicities (e.g., published in literature), you should submit studies to demonstrate the safety of the NDI or dietary supplement containing the NDI, with special consideration to the known risks or toxicities (e.g., studies to assess immunotoxicity, neurotoxicity, or other toxicity endpoints). If your NDIN does not contain data addressing known risks and toxicity associated with your NDI, FDA may request that you provide it. Without these data, we may be unable to determine whether the NDI or dietary supplement containing the NDI has a reasonable expectation of safety.

16. Are you required to conduct human clinical studies to assess the safety of the NDI or the dietary supplement containing the NDI?

The FD&C Act does not expressly require you to conduct human clinical studies before submitting an NDIN. However, there may be circumstances where performing such studies is recommended because the existing history of safe use data, safety data, and data on population exposure do not provide a sufficient basis for you to conclude that the dietary supplement containing the NDI will reasonably be expected to be safe under its proposed conditions of use.

17. What kinds of human clinical studies are recommended to assess the safety of an NDI or dietary supplement containing an NDI?

Where appropriate, human studies can be used alone or in conjunction with animal studies to provide other evidence of safety pursuant to 21 CFR 190.6(b)(4). In general, FDA recommends using ADME studies and short-term tolerability studies to assess safety. Where possible, FDA recommends the test article used should be identical in form, identity, and composition (including ingredients used in combination with the NDI) to the NDI or dietary supplement composition described in your notification. For studies where the test article is a related substance or product to the NDI, FDA recommends that you include a qualitative and quantitative comparison of the test article(s) and the NDI. If you choose to conduct human studies, they should be well-designed and reflect the target population proposed for the NDI or dietary supplement containing the NDI. Human studies should be evaluated for population size and the power to measure adverse events, serving size and its relation to the proposed conditions of use for the NDI or dietary supplement containing the NDI, and study duration.

Human ADME studies provide information on exposure characteristics following human ingestion. When human ADME studies are done in conjunction with ADME studies conducted in the animal species used for toxicological testing, the relevance of the animal data to humans can be demonstrated and the safety factors used to calculate the margin of safety can be reduced.

When animal toxicity studies or history of safe use data do not document an adequate margin of safety between the NOAEL for the NDI or dietary supplement containing the NDI and the

expected intake from its proposed conditions of use, we recommend a human study consisting of a repeat-dose study. Tolerability studies identify acute toxicity, such as that associated with toxins or with indigestible nutrients at very high serving levels of ingredients like fats and oils. Human repeat-dose studies (i.e., giving test subjects the test articles more than once, over a specific duration, such as 28 days) can be used to directly demonstrate the safety of the test article in humans. However, they can also address specific safety concerns raised by animal studies or history of safe use information, or establish a margin of safety for an NDI where administration to animals is problematic (e.g., solubility, undesirable taste/smell, extremely large serving size).

18. Where can you find more information and examples of clinical protocols that can be used in conducting human studies for NDIs and dietary supplements containing an NDI?

There are a number of sources with examples of clinical protocols that could be used in human studies for NDIs and dietary supplements containing an NDI.³⁴ FDA recommends consulting “Principles and Methods for the Risk Assessment of Chemicals in Food”³⁵ for a general discussion of this topic. This reference, which recognizes the value of the experience gained from pharmaceutical safety studies in designing food safety studies, also includes a discussion of the similarities and differences between clinical studies conducted for foods and those for pharmaceuticals.

In addition, “Guidance for Industry—M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals”³⁶ contains a useful discussion of selecting an appropriate dose for subchronic oral studies in animals and clinical trials in human volunteers.

When you choose a protocol, FDA recommends that you ensure that the method you use is a recognized protocol, developed by consensus, and reproducible.

19. How do you select the highest dose for use in human studies?

The highest dose in clinical studies should be governed by safety considerations, as determined by an Institutional Review Board. However, within the limits of safety, the highest dose in clinical studies should be as high as feasible. At a minimum, the highest dose or total daily intake level in a clinical trial of an NDI should be as high as the highest dose or total daily intake

³⁴ The NDI safety standard is different than the standard for food additives, drugs, and other products. Recommendations in guidance documents that are tailored to the safety assessment needs of other products may not always be appropriate for dietary ingredients or dietary supplements.

³⁵ Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. 2009, updated in 2020. Available at: <https://www.who.int/publications/i/item/9789241572408> (accessed December 17, 2025).

³⁶ FDA, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research. Guidance for Industry: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, Revision 1. January 2010. Available at: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073246.pdf> (accessed December 17, 2025).

level of the NDI under the proposed conditions of use. Preferably, the highest total daily intake level in the trial should be higher than the highest total daily intake level of the NDI.

VIII. Use of Data from Emerging Scientific Disciplines

1. What information should you submit to demonstrate the safety of a microbial NDI?

A live microbial NDI is one that is produced by fermentation of microorganisms like bacteria or yeast that are intended to be viable when consumed. As a living entity, the behaviors and characteristics of a live microbial NDI differ significantly from other dietary ingredients (e.g., contribution of media constituents, possibility for genetic changes or drift). Therefore, the data needs for microbial NDINs to demonstrate identity and safety differ from information in other NDINs. If you have a live microbial NDI, your safety narrative should:

- identify the microorganism using scientifically valid nomenclature for the genus, species, and the name of the strain;
- discuss the history of safe use of the organism or related organisms as food or to produce food;
- identify any human pathogens that are phylogenetically related to the fermentation microorganism at the species or genus level;
- characterize the ability of your microbial NDI to produce toxic products such as hemolysins, cytotoxins, virulence factors, and/or biogenic amines;
- identify any toxins, classes of toxins, or other deleterious substances known to be present in the same species as the microorganism or in a genus or species that is phylogenetically related to the microorganism;
- document the absence (or the amount, if present) of such toxins or other deleterious substances³⁷ in the microorganism; and
- document resistance to any clinically relevant antibiotics, and if applicable, the genetic nature of the resistance. If the microbial NDI is resistant to any clinically relevant antibiotics, we also recommend that you perform an assessment of the ability of the antibiotic resistance genes to mobilize and transfer to human pathogens under the proposed conditions of use.

If your NDIN cites the history of safe use of a live microorganism as evidence of safety, you should describe how the historical article is related to your NDI based on species and strain. FDA also recommends conducting a careful assessment of the relative level of historical exposure compared to the proposed conditions of use of the NDI, including a discussion of how the form of the dietary supplement and any non-dietary ingredients (e.g., binders, fillers) used affect availability of the NDI in the human gastrointestinal tract.

If history of safe use data are inadequate to support the safety of the microbial NDI, you should include safety studies in appropriate animal models or humans in your notification. Human or

³⁷ The absence of unsafe levels of deleterious substances in microbial supplements should be demonstrated by an appropriate combination of specifications for the NDI, safety testing in humans, and/or safety testing in an appropriate animal model.

animal safety studies should include measurements of the persistence of the organism in the body after administration, the ability of the organism to translocate outside of the gastrointestinal tract, and tolerance of the ingredient using the proposed serving form.

For microbial ingredients intended to be non-viable when consumed (e.g., heat-killed, pasteurized), the safety narrative should include the full microbial characterization described above for viable microorganisms, in addition to the full description of the kill step and demonstration that the microorganism is no longer viable.

Because this is a rapidly evolving scientific discipline, FDA recommends that you familiarize yourself with the state of the recent scientific literature at the time the notification is submitted.

More details about what identity information your NDIN should include when your NDI is a live microbial are included in question IV.17.

2. How should an NDI that contains nanomaterials or otherwise involves the application of nanotechnology demonstrate safety?

Nanomaterials and nanotechnology are a developing field, and the scientific literature is still emerging. An NDI or dietary supplement containing an NDI that contains nanomaterials or otherwise involves the application of nanotechnology generally would be engineered to:

- have at least one external dimension, or an internal or surface structure, in the nanoscale range (approximately 1 nanometer (nm) to 100 nm); and
- exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to 1 micrometer (1,000 nm).³⁸

Like other NDIs, the demonstration of safety relies on a history of safe use or other evidence of safety. However, because the nanotechnology version of an ingredient may have different biological and physical effects than the non-nanotechnology version of an ingredient, the non-nanotechnology data alone are unlikely to address safety concerns specific to the nanomaterial- or nanotechnology-modified version. The safety narrative should address the nanomaterial or nanotechnology's biological and physical effects on the ingredient, including a discussion of how the use of nanomaterials or nanotechnology impacts the safety of the ingredient.

FDA recommends that you contact FDA³⁹ before submitting an NDIN for an NDI that contains

³⁸ See FDA, Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology; June 2014. Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considering-whether-fda-regulated-product-involves-application-nanotechnology> (accessed December 17, 2025).

³⁹ Contact information for the Office of Dietary Supplement Programs can be found on our webpage at <https://www.fda.gov/food/dietary-supplements>. Parties requesting a meeting should be aware that meeting materials, like other FDA records, may be requested under the Freedom of Information Act (FOIA) (5 U.S.C. 552), and, unless the information being requested is trade secret, CCI, or is otherwise exempt from disclosure under FOIA and our regulations, it will be released to requesters.

nanomaterials or otherwise involves the application of nanotechnology.⁴⁰

IX. Safety Calculations and Conclusions

1. Should your NDIN discuss safety endpoints for all studies?

The safety narrative should summarize the safety endpoints from all studies and describe their relevance to an overall safety determination. Conclusions from *in vitro/in silico* studies or alternative methods may be difficult to directly extrapolate to a proposed conditions of use, but the safety narrative should describe the value of these studies to support an expectation of safety. When applicable, animal studies should select a no observed adverse effect level (NOAEL) value based on the most sensitive animal species and complete one set of safety calculations in support of an expectation of safety for the NDI or dietary supplement containing the NDI. If your notification includes more than one study that contains a NOAEL value, your safety narrative should select one NOAEL and provide a justification to support your selection of it for use in subsequent safety calculations (such as the margin of safety). However, as part of our safety assessment, FDA will review all studies included in the NDIN in addition to the selected study. In addition, FDA may ask questions or perform additional calculations related to the other submitted studies.

Clinical studies should describe the conditions of use in the study compared to the proposed conditions of use for the NDI or dietary supplement containing the NDI. The safety narrative should describe how the population of the clinical study reflects the target population for the NDI. The collection of safety endpoints is recommended to make safety conclusions from clinical studies; clinical studies collecting efficacy endpoint(s) alone often do not provide substantive data for conclusions on safety.

2. Does FDA recommend including margin of safety discussions in NDINs?

Yes, we recommend including margin of safety calculations in your safety narrative. The margin of safety is a calculation derived from the level of the NDI shown to have no adverse effect in animal or human studies (the NOAEL) and the intake level that would result from the proposed conditions of use of the NDI (i.e., the estimated daily intake, or EDI). For purposes of this guidance, the EDI for the NDI or dietary supplement containing the NDI is the highest total daily intake level under the proposed conditions of use. A margin of safety is a measure of how close the EDI is to the NOAEL. To conclude that an NDI or dietary supplement containing an NDI is reasonably expected to be safe based on animal or human safety studies, FDA recommends that you determine the margin of safety demonstrated by the proposed ingredient.

⁴⁰ FDA, Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives. Docket No. FDA-2011-D-0490. June 2014. Available at: <http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ucm300661.htm> (accessed December 17, 2025). See also FDA, Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology (June 2014). Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considering-whether-fda-regulated-product-involves-application-nanotechnology> (accessed December 17, 2025). Additional information may also be found in Docket No. FDA-2010-D-0530, available at: <https://www.regulations.gov/docket/FDA-2010-D-0530> (accessed December 17, 2025).

3. What is the difference between a safety factor and a margin of safety?

Safety factors are used to account for uncertainty about the extent to which data gathered in one context can be used to predict the safety of a substance in other contexts. For example, safety factors attempt to account for differences between animals and humans and differences in sensitivity among humans. The use of safety factors is based on the observation that toxic substances usually have thresholds below which toxic effects cannot be detected. Safety factors are used in calculating an ADI—i.e., the maximum amount of the NDI that can be ingested daily over a lifetime without an appreciable health risk—for various FDA-regulated products, including color additives, food additives, and new animal drugs. Safety factors can be combined multiplicatively to predict toxicity in the human population.

- $ADI = NOAEL / \text{combined safety factors}$

See question VIII.4 for additional discussion of safety factors.

In contrast, the margin of safety is a calculation derived from the NOAEL in a single study and the highest total daily intake level determined from the proposed conditions of use, the EDI. A margin of safety is a measure of how close the EDI is to the level that has been shown to have no adverse effect in animal or human studies (the NOAEL). The NOAEL and EDI should be described in the same units (e.g., either be in milligrams (mg) or milligrams per kilogram (mg/kg) unit).

- $\text{Margin of safety} = NOAEL / EDI$

A margin of safety of 100-fold means that the doses shown to be without adverse effects in animals or humans are 100 times greater than the levels that would be consumed from the use of the dietary supplement. The same calculation should be used whether you are calculating a margin of safety for individual ingredients in a combination or for the finished dietary supplement.

4. Should you submit margin of safety information if the NDI has a history of safe use in humans?

Even if a history of safe use is the basis of your safety evaluation, a margin of safety for NDI intake should be calculated, and the method of calculation explained and justified in the safety narrative.

When your notification relies on a history of safe use, the margin of safety should be calculated based upon the historical levels of the NDI that were safely consumed and the NDI intake levels that would result from the proposed conditions of use.

$$\text{Margin of safety} = [\text{historical level safely consumed}] / EDI$$

A margin of safety of greater than or equal to 1 corresponds to the argument that a history of safe use alone is sufficient to demonstrate the safety of the proposed use based on conditions of use that are the same as or lower than the conditions of historical use.

5. What safety factors should be used for safety calculations from animal toxicity studies?

In addition to the NOAEL, you should determine the ADI. The ADI is calculated as follows: the NOAEL, expressed on a body weight basis (e.g., mg/kg/day), is divided by a safety factor.

If the NOAEL is derived from a chronic toxicity study (one-year duration or longer) in animals, the combined safety factor is usually 100. This number is calculated using a factor of 10 to account for interspecies variation between animals and humans and another factor of 10 to account for the variation in sensitivity within the human population.

When extrapolating from subchronic toxicity studies to chronic use of an NDI or dietary supplement containing an NDI, you should use an additional safety factor. In this situation, FDA recommends referring to at least two subchronic toxicity studies, at least one of which was conducted in a non-rodent species and the other in a rodent species, and introducing another safety factor of 10 for a combined safety factor of 1,000. In the absence of supporting history of safe use data, using only a single rodent subchronic toxicity study as a basis to conclude that chronic use of an NDI in humans will be safe is strongly discouraged, but might be acceptable if a safety factor of 2,000 is used and there is no toxicity to the rodents at the MTD. The additional safety factor of 2 is used to account for the missing non-rodent subchronic study because a complete animal toxicology assessment includes two subchronic (90-day) animal studies, both rodent and non-rodent.

The safety factors in these examples are approximate values, which can vary with the specific data that are available. For example, a higher value may be appropriate if toxicity is particularly severe or the variation in human sensitivity is expected to be great. On the other hand, a lower value may be appropriate if subchronic studies in both rodent and non-rodent species showed no adverse effects. If human data from chronic toxicity or ADME studies are available, a safety factor lower than 100 may be appropriate. While FDA does not consider the ADI to be a sharp dividing line between safe and unsafe levels, the ADI may provide a useful benchmark for determining a safe serving size for the consumer.

In summary, safety factors represent uncertainty and are used multiplicatively to arrive at the combined safety factor that is applied to a particular dataset provided in a notification. This combined safety factor is used to calculate the ADI.

$$\text{ADI} = \text{NOAEL} / \text{combined safety factor} = \text{NOAEL} / (U_{\text{fintra}} \times U_{\text{fextrap}} \times U_{\text{finter}})$$

- *U_{fintra}*: An uncertainty factor to account for *intraspecies* variation is introduced to protect sensitive members of the population when clinical trials include only healthy subjects, since dietary supplements may be consumed by anyone—the young, the aged, the healthy, and the infirm. A value of 10 is usually used. The size of the *intraspecies*

uncertainty factor should be smaller when there is a long history of food use by a large, diverse population. The size of the intraspecies uncertainty factor should be larger when toxicity is severe or when a notification relies on studies with limited duration or small populations.

- *U_{finter}*: Extrapolation from animal to human requires an uncertainty factor for interspecies variation. A factor of 10 is usually used to capture the uncertainty associated with using animal studies to predict the safety of human exposure. An alternative extrapolation factor of less than 10 for an NDIN safety review may be supported by references and justification.
- *U_{fextrap}*: Extrapolating from a set of two subchronic toxicity studies in different animal species to chronic exposure in humans is not recommended, but the associated uncertainty may be approximated by an additional safety factor of 10 to account for the use of subchronic data to predict chronic use. If subchronic toxicity data are available in only a single animal species, an additional safety factor should be used. Usually, this additional safety factor is approximately 2.

6. What is the ratio of the EDI to the ADI (the EDI/ADI ratio), and how is it used?

The EDI/ADI ratio is the quantitative comparison of the estimated versus the acceptable daily intake. For purposes of this guidance, the EDI for the NDI or dietary supplement containing the NDI is the highest total daily intake level under the proposed conditions of use. The ADI is calculated from the NOAEL value and the application of a combined safety factor.

The ratio of the EDI to the ADI should be less than or equal to 1 ($EDI/ADI \leq 1$) to support a conclusion that the proposed use of the NDI in the dietary supplement will reasonably be expected to be safe under the proposed conditions of use. The size of the EDI/ADI ratio will vary in accordance with the nature and extent of data available and the circumstances of use of the NDI. For example, a ratio of 1 ($EDI/ADI = 1$), where the proposed dose (EDI) is equal to the safe dose (ADI), could be adequate if the levels of historical chronic safe use of the ingredient are the same as the levels proposed in the dietary supplement. If the ratio of the EDI to the ADI is greater than 1 ($EDI/ADI > 1$), then the study does not support a reasonable expectation of safety for the NDI under the proposed conditions of use.

For example, where safety evidence is based on a single subchronic rat study during which no adverse effects were noted at the highest dose (e.g., 3,000 mg/kg body weight), and the top dose was limited by the fact that larger volumes could not be humanely administered to the animals, the safety calculations for ADI, EDI, and the EDI/ADI ratio are as follows:

- If the proposed conditions of use for the ingredient are 1 mg/person per day in adults, the EDI is $(1 \text{ mg/person})/70 \text{ kg average adult} = 0.014 \text{ mg/kg}$; this transforms the serving level into the same units as the NOAEL.
- The margin of safety is the $NOAEL/EDI$ or $3,000/0.014 = 2.1 \times 10^5$ or 210,000.
- The safety factors chosen are $U_{fintra} \times U_{fextrap} \times U_{finter} = 10 \times 20 \times 10 = 2,000$.
- The ADI is the $NOAEL/\text{combined safety factors}$ or $3,000/2,000 = 1.5 \text{ mg/kg}$.
- The EDI/ADI ratio is $0.014/1.5 = 0.01$ (this is a unitless number).

In the example, the EDI/ADI ratio is much less than 1, which suggests that, if these safety factors were defined appropriately, the test article is likely to have a reasonable expectation of safety at the proposed daily intake level. An intake level of 1g per day (1,000 times greater) would result in an EDI/ADI ratio of close to 10. More studies would be needed to justify the higher serving level.

There are several sources that you may consult for assistance with calculation of ADI and EDI and how they are used in safety evaluations. For example:

- Pressman P, Clemens R, Hayes W, Reddy C. Food additive safety: A review of toxicologic and regulatory issues. *Toxicology Research and Application*. 2017;1.
- World Health Organization. Principles for the Safety Assessment of Food Additives and Contaminants in Food. Environmental Health Criteria. Geneva, Switzerland: 1987.⁴¹
- Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes Collection. Washington, DC: National Academy Press.⁴²

⁴¹ Available at: <https://www.inchem.org/documents/ehc/ehc/ehc70.htm> (accessed December 17, 2025).

⁴² Available at: <https://nap.nationalacademies.org/collection/57/dietary-reference-intakes> (accessed December 17, 2025).

Appendix A – Standard Safety Testing Table and Associated Questions and Answers

FDA has developed the Standard Safety Testing Table below to illustrate the safety studies that could be used to fill any data gap between historical use and proposed conditions of use. The Standard Safety Testing Table contains scientifically recognized genetic toxicity, pre-clinical animal and human studies that have traditionally been used to test the safety of a variety of other regulated products or chemicals. However, where appropriate, alternative methods are encouraged to inform a safety conclusion in the context of a scientifically valid, fit-for-purpose approach. If you choose to include NAMs data on your NDI or dietary supplement containing your NDI to support a safety conclusion, your safety narrative should provide information demonstrating its relevance to the safety endpoint of interest and for the dietary supplement context.

The Standard Safety Testing Table is designed to demonstrate that the greater the differences in the proposed conditions of use for the NDI or dietary supplement containing the NDI and the conditions of use for the historically consumed article, the more “other evidence of safety” would be recommended to fill a data gap. This recommendation to submit other evidence of safety is especially true for those ingredients with little to no documentation of historical consumption or circumstances where the proposed conditions of use for the NDI or dietary supplement containing the NDI differ significantly from that of the historically consumed article.

For ease of use, the Standard Safety Testing Table includes only those circumstances where the proposed conditions of use results in *greater exposure* (from a larger serving size or a change in frequency/duration) compared to the historical conditions of use, and therefore other evidence of safety is recommended. Where the proposed use of an NDI or dietary supplement containing an NDI results in *less exposure* than its documented historical use, FDA believes history of use data should be sufficient for purposes of the data provided in the NDIN.

History of use data alone should be sufficient in the following circumstances:

Documented Historical Use	Proposed Use of NDI	Proposed NDI Serving
Daily Chronic	Intermittent	Less than historical use
Daily Chronic	Daily Chronic	Less than historical use
Intermittent	Intermittent	Less than historical use

The information provided in the Standard Safety Testing Table should not be considered to be a comprehensive or exhaustive list of all relevant traditional safety studies, not to mention more novel methods under current or future development. In addition, you are not required to complete and submit all the studies listed in a given row of the Standard Safety Testing Table. FDA recommends that you perform additional studies to address any known toxicities specific to the NDI (e.g., immunotoxicity, neurotoxicity).

STANDARD SAFETY TESTING TABLE

		Genetic Toxicity		Pre-clinical Animal Studies				Human Studies	
Documented Historical Conditions of Use	Proposed Conditions of Use of the NDI	Two-Study Battery	Three-Study Battery	Single-dose Acute and/or ADME Study in Animals	14-Day Repeat-dose Oral Study in Animals	90-Day Subchronic Oral Study in Animals	One-Generation Rodent Reproductive Study [^]	Single-Dose Tolerability and/or ADME Study in Humans*	Repeat-Dose Tolerability and/or ADME Study in Humans*
Daily Chronic	<u>Intermittent</u> , Serving Greater Than Historical Use	X		X ^a		X		X ^a	
Daily Chronic	<u>Daily Chronic</u> , Serving Greater Than Historical Use	X		X ^a		X	X	X ^a	
Intermittent	<u>Intermittent</u> , Serving Greater Than Historical Use	X		X ^a		X	X	X ^a	
Intermittent	<u>Daily Chronic</u> , Serving Less Than Historical Use		X		X	X	X		
Intermittent	<u>Daily Chronic</u> , Serving Greater Than Historical Use		X		X	X	X		X
No History	<u>Daily Chronic</u>		X		X	X	X		X
No History	<u>Intermittent</u>		X		X	X	X		X

[^] - Reproductive study is only relevant if the NDI or dietary supplement containing the NDI is intended for use in infants, children or those that are pregnant/lactating.

* Single- and Repeat-dose tolerability or ADME studies in humans should be conducted on a case-by-case basis, as appropriate to address existing data gaps.

a – Indicates where animal or human studies are equally as relevant; you could perform a single-dose animal study OR a single-dose human study.