Microbiological Considerations for Antimicrobial Agents Used in Food Applications: Guidance for Industry

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Microbiological Considerations for Antimicrobial Agents Used in Food Applications: Guidance for Industry[1]

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 it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. Introduction

This guidance will assist submitters of food additive petitions (FAPs), food contact notifications (FCNs), generally recognized as safe (GRAS) notices (GRNs), and threshold of regulation (TOR) exemption requests in providing data to demonstrate that an antimicrobial agent (defined in Section II.A, below) achieves its intended technical effect in or on food or food contact articles. Also, this guidance discusses microbiological data that may be necessary to demonstrate that an antimicrobial agent is safe for the intended use and achieves its intended effect. This guidance replaces a guidance previously issued as "Microbiological Considerations for Antimicrobial Food Additive Submissions" (September 2007; Revised June 2008) by expanding the scope to include GRNs, by using more consistent terminology, and by eliminating portions of the guidance that reiterate information about regulatory processes addressed in regulations or other guidance documents.

Given the complexity and variety of antimicrobial agents and the diverse conditions of use, no single document can anticipate and address all microbiological issues. Therefore, this guidance is intended to answer common questions associated with microbiological data needed to establish that an antimicrobial agent accomplishes the intended technical effect and is safe for use in or on food or food contact articles. We recommend that you discuss any proposed experimental protocols with FDA before initiating them to ensure that the

[1] This guidance has been prepared by the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration.

data will address our safety questions, including those related to achieving the intended technical effect.[2] In addition, we recommend that you meet with us early during development of your products to avoid studies that may be unnecessary or that may fail to address all issues.

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

II. Questions and Answers

A. What is an antimicrobial agent?

The term "antimicrobial agent," as used in this guidance, refers to a substance (including other microorganisms) or a source of radiation used to control microorganisms such as bacteria, viruses, fungi, protozoa, or other microorganisms in or on food or food contact articles.

B. What is FDA's statutory authority with respect to antimicrobial agents used in food or in contact with food?

FDA regulates the uses of antimicrobial agents in food applications, including use in food contact materials, under the Federal Food, Drug, and Cosmetic Act (FD&C Act). You can find details on resources for participating in FDA's pre-market submission programs for substances used in food applications in Table 1. We encourage submitters to consult with us to determine which program is appropriate for the substance, its intended conditions of use, and the types of available information supporting its safe use.

Table 1. Resources for Premarket Submissions

Type of Submission	Applicable Regulations	Regulatory Guidance Documents
Food Additive Petition	21 CFR 171.1	Guidance for Industry: Questions and Answers About the Food Additive or Color Additive Petition Process (April 2011) Guidance for Industry: Antimicrobial Food Additives (July 1999)

[2] "We," "us," and "our" refers to FDA. "You," "I," and "my" refer to the responsible person (developer, sponsor, or proponent) who conducts a food safety evaluation and submits such information to FDA.

Type of Submission	Applicable Regulations	Regulatory Guidance Documents
Food Contact Notification	21 CFR part 170 – Subpart D	Guidance for Industry: Preparation of Food Contact Notifications (Administrative) (May 2002)
GRAS Notice	21 CFR part 170 – Subpart E	Guidance for Industry: Frequently Asked Questions About GRAS for Substances Intended for Use in Human or Animal Food (October 2016)
Threshold of Regulation	21 CFR 170.39	Guidance for Industry: Submitting Requests under 21 CFR 170.39 Threshold of Regulation for Substances Used in Food-Contact Articles (April 2005)

C. **Which other Federal agencies have authority over certain uses of antimicrobial agents in food applications**?

For details about regulatory authority over uses of antimicrobial agents in food applications, see our Guidance for Industry entitled Antimicrobial Food Additives (July 1999). Depending on the proposed conditions of use, an antimicrobial agent may also be a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). As such, it may be subject to registration as a pesticide by the U.S. Environmental Protection Agency as well as regulation by FDA.

The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) is responsible for determining the suitability of the use of food ingredients used in the production of meat,[3] poultry, or certain egg products. FSIS derives its authority to regulate the suitability of food ingredients from the Federal Meat Inspection Act,[4] the Poultry Products Inspection Act, and the Egg Products Inspection Act. According to FSIS, "suitability relates to the effectiveness of the substance in performing the intended technical purpose of use, at the lowest level necessary, and the assurance that the conditions of use will not result in an adulterated product or one that misleads consumers."[5] FDA and FSIS

[3] Including Siluriformes fish, see footnote 4.

[4] As a result of amendments to the Federal Meat Inspection Act in 2008 and 2014, as of March 1, 2016, FSIS has jurisdiction over all wild-caught and farm-raised Siluriformes fish that are harvested and sold for human food in the United States. This includes Siluriformes fish and fish products that are imported into the United States.

[5] *Guidance on the Procedures for Joint FSIS and Food and Drug Administration (FDA), Approval of Ingredients and Sources of Radiation Used in the Production of Meat and Poultry Products*, U.S. Department of Agriculture, Docket No. 00-022N, *E. coli* O157:H7 Contamination of Beef Products.

currently have a Memorandum of Understanding (MOU) establishing each agency's responsibilities in the evaluation of food ingredients and sources of radiation used in the production of meat, poultry, and egg products. Under this MOU, we collaborate with FSIS on the review of antimicrobial agents intended for use in the production of these products.

D. **Why does FDA request microbiological data on the use of an antimicrobial agent?**

Microbiological data address certain issues related to the use of an antimicrobial agent, regardless of the regulatory status of the substance. The legal bases for such requests are described below.

- Under section 409(c)(3)(B) of the FD&C Act, a food additive regulation will not be established if an evaluation of the data "shows that the proposed use of the additive would promote deception of the consumer in violation of this Act or would otherwise result in adulteration or in misbranding of food within the meaning of this Act." Here, the microbiological data may be used to demonstrate that an antimicrobial agent does not promote consumer deception, such as making a food product appear to be fresher or of greater value.
- Under section 409(c)(4) of the FD&C Act, if "a tolerance limitation is required in order to assure that the proposed use of an additive will be safe, the Secretary shall not fix such a tolerance limitation at a level higher than he finds reasonably required to accomplish the physical or other technical effect for which such additive is intended; and, shall not establish a regulation for such proposed use if he finds upon a fair evaluation of the data before him that such data do not establish that such use would accomplish the intended physical or other technical effect."
- When a tolerance limitation is required, the microbiological data may be used to demonstrate that the use of an antimicrobial agent achieves the intended technical effect and that the maximum intended use level is not higher than the level reasonably required to achieve this effect.
- Microbiological data may be needed to supplement the safety assessment of an antimicrobial agent that is a food additive under section 409(c)(3)(A) of the FD&C Act, which states that "No such regulation shall issue if a fair evaluation of the data before the Secretary fails to establish that the proposed use of the food additive, under the conditions of use to be specified in the regulation, will be safe…" Circumstances when microbiological data may be used to supplement the safety assessment are described in Items F, J, and K below.
- Section 409(b)(2)(C) of the FD&C Act requires that an FAP contain all relevant data bearing on the physical or other technical effect the additive is intended to produce and the quantity of such additive required to produce such effect. Therefore, a petition for a food additive that is used as an antimicrobial agent requires data on the intended technical effect.
- 21 CFR 170.230(d)[6] on GRAS Notices states that "when necessary to demonstrate

[6] In the preamble to the GRAS Final Rule, Under XIII. Comments on Part 2 of a GRN: Identity, Method of Manufacture, Specifications, and Physical or Technical Effect (Substances Generally Recognized as Safe, Final Rule 81 FR 54960 at 55001, August 17, 2016), D. Data and Information Bearing on the Physical or Other Technical Effect of the Notified Substance, we stated, "An example of when such data and information would be relevant to safety is when the intended use of the notified substance is as an antimicrobial agent."

safety," Part 2 of a GRAS Notice must include: "relevant data and information bearing on the physical or other technical effect the notified substance is intended to produce, including the quantity of the notified substance required to produce such effect."

E. **What types of data and information should be included in a submission to FDA to demonstrate that the use of an antimicrobial agent achieves the intended technical effect?**

To demonstrate that the use of an antimicrobial agent achieves the intended technical effect and that the proposed use level is the minimum level necessary to accomplish the intended technical effect, we recommend that you provide the following (at a minimum):

- The chemical or biological identity of the antimicrobial agent, whichever is appropriate;
- A detailed description of the intended antimicrobial effect and identification of individual or groups of targeted microbes, if appropriate;
- A description of the conditions of use and any limitations on conditions of use, e.g.:
  - types of foods or other substances for treatment;
  - proposed use level or range;
  - temperature range of use;
  - method of application, such as spraying, dipping or fumigating, when applicable;
  - post-processing steps (e.g., potable water rinse, cooking by the consumer);
- Antimicrobial effect data, including methods used and results;[7]
- Directions, recommendations, and suggestions regarding the proposed use, and for FAPs, a sample of the label proposed for the food additive and any labeling that will be required on the finished food because of the use of the food additive; and
- For an FAP, wording for the proposed regulation or changes to an existing regulation. This proposed language will clarify our understanding of the petitioner's intent.

F. **What factors should be taken into consideration when developing an experimental protocol to show that the use of an antimicrobial agent is safe and achieves the intended technical effect?**

You should consider certain factors when developing an experimental protocol to show that the use of an antimicrobial agent achieves the intended technical effect. These are described below:

- Simulate, to the extent feasible, the intended conditions of use of the antimicrobial agent;
- Use samples of the relevant matrix (e.g., food, process water, food packaging) treated under the intended conditions of use, and include appropriate controls;
- Consider the typical organisms expected to be observed and the conditions under which they would be present in the food. For example, the commonly consumed

[7] For GRAS notices, generally available methods are typically used to generate antimicrobial effect data.

portions of meat (beef, lamb, pork) and poultry are generally considered uncontaminated with pathogens prior to slaughter. Contamination with enteric pathogens may occur because of improper slaughtering techniques and subsequent processing; therefore, enteric pathogens are the organisms of concern for meat and poultry and should be the subjects of any technical effect experiments. (NOTE: We recommend that you consult with FSIS regarding the development of a protocol where the intended use includes the treatment of USDA-regulated products);

- Consider the pertinent microorganisms that may be present in a food. For purposes of this guidance, the "pertinent microorganism" is the most resistant microorganism of public health concern that may occur in a food.[8] The protocol should include the pertinent microorganism(s) that the antimicrobial agent is intended to target, especially those associated with a given food (e.g., *Salmonella* spp. and *Campylobacter jejuni* for poultry, *Escherichia coli* for beef, *Vibrio* spp. for seafood, *Salmonella* spp. for juices, *Salmonella* spp. and *Cronobacter sakazakii* for infant formulas, etc.). The pertinent microorganism(s) vary among different food types;
- Consider indigenous vs. introduced food pathogens. Seafood, fruits, and vegetables may have indigenous food pathogens present at harvest; other pathogens may be introduced during harvest or processing. We recommend that notifiers/petitioners consider whether their antimicrobial agent is equally effective against both types of pathogens;
- Consider the value of spoilage. Organoleptic changes caused by spoilage organisms are indicators that consumers may use to gauge the freshness of meat and poultry, seafood, and produce, and indirectly, their safety. As such, an antimicrobial agent that preferentially eliminates spoilage organisms over pathogens might allow pathogens to proliferate while suppressing spoilage organisms and their effects. Under such conditions, a consumer's senses may not reliably identify or inform an individual about spoiled and contaminated products, and the use of an antimicrobial agent under those conditions might not be safe. We recommend submitters provide data on the effects of their antimicrobial agent on specific pathogens as well as general (spoilage) populations for comparison; typically, aerobic plate counts are sufficient to characterize general populations;
- Consider unintended consequences. Experimental design should consider and address whether the use of the antimicrobial agent may result in unintended consequences. For example, an antimicrobial agent may change the microbiological profile of food such that it suppresses one group of pathogenic microorganisms while allowing others to proliferate, thereby creating a potential health problem.

G. How should data be generated to demonstrate that the use of an antimicrobial agent achieves the intended technical effect?

We recommend that all protocols designed to demonstrate the intended technical effect(s) of the use of an antimicrobial agent:

[8] See, e.g., 21 CFR 120.24.

- Use a direct method to enumerate microorganisms, such as plate counting or visual microscopic counts, as opposed to measuring biomass; evaluate the reduction (or suppression) of microorganisms affected by the treatment;
- When available, use enumeration methods that capture damaged or stressed microbial cells that survive the antimicrobial treatment;
- For all data, collect and analyze replicate samples and describe the variability of the data; and
- Use statistical analyses to compare data from the treated samples to data from controls.

We recommend that submitters consult with FDA in designing experimental protocols prior to the initiation of any experiments. It is our experience that these consultations lead to consensus between FDA and the submitter prior to conducting experiments. We believe these consultations result in data that are more likely to address FDA's concerns, leading to a more efficient regulatory process.

H. Does FDA recommend any specific experimental methods to perform a safety assessment?

We recognize that you can address relevant safety issues for an antimicrobial agent in multiple ways. However, we recommend several resources specific to microbiological methods. FDA publishes an online version of the Bacteriological Analytical Manual which presents useful laboratory procedures (used by FDA labs) for microbiological analyses of foods and cosmetics. Furthermore, FDA maintains a website with links to related resources on Microbiological Methods. Also, FSIS maintains a Microbiology Laboratory Guidebook of current protocols for analytical tests FSIS uses in USDA regulated products; these test procedures may be useful in developing protocols to assess technical effect. However, a submission will not be treated differently simply because alternative protocols were used.

I. Does FDA have a performance standard for the use of a new antimicrobial agent?

We do not have a performance standard requirement for the efficacy of an antimicrobial agent for use in or on food or food contact articles in general; however, data are needed to demonstrate that an antimicrobial agent achieves its intended technical effect and that the intended use level is the minimum level necessary to achieve the intended technical effect. Therefore, efficacy data should show a quantifiable reduction of target microbes in the samples treated with the antimicrobial agent when compared to corresponding negative controls (e.g., the treatment absent the active antimicrobial agent) and that the intended use level is the minimum level necessary to achieve such a reduction. However, certain regulations may specify performance standards for some types of food (e.g., reductions as specified in the Juice Hazard Analysis and Critical Control Point regulation (21 CFR 120.24)).

J. **What information may be helpful to provide to FDA to demonstrate that a source of radiation achieves the intended technical effect as an antimicrobial treatment for food?**

In addition to the data and information typically submitted in an FAP, a petition for the use of a source of radiation as an antimicrobial treatment for food must include additional information related to antimicrobial effects (21 CFR 171.1(c)). Examples of this information include:

- Information describing pertinent pathogens and other microorganisms of concern in the targeted foods; this includes the occurrence and levels of pathogenic and non-pathogenic microorganisms; and
- Information describing the effects of the proposed irradiation on microorganisms in or on the targeted food, including the radiation sensitivity of the microbes of concern, how the change in microbial population could affect and permit the outgrowth of pathogens without competing spoilage organisms, and the comparison of the microbiological safety of the irradiated food to the untreated food. For example, the pathogenic spore-forming bacterium *Clostridium botulinum* producing type E toxin is associated with coastal water fish in certain areas. If a petitioner wished to irradiate fish that may be contaminated with *C. botulinum*, they would need to demonstrate that naturally occurring spoilage organisms would be able to outcompete the pathogen and spoil the fish before toxin production by *C. botulinum* occurs in the irradiated fish.

K. **What safety information should I provide to FDA if my antimicrobial agent is derived from a microorganism or is a microorganism?**

If an antimicrobial agent is derived from a microorganism or is itself a microorganism, we recommend the following:

- Describe the biological identity of the specific strain of microorganism to be used for production. If the strain has been genetically altered, regardless of method, describe how the strain was derived, including information about the biological sources contributing genetic material to the production strain, focusing largely on the safety of the production strain or the microbe itself;
- Describe the quality control procedures used during production, including how you ensure pure cultures of the production organism and the procedures you follow when contamination is observed in the starter cultures or during production;
- Describe the methods used to verify that the microorganism does not produce clinically relevant antibiotics or reasons why this would not be needed. **The production microorganism should not produce clinically relevant antibiotics in preparations of the antimicrobial agent;**
- Demonstrate that the production strain is nonpathogenic and does not express toxins, or if you use a pathogenic or toxigenic strain, demonstrate that toxins are not present in the final food product at a toxicologically relevant level; and,
- Describe the methods and relevant quality control procedures to ensure that no viable cells of the production strain are found in the product when those cells present a safety concern.

We understand that for some newly developed antimicrobial agents, considerations may differ. We will consider whether the information you provide in antimicrobial agent submissions establish the safety of the intended use on a case-by-case basis.

The document above supersedes a previous guidance issued in June 2008.