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Guidance for Industry

ASSESSMENT OF THE EFFECTS OF ANTIMICROBIAL DRUG RESIDUES FROM FOOD OF ANIMAL ORIGIN ON THE HUMAN INTESTINAL FLORA

Final Guidance

This guidance discusses a recommended pathway approach for assessing the effects of antimicrobial drug residues in food on the human intestinal flora. This document supercedes the current guidance #52, Guideline for Microbiological Testing of Antimicrobial Drug Residues in Food, published in January of 1996.

Comments and suggestions regarding this document should be sent to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with the Docket No. 93D-0398. Submit electronic comments at <http://www.fda.gov/dockets/ecomments>.

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Additional copies of this final guidance may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855 and may be viewed on the Internet at <http://www.fda.gov/cvm>.

Paperwork Reduction Act Public Burden Statement

According to the Paperwork Reduction Act of 1995, a collection of information should display a valid OMB control number. The valid OMB control number for this information collection is 0910-0521. It expires 1/31/07. The time required to complete this information collection is estimated to average 70,550 hours per response, including the time to review instructions, search existing data resources, gather the data needed, and complete and review the information collection.

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
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ASSESSMENT OF THE EFFECTS OF ANTIMICROBIAL DRUG RESIDUES FROM FOOD OF ANIMAL ORIGIN ON THE HUMAN INTESTINAL FLORA¹

This guidance represents the agency's current thinking on the approach that should be used to assess the microbiological safety of antimicrobial drug residues in food of animal origin. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

The human intestinal flora is a balanced ecosystem that is very important in maintaining an individual's health. Although this system is generally stable, clinical studies have shown that therapeutic doses of antimicrobials may change the balance.^(1,2,3) The type or extent of change in the system will depend on the spectrum of action of the antimicrobial drug, its dose, and the length of an individual's exposure to the drug. The lowest concentration of any antimicrobial drug that can affect the intestinal flora is not clear. However, studies in *in vitro* (continuous or semi-continuous flow culture systems) and *in vivo* human flora-associated rodent (rodents implanted with human fecal flora) test systems and in human volunteers have shown that low levels of antimicrobial drugs are capable of altering different parameters of the intestinal flora depending on the spectrum of action and concentration of drug.^(4, 5, 2, 3)

The main adverse effects of antimicrobial drugs on the human intestinal flora are selection of resistant bacteria and disruption of the colonization barrier (or barrier effect) of the resident intestinal flora. Colonization barrier or barrier effect is the "limiting action" of the normal flora on colonization of the bowel by exogenous or indigenous potentially pathogenic microorganisms.⁽⁶⁾ Other effects, such as alteration of the metabolic activity of the flora, may be important, also.

Regulators and sponsors of new animal drugs have an interest in establishing relevant and validated methods for determining the effects of microbiologically active animal drug residues on the human intestinal flora. Any such effects should be assessed in the human food safety evaluation of new animal drugs intended for use in food-producing animals. Among the *in vitro* and *in vivo* approaches currently used to study the effect of antimicrobial drugs on the human intestinal flora are quantitative *in vitro* antimicrobial drug susceptibility testing, static batch cultures, semi-continuous and continuous flow

¹ This guidance has been prepared by the Office of New Animal Drug Evaluation in the Center for Veterinary Medicine at the Food and Drug Administration.

culture systems, simulated gut models, human volunteers, conventional animals, gnotobiotic rodents, and human flora-associated rodents.

CVM has decided to modify Guidance No.52 “Microbiological Testing of Antimicrobial Drug Residues in Food” published in January of 1996 ⁽⁷⁾ based on information made available after that time concerning the effects of low doses of different classes of antimicrobial drugs on the human intestinal flora. Therefore, CVM is recommending that sponsors use a “pathway approach” (described below) for addressing the human food safety of antimicrobial drug residues rather than the approach described in the 1996 version of the guidance. The scientific rationale for this decision and the FDA/CVM regulatory history on this issue are provided in a separate document available to the public at the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. (“History and Scientific Issues Related to Guidance #52”)

The pathway approach presented in this guidance document represents a general approach for assessing the microbiological safety of antimicrobial drug residues in food. If further microbiological studies are warranted for determining the Acceptable Daily Intake (ADI) for a new animal antimicrobial drug, the sponsor of that drug is encouraged to contact the Center to discuss the appropriate test systems and protocols for the studies.

CVM is aware that the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) is currently drafting a related guideline and that this guidance may be superceded at a later date by a guidance document published by the VICH.

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

II. PATHWAY APPROACH FOR ADDRESSING THE EFFECTS OF ANTIMICROBIAL DRUG RESIDUES FROM FOOD OF ANIMAL ORIGIN ON THE HUMAN INTESTINAL FLORA

The conditions and rationale for addressing the microbiological safety of antimicrobial drug residues in food are simplified in a chart at the end of this section.

The microbiological safety of antimicrobial drug residues in food is an important issue that should be addressed by the sponsor of a new animal drug. An assessment of the safety of antimicrobial drug residues in food should be part of the human food safety component of new animal drug applications for antimicrobial drugs. If these residues have no antimicrobial activity against representatives of the human intestinal flora (*E.*

coli, and species of *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium* (*Collinsella*), *Fusobacterium*, *Lactobacillus*, *Peptostreptococcus/Peptococcus*), an ADI should be calculated based on traditional toxicology studies. However, if the residues have antimicrobial activity, the sponsor should address the potential availability of these microbiologically active residues in the human colon. It should be assumed that the human colon would be exposed to all residues present in the edible tissues, unless the sponsor can demonstrate through reference to controlled experimentation in humans or animals (e.g., pharmacokinetic studies, bioavailability studies, etc.) that some or all of the residues have no potential to enter the colon.

If it is determined that microbiologically active residues can enter the colon, the sponsor should assess the potential of these residues to select for resistant bacteria, disrupt the protective barrier effect provided by the intestinal flora, or otherwise alter the balance of intestinal flora. The sponsor may attempt to demonstrate that the residues are metabolized rapidly to microbiologically inactive compounds or are rapidly bound to intestinal contents and rendered microbiologically unavailable in the human colon. Alternatively, if the antimicrobial residues are not metabolized or bound such that they are microbiologically inactive, the sponsor should determine if these residues would cause adverse effects on the intestinal flora. This could be done using appropriate human data, if available, or data from *in vitro* or *in vivo* test systems. The adverse effects of human health concern that should be considered are the potential of these residues to select for resistant bacteria, disrupt the protective barrier effect provided by the intestinal flora, or otherwise alter the balance of intestinal flora. If no information is available, the sponsor should perform studies using an *in vitro* or an *in vivo* test system to determine the endpoint(s) of human health concern.

The sponsor may wish first to perform preliminary studies such as batch cultures with fecal suspensions or an *in vivo* preliminary study to determine which microbiological endpoint is suspected to be altered by the drug. The sponsor may also choose to perform a definitive study using an *in vitro* or an *in vivo* test system to study the effect of the drug on the endpoints of human health concern and determine the no-observable effect concentrations/no-observable effect levels (NOECs/NOELs) for these endpoints. However, if the endpoint(s) of concern have been determined, definitive studies using *in vitro* or *in vivo* test systems should be performed to determine the NOEC/NOEL for the drug on the chosen endpoint(s).

If disruption of the colonization barrier is the endpoint of concern, either *in vitro* (e.g., continuous or semi-continuous culture systems) or *in vivo* test systems (e.g., human flora-associated rodent test systems) are preferable for determining a NOEL for this endpoint, as opposed to *in vitro* antimicrobial susceptibility testing to generate minimum inhibitory concentration (MIC) data. This is because these systems have the potential to better approximate the effects of microbial interactions and high bacterial densities than MIC data. However, the sponsor could use MIC data as an option to estimate a conservative microbiological ADI. MIC data indirectly assess changes in bacterial populations. If the antibiotic concentration is below the levels that inhibit cell growth, it could be assumed

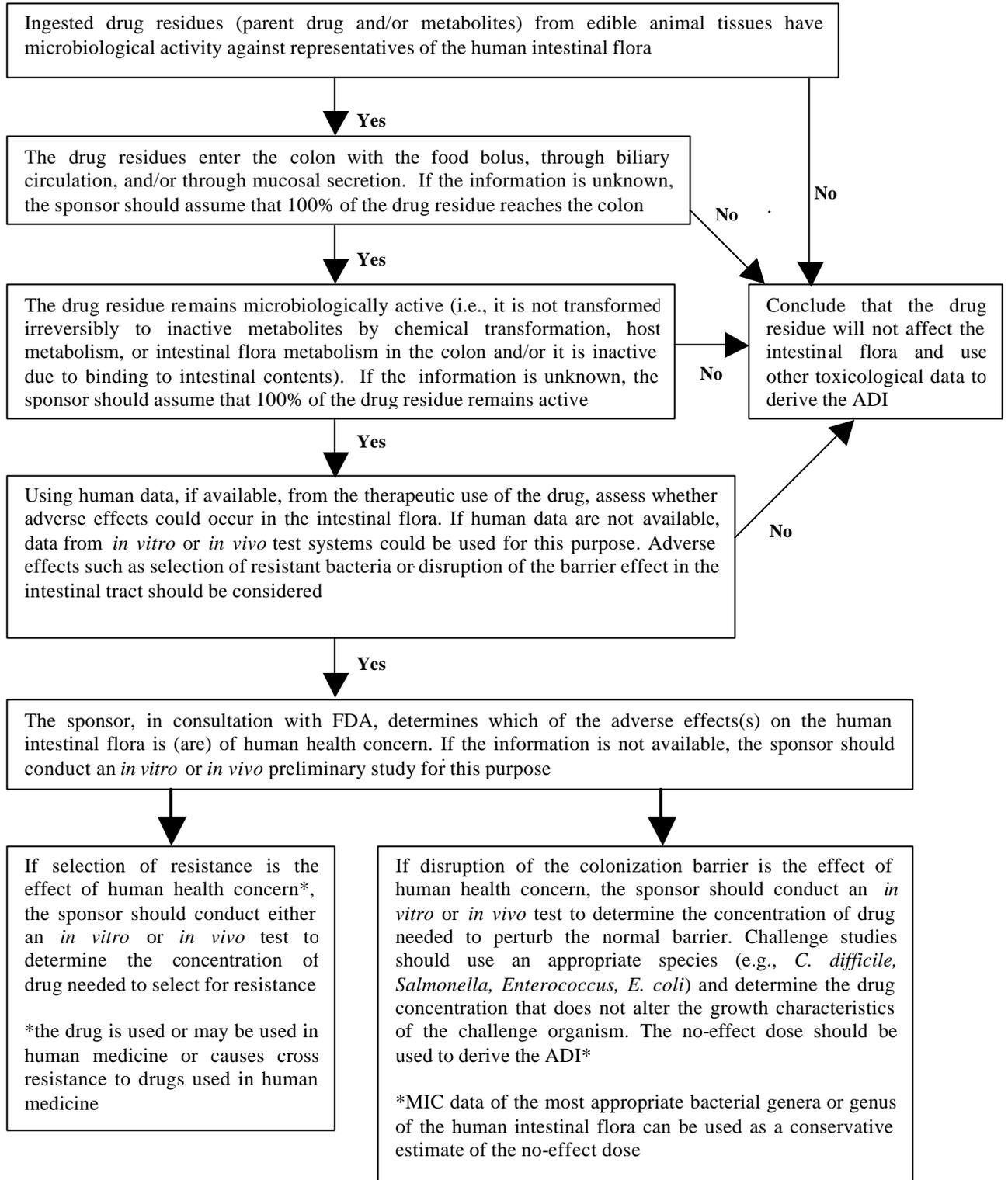
that the bacteria responsible for the barrier effect would not be affected. Vice versa, if bacteria are not allowed to grow due to low concentrations of antimicrobials, an unbalanced flora could allow the establishment of a pathogen that is not sensitive to the antibiotic in study.

The ADI derived from MIC data is conservative because the inoculum density used for testing is orders of magnitude lower than the bacterial population of the colon. In addition, the growth conditions in MIC testing (growth medium, pH, lack of fecal solids, lack of microbial interactions and drug metabolism, etc.) minimize the potential of drug inactivation. If MIC testing is used to derive an ADI, the median MIC obtained by standard methods such as those of the National Committee for Clinical Laboratory Standards (NCCLS) should be used to determine a NOEC. It is recommended that at least 10 isolates from each of the most representative bacteria listed on pages 4 and 5 of this guidance be obtained from healthy human volunteers.

Although the use of MIC data may be an option to derive an ADI, CVM does not encourage the use of this data for determining the NOEC for disruption of the colonization barrier of the human intestinal flora. Quantitative *in vitro* determinations of antimicrobial susceptibility do not reflect or account for factors such as bacterial population density, pH, intestinal growth conditions, bacterial metabolism, bacterial antagonism, or other factors of relevance to the human colonic flora. A more appropriate NOEC may be obtained through other test systems that better model the intestinal flora.

If the endpoint of concern is the selection of resistant bacterial strains, the sponsor should conduct *in vitro* or *in vivo* studies in test systems to determine a NOEL for this endpoint. Quantitative *in vitro* determinations of antimicrobial susceptibility, leading to the generation of MIC data that is coupled to the effects generated in the test system(s), should be an element of this analysis.

**PATHWAY APPROACH FOR ADDRESSING THE EFFECTS OF ANTIMICROBIAL
DRUG RESIDUES FROM FOOD OF ANIMAL ORIGIN ON THE HUMAN
INTESTINAL FLORA**



III. REFERENCES

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