#3

Guidance for Industry

General Principles for Evaluating the Human Food Safety of New Animal Drugs Used In Food-Producing Animals

DRAFT REVISED GUIDANCE

This draft revised guidance document is for comment purposes only and makes revision to the final guidance that was made available July 2006.

Submit comments on this draft revised guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For further information regarding this document, contact Julia Oriani, Center for Veterinary Medicine (HFV-151), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 240-402-0788, e-mail: julia.oriani@fda.hhs.gov.

Additional copies of this draft revised guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
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Draft Revised Guidance for Industry

General Principles for Evaluating the Human Food Safety of New Animal Drugs Used In Food-Producing Animals

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

I. Introduction and Scope

This draft revised guidance describes the type of information that the Food and Drug Administration's (FDA's) Center for Veterinary Medicine (CVM) recommends sponsors provide to address the human food safety of new animal drugs used in food-producing animals.

The human food safety evaluation of new animal drugs used in food-producing animals helps ensure that food derived from treated animals is safe for human consumption. Sponsors are required to furnish to CVM scientific data or information necessary to demonstrate that residues of the new animal drug in the edible tissues of treated animals are safe (see section 512(b)(1) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act)). In general, studies conducted to provide this information must be conducted in accordance with FDA's Good Laboratory Practice (GLP) regulations. See Title 21, Code of Federal Regulations, Part 58 (21 CFR 58).

FDA conducts a human food safety assessment of new animal drugs for use in food-producing animals through hazard identification, hazard characterization and mitigation to reduce human exposure to residues in food derived from treated animals. CVM developed this draft revised guidance to inform sponsors of the scientific data and/or information that may provide an acceptable basis to determine that the residue of a new animal drug in or on food, when consumed, presents a reasonable certainty of no harm to humans. This guidance describes a recommended approach for providing human food safety scientific data and/or information. CVM acknowledges that alternate approaches also may be appropriate and encourages sponsors to discuss with CVM whether an alternate approach may be appropriate for specific new animal drugs.

This draft revised guidance provides, in one document, an overview of the overall process for the human food safety evaluation of new animal drugs used in food-producing animals, including:

- Determining an acceptable daily intake (ADI);
- Calculating safe concentrations;
- Assignment of a tolerance;
- Calculation of a withdrawal period and a milk discard time; and
- Evaluation of carcinogenic compounds

It is intended to be used in conjunction with other CVM Guidance for Industry (GFI) documents that are cited in this draft guidance or listed on FDA's website.[1]

FDA's guidance documents, including this draft revised 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's guidances means that something is suggested or recommended, but not required.

II. Legal Authority

FDA is required by sections 512(d)(1)(A), (B), (D), and (F) of the Federal Food, Drug, and Cosmetic Act (the FD&C Act) to determine whether the edible tissues derived from treated animals are safe for human consumption. The pertinent regulations implementing these statutory provisions are found at 21 CFR 514 and 556.

Section 512(d)(2) of the act explicitly provides that, in determining whether a new animal drug is safe, FDA consider the probable consumption of any substance formed in or on food because of the use of such new animal drug. Safety of these formed compounds typically is addressed by evaluating the toxicology (including the impact of residues on human intestinal flora), residue chemistry and, if the new animal drug is of an antimicrobial drug class important to human medicine or is recognized to have certain properties that would promote antimicrobial resistance among bacteria of public health concern, microbial food safety.

III. Overview of the Human Food Safety Evaluation

FDA's evaluation of human food safety is a risk-based approach that may include hazard identification, hazard characterization, exposure assessment, and exposure mitigation. The assessment is conducted from the perspectives of toxicology, residue chemistry, and antimicrobial resistance as outlined in the following flow diagram (Figure 1).

[1] All FDA guidances mentioned in this document are available at http://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

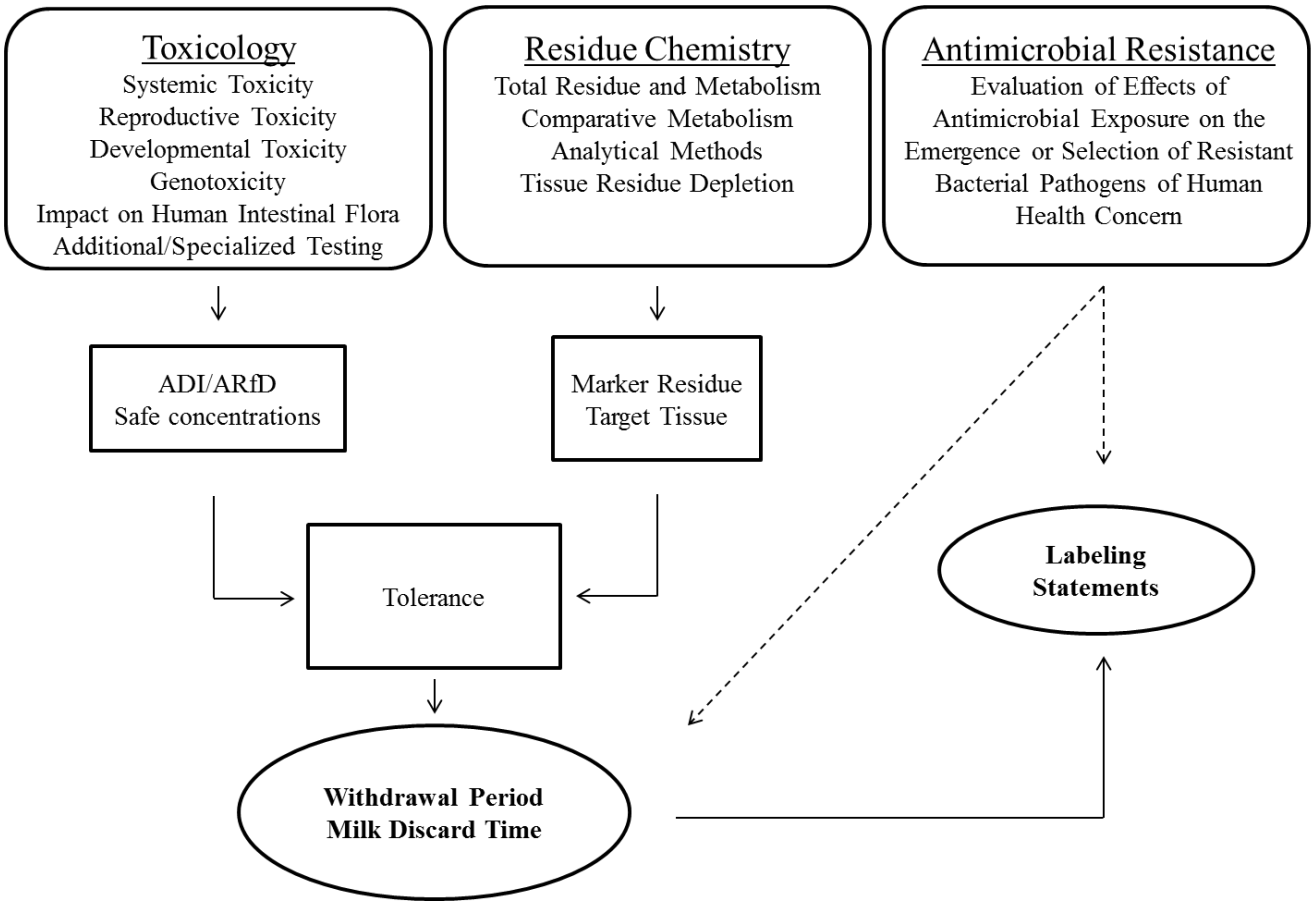

Figure 1: Flow Diagram Illustrating the General Approach for the Human Food Safety Evaluation. The dashed lines indicate that the component applies to new animal drugs with antimicrobial activity. ADI means Acceptable Daily Intake. ARfD means Acute Reference Dose.

IV. Microbial Food Safety (Antimicrobial Resistance)

The Agency routinely evaluates the ability of antimicrobial new animal drugs to exert pressure towards the emergence or selection of antimicrobial resistant bacteria of human health concern.

In GFI #152, "Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern," CVM outlines two potential approaches to address the risk associated with new animal drugs possessing antimicrobial activity: 1) a hazard characterization, and 2) a complete, qualitative antimicrobial resistance risk assessment.

Sponsors electing to use these approaches should first characterize the hazard and the conditions that influence the occurrence of that hazard. CVM recommends sponsors submit the hazard characterization as a distinct, stand-alone document, separate from the complete, qualitative risk assessment. This hazard characterization will enable CVM to advise the sponsor about the

information that should be included in a complete, qualitative risk assessment. It also may enable CVM to determine that completion of a complete, qualitative risk assessment is not necessary for certain new animal drugs possessing antimicrobial activity.

Concerns outlined in GFI #152 may not need to be addressed if the new animal drug, including the drug's metabolites and excipients, are not:

- regularly considered to have properties that would exert pressure towards the emergence or selection of bacteria of public health concern;
- used to treat zoonotic gastroenteritis or other bacterial diseases in humans;
- under development to treat bacterial diseases in humans; or
- indicated for a bacterial disease in food-producing animals (i.e., indication is instead antifungal, antiprotozoal, anthelminthic, etc.).

Please contact the Director, Division of Human Food Safety, for further guidance should you think these conditions are attributable to your new animal drug product.

V. Toxicology

A. Introduction

For new animal drugs used in food-producing animals, CVM is concerned with both intermittent and chronic exposure of people to relatively low concentrations of residues. The toxicity studies described in this guidance document are a standard set of studies designed to evaluate the oral toxicity to humans who may be exposed to new animal drug residues through the consumption of food derived from animals treated with it. CVM recognizes, however, that this standard toxicological testing paradigm may not always be the most appropriate approach to evaluate the safety to humans from the consumption of a particular new animal drug. CVM considers alternative and new approaches to address toxicological concerns when such approaches are scientifically justified. For such approaches, CVM's recommendations for the type and duration of toxicological studies and other information needed to demonstrate human food safety are specific to each new animal drug. In making its recommendations for alternative approaches to addressing toxicological concerns, CVM considers, for each new animal drug and its residues:

- Physiochemical properties
- Proposed use
- Mode of action
- Probable amounts and patterns of human exposure under the proposed conditions of use
- Possible biological effects of the residues as deduced through structure-activity comparisons; and
- Observed effects when new animal drugs are tested in toxicological and biological model systems

This information can be used to identify a more targeted approach to the design of individual toxicology studies, such as a recommendation to use fewer test animals or to use alternative toxicological endpoints in specific studies. It can also lead to modifications to the overall toxicological testing strategy for a particular new animal drug, such as recommending that a non-traditional toxicology study be conducted to help understand the results of traditional studies or recommending that one or more standard toxicology studies may not be needed to determine the human food safety for a particular new animal drug.

The purpose of toxicological studies is to define the biological effect(s) of the new animal drug and to establish the threshold dose for the effect(s). CVM typically requests testing of the new animal drug, with particular focus on the test new animal drug compared to a control article. In addition to the information regarding the test new animal drug, separate information on the toxicology of a metabolite and/or excipient(s) may be considered necessary to assess human food safety. Consult GFI #100, "Impurities: Residual Solvents in New Veterinary Medicinal Products, Active Substances, and Excipients (Revision)" for additional information regarding the toxicity information recommended for determining the human food safety of residual solvents and excipients.

B. **Generally Recommended Testing Approach**

One recommended testing approach to assure the safety of human food derived from animals treated with new animal drugs is outlined in GFI #149, "Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Testing." However, CVM recommends that sponsors discuss their proposed approach with CVM for determining human food safety prior to conducting studies. GFI #149 references additional human food safety toxicology guidance documents and the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines (GL),[2] as listed in the following table.

Table 1: Guidance Documents for Toxicology Studies for the Human Food Safety Evaluation

Title	GFI #	VICH GL #
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Repeat-Dose (90-Day) Toxicity Testing	147	31
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Repeat-Dose (Chronic) Toxicity Testing	160	37
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Developmental Toxicity Testing	148	32
Safety Studies for Veterinary Drug Residues in Human Food: Reproduction Toxicity Testing	115	22
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Genotoxicity Testing	116	23

[2] http://www.vichsec.org/

Title	GFI #	VICH GL #
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: Carcinogenicity Testing	141	28
Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI	159	36

Sponsors should consult the referenced guidance documents if they intend to design and conduct repeat-dose (90-day) subchronic toxicity studies, repeat-dose chronic toxicity studies, developmental toxicity studies, reproduction studies, genotoxicity studies, or carcinogenicity studies. Sponsors should consult GFI #159 if they need to determine the acute adverse effects of antimicrobial residues on the human intestinal flora (see section V.E.2. below). The studies and approaches described in these guidance documents recommend standard testing protocols that are likely to satisfy the toxicology requirements for the human food safety evaluation of a wide range of new animal drugs. Alternative, scientifically justified protocols may also be appropriate for some new animal drugs.

The specific recommendations to demonstrate safety with reasonable certainty of no harm with respect to potential residues in the edible tissues of animals that humans may consume are determined on a case-by-case basis. In all cases, sponsors should contact CVM to discuss the types of studies recommended to demonstrate human food safety of a particular new animal drug, the details of any human food safety experimental protocol, or to receive additional information for any other questions they have about the details of a particular study.

C. **Evaluation of Non-traditional New Animal Drugs**

CVM recognizes that the traditional toxicological testing paradigm described in guidance documents is often the most appropriate approach for new animal drugs that are small molecules, but may not be the most appropriate approach to evaluate the safety to humans from the consumption of food derived from animals that have been treated with other, "non-traditional" new animal drugs. For non-traditional new animal drugs, CVM considers appropriate alternative approaches to address toxicological concerns. When evaluating such new animal drugs, CVM adopts a case-by-case approach that is often based on their unique biological and chemical properties or mode of action. Examples of "non-traditional" new animal drugs that could be evaluated by an alternative toxicological approach are biotechnology-derived new animal drugs, including some enzymes, fusion proteins, hormones, and antibodies.[3]

[3] This document does not cover animals with heritable or non-heritable rDNA constructs as defined and discussed in GFI 187. The document also does not apply to rDNA vaccines and live vaccines that stimulate a protective immune response that are regulated by the United States Department of Agriculture under the Virus-Serum-Toxin Act (21 U.S.C. 151-159 et seq.).

Sponsors of such "non-traditional" new animal drugs for use in food-producing animals should provide sufficient information to demonstrate that the residues are safe for human consumers. Scientific rationale should be provided to justify alternative approaches for evaluation. Because the approaches are non-standard, it is recommended that the sponsor contact CVM early in the development process to discuss approaches and specific studies to address toxicological concerns for these "non-traditional" new animal drugs for use in food-producing animals.

D. Additional or Specialized Toxicological Studies

Occasionally, on a case-by-case basis, CVM will recommend that sponsors conduct additional or specialized toxicological studies to address unanticipated or unusual toxicological or pharmacological findings from the initial set of recommended toxicology studies. The additional, specialized studies may be requested to address a particular toxicity concern (e.g., adverse nutritional effects, immunotoxicity, cardiotoxicity, neurotoxicity), or to investigate specific, anticipated biochemical effects not effectively evaluated in traditional toxicology tests, such as effects on hormones or neurotransmitters.

E. Establishing the Acceptable Daily Intake (ADI), Acute Reference Dose (ARfD), and Safe Concentrations

1. Determination of the Toxicological Acceptable Daily Intake

The safety of residues in foods is usually addressed through calculation of an acceptable daily intake (ADI). The ADI is the daily intake which, during up to an entire life of a human, appears to be without adverse effects or harm to the health of a consumer. The toxicological ADI is based on the new animal drug's toxicological or pharmacological properties as determined through recommended toxicology studies and other applicable scientific information and reflects the uncertainties with the information. It is usually expressed in micrograms or milligrams of the new animal drug per kilogram of body weight per day (µg/kg bw/day) or (mg/kg bw/day). CVM uses a weight of evidence approach for this hazard characterization, which means that all data from available studies and other applicable scientific information are considered.

Traditional toxicology studies are designed to determine if the new animal drug produces an adverse effect in a biological test system and to identify the highest dose of the new animal drug that produces no observable effect, which may be a no-observed-effects dose (NOEL), a no-observed-adverse-effects dose (NOAEL) or a benchmark dose (BMD).[4] The BMD is the dose associated with a specified

[4] See Benchmark Dose Software (BMDS) available from the US Environmental Protection Agency [https://www.epa.gov/bmds] and PROAST available from the National Institute for Public Health and the Environment of the Netherlands (RIVM) [http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST].

low incidence of response, generally in the range of 1 to 10%, of a health effect, or a dose associated with a specified measure or change in a biological effect. Because it is often difficult to determine whether a particular observed effect in an animal toxicology study is adverse to humans or not, it is generally assumed that such effects are adverse, and that a new animal drug's NOAEL is equivalent to its NOEL.

When establishing the ADI, the toxicities of the new animal drug and of its principal metabolites are considered, and the ADI is based on the toxicological end-point of most concern. For non-carcinogenic endpoints, the NOAEL/NOEL or the BMDL (lower one-sided confidence limit of the BMD) from the most appropriate toxicological study and toxicological endpoint from that study will be used as the point of departure (POD) for calculating the toxicological ADI for the total residues of the new animal drug. The toxicological ADI is derived by dividing the POD by an appropriate safety factor, using the following formula:

$$\text{Toxicological ADI} = \frac{\text{NOAEL/NOEL or BMDL}}{\text{Safety Factor}}$$

The safety factor used in this calculation reflects uncertainties associated with the extrapolation of data and information from toxicology studies to humans, including extrapolation of long-term, chronic effects from laboratory studies with shorter-term exposures, extrapolation of animal data to humans, and variability in sensitivity to the toxicity of the new animal drug among humans. Generally, the safety factor consists of multiples of 10, with each factor representing a specific uncertainty inherent in the available data. For example, CVM has historically applied a safety factor of 100 for an ADI based on the NOAEL/NOEL from a chronic toxicity study (10-fold for extrapolating animal data to humans and 10-fold for variability in sensitivity to the toxicity of the new animal drug among humans). Thus, the overall safety factor used in the calculation of a toxicological ADI is dependent on the interplay among many factors, including the type of study from which the NOEL/NOAEL was derived, the species of animal used in the study, and the endpoint(s) observed in the study. The toxicological ADI is expressed with the appropriate number of significant figures as justified by the underlying scientific information used in these calculations (usually one significant figure). This avoids any inference of inappropriate precision, and is consistent with mathematically acceptable rounding procedures.

For compounds of carcinogenic concern used in food-producing animals, see section VII.

2. Impact of Residues on Human Intestinal Flora - Determining the Microbiological Acceptable Daily Intake (mADI)

   VICH Guideline (GL) 36/FDA GFI #159, "Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a

Microbiological ADI" aims to ensure that residues in the edible tissues of food-producing animals are safe for humans to consume. This guidance describes a harmonized step-wise approach to determine if residues of antimicrobial new animal drugs, their metabolites or any excipients reaching the human colon remain microbiologically active, affect intestinal bacteria, and whether a determination of a mADI is necessary.

The microbiological endpoints of current public health concern that should be considered when establishing a mADI are the disruption of the colonization barrier and an increase of the population(s) of resistant bacteria. The final mADI for total residues in edible tissues is the lesser of these two microbiological endpoints. Evaluations of these assessments are performed by expert microbiologists in the Division of Human Food Safety's Microbial Food Safety Team.

3. Establishing the Final ADI

   New animal drugs with antimicrobial activity may be assigned both a microbiological ADI and toxicological ADI. The final ADI is the more appropriate of these two ADIs, which is usually the lower of the toxicological and microbiological ADIs. In some instances, CVM may not establish an ADI for a new animal drug if it does not present a hazard to human health. In these situations, CVM will conclude that an ADI is not needed for the new animal drug.

4. Acute Reference Dose (ARfD)

   There is the potential for some new animal drugs to cause acute toxicity to the human consumer following consumption of a single meal or consumption of food over a single day. Some toxicological tests may suggest the need for a safe intake value in addition to the ADI to account for new animal drugs that cause acute toxicity. In these cases, the ADI is not the appropriate safe intake value for quantifying the dose above which exposure from a single meal or over a single day can produce acute adverse effects; instead, determining an acute reference dose (ARfD)[5] is the most appropriate approach.

   The ARfD is an estimate of the amount of residues, expressed on a body weight basis, which can be ingested in a period of 24 hours or less without adverse effects or harm to the health of the human consumer. The ARfD is expressed with the appropriate number of significant figures, as justified by the underlying scientific information used in such calculations (usually one significant figure).

   Please consult CVM or GFI #232, "Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish an Acute Reference Dose (ARfD) – VICH GL54" for additional information on the

[5] Prior to GFI #232 (VICH GL 54), FDA referred to this value as the Acceptable Single-Dose Intake (ASDI).

derivation of the ARfD and implementation of this approach for new animal drugs that can produce acute adverse effects. The need for an ARfD is determined on a case-by-case basis. If CVM determines that an ARfD is needed, sponsors should contact CVM to discuss the type(s) of study(ies) and the experimental protocol(s) for those studies prior to conducting any study.

5. ADI Partitioning

Partitioning refers to allocating portions of the ADI across edible tissues when consumption of those tissues may contribute to the total human exposure to a new animal drug and/or its residues. In this situation, the ADI is partitioned across applicable edible tissues based on the expected use of the new animal drug and the food consumption values for each tissue (discussed below). Edible tissues include muscle, liver, kidney, fat or skin with fat in natural proportions (as appropriate), whole eggs, whole milk, and honey. For some products (e.g., products not used in lactating dairy cows or laying hens) the ADI can be allocated to edible muscle, fat, kidney or liver without partitioning. The partitioning of the ADI across edible tissues is based on the requested allocation of the new animal drug's sponsor and on information on residues in edible tissues. For example, for products approved in lactating dairy cows or for products approved in lactating dairy cows and other meat-producing species, a part of the ADI would be reserved for milk. The remaining part of the ADI would be partitioned among the animals' edible tissues, as described above.

6. Food Consumption Values for Edible Tissues

When estimating the daily consumption for edible tissues, CVM assumes that, when an individual consumes a full portion of edible muscle, liver, kidney, or fat tissue from one species, that individual would not consume a full portion of the same edible tissue from another species on the same day.

The default consumption values (quantity consumed per day) for edible tissues are listed in Table 2. These food consumption values are used for all applicable species.

Table 2: Food Consumption Values

Edible Tissue	Quantity Consumed per day
Muscle	300 g
Liver	100 g
Kidney	50 g
Fat	50 g
Milk	1500 mL
Eggs	100 g
Honey	20 g

CVM regulates milk, eggs and honey as independent food commodities; this means that CVM assumes these edible tissues are consumed in addition to the consumption of muscle, fat, kidney or liver. Therefore, CVM assumes that a person consumes a full portion of milk, a full portion of eggs and a full portion of honey in addition to a full portion of other edible tissues each day.

7. Calculation of the Safe Concentrations

The safe concentration is the amount of total residue of a new animal drug that can be consumed from each edible tissue every day for the lifetime of a human without exposing the human to residues in excess of the ADI. The calculation of each edible tissues' safe concentration reflects the partition of the ADI described above (section V.E.5). CVM calculates the safe concentration for each edible tissue using the ADI, the weight in kilograms (kg) of an average adult human body (approximated at 60 kg),[6] and a conservative estimate of the amount of the edible tissue eaten per day in grams (see Table 2). The safe concentration of total residues of the new animal drug in each edible tissue is calculated using the following formula:

$$\text{Safe Concentration (edible tissue)} = \frac{\text{ADI} \times \text{Human Body Weight}}{\text{Food Consumption Value}}$$

For example, the safe concentration for milk equals the ADI reserved for milk (μg/kg bw/day) times 60 kg divided by 1500 mL/day; safe concentrations for other tissues are calculated in a similar manner.

Safe concentration for injection sites:

Human consumption of all or part of a new animal drug residue in a single meal is a relatively rare event, likely limited to occasional consumption of a site of injection from which the new animal drug did not yet distribute to other tissues in the animal. In order to ensure human food safety for this possible acute exposure, the safe concentration of residues in the injection site tissue is calculated for injectable new animal drug products. Typically, injection site residues should be less than 10x the muscle safe concentration, or the injection site safe concentration calculated using the ARfD.

- When no specific acute toxicological concern has been identified, the safe concentration for injection sites is estimated by multiplying the safe concentration for muscle by a factor of 10.

[6] FDA chose 60 kg to act as the surrogate body weight for all life stages ranging from a two-year-old toddler to the eldest consumer. The weight adjustment assumes an arbitrary human body weight for either sex of 60 kg. While making this assumption, FDA recognizes that some humans weigh less than 60 kg; these consumers are considered to be accommodated by the built-in safety factors used to determine an ADI.

- When a specific acute toxicological concern has been identified for an injected new animal drug product, the safe concentration for the injection site is calculated using the ARfD as follows:

$$\text{Safe Concentration (Injection Sites)} = \frac{\text{ARfD x Human Body Weight}}{\text{Consumption Value for Injection Sites}}$$

CVM applies the muscle consumption value for injection site consumption (300 g) to determine the safe concentration for injection sites for most new animal drugs. Thus, a conservative estimate of the amount of the injection site tissue consumed in a single meal is estimated to be 300 g/day. CVM may accept an alternative consumption value or approach for calculating the safe concentration of injection sites with appropriate scientific justification.

VI. Residue Chemistry

A. Introduction

The purpose of the residue chemistry studies is to assess the quantity and nature of residues in tissues derived from animals treated with new animal drugs. Sponsors should consult the referenced guidance documents below in Table 3 prior to design and conduct of metabolism studies, residue depletion studies, or analytical method validation.

The sponsor should present the results of each study in a format that will facilitate CVM review and should include individual animal data from analyses of each sample derived from treated animals.

Table 3: Guidance Documents for Residue Chemistry Studies for the Human Food Safety Evaluation

Title	GFI #	VICH GL #
Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues	205	46
Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Comparative Metabolism Studies in Laboratory Animals to Determine the Quantity and Identify the Nature of Residues	206	47
Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods	207	48

Title	GFI #	VICH GL #
Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Validation of Analytical Methods used in Residue Depletion Studies	208	49

B. **Total Residue and Metabolism Study**

The objective of the total residue and metabolism study is to develop information on the amount, persistence, and chemical nature of the total residues, and the metabolic fate of the new animal drug in the treated target animals. The recommended study design for a total residue and metabolism study is described in GFI #205, "Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues VICH GL 46."

While GFI #205 describes administering the dose to achieve steady state in edible tissues for continuously administered new animal drugs, the dosing period for laying hens and the sampling timepoints for eggs are not provided. For new animal drugs intended for continuous use in laying hens, a twelve-day dosing regimen after steady state has been reached should be used to approximate the time required for complete development of the yolk. For continuous use new animal drugs, recommended sampling timepoints for eggs are from 2 days before cessation of treatment through 2 days after cessation of treatment. For new animal drugs administered to laying hens on a noncontinuous basis, we recommend consulting with CVM about the appropriate dosing and sampling periods.

The sponsor may use tissues from the total residue depletion study to determine the metabolic fate of the new animal drug. If the tissues are stored frozen for long periods, CVM would ask the sponsor to demonstrate that the metabolites are stable in the frozen state, such as by demonstrating that metabolite profiles do not change.

C. **Comparative Metabolism Study**

The purpose of the comparative metabolism study is to determine whether the metabolites to which people consuming tissues of treated animals will be exposed are also produced by metabolism in the laboratory animals used for the toxicological testing (i.e., autoexposure). Autoexposure, in the context of the current document, refers to exposure of the test animal to the administered test article, its metabolites and/or breakdown products. The test animal serves as a surrogate for the human consumer. The recommended study design for a comparative metabolism study is described in GFI #206.

CVM may ask for separate toxicological studies on a metabolite if it is not tested through autoexposure and it is likely to have toxicological potency significantly greater than the parent new animal drug. CVM would use the information obtained from target animals on the concentration, persistence, and chemical structure or characterization of that metabolite to determine whether separate toxicological testing is desirable.

D. **Selection of Target Tissue and Marker Residue**

The marker residue refers to the residue whose concentration is in a known relationship to the concentration of total residue in an edible tissue. The target tissue refers to the edible tissue selected to monitor for residues in the target animal. The total residue and metabolism data should be presented so that it is possible to determine the marker residue, the target tissue, and the marker residue to total residue ratio (M:T ratio). The target tissue is usually, but not necessarily, the last tissue in which residues deplete to the permitted concentration. The target tissue and marker residue are selected so that the absence of marker residue above the tolerance would confirm that each edible tissue has a concentration of total residue at or below its safe concentration. Typically, for terrestrial animals, the target tissue is liver or kidney but it could also be fat. Although residues persist in injection sites from certain formulations, injection site is not an appropriate target tissue because it is not always identifiable at the slaughter plant.

To facilitate international harmonization, CVM recommends that sponsors provide total residue and metabolism data that would allow the determination of the M:T ratio and the marker residue in a nontarget tissue (often muscle or fat for terrestrial animals) for purposes of assigning a nontarget tissue tolerance value.

When a new animal drug is intended for use in milk- or egg-producing animals, milk or eggs may be a target tissue in addition to one tissue selected by CVM to monitor for residues in the edible carcass. Milk or eggs may be a target tissue because milk or eggs enter the food supply independently. In these cases, it may be necessary to identify a marker residue for milk or eggs and one additional edible carcass tissue. The marker residue in milk or eggs may be different from the marker residue selected for the target tissue representing the edible carcass, where appropriate.

E. **Assignment of Tolerance**

Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined. Tolerance is the maximum concentration of a marker residue, or other residue indicated for monitoring, that can legally remain in a specific edible tissue of a treated animal. The tolerance is determined by examining depletion data consisting of total residue concentrations (typically from radiolabel studies as described in GFI #205) and marker residue concentrations measured by the proposed analytical method. Tolerances for new animal drugs approved for use in food-producing animals are listed in 21 CFR 556.

CVM recommends that sponsors provide data that will allow assignment of a tolerance in one or more edible tissues of the target animal. CVM recommends providing data for establishing a tolerance in the target tissue as well as other edible tissues, as needed. A practicable method is required to be submitted by the sponsor when a tolerance is assigned to ensure the safe use of the new animal drug (21 CFR 514.1(b)(7)).

CVM may assign the tolerance by harmonizing with previously established international safety numbers (MRLs, maximum residue limits). Tolerances and MRLs are both numbers that describe the limits of residues. However, tolerances and MRLs are not derived in the same manner and are used for different purposes. At times, it may be appropriate to harmonize the tolerance with an already established MRL.

For injectable products, there may be a need to address safety of residues in injection site tissue. Typically, injection site residues should be less than 1) 10x the muscle safe concentration, or 2) the injection site safe concentration calculated using the ARfD (see section V.E.4 above) at the timepoint at which total residues at the injection site reach a concentration that is safe.

Safety of injection site residues may be addressed by lowering the target tissue tolerance with a resultant extension of the withdrawal period to ensure that injection site residues are less than the injection site safety number. One procedure that may be used to address safety of residues in injection site tissue is to do the following:

1. Determine the concentration of the marker residue in the target tissue at the timepoint at which total residues at the injection site reach a concentration that is safe.
2. Assign a tolerance for the marker residue in the target tissue.
3. Confirm that the analytical method performs at the new target tissue tolerance.
4. Conduct a tissue residue study to assign the withdrawal period based on the new target tissue tolerance.
5. Confirm that residues at the injection site at the new withdrawal period are safe.

F. **Analytical Methods for Residues**

1. Introduction

Sponsors must provide a practicable method for analyzing tissue residues as described in 21 CFR 514.1(b)(7) except when it is not reasonable to expect the new animal drug to become a component of food at concentrations considered unsafe. For purposes of this guidance, a practicable method means an analytical method that meets the criteria in 21 CFR 514.1(b)(7). Typically, the analytical method is developed to analyze the marker residue in the target tissue. The sponsor usually develops a method for use in residue chemistry studies.

A method for analyzing the marker residue in the target tissue consists of two procedures: the determinative (quantitative) and confirmatory procedures. The determinative and confirmatory procedures may be combined into a single procedure as instrumentation allows.

2. Evaluation Criteria

Criteria for an analytical method for use in residue chemistry studies are outlined in GFI #208, "Validation of Analytical Methods Used in Residue Depletion Studies."

Criteria for the determinative and confirmatory method are described in this section. The method should have the ability to be transferred easily between laboratories and performed by analysts unfamiliar with the method. The process of obtaining the acceptance of the method by CVM typically consists of up to three steps. It is strongly recommended that the presumptive tolerance be available prior to completing the following three steps to assure that the method performance meets regulatory needs:

1. The sponsor develops and validates the method.
2. CVM reviews the sponsor's validation data (the desk review) to determine that the method satisfies the performance criteria (a)(1) through (12) below). In some cases, a meeting dedicated to the analytical method may be held with the sponsor to determine the suitability of the method for an inter-laboratory study (method trial).
3. The inter-laboratory study (section VI.F3.b of this guidance) is conducted to determine whether the method performs as claimed and thus can be accepted as a practicable method.

a. Determinative Procedure

The determinative procedure quantifies the amount of new animal drug residue present in the tested matrices. The sponsor's validation data are evaluated by CVM based on the following performance criteria:

(1) Calibration Range and Linearity

CVM recommends the use of solvent-based calibration standards. However, CVM also will accept standards prepared in extracted matrix provided there is acceptable justification for the use of a matrix standard curve. CVM recommends that the linearity of the non-weighted calibration curves range from approximately 30 to 250% of the tolerance. The concentrations of the solvent-based standards for the calibration curve should extend beyond the equivalent tissue fortification concentrations because CVM allows recoveries to range from 60-110% for tolerance <0.1 ppm, or 80-110% for tolerance ≥0.1 ppm. If a weighting factor is used for the calibration curve, justification for weighting should be provided.

(2) Bias (Systematic Error)

Bias, or systematic error, is the difference of the measured value from the true, assigned, or accepted value. Bias is generally expressed as the percent difference between the measured mean concentration and the nominal concentration.

Recovery refers to the percentage of the measured analyte concentration compared to the known concentration of a spiked sample. Recovery also may be measured for specific parts of a method such as an extraction step (extraction recovery). Method recovery is assessed using control tissue fortified with the proposed marker residue. Assuming acceptable precision, an average recovery of 80 to 110% should be obtained when the designated concentration of marker residue (tolerance) is 0.1 ppm or greater. An average method recovery of 60 to 110% is acceptable when the designated concentration of marker residue (tolerance) is less than 0.1 ppm. Higher recoveries (greater than 110%) may indicate a lack of specificity.

(3) Precision and Accuracy

Precision is a measure of the variability of repetitive measurements. Contributions to variability from numerous sources affect precision, but the major components are those from different laboratories (reproducibility) and those from within a laboratory (repeatability). Precision is usually expressed as a standard deviation or coefficient of variation (relative standard deviation). CVM recommends expressing precision as the coefficient of variation (CV) because it is relatively constant over a considerable concentration range.

The within laboratory CV should not exceed 10% where the designated concentration of marker residue (tolerance) is greater than or equal to 0.1 ppm. Where the designated concentration of marker residue (tolerance) is less than 0.1 ppm, the within laboratory CV should not exceed 20%.

Accuracy is defined as the closeness of agreement between the measured value and an accepted reference value. CVM will evaluate accuracy of the method based on both bias and precision (repeatability).

(4) Limit of Detection (LOD) and Lower Limit of Quantitation (LLOQ)

The method should be capable of reliable detection and measurement of concentrations around the tolerance of the analyte (drug marker residue). In order to estimate the LOD signal, the standard deviation of the signal (analytical response) from control samples is multiplied by a factor related to the acceptable probability of false positive and false negative (refer to GFI #208). A factor of 3.3 is commonly used to estimate the LOD signal. The factor of 3.3 corresponds to a 5% probability of both false positive and false

negative. In order to estimate the LLOQ signal, the standard deviation of the signal from control samples may be multiplied by a factor of 10. For a calibration-based method, the standard error of the calibration curve intercept is an acceptable substitute for the standard deviation of the response from control samples. The respective LOD and LLOQ signals are then converted to concentrations using the slope of the calibration function.

(5) Robustness and Ruggedness

The final version of the method should be optimized by robustness and ruggedness experiments. For example, robustness of a LC-MS/MS method is demonstrated by using different lots of Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) columns. *Ruggedness* is a measure of the transferability of the method between operators, laboratories, instrumental platforms, or long intervals of time. Examples in demonstrating ruggedness of a LC-MS/MS method can include the use of different MS platforms from multiple manufacturers and different LC systems. Ruggedness is assessed by performing the analyses on identical samples. The method should be performed by an analyst who was not involved in the development of the method in order to verify the adequacy of the method's description and to identify critical points.

(6) Specificity and Selectivity

Specificity is the ability of the method to respond only to the substance being measured, irrespective of other substances and materials present in the matrix. Selectivity of the analytical method is the extent to which substances affect the determination of the marker compound of interest according to the procedure. The method should demonstrate the absence of the following aspects of interference:

- Matrix Interference: No interference from the control tissue greater than 10% of the tolerance.
- Interference from other new animal drugs: No interference from other compounds of similar chemical structure approved for use in the same target species.
- Carryover: Method should be free of carryover during or between runs. No interference from carryover peak greater than 10%.

(7) Parallelism

Parallelism is the ability to dilute samples with analyte concentrations above the upper limit of quantitation (ULOQ). The sponsor should provide data to demonstrate that using control matrix to dilute samples with initial concentration above ULOQ does not compromise the accuracy and precision of the method following re-analysis of the diluted sample.

(8) Stability

Data should be provided in support of the following aspects of stability of the analyte (marker residue):

- Stability of stock solutions of standards of the analyte and internal standard.
- Stability of analyte in the matrix (short-term stability, long-term stability, and freeze-thaw stability).
- Stability of the analyte in extracts.

(9) System Suitability

CVM recommends that the method protocol include system suitability criteria that will evaluate instrument performance prior to an analytical run. In particular, to aid the transferability of the method between the different LC-MS platforms in government laboratories, CVM recommends that the sponsor include in their system suitability criteria the requirement that the CV for the ratios of analyte area to internal standard peak area for the suitability solutions should be below 6%.

(10) Additional Criteria

The method should:

(a) Utilize commercially available reagents, supplies, instruments (except that new or unusual reagents or standards may be supplied by the sponsor on request);
(b) Be capable of being performed by reasonably experienced analysts;
(c) Be capable of being completed within reasonable time periods consistent with regulatory objectives (usually no more than 48 hours of total elapsed time);
(d) Not need unique instrumentation, large quantities of solvents, reagents, and supplies which would render the method economically impractical; and
(e) Be capable of being performed safely.

(11) Confirmatory Procedure

The confirmatory procedure unequivocally identifies the marker residue present in the tested matrices.

As with the determinative procedure, the performance criteria for the confirmatory procedure are evaluated using tolerance-related concentrations. CVM recommends the use of solvent standards for comparison with sample extracts during the confirmation procedure. Whenever possible, the

confirmatory procedure should be performed on the same sample extracts used for the determinative portion of the method. Details of the performance criteria for a confirmatory procedure for a marker residue are described in GFI #118, "Mass Spectrometry for Confirmation of Identity of Animal Drug Residues."

(12) Adaption of a Previously Validated Method

Instead of developing and validating a new method for the marker residue, the sponsor may elect to adapt a method that was validated in a government laboratory (e.g., FDA/ORA, USDA/FSIS), or a method designated as an "Official Method" by the Association of Analytical Chemists (AOAC). Typically, an AOAC method is not validated at the tolerance while the practicable method is validated around the tolerance. When adapting a previously validated method, the sponsor will need to provide only partial validation data to demonstrate that the method can be satisfactorily performed in the sponsor's laboratory. CVM recommends that, before developing and validating a new method for the marker residue, the sponsor should determine if there is a government laboratory or AOAC previously validated method that they can adapt. Adaption of an FDA or AOAC validated method may limit the extent and expense of the method trial study that is part of the method approval process.

3. Specific Data Needed

a. Sponsor's Submission

In the development of a method, sponsors should collect data from three types of samples: (a) "control" target tissue from untreated animals; (b) "fortified" target tissue containing known concentrations of the marker residue added to the sample of control target tissue; and (c) "incurred" target tissue from animals of the target species that have been treated with the new animal drug.

In presenting a submission for evaluation of a proposed method, the sponsor should provide:

- A complete description of the method (method SOP) including sampling, preparation of analytical samples, storage conditions, reagents, instrumentation, standards, identification of critical steps, and stopping points.
- Quality control criteria necessary to verify and maintain method performance.
- A typical solvent standard curve prepared from marker residue of known purity.
- Data derived from control, fortified, and incurred tissue samples showing that the method meets the specificity, precision, and bias attributes.
- Relevant worksheets, calculations, statistical analyses, chromatograms, etc., from the analyses of control, fortified and incurred tissue samples.

- Using the proposed determinative procedure of the method, results of analyses of the following samples (as a minimum):

    - 5 control tissues
    - 5 control tissues fortified with marker residue at 0.5X tolerance
    - 5 control tissues fortified with marker residue at 1X tolerance
    - 5 control tissues fortified with marker residue at 2X tolerance
    - 5 tissues containing biologically incurred marker residue at approximately its tolerance

- Using the proposed confirmatory procedure, results of analyses to verify the identity of the marker residue with the following samples (as a minimum):

    - 5 control tissues to ensure the absence of false positives
    - 5 control tissues fortified with marker residue at tolerance
    - 5 tissues containing biologically incurred marker residue at approximately its tolerance

b. Inter-laboratory Study

After the method has passed desk review (section VI.F2, step 2, above), FDA may conduct an inter-laboratory method transfer trial of the proposed method. The inter-laboratory study, also termed a method trial, is designed to validate the reproducibility (ruggedness) component of the precision of the method. The method will be tested in one FDA laboratory, the sponsor's reference laboratory that developed and validated the procedure, and two independent testing (contract) laboratories selected by the sponsor. One USDA laboratory may optionally participate in the method trial. The method trial is conducted using target tissues supplied by the sponsor. The method trial participants are the independent laboratories and the FDA laboratory that are naïve laboratories with no previous experience with the method. In addition, only the FDA laboratory conducts a validation of the confirmatory procedure. To evaluate the performance of the determinative procedure, the data from the naïve laboratories are compared to the data obtained in the sponsor's reference laboratory. During the method trial, the results for each day of analysis in the sponsor's reference and testing laboratories are sent to the sponsor and to the CVM method trial coordinator for review. The results from the FDA laboratory are sent only to the CVM method trial coordinator. The inter-laboratory study is supervised by CVM. Adaption of an FDA or AOAC validated method may limit the extent of the method trial study.

Each of the three laboratories taking part in the method trial will analyze the following samples by the determinative procedure:

- 5 control tissues
- 5 control tissues fortified with marker residue at 0.5X tolerance
- 5 control tissues fortified with marker residue at 1X tolerance
- 5 control tissues fortified with marker residue at 2X tolerance
- 5 tissues containing biologically incurred marker residue at each of two concentrations at approximately 0.5X and 1X, respectively.

The samples analyzed by the participating laboratories are blinded and randomized. Both batch sequence and individual sample analysis within an analytical batch are randomized.

The confirmatory procedure is performed in the FDA laboratory on the same extracts used for the determinative portion of the method.

If the sponsor elects to adapt a method that was developed in a government laboratory, the sponsor may elect to conduct a collaborative study with that government laboratory by providing the aforementioned tissues to the government laboratory (single lab validation (SLV)). If the sponsor elects to adapt a method that is being validated, or already designated as an "Official Method" by the AOAC, then the sponsor may have the option to conduct a collaborative study with the AOAC following validation experiments recommended by CVM.

G. **Tissue Residue Depletion Study**

The purpose of the tissue residue depletion study is to provide data to demonstrate the depletion of the marker residue post-treatment to below the tolerance and to provide data suitable for calculating a withdrawal period or milk discard time. The recommended study design for a tissue residue depletion study is described in GFI #207, "Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods VICH GL 48."

Ideally, tissues from treated animals should be collected to provide residue data above the tolerance at a minimum of two sampling timepoints and residue data below the tolerance (but above the LOQ) at a minimum of one sampling timepoint. The purpose for providing residue data below the tolerance is to avoid excessive extrapolation of the linear regression to the proposed withdrawal period.

Depletion studies in laying hens should be designed to provide residue data to demonstrate that the tolerance is compatible with zero withdrawal. Layers should be administered the new animal drug as proposed on the labeling. For continuous use new animal drugs, recommended sampling timepoints for eggs are from 2 days before cessation of treatment through 2 days after cessation of treatment. For new animal drugs administered to laying hens on a noncontinuous basis, we recommend consulting with CVM about the appropriate dosing and sampling periods.

H. **Establishing a Withdrawal Period**

The withdrawal period or the milk discard time is the interval between the time of the last administration of a new animal drug and the time when the animal can be safely slaughtered for food or the milk can be safely consumed. When a tissue withdrawal period or milk discard time is assigned pursuant to an approved application, neither tissues nor milk collected during treatment and during the withdrawal period or milk discard time are safe and, as a result, cannot be used for human consumption. See Section 512 of the FD&C Act [21 U.S.C. 360b]. For convenience, this guidance will use the "withdrawal period" to refer to both the withdrawal period and the milk discard time.

This guidance describes a procedure for establishing a withdrawal period that is based on a statistical tolerance limit procedure (De Gryze et al., 2007). Tolerance limits explicitly account for the degree of variation in the background population and the size of the sample of measurements used. When determining the withdrawal period, CVM uses the 99^{th} percentile tolerance limit with a 95% confidence (ITRC 2013). The withdrawal period is determined when the tolerance limit on the residue concentration is at or below the permitted concentration. A tolerance limit provides an interval within which a given percentile of the population lies, with a given confidence that the interval in fact contains that percentile of the population. This means that 99% or more of tissue or milk residue samples at and beyond the withdrawal period (or discard time) are expected to fall below the tolerance.

The statistical program using "R" to calculate the withdrawal period is available upon request by contacting AskCVM@fda.hhs.gov.

If the calculated withdrawal period is a fraction of a day or milking, CVM will establish the withdrawal period as the next day or milking.

A zero withdrawal period usually will be assigned when total residues from a 1X study are below ½ the safe concentrations or when total residues from a 1.5X study are below 1X the safe concentrations. However, CVM recommends that the sponsor provide residue depletion data to ensure that the tolerance assignment is consistent with a zero withdrawal period determined from the radiolabeled study. A single timepoint residue depletion study may be conducted to demonstrate that the marker residue is less than the tolerance at a zero withdrawal period.

I. **Statistical Analysis of the Residue Data**

1. Theory

The depletion of new animal drug residues in an animal is assumed to follow exponential elimination kinetics. This means that the concentration of residues as a function of time after stopping treatment of a drug can be described as the sum of one or more exponential terms (Equation 1).

$$C_t = \sum_{i=1}^{n} C_{0,i}\, e^{-\lambda_i t}$$

where:

C_t is the concentration at time t,
$C_{0,i}$ is the extrapolated concentration at time=0 and the ith exponential term, and
λ_i is the rate constant corresponding to the ith exponential term.

CVM makes a simplifying assumption for analysis of residue data that the depletion curve, during the phase of the depletion closest to the established tolerance, can be represented by a single exponential equation. This assumption enables the use of ordinary least squares (OLS) regression methods to estimate the intercept (b_0) and slope (b_1) of log-linear depletion curves in either groups or individual animals (Equation 2).[7] The intercept and slope of the line are used to estimate the time at which the concentration in tissue or milk achieves the target tolerance concentration. The straight-line fit of concentration data relies on natural logarithm transformation of the dependent variable (Equation 2).

$$\ln(y)_i = \ln(b_0) - b_1 x_i$$

where:

$\ln(y)_i$ = is the natural log of the regression-estimated concentration at x_i ;
x_i = the ith sampling period (in units of time) for either tissue or milk samples;
b_0 = regression-estimated intercept of the elimination curve; and
b_1 = the estimate of the slope (regression coefficient) from the OLS regression.

Several measures of statistical confidence are used to describe the possible range of values given the variability among the observations. These confidence *limits* include the confidence limits on the regression line and the confidence limits for a new estimate of concentration (taken as $\ln(\hat{y})$) given a new value of x (also known as "prediction limits" Myers, 2000), and the tolerance limits for the new estimates of concentration. The tolerance limits describe an interval in which a specified proportion (P) of the sampled population is expected to fall given the current observations (De Gryz et al., 2007).[8] If the true regression parameters for the intercept and slope (β_0, β_1) are known, then the tolerance limits and confidence limits on the new estimate of concentration are the same. Because the true regression parameters are seldom known and must be estimated from the observations, the tolerance and prediction (confidence) limits differ. Tolerance intervals may be either two-sided (for which the estimated parameter is expected to lie between a lower and

[7] A variety of notation is used to describe the estimates of intercept and slope (*intercept, slope*) from linear regression including (b_0,b_1), (a,b) and (b,m).
[8] See De Gryz,S; Langhans, I; Vandebroek, M (2007) for a discussion of tolerance intervals from linear regression.

upper limiting value) or one-sided, (in which the estimated parameter is expected to lie above or below a specified limit). CVM uses a one-sided tolerance limit to set an upper limit on the concentration of residue in tissue or milk.

CVM uses a 99th percentile tolerance with 95% confidence as an upper limit of residue concentration in either tissue or milk. In general terms, this means that a new individual value of concentration, randomly sampled from animals at the proposed withdrawal or discard period, is expected to have only a 1% chance of exceeding the established tolerance limit. The purpose of the regression error (variance) analysis is to calculate uncertainty (e.g., $y \pm s$) in the estimated concentration near the proposed tissue withdrawal period or milk discard time. CVM solves for a factor, *k*, that adjusts the regression-estimated error (*s*) for the 99th percentile tolerance with 95% confidence. In the form of a linear equation (y = b + mx) the residue calculation to estimate *y* from a new value of $\boldsymbol{x}_0$ (Equation 3):

$$\ln(y)|\boldsymbol{x}_0 = (\ln(b_0) - b_1 x_0) + k$$

The modifying factor, *k*, is derived from the noncentral *t*-distribution, and it is a function of degrees of freedom, the distance from the expected value of $\ln(y)$, the desired proportion of coverage for future values (P=0.99) and the confidence level for the difference ($1 - \alpha = 0.95$). The noncentral *t*-distribution can be obtained from tables of the noncentral *t*-distribution (Owen 1968) or statistical software.

CVM solves Equation 3 for the value of $x = T$, the tissue withdrawal period or milk discard time. Given simultaneous unknown values in Equation 3, the value of *x*, such that concentration estimated at $x = T$ satisfies the 99/95 tolerance condition, is found iteratively either by hand or computer program. The general location of the tolerance limit, the withdrawal period or discard time (T) and the various OLS regression results are shown in
Figure 2.

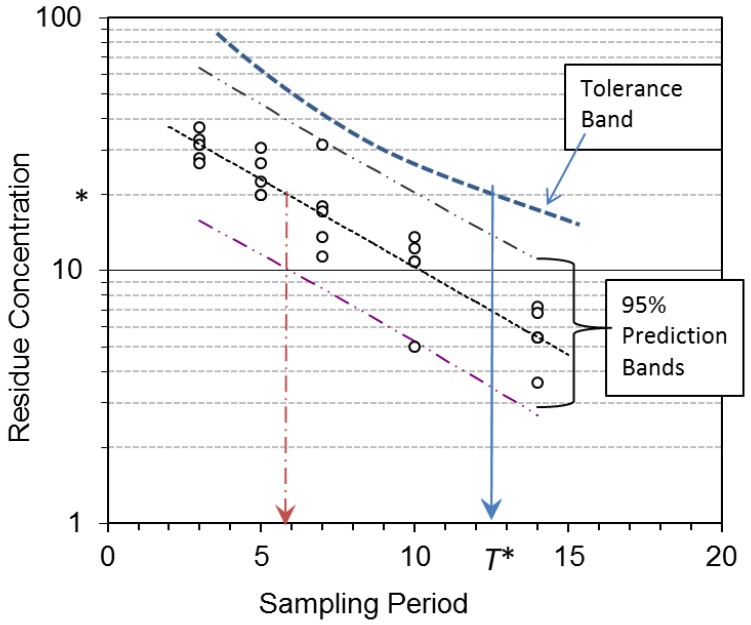

Figure 2. Hypothetical Residue Depletion Curve and Withdrawal Period Estimate.
A least squares fit of the data (circles) is shown as a decreasing, straight and small-dashed line through the data. The 95% prediction bands and the 99th percentile tolerance for the upper 95% confidence limit are shown. If the regulatory limit is the value of concentration marked by the asterix (*=2 units), then the regression analysis predicts that the withdrawal period (T*) is about 12.5 sampling period units determined by where the tolerance band crosses the desired limit. Note that the tolerance band is not drawn to scale for ease of presentation: the tolerance generally lies much closer to the upper 95% prediction band.

Calculation of the tolerance from the linear regression data relies on first calculating the amount of variation among the observations (samples). The sources of variation include both the within-animal variation as a function of the sampling time, and variation from the difference among the animals. Laboratory analytical variability also contributes to overall variability. The assumptions for OLS regression include that the samples are independent from each other; the residue assays are independent from each other and from the animal in question; the depletion of the concentration of residue is log-linear with time; and the measured log(concentration)s of residue are distributed normally and have a constant variation over the time periods. The validity of the assumptions for an experiment can be examined using analysis of variance for regression and various diagnostic statistics (Draper and Smith 1998; Myers 2000). Generally, statistical software or spreadsheets with statistical functions can be used to calculate the intercept, slope, and regression diagnostics.

2. Statistical Software: Residues in Tissue and Milk

Most commercial, off-the-shelf statistical programs include tools for ordinary linear regression (OLS), generalized linear models (GLM) and analysis of variance (AOV). Commercial spreadsheet software generally includes OLS and AOV functions. Open-source software, such as R statistical software and Python Scipy, is freely available and has extensive libraries of "packages" for OLS, GLM, and AOV (R Core Team, 2013; Python, 2015). Although the formulae used in regression and AOV might appear complex, they all derive from elementary sample statistics that can be calculated manually or using built-in software functions. CVM has developed statistical programs for fitting tissue and milk residue data according to the rationale discussed in this guidance and in Martinez et al. (2000). The programs (in R or SAS®) are available freely upon request as open-source programs and without any expressed warranties for their use.

In contrast to carcasses, which are individually sampled by USDA in the residue monitoring program, milk from an entire dairy herd is pooled in the bulk tank before sampling in a compliance program. Therefore, the statistical procedure for calculating the milk discard time must contain a term for the expected number of cows contributing milk to the pooled sample. To approximate the size of a small dairy operation, CVM uses 10 for that value.

Alternative methods of analysis for residues are well known, and alternatives are recommended by the European Union and others (FAO, 2003). The approach described in this guidance was discussed in detail as described in Martinez, et al., 2000. CVM believes that the default methods described here are adequate for setting operational withdrawal periods or milk discard times. Essentially, CVM addresses the milk discard time analysis more as decision analysis than as a search for the best statistical fit of the entire residue depletion curve. The latter is focused on statistical rationale for the optimal statistical fit of all the data while CVM methods seek a reasonable estimate of the time at which the concentration falls below a 99^{th} percentile tolerance.

VII. Carcinogenic Compounds

FDA cannot approve a compound for use as a new animal drug in food-producing animals when the compound or any of its metabolites has been found to cause cancer in animals or humans, unless: 1) the compound will not adversely affect the animals for which it is intended, and 2) no residue of the compound will be found by approved regulatory methods in any edible tissues (see section 512(d)(1)(I)) of the FD&C Act). The regulations in 21 CFR part 500, subpart E entitled "Regulation of Carcinogenic Compounds Used in Food-Producing Animals" (§§ 500.80 through 500.92) describe the requirements for demonstrating that no residue of the new animal drug will be found by an approved regulatory method in any edible tissues of or in any foods yielded from the animal. Contact CVM for additional information regarding carcinogenic compounds used in food-producing animals.

VIII. References

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