

Guidance for Industry

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 (MRK)

VICH GL46

Submit comments on this guidance at any time. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. Submit electronic comments on the guidance at <http://www.regulations.gov>. All written comments should be identified with the Docket No. FDA-2010-D-8228.

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, 240-276-8204, e-mail: julia.oriani@fda.hhs.gov

Additional copies of this guidance document 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 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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**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**

Adopted at Step 7 of the VICH Process

by the VICH Steering Committee

in February 2011

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This Guidance has been developed by the appropriate VICH Expert Working Group and is subject to consultation by the parties, in accordance with the VICH Process. At Step 7 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and the USA.

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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

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. 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 appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. INTRODUCTION

1.1. Objective of the guidance

Drug sponsors should perform a comprehensive set of metabolism, residue depletion and pharmacokinetic studies to establish the safety of veterinary drugs in food. The objective of this guidance is to provide recommendations for internationally-harmonized test procedures to study the quantity and nature of residues of veterinary drugs in food-producing animals.

1.2. Background

This guidance is one of a series developed to facilitate the mutual acceptance by national/regional regulators of residue chemistry data for veterinary drugs used in food-producing animals. This guidance was prepared after consideration of the current national/regional requirements and recommendations for evaluating veterinary drug residues in the European Union, Japan, United States, Australia, New Zealand and Canada.

Although this guidance recommends a framework for metabolism testing, it is important that the design of the studies remain flexible. It is recommended that studies be tailored to sufficiently characterize the components of the residue of concern.

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's guidances means that something is suggested or recommended, but not required.

2. GUIDANCE

2.1. Purpose

The human food safety evaluation of veterinary drug residues helps ensure that food derived from treated food-producing animals is safe for human consumption. As part of the data collection process, studies should be conducted to permit an assessment of the quantity and nature of residues in food derived from animals treated with a veterinary drug. These metabolism studies provide data on (1) the depletion of residues of concern from edible tissues of treated animals at varying times

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after drug administration, (2) the individual components, or residues, that comprise the residue of concern in edible tissues, (3) the residue(s) that can serve as a marker for analytical methods intended for compliance purposes (i.e., monitoring of appropriate drug use), and (4) the identification of a target tissue or tissues, as applicable to national or regional programs.

2.2. Scope

Metabolism studies in food-producing animals most often are accomplished using radiolabeled drugs. These studies are sometimes referred to as total residue studies because they are capable of monitoring all (i.e., “total”) drug-derived residues resulting from the administration of test material. This guidance recommends procedures for metabolism studies conducted with radiolabeled drugs, when such studies are performed.

Alternative approaches (i.e., not using radiolabeled drug) to characterize the components of the residue in food derived from treated animals might be suitable.

In any case, testing should be conducted in compliance with applicable Good Laboratory Practice (GLP).

2.3. Study to Determine the Quantity and Identify the Nature of Residues

2.3.1. Test Materials

2.3.1.1. Drug

The chemical identity (including, for example, the common name, chemical name, CAS-number, structure and molecular weight) and purity of the drug substance should be described. The chemical name and structure should indicate the position(s) of the radiolabel. Handling and disposal of radiolabeled materials should be in compliance with applicable laws and regulations.

2.3.1.2. Radiolabeled Drug

2.3.1.2.1. Nature and Site of Label

Carbon-14 (¹⁴C) is the label of choice because intermolecular exchange is not an issue. Other isotopes, such as ³H, ³²P, ¹⁵N or ³⁵S, might be appropriate. Tritium (³H) might be considered suitable if a rigorous demonstration of the stability of the tritium label is provided; for example, the extent of exchange with water is assessed and found to be ≤5%.

The drug should be radiolabeled in a site, or in multiple sites, to assure that the portions of the parent drug that are likely to be of concern are suitably labeled. The radiolabel should be placed in a metabolically stable position(s).

2.3.1.2.2. Purity of Radiolabeled Drug

Radiolabeled drugs should have a high level of purity, preferably of approximately 95%, in order to minimize artifactual results. Radiochemical purity should be demonstrated via appropriate analytical techniques (e.g., using two chromatographic systems).

2.3.1.2.3. Specific Activity

The specific activity of the synthesized radiolabeled drug should be stated in the study report. The specific activity should be high enough to permit tracking of the residue of concern in edible tissues. The sensitivity should be determined by the potency of the drug.

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The specific activity can be adjusted by mixing radiolabeled drug with unlabeled drug. To facilitate analytical measurements and conserve radiolabeled drugs, animals to be euthanized at early withdrawal periods can be dosed with drug of lower specific activity, while animals to be euthanized at later withdrawal periods can be dosed with drug of higher specific activity.

2.3.1.3. Analytical Standards

Analytical standards should be available for the parent drug and, if possible, for putative metabolites for use in the chromatographic characterization of drug residues.

2.3.2. Test Systems

There are some national/regional differences regarding the designation of major and minor species, particularly for turkeys and sheep. These differences can affect national/regional data collection requirements and recommendations. In certain instances, the total residue and metabolism data for a drug's use in a major species might be extrapolated to the minor species. When a national/regional authority calls for a total residue and metabolism study for a minor species or for a species considered to be major in one region but not another, the study design outlined in this guidance should be acceptable.

2.3.2.1. Animals

Animals used in the metabolism study should be representative of commercial breeds and representative of the target population. The source of the animals, their weights, health status, ages and sex should be provided.

Ordinarily, a single study can be performed in swine (~40 to 80 kg), sheep (~40 to 60 kg) and poultry. For cattle, a single study in beef cattle (~250 to 400 kg) could apply to dairy cattle, and vice versa. Generally, the results of a metabolism study in adult cattle and sheep can be extrapolated to calves and lambs, respectively. However, a second study might be appropriate for pre-ruminating animals if there is sufficient reason to believe the pre-ruminating animal will have significantly different metabolism than the adults. A separate study should be performed to demonstrate the total residue in milk of dairy cows.

If the study is intended to support a withdrawal period, the study parameters should address the worst-case study conditions (for example, animal weights and associated maximum injection volume).

2.3.2.2. Animal Handling

Animals should be allowed time to acclimatize. Normal husbandry practices should be applied to the extent possible. It is recognized that these studies might call for metabolism cages, a departure from "normal" practices; therefore metabolism cages should only be used if the study is intended to collect urine and excreta or other specification. Animals should be healthy and, preferably, should not have been previously medicated. The feed and water supplied to the animals should be free from other drugs and/or contaminants and adequate environmental conditions should be ensured to be consistent with animal welfare, in accordance with applicable national and regional regulations. However, it is recognized also that animals might have received biological vaccinations or other treatment, for example with anthelmintics. In any case an appropriate wash-out time should be observed for the animals prior to their being put on study. Animals should have a known history of medication.

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Handling and disposal of animals and tissues from animals treated with radiolabeled materials should be in compliance with applicable laws and regulations.

2.3.3. Test Procedures

2.3.3.1. Drug Formulation

The drug formulation, method of dose preparation, and stability of the drug in the formulation during the dosing period should be described in the study report. Although it is recognized that metabolism studies can be conducted well in advance of definitive formulation decisions, the drug should be administered to test animals via the intended final formulation whenever possible; otherwise, representative or prototype formulations can be considered appropriate.

2.3.3.2. Route of Administration

The drug should be administered via the intended route of administration (e.g., orally, dermally, intramuscularly, subcutaneously). For drugs that are intended for oral administration, especially via feed or drinking water, gavage or bolus dosing can be employed to ensure that animals receive the complete dose and to minimize environmental concerns. For drugs that are intended for oral and parenteral administrations, usually separate metabolism studies should be performed. Ordinarily, a single study with a parenteral route will be applicable to cover all parenteral routes including intramuscular, intramammary, subcutaneous and topical. Similarly, a single study with an oral route ordinarily will be applicable to all potential oral formulations (e.g., drinking water, in-feed and quick release tablets).

2.3.3.3. Dosing

The dose should be the highest intended treatment concentration and should be administered for the maximum intended duration or for the time required for steady state to be achieved in edible tissues. Predosing of animals with unlabeled drug, followed by administration of radiolabeled drug, is not recommended.

For continuously administered drugs, a separate study to determine the time for residues to reach steady state in edible tissues might be appropriate. When a drug administered in a single dose is intended to have zero withdrawal, completion of the absorption phase should be demonstrated.

When gavage dosing for the feed and water routes, the dose should be divided and given in the morning and afternoon to better approximate actual use conditions.

2.3.3.4. Number of Animals and Number of Euthanasia Intervals

At least four groups of animals, evenly-mixed as to sex if the drug is intended for use in both males and females, should be euthanized at appropriately spaced time points. The following numbers of animals are recommended:

Large animals (cattle, swine, sheep): ≥ 3 per euthanasia time

Poultry: ≥ 3 per euthanasia time

Lactating cattle for milk collection: ≥ 8 multiparous cattle representative of high and low milk production

Laying birds for egg collection: a number sufficient to collect ≥ 10 eggs

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For drugs for which a withdrawal period is not anticipated, euthanasia timepoints should reflect geographical distances in the amount of time to transport animals to the abattoir.

Typical timepoints for practical zero withdrawal would be:

0-2, 3-4, and 6 hours for poultry

0-3, 6-8, and up to 12 hours for large animals

Up to 12 hours for milk

A sufficient amount of control tissues should be available to permit a determination of background concentrations and combustion efficiency, and to provide tissue for related analytical methods testing.

2.3.3.5. Animal Euthanasia

Animals should be euthanized using commercially applicable procedures, making certain to observe appropriate exsanguination times. Chemical euthanasia can be used unless it will interfere with analysis of metabolites of interest.

2.3.3.6. Sample Collection – Edible Tissues

Following euthanasia, samples of sufficient amounts of edible tissues should be collected, trimmed of extraneous tissue, weighed, and divided into aliquots. If the analysis cannot be completed immediately, the samples should be stored under frozen conditions pending analysis. If samples are stored after collection, the sponsor should ensure that the radiolabeled compound remains intact throughout the storage period.

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Recommended samples are shown in Table 1.

Table 1. Recommended samples to be taken from animals in the metabolism study

Edible Tissue Type	Species / Sample Description		
	Cattle/Sheep	Swine	Poultry
Muscle	Loin	Loin	Breast
Injection Site Muscle	Core of muscle tissue ~ 500 g 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	Core of muscle tissue ~ 500 g 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	Collect sample from entire site of injection, e.g., chicken: whole neck, whole breast, or whole leg. Larger birds: not to exceed 500 g
Liver	Cross-section of lobes	Cross-section of lobes	Entire
Kidney	Composite from combined kidneys	Composite from combined kidneys	Composite from combined kidneys
Fat	Peri-renal	NA	NA
Skin/Fat	NA	Skin with fat in natural proportions	Skin with fat in natural proportions
Milk	Whole milk	NA	NA
Eggs	NA	NA	Composite from combined white and yolk

NA: Not Applicable

The tissues shown in Table 1 should be analyzed. Additional tissues should be collected and analyzed to provide information on the one additional tissue to be analyzed in the marker residue depletion study. (see VICH GL 48 “Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Marker Residue Depletion Studies to Establish Product Withdrawal Periods,” section 2.2.6.1) As appropriate to species, the additional tissues might include heart (cattle, swine, sheep, poultry), small intestines (cattle and swine) and gizzard (poultry); furthermore, it might be appropriate to collect and analyze other edible offal from the various species if it is deemed important for a safety assessment (e.g., offal with expected high residue concentrations or with residues having slow depletion rates).

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2.3.3.7. Sample Collection – Excreta and Blood

Excreta and blood are not typically collected. However, analyses of these samples can be useful from several perspectives: first, analyses of the excreta and blood allow an estimate of the mass-balance, a valuable tool in assessing the quality of the study; second, the samples of excreta can be a good source of metabolites; and third, the samples can be of use in conducting an Environmental Risk Assessment. If the decision is made to collect such data, it is recommended that urine and excreta be collected from selected animals on a daily basis.

Blood samples can be taken from selected animals at various time points and at euthanasia. Data on the total residue in blood can provide valuable pharmacokinetic information.

2.3.3.8. Determination of Total Radioactivity

Total radioactivity in samples should be determined by established procedures which might include, for example, combustion followed by liquid scintillation counting, solubilization and counting, or direct counting, depending on the nature of the sample. The details of the radioassays, including preparation of analytical samples, instrumentation, and data from standards, control tissues, fortified tissues, and incurred tissues, should be completely described. The ability of the procedure to recover radioactivity added to control tissues should be demonstrated.

The results of analyses of samples for radioactivity should be reported on a wet weight basis and on a weight/weight basis, with $\mu\text{g}/\text{kg}$ the preferred units. Sample calculations showing conversion from cpm/weight or dpm/weight to the weight/weight basis should be described in the study report.

2.3.4. Separation and Identification of Metabolites

Commonly available analytical technology (including, for example, thin layer chromatography, high performance liquid chromatography, gas chromatography, and mass spectrometry) enable the separation of the total residue into its components and the identification of the drug-derived residues.

2.3.4.1. Analytical Method

A complete description of the analytical method should be provided in the study report. The description should include the preparation of standards, reagents, solutions, analytical samples; the extraction, fractionation, separation and isolation of the residues; the instrumentation; and the data derived from standards, control tissues, fortified tissues and incurred tissues. The analytical method should be validated at least to demonstrate the recovery, the limit of detection and the variability.

2.3.4.2. Extent of Characterization/Major Metabolites

The degree of characterization and structural identification depends on several factors which include the amount of residue present, the concern for the compound or for the class of compounds to which it belongs, and the suspected significance of the residue based on prior knowledge or experience.

In general, characterization and structural identification of major metabolites should be accomplished using a combination of techniques and might include chromatographic comparison to standards or mass spectrometry. As a point of reference, major metabolites are those comprising

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≥100 µg/kg or ≥10% of the total residue in a sample collected at the earliest euthanasia interval (or following attainment of steady-state or at or near the end of treatment for continuous-use drug products). In some cases, chemical characterization rather than unequivocal structural identification for a major metabolite will be appropriate (e.g. when a conjugate is present or if mass spectrometry information indicates the likely biotransformation pathway (for example M+16 for hydroxylation)). Ordinarily, no differentiation of the radioactivity below these levels (i.e., of the minor metabolites) would be recommended unless there are concerns over residues occurring at the lower levels.

2.3.4.3. Characterization of Bound and Nonextractable Residues

An investigation into the nature of bound residues is usually optional. However, the information obtained from such an investigation might warrant the discount of some of the residues from the total residue of concern.

2.3.4.3.1. General Comments

The use of veterinary drugs in food-producing animals can result in residues that are neither extractable from tissues using mild aqueous or organic extraction conditions nor easily characterized. These residues arise from (a) incorporation of residues of the drug into endogenous compounds, (b) chemical reaction of the parent drug or its metabolites with macromolecules (bound residues), or (c) physical encapsulation or integration of radioactive residues into tissue matrices.

Those nonextractable residues shown to result from incorporation of small fragments of the drug (usually one or two carbon units) into naturally occurring molecules are not of significance.

Characterization of the bound residues of a veterinary drug is usually prompted when the bound residue comprises a significant portion of the total residue or when the concentration of bound residue is so high as to preclude the assignment of a practicable withdrawal period for the drug (i.e., the total residue does not deplete below the residue of concern because of the amount of bound residue). The extent of data collection on the bound residue depends on a number of factors, including the amount of bound residue, the nature of the bound residue and the potency of the parent drug or metabolite on which the Acceptable Daily Intake (ADI) is based.

2.3.4.3.2. Characterization of the Bound Residue

The characterization of bound residues is usually difficult, involving vigorous extraction conditions or enzymic preparations that can lead to residue destruction or artifact formation.

However, the biological significance of residues of veterinary drugs in foods usually depends on the degree to which those residues are absorbed when the food is ingested. Therefore, the determination of the bioavailable residues that result when tissue containing bound residue is fed to test animals can be a useful characterization tool (the method of Gallo-Torres (*Journal of Toxicology and Environmental Health*, 2: 827-845 (1977))) might be an appropriate procedure for demonstrating bioavailability).

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The data should be presented so that it is possible to determine the marker residue to total residue ratio, the marker residue, and the target tissue if these concepts are called for by national/regional regulators. The total residue concentration for each tissue should be reported for each collection timepoint. The amounts of total residue radioactivity extracted (percentage extractable) using various treatments (enzyme, acid) should be provided. The target tissue is the edible tissue selected to monitor for the total residue in the target animal. The target tissue is usually, but not necessarily, the tissue with the slowest depletion rate of the residues.

The components of the total residues should be reported for each collection timepoint for comparison to the total residue concentrations. The components of the total residues (parent drug plus metabolite(s)) should be examined to select the marker residue. The marker residue might be the parent compound. However, the marker residue might also be defined as a combination of parent compound plus a metabolite(s) or as a sum of residues that can be chemically converted to a single derivative or fragment molecule.

An appropriate marker residue has the following properties:

- 1) there is a known relationship established between the marker residue and the total residue concentration in the tissue of interest;
- 2) the marker residue should be appropriate to test for the presence of residues at the time point of interest, i.e. adherence to the withdrawal period; and
- 3) there should be a practicable analytical method to measure the marker residue at the level of the MRL.

4. GLOSSARY

The following definitions apply for purposes of this document.

Acceptable daily intake (ADI) of a chemical is the daily intake which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer. The ADI most often will be set on the basis of the drug's toxicological, microbiological or pharmacological properties. It is usually expressed in micrograms or milligrams of the chemical per kilogram of body weight.

Bound residues are residues formed by covalent binding of the parent drug or its metabolites with macromolecules in food-producing animals.

Edible tissues are tissues of animal origin that can enter the food chain and include, but are not limited to muscle, injection site muscle, liver, kidney, fat, skin with fat in natural proportions, whole eggs and whole milk.

Good laboratory practice (GLP) is the formalized process and conditions under which laboratory studies on veterinary drugs are planned, performed, monitored, recorded, reported and audited. Studies performed under GLP are based on national or regional requirements and are designed to assure the reliability and integrity of the studies and associated data.

Major metabolites are those comprising ≥ 100 $\mu\text{g}/\text{kg}$ or $\geq 10\%$ of the total residue in a sample collected from the target animal species in the metabolism study (VICH GL 46).

Marker residue is that residue whose concentration is in a known relationship to the concentration of total residue in an edible tissue.

Maximum residue limit (MRL) is the maximum concentration of a veterinary drug residue that is legally permitted or recognized as acceptable in or on a food as set by a national or regional regulatory authority. The term 'tolerance,' used in some countries, can be, in many instances, synonymous with MRL.

Metabolism, for purposes of this guidance, is the sum total of all physical and chemical processes that occur within an organism in response to a veterinary drug. It includes uptake and distribution of the drug within the body, changes to the drug (biodegradation), and elimination of drugs and their metabolites.

Practical zero withdrawal is representative of the shortest time interval between administration of the last dose of the drug (e.g. at the farm) and slaughter (including transport from the farm).

Residue means the veterinary drug (parent) and/or its metabolites.

Residue of concern refers to the total amount of residues that have relevance to the ADI established for the veterinary drug.

Total residue of a drug in edible tissues is the sum of the veterinary drug (parent) and all metabolites as determined in radiolabeled studies or other equivalent studies.

Wet weight basis means that samples are analyzed fresh, with no allowance made for the moisture content.