

Guidance for Industry

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 GL48

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-8231.

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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
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Center for Veterinary Medicine
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**STUDIES TO EVALUATE THE METABOLISM
AND RESIDUE KINETICS OF VETERINARY
DRUGS IN FOOD-PRODUCING ANIMALS:
MARKER RESIDUE DEPLETION STUDIES TO
ESTABLISH PRODUCT WITHDRAWAL PERIODS**

Adopted at Step 7 of the VICH Process

by the VICH Steering Committee

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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 will be recommended for adoption to the regulatory bodies of the European Union, Japan and the USA.

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Table of Contents

1. INTRODUCTION	2
1.1. Objective of guidance	2
1.2. Background.....	2
2. GUIDANCE	2
2.1. Purpose.....	2
2.2. Scope.....	3
2.3. Marker Residue Depletion Studies	3
2.3.1. Test Article.....	3
2.3.2. Animals and Animal Husbandry	3
2.3.2.1. Intramammary Studies	3
2.3.2.2. Other Parameters	4
2.3.3. Number of animals for the study.....	4
2.3.3.1. Cattle, pigs and sheep for tissue residue studies	4
2.3.3.2. Dairy animals for milk residue studies	4
2.3.3.3. Poultry	4
2.3.4. Dosing and Route of Administration	5
2.3.4.1. General guidance	5
2.3.4.2. Considerations for products intended for intramammary administration	5
2.3.4.3. Considerations for products intended for multiple routes of administration.....	5
2.3.4.4. Considerations for Use of Multiple Injection Sites per Animal	6
2.3.5. Animal Euthanasia.....	6
2.3.6. Sampling.....	6
2.3.6.1. General Considerations.....	6
2.3.6.2. Injection Sites	8
2.3.6.3. Other Considerations.....	9
2.3.6.4. Milk Sampling	9
2.3.6.5. Egg Sampling	9
2.3.7. Recommendations for Products Proposed for 0-Day Withdrawal Periods (Single Time-Point Studies).....	10
2.4. Analytical Method for Assay of Marker Residue	10
3. GLOSSARY	11

STUDIES TO EVALUATE THE METABOLISM AND RESIDUE KINETICS OF VETERINARY DRUGS IN FOOD-PRODUCING ANIMALS: MARKER RESIDUE DEPLETION STUDIES TO ESTABLISH PRODUCT WITHDRAWAL PERIODS

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 guidance

As part of the approval process for veterinary medicinal products in food-producing animals, national/regional regulatory authorities require data from marker residue depletion studies in order to establish appropriate withdrawal periods in edible tissues including meat, milk and eggs. The objective of this guidance is to provide study design recommendations which will facilitate the universal acceptance of the generated residue depletion data to fulfill the national/regional requirements.

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.

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

Marker residue depletion studies for registration or approval, as applicable, of a new veterinary medicinal product in the intended species are recommended to:

- demonstrate the depletion of the marker residue upon cessation of drug treatment to the regulatory safe level (*e.g.* maximum residue limit or tolerance).
- generate data suitable for elaboration of appropriate withdrawal periods/withholding times to address consumer safety concerns.

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2.2 Scope

The intent is that one residue depletion study (per species), conducted within any VICH region, would satisfy the data recommendations for establishment of appropriate withdrawal periods for a specific product in food-producing animals.

The guidance encompasses the most common species, namely cattle, pig, sheep and poultry; however, the principles of this guidance can also be applied to related species not mentioned in this core group (e.g., cattle vs. all ruminants). The guidance does not provide study design recommendations for fish or honey bees (as producers of honey).

Studies should be conducted in conformity with the applicable principles of Good Laboratory Practice (GLP).

2.3. Marker Residue Depletion Studies

2.3.1. Test Article

The test article used for the study should be representative of the commercial formulation. Use of final GMP manufactured material (pilot scale or commercial scale) is the preferred source of test article; however, laboratory scale preparations characterized with respect to GLP could also be appropriate.

2.3.2. Animals and Animal Husbandry

Ordinarily, one marker residue depletion study (for tissues) should be performed in swine, sheep and poultry. For cattle, a single study in ruminating beef cattle could be applied to dairy cattle (or vice versa). However, because of differences in ruminant and pre-ruminant physiology, separate studies are recommended when the target species encompasses both adult and pre-ruminating animals. A separate study should be performed to demonstrate the residue depletion profile in milk of dairy animals or in eggs produced by laying hens.

Animals should be healthy and, preferably, should not have been previously medicated. However, it is recognized that animals might have received biological vaccinations or prior treatment, for example, with anthelmintics. In the latter case, an appropriate wash-out time should be observed for the animals prior to enrollment in the actual trial. Study animals should be representative of the commercial breeds and representative of the target animal population that will be treated. The source of the animals, their weights, health status, ages and sex should be reported.

Animals should be allowed adequate time to acclimatize and normal husbandry practices should be applied to the extent possible. 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.

2.3.2.1. Intramammary Studies

For studies with intra-mammary products, all animals should have visibly healthy udders free from effects from chronic mastitis. For pre-parturition studies, pregnant animals with a predicted parturition date should be introduced into the study facility well in advance of study enrollment.

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2.3.2.2. Other Parameters

The marker residue depletion study should take into account all factors that might contribute to the variability of residue levels in animal commodities in the planning and conduct of trials. The intent here is that these “other factors” (*e.g.*, animal breeds, physical maturity, etc) be considered within the pool of animals to be included in the marker residue depletion study without warranting an increase in the number of animals as recommended in 2.3.3. For example, if a milk residue depletion study recommends 20 animals, all “other factors” should be represented within the 20 initially selected animals (not an additional 20 animals representing each “other factor”).

2.3.3. Number of animals for the study

The number of animals used should be large enough to allow a meaningful assessment of the data. From a statistical point of view, residue data from a minimum of 16 animals with four animals being euthanized at four appropriately distributed time intervals are recommended. Higher numbers of animals should be considered if the biological variability is anticipated to be substantial as the increased numbers might result in a better defined withdrawal period. Control (non-treated) animals are not necessarily called for as part of the actual marker residue depletion study; however, sufficient amounts of control matrices should be available to provide for related analytical methods testing. The following section provides a general recommendation for numbers of animals to be included in the study design.

2.3.3.1. Cattle, pigs and sheep for tissue residue studies

At least 4 (evenly mixed as per sex) per each euthanasia time are recommended. The suggested bodyweight ranges are ~40 to 80 kg for swine, ~40 to 60 kg for sheep and ~250 to 400 kg for beef cattle. Consistent with Section 2.3.2, non-lactating dairy cows could also be used for these tissue residue studies.

2.3.3.2. Dairy animals for milk residue studies

For lactating animal studies, at least 20 animals, randomly selected from a herd where all lactation stages are represented, are recommended. High yielding animals at an early lactation stage and low yielding animals at a late lactating stage should be included in the group of animals but specific numbers of each are called for.

For pre-parturition (*i.e.* dry-cow) studies, a minimum of 20 animals is recommended. The study should include randomly selected cows representative of commercial dairy practices.

2.3.3.3. Poultry

A sufficient number of birds should be used to obtain at least 6 samples at each euthanasia time for tissue residue studies.

For egg residue studies, a sufficient number of birds should be used to collect 10 or more eggs at each interval time point.

2.3.4. Dosing and Route of Administration

2.3.4.1. General guidance

Animal treatment should be consistent with the intended product label including, for injectable products, the location and injection method. For multiple treatments, the injections should be given alternately between left and right sides of the animal.

The highest intended treatment dose should be administered for the maximum intended duration. If an extended drug administration period is intended, duration of treatment sufficient to reach steady state in target tissue(s) can be used instead of the full length of the treatment. The time to steady-state data are often obtained as part of the total residue study, see VICH GL 46, “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.”

2.3.4.2. Considerations for products intended for intramammary administration

Drug products intended for intra-mammary administration either for lactating animals or for pre-parturition (*i.e.*, dry cow treatment) studies should be given to all quarters (*i.e.*, normally four quarters in bovine). Although it is unlikely that all quarters will be treated with an intramammary product during commercial practice, for residue studies this study design represents a worst-case scenario.

For pre-parturition (*i.e.*, dry cow treatment) studies, the test article should be administered after the last milking (dry-off) and consistent with the desired pre-calving interval. It is recognized that these (dry-cow) studies comprise both a milk residue depletion phase (post-calving) as well as a decision on a desired pre-calving treatment interval. In order to reduce variability in the residue data, the pre-calving period should be tightly controlled and the study should be designed such that a sufficient number of animals give birth in a limited time interval (*i.e.*, the differences between dry periods among animals within an experiment should be kept as small as possible). For instance, to target a pre-calving treatment interval of 30 days, data should be collected from at least 20 cows calving between, for example, 20-30 days after treatment. For a pre-calving treatment interval of 60 days, data should be collected for at least 20 cows calving between, for example, 40-60 days after treatment. This can be accomplished by drying-off and infusing animals with the test formulation based on the expected calving date.

2.3.4.3. Considerations for products intended for multiple routes of administration.

If the drug product is intended to be administered via more than one parenteral route (intramuscular (IM), subcutaneous (SC) or intravenous (IV)), a separate marker residue depletion study for each route of administration should be provided. If the withdrawal period is clearly defined by depletion of residues from the injection site following SC or IM dosing, a separate intravenous residue study (at the same dose) is not recommended provided the same withdrawal period (for SC or IM) can be applied to the IV route.

A single marker residue depletion study can be conducted for drug formulations containing the same active substance but which are applied via different dermal routes (*e.g.*, dipping, spray or pour-on). However, the methodology used in the study should represent delivery of the highest possible dose and this should be appropriately justified. The consequence of this

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approach is that the same withdrawal time would be applied to all approved dermal application routes. Separate residue studies are recommended if differentiation among these routes of administration is desired.

2.3.4.4. Considerations for Use of Multiple Injection Sites per Animal

Where the withdrawal period will clearly be determined by residue depletion at the site of injection, the Sponsor generally has the option of collecting data from two injection sites per animal (and using the data from both sites in a determination of the withdrawal time). This practice can have a positive impact on study design with respect to animal welfare by reducing animal numbers. An example of where this approach is applicable is described below:

- For a product that utilizes only a single injection, treatment can be given on the right side of the neck on Day 0 and then on the left side of the neck on Day 4. Euthanasia on Day 7 following the final treatment would provide depletion data at 7-days (left injection site (IJS)) and 11 days (right IJS) withdrawal. In this case, however, collection and assay of the other tissues would not be warranted since the product was administered contrary to the label (two injections vs. one injection) and residues could be excessively elevated. Such a dosing regimen is designed specifically for determination of injection site residue depletion.

2.3.5. 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 the analysis of the marker residue.

2.3.6. Sampling

2.3.6.1. General Considerations

Following euthanasia, edible tissue samples in sufficient amounts should be collected, trimmed of extraneous tissue, weighed and divided into aliquots. If the analysis can not be completed immediately, the samples should be stored under frozen conditions pending analysis. If samples are stored after collection, the Sponsor generally bears the responsibility for demonstrating residue stability through the time of assay.

The tissue sampling protocol encompasses two sections; (1) those tissues that are recommended in support of registration or approval, as applicable, in all VICH regions and (2) additional tissues that can be collected to address specific national/regional consumption habits and/or legal concerns. Table 1 indicates the recommended samples for collection for all VICH regions. Table 2 indicates the recommended additional samples for collection.

For purposes of this guidance, one of the additional tissues from Table 2 (per species) should be selected for assay, based on the results of the total radioactive residue (TRR) study. This would typically be the additional tissue with the highest residues or the slowest depletion rate. It is important to emphasize that collection of only one additional tissue is recommended. For example, if the TRR study indicates that cattle heart has the slowest depletion rate, that additional tissue should be selected for assay in the marker residue depletion study, but cattle small intestine marker residue data are not recommended. Similarly, if poultry gizzard has the highest residues, assays of poultry heart are not recommended. If no TRR data are available

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for the additional tissues, it is suggested that the Sponsor discuss with the appropriate national/regional authorities how best to conduct the marker residue depletion study to satisfy the specific national/regional consumption habits and/or legal concerns, if any.

Table 1. Sample Collection from Animals in the Marker Residue Depletion Study (All Regions)

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

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Table 2. Additional Tissues that can be Collected to Address Specific National/Regional Consumption and/or Legal Concerns in the Marker Residue Depletion Study

Edible Tissue Type	Species / Sample Description			
	Cattle / Sheep		Swine	Poultry
Gizzard	NA		NA	Entire
Heart	Cross-section		Cross-section	Entire
Small Intestine	Composite, rinsed of content		Composite, rinsed of content	NA
Other Tissues	Composite	Composite	Composite	Composite

NA: not applicable

2.3.6.2. Injection Sites

For parenteral preparations (IM or SC), residue depletion data from the injection site(s) should be included. Injection site residues are local residues (*i.e.* those that do not arise via the systemic circulation) that might or might not remain localized at the site of administration. As such, it is important for the Sponsor to develop appropriate quality control sampling procedures which ensure that the collected tissue actually encompasses the injection site. Any approach taken by the Sponsor should be justified on a case-by-case basis, taking into account the data available and the formulation characteristics. The following methodologies should be considered, however, this list is not to be considered comprehensive. Regardless of the option selected, the primary core sample should target 500 g \pm 20%.

- Collection of an additional ring sample (300 g \pm 20%) around the primary core sample (500 g \pm 20%). These tissue amounts would generally not apply to small animals that do not allow sampling of 500 g. For these situations, the optimum sampling strategy should be defined on a case by case basis and should be justified. However, collection of two samples (a core and surrounding sample) remains appropriate.
- Collection of an elliptical (or other appropriate shape) sample along the injection track and/or the site of irritation. The Sponsor should provide evidence that this method correctly targets the injection site residues, such as with accompanying photographs of the site(s) of sampling.
- Provide data on the migration potential of injection site residues based on information obtained from the TRR study. For example, a circular core (or elliptical) sample would be taken along the injection track and/or site of irritation as well as several adjacent samples for TRR comparisons. If this protocol demonstrates an appropriate sample collection technique, only the primary sample should be collected during the marker residue depletion study. It might be constructive to include an additional time point (*i.e.*, at a longer withdrawal time) in this study.

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- Provide data on the migration potential of injection site residues based on information obtained from a target animal safety study (*i.e.* pathological examinations of the physical injection site).
- Conduct one of the above study designs using a colored dye to provide a visual assessment of the migration potential of injection site residues.

Samples should be collected from the last injection site (or sites), as appropriate (see Section 2.3.4.4, Considerations for Use of Multiple Injection Sites per Animal). In the case of products requiring multiple injections, the study design should be such that the last injection site will occur on the side of the animal receiving the higher number of injections. When a circular cores sample is indicated, collection of the injection site muscle tissue (from large animals) should be centered on the point of injection and consistent with the recommendations shown in Table 1.

2.3.6.3. Other Considerations

- For formulations that are able to leave local residues, such as dermal pour-on products, samples of relevant tissues (*e.g.*, muscle, subcutaneous fat or skin/fat from the application site) should be harvested for analysis (in addition to those specified in Table 1).
- For clarity, if two or more of the tissues are assayed as composite tissues such as skin plus fat in natural proportions (pig and poultry), it is not recommended to assay separate samples of skin and fat.
- Muscle samples can be obtained from skeletal (striated) muscles that include intramuscular fat in natural proportion.

2.3.6.4. Milk Sampling

Milk samples should be obtained from all animals at evenly spaced milking times (*e.g.*, every 12 hours) after the final dose of the medication. Four-quarter composite samples should be collected from individual cows at each time point. For multiple dosed products used in dairy animals, samples should be taken after the last treatment. For products that might qualify for 0-day withdrawal, samples should also be collected during treatment. There is no standard number of sampling times. Milk collections should continue until the residues fall below the appropriate reference point (*e.g.*, MRL, tolerance, LOQ, etc) as determined by the chemical properties of the drug product.

Although beyond the scope of this guidance, the Sponsor might be requested to assess residues in calves fed milk (including colostrum) from treated adults (*i.e.*, mothers), if these animals are intended for human consumption (such as for veal calves).

2.3.6.5. Egg Sampling

Egg samples should be obtained from 10 or more laying hens at every laying time point during the medication period and after the final medication. Egg samples should be collected after the period necessary to complete egg yolk development, which is usually up to 12 days. Egg white and yolk should be combined for analysis.

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2.3.7. Recommendations for Products Proposed for 0-Day Withdrawal Periods (Single Time-Point Studies)

For products administered as one treatment or as several treatments (*i.e.*, daily for 3-5 days), or for continuous use products in which residues have reached steady state, a single time point study should be sufficient to qualify for 0-day withdrawal, provided that the total residue depletion characteristics of the drug have been adequately described as indicated in VICH GL 46. If such data are available, then a single time point study conducted with the specified minimum number of animals is recommended to demonstrate acceptability of 0-day withdrawal.

- Poultry 12 birds (to provide at least 6 individual samples for assay)
- Large Animals: 6 animals
- Milk: 10 animals

The sampling time chosen for this study should be consistent with the peak concentrations observed during the total residue depletion study, a minimum transit time (practical zero withdrawal; *e.g.*, not less than 3 hr) and a maximum time that would still qualify as 0-day withdrawal (*e.g.*, ≤ 12 hr).

The increased number from that recommended in Section 2.3.3 is generally appropriate for the single time point. However, for dairy animals, a minimum of 10 animals is recommended to define the milk concentration at the single (0-day) time point. Drug concentrations, which remain below the appropriate reference point (*e.g.*, MRL, tolerance) generally would be considered for the 0-day withdrawal designation.

While a 0-day milk withdrawal designation could be possible based on a single time point (*i.e.* 12 hours) sampling protocol, it is strongly recommended that additional samples (*e.g.*, over 1-4 milkings) be collected for full assessment of the residue profile. As milk studies do not call for terminal euthanasia for sample collection, compliance with this recommendation is straightforward.

2.4. Analytical Method for Assay of Marker Residue

The Sponsor should submit a validated analytical method for the determination of the marker residue in samples generated from the residue depletion studies in the edible tissues and where applicable, in milk and eggs. The method(s) should be capable of reliably determining concentrations of marker residue which encompass the appropriate reference point (*i.e.*, MRL / Tolerance) for the respective tissues or products.

The parameters to be included in the method validation are fully discussed in VICH GL49, “Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies.”

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3. GLOSSARY

The following definitions are applied 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.

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.

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.

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 euthanasia (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.

Zero-day withdrawal refers to a label indication that allows entry of edible tissues into the food chain without regard to the time of last drug administration.