



European Medicines Agency  
Veterinary Medicines and Inspections

London, 14 December 2009  
Doc. Ref. EMEA/CVMP/VICH/463072/2009-CONSULTATION

**VICH Topic GL46**

**at step 4**

**GUIDELINE ON 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**

<b>DRAFT AGREED BY VICH STEERING COMMITTEE</b>	November 2009
<b>ADOPTION BY CVMP FOR RELEASE FOR CONSULTATION</b>	10 December 2009
<b>END OF CONSULTATION (DEADLINE FOR COMMENTS)</b>	20 May 2010

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**VICH GL 46 (MRK) – NATURE OF RESIDUES**

**November 2009**

**For consultation at Step 4 - Draft 1**

**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**

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Recommended for Consultation

at Step 4 of the VICH Process

on 6 November 2009

by the VICH Steering Committee

This Guideline 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 USA.

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## **1. INTRODUCTION**

### **1.1. Objective of the guidance**

A comprehensive set of metabolism, residue depletion and pharmacokinetic studies is performed 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 of residue chemistry data for veterinary drugs used in food-producing animals. This guidance was prepared after consideration of the current requirements 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 remains flexible. It is recommended that studies be tailored to sufficiently characterize the components of the residue of toxicological concern.

## **2. GUIDANCE**

### **2.1. Purpose**

The human food safety evaluation of veterinary drugs assures that food derived from treated animals is safe for human consumption. As part of the data collection process, studies are 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 toxicological concern from edible tissues of treated animals at varying times after drug administration, (2) the individual components, or residues, that comprise the residue of toxicological concern in edible tissues, (3) the residue(s) that may serve as 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, therefore, recommends procedures for metabolism studies conducted with radiolabeled drugs. However if the drug sponsor generates new approaches other than radiolabeled studies and those approaches are considered as equivalent to radiolabeled studies, those studies can be accepted instead of radiolabeled total residue studies. A alternate approach to a radiolabel total residue study may be acceptable if the approach is considered to be equivalent to a radiolabel study.

For drugs that are intended only for regional registration, alternative approaches (i.e., not using radiolabeled drug) to characterize the components of the residue in food derived from treated animals may be suitable.

In any case, testing should be conducted pursuant to international standards or 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 or regulations.

#### **2.3.1.2. Radiolabeled Drug**

##### **2.3.1.2.1. Nature and Site of Label**

Carbon-14 ( $^{14}\text{C}$ ) is the label of choice because intermolecular exchange is not an issue. Other isotopes, such as  $^3\text{H}$ ,  $^{32}\text{P}$ ,  $^{15}\text{N}$  or  $^{35}\text{S}$ , may be appropriate. Tritium ( $^3\text{H}$ ) will be considered acceptable if a rigorous demonstration of the stability of the tritium label is provided; that is, the extent of exchange with water must be assessed and found to be  $\leq 5\%$ .

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 toxicological 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 about 98%, 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 reported. The specific activity must be high enough to permit tracking of the residue of toxicological concern in edible tissues. The required sensitivity will be determined by the toxicological potency of the drug.

The specific activity may be adjusted as necessary by mixing with unlabeled drug. To facilitate analytical measurements and conserve labeled drugs, animals to be sacrificed at early withdrawal periods may be dosed with drug of lower specific activity, while animals to be sacrificed at later withdrawal periods may be dosed with drug of higher specific activity.

#### **2.3.1.3. Analytical Standards**

Analytical standards should be available for 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, sheep and fish. These differences may affect national/regional data collection requirements. In certain instances, the total residue and metabolism data for a drug's use in a major species may be extrapolated to the minor species. The study designs outlined in this guideline are acceptable if a total residue and metabolism study is required for a minor species or for a species considered to be major in one region but not another.

#### **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, one study may 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) would apply to dairy cattle (or vice versa). Generally, the results of the metabolism study in adult cattle and sheep may be extrapolated to veal calves and lambs, respectively. However, a second study may be necessary for veal calves or lambs if there is reason to believe the nonruminating 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.

#### **2.3.2.2. Animal Handling**

Animals should be allowed adequate time to acclimatize. Normal husbandry practices should be applied to the extent possible (it is recognized that these studies require metabolism cages, a departure from “normal” practices). Animals should be healthy and, preferably, should not have been previously medicated. However, it is recognized also that animals may be permitted biological vaccinations and may have received prior treatment, for example with anthelmintics. In the latter case an appropriate wash-out time should be observed for the animals prior to their being put on study. In short, animals should have a known history of medication.

Handling and disposal of animals and tissues from animals treated with radiolabeled materials should be in compliance with applicable laws or 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. Although it is recognized that metabolism studies may 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 are acceptable.

##### **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 is permitted to ensure that animals receive the complete dose and to minimize environmental concerns. For drugs that are intended for oral and parenteral administrations, separate metabolism studies should be performed. Ordinarily, a single study with a parenteral route will be valid to cover all parenteral routes including intramuscular, intramammary, subcutaneous and topical.

##### **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 permitted.

For continuously administered drugs, a separate study to determine the time required for residues to reach steady state in edible tissues may be necessary. When a drug administered

in a single dose is intended to have zero withdrawal it will be necessary to demonstrate when the absorption phase is completed.

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

#### **2.3.3.4. Number of Sacrifice Intervals and Number of Animals**

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

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

Poultry: 6 per sacrifice time

Fish: 6 per sacrifice 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

For drugs anticipated not to require a withdrawal period, sacrifice timepoints should reflect regional differences 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 Sacrifice**

Animals should be sacrificed using commercially applicable procedures, making certain to observe appropriate exsanguination times. Chemical sacrifice should be avoided.

#### **2.3.3.6. Sample Collection – Edible Tissues**

Following sacrifice, samples of sufficient amounts of edible tissues 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 bears the responsibility for demonstrating that the radiolabeled compound remains intact throughout the storage period.

Recommended samples are shown in Table 1.

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

Sample	Cattle	Swine/Sheep	Poultry	Fish
	Description	Description	Description	Description
Muscle	Loin	Loin	Breast	Muscle with skin in natural proportions
Injection Site Muscle	Core of muscle tissue ~0.5 kg 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	Core of muscle tissue ~0.5 kg 10 cm diameter x 6 cm deep for IM; 15 cm diameter x 2.5 cm deep for SC	---	---
Liver	Cross-section of lobes	Cross-section of lobes	Entire	---
Kidney	Composite from combined kidneys	Composite from combined kidneys	Composite from combined kidneys	---
Fat	Omental	Omental		---
Skin	---	Skin with fat in natural proportions	Skin with fat in natural proportions	---
Milk	Whole milk	---	---	---
Eggs	---	---	Clean shell, break egg, combine white and yolk	---

The tissues shown in Table 1 should be analyzed for registrations in all regions. However, besides the tissues shown in Table 1, additional tissues may need to be collected and analyzed to address specific regional requirements. As appropriate to species, the additional tissues may include heart (cattle, swine, sheep, poultry), small intestines (cattle and swine), gizzard (poultry) and organs of fish; furthermore, other

edible offal from the various species may need to be collected and analyzed if they are deemed important for a safety assessment (e.g., offal with expected high residue concentrations or with residues having slow depletion rates).

#### **2.3.3.7. Sample Collection – Excreta and Blood**

The collection of excreta and blood is optional. 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 may be a good source of metabolites; and third, the samples may be of use for 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 may be taken from selected animals at various time points and at sacrifice. Data on the total residue in blood can provide valuable pharmacokinetic information.

#### **2.3.3.8. Determination of Total Radioactivity**

Total radioactivity in samples may be determined by established procedures which 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.

#### **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, permit the separation of the total residue into its components and identification of the drug-derived residues.

##### **2.3.4.1. Analytical Method**

A complete description of the analytical method should be provided. The description should include 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 toxicological 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 which may include chromatographic comparison to standards or mass spectrometry. Major metabolites are those comprising  $\geq 100 \mu\text{g}/\text{kg}$  or  $\geq 10\%$  of the total residue in a sample collected at the earliest slaughter interval (or following attainment of steady-state or at or near the end of treatment for continuous-use drug products). Ordinarily, no differentiation of the radioactivity below these levels (i.e., of the minor metabolites) would be required unless there are toxicological concerns over residues occurring at the lower levels.

#### **2.3.4.3. Characterization of Bound and Nonextractable Residues**

The investigation into the nature of bound residues is optional. However, the information obtained from the investigation may permit the discount of some of the residues from the total residue of toxicological 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 toxicological significance.

The need to characterize 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 toxicological concern because of the amount of bound residue). The extent of data collection on the bound residue will depend on a number of factors, including the amount of bound residue, the nature of the bound residue and the toxicological potency of the parent drug or metabolite on which the ADI is based.

##### **2.3.4.3.2. Characterization of the Bound Residue**

The characterization of bound residues usually is difficult, involving vigorous extraction conditions or enzymic preparations that may 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)) may be an acceptable procedure for demonstrating bioavailability).

### 3. Reporting of Data

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 needed for registration. The total residue concentration for each tissue should be reported for each collection timepoint. The amounts of total residue radioactivity that were possible to extract (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 to allow comparison to the total residue concentrations. The components of the total residues (parent drug plus metabolite(s)) are examined to select the marker residue. The marker residue may be parent compound. However, the marker residue may 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

**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. 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** mean muscle, injection site muscle, liver, kidney, fat, skin with fat in natural proportions, whole eggs, whole milk and honey.

**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 the national regulations of a country and are designed to assure the reliability and integrity of the studies and associated data.

**Major metabolites** are those comprising  $\geq 100 \mu\text{g}/\text{kg}$  or  $\geq 10\%$  of the total residue in a sample at the time the total residue peaks.

**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 regulatory authority. The term tolerance used in some countries is, in many instances, synonymous with MRL.

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

**Minor Metabolites** are those comprising  $< 100 \mu\text{g}/\text{kg}$  or  $< 10\%$  of the total residue.

**Practical zero withdrawal** is considered to be up to 12 hours post last drug administration for large animals and up to 6 hours for poultry to allow for transfer to abattoir. For milk, practical zero withdrawal is up to 12 hours.

**Residue** means the veterinary drug, its metabolites, derivatives or related compounds.

**Residue of toxicological concern** refers to the total amount of residues that have relevance to the ADI established for the veterinary drug. The ADI most often will be set on the basis of the drug's toxicological, microbiological or pharmacological properties.

**Tolerance** is the maximum amount of a veterinary drug residue that may lawfully be present in an edible tissue.

**Total residue** of a drug in edible tissues is the sum of the parent drug, all metabolites and all drug-related compounds, including drug contaminants 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.