European Medicines Agency
Veterinary Medicines and Inspections

London, 14 December 2009
Doc. Ref. EMEA/CVMP/VICH/463199/2009-CONSULTATION

VICH Topic GL48

at step 4

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

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

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VICH GL 48 (MRK) – MARKER RESIDUE DEPLETION STUDIES

November 2009

For consultation at Step 4 - Draft 1

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

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 guidance

As part of the approval process for veterinary medicinal products in food-producing animals, regulatory authorities require data from marker residue depletion studies in order to establish appropriate withdrawal periods in edible products including meat, milk, eggs and honey. 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 this requirement.

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.

2. GUIDANCE

2.1. Purpose and Scope

Marker residue depletion studies for registration 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.

The intent is that one residue depletion study (per species), conducted within any global region, will be sufficient to satisfy the data requirements for establishment of appropriate withdrawal periods for a specific product in food-producing animals. The guideline encompasses the most common species, namely cattle, pig, sheep, chicken and honey bees (as producers of honey); however, the principles of this guideline allow sufficient flexibility for application to related species not mentioned in this core group (*e.g.* cattle *vs*. all ruminants, chickens *vs.* all poultry).

For fish, the appropriate regional authorities should be consulted for advice on selection of the appropriate species for conduct of the marker residue study.

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

2.2. Marker Residue Depletion Studies

2.2.1. 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 guidelines are also acceptable.

2.2.2. Animals and Animal Husbandry

Ordinarily, one marker residue depletion study (for tissues) may be performed in swine, sheep and chicken. For cattle, a single study in ruminating beef cattle would apply 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 beef cattle and veal calves (or adult sheep and lambs). 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 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 enrollment in the actual trial. Study animals must 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 provided.

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.

2.2.2.1. 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.

2.2.2.2. Special Considerations for Aquaculture (studies in fish)

It is recognized that fish-farming practices may vary widely among regions. Thus, it is appropriate to seek further guidance from regional authorities to ensure appropriate study design. This additional guidance may include information with respect to regional practices for fish-farming and/or specific fish species as well as considerations based on the known or predicted chemical properties (*e.g.* metabolism and residue kinetics) of the test article.

2.2.2.3. 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 study without the necessity for increasing the number of animals as recommended in 0. 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”).

2.2.3. 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 slaughtered at four appropriately distributed time intervals are recommended. Higher numbers of animals may be considered if the biological variability is anticipated to be substantial as the increased numbers may result in a better defined withdrawal period. Control (non-treated) animals are not necessarily required 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.

2.2.3.1. Cattle, pigs and sheep for tissue residue studies

At least 4 (evenly mixed as per sex) per each slaughter 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 0, dairy cows may also be used for these tissue residue studies.

2.2.3.2. 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 not required.

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. [Note: further information on this topic may be found in a guideline (including relevant updates when issued) developed with respect to this topic by the European Union (reference: EMEA/CVMP/473/98, section 2.1.10).

2.2.3.3.Poultry

A sufficient number of birds should be used to obtain at least 6 samples at each slaughter 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.

2.2.3.4. Fish:

The appropriate regional authorities should be consulted for advice on the number and nature of samples to be collected. A general recommendation is that a sufficient number of fish (15 to 20) be used to obtain at least 10 composite samples per each sampling time for conduct of the marker residue study.

2.2.3.5. Honey Bee

The collection of five or more samples from each of five hives is recommended. The time points should be consistent with the period of treatment and standard honey production practices. Honey samples should be harvested only from super honey.

2.2.4. Route of Administration

2.2.4.1. 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 dose should be the highest intended treatment concentration and 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. [*Note: the time to steady-state data are often obtained as part of the total residue study, see VICH guideline for conduct of the "Metabolism Study to Determine the Quantity and Identify the Nature of the Residues"*].

Drug products intended for intra-mammary administration should be given to all 4-quarters of each cow.

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.

This guideline recommends use of a 1X dose, and Sponsors conducting marker residue depletion studies using a 1X dose can be assured of global study acceptance and subsequent elaboration of withdrawal periods using commonly accepted statistical principles.

2.2.4.2. 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, subcutaneous or intravenous), a separate marker residue depletion study for each route of administration should be provided. *[Note: 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 study may 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). To be acceptable, however, the methodology used in the study must represent delivery of the highest possible dose and this should be adequately justified. The consequence of this 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.

2.2.4.3. 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 is given an option of collecting data from two injection sites per animal (and using the data from both sites in a statistical analysis 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. Sacrifice on Day 7 following the final treatment would provide depletion data at 7-days (left 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 may be excessively elevated. Such a dosing regimen is designed specifically for determination of injection site residue depletion.

As this represents a new concept for residue study design, it is suggested that as additional experience is gained, the premise be reviewed in conjunction with guideline revisions that may occur in the future.

2.2.5. Animal Sacrifice

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

2.2.6. Sampling

2.2.6.1. General Considerations

Following sacrifice, 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 bears the responsibility for demonstrating residue stability through the time of assay.

Table 1 indicates the recommended samples for collection at sacrifice.

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

Edible Tissue Type	Species / Sample Description			
	Cattle	**Swine/Sheep**	**Poultry**	**Fish***
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 (sheep only)	---	---
Skin	---	Skin with fat in natural proportions (swine only)	Skin with fat in natural proportions	---
Milk	Whole milk	Whole milk (sheep only)	---	---
Eggs	---	---	Clean shell, break egg, white and yolk may be combined	---

* See Section 0 for further guidance

** For specific order or family classification of fish species within specific regions, muscle and skin should be assayed separately. The Sponsor is encouraged to seek regional advice.

The tissues shown in Table 1 should be analyzed for registrations in all regions. However, the VICH guideline for conduct of the *"Metabolism Study to Determine the Quantity and Identify the Nature of the Residues"* recommended collection of additional tissues to quantify the total residues to address specific regional concerns. The additional tissues suggested for collection are shown in Table 2.

Table 2. Additional Tissues to be Collected to Address Regional Concerns in the Marker Residue Depletion Study

Edible Tissue Type	Species / Sample Description			
	Cattle	**Swine/Sheep**	**Poultry**	**Fish**
Gizzard	---	---	Entire	---
Heart	Cross-section	Cross-section	Entire	---
Small Intestine	Composite, rinsed of content	Composite, rinsed of content (swine only)	---	---
Other Organs	Composite	Composite	Composite	Individual or Composite

For purposes of this guidance, one of the additional tissues (per species) should be selected for assay of the marker residue to address the regional concerns. The additional tissue selected is based on the results of the total residue (TRR) study and is typically 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 will be selected for assay in the marker residue study, but cattle small intestine marker residue data need not be generated. Similarly, if poultry gizzard has the highest residues, assays of poultry heart are not recommended. [Note: if no total residue data are available, all tissues indicated in Table 2 are recommended for marker residue assay].

The Sponsor is encouraged to seek regional advice if it becomes necessary to sample the "other organs" from fish.

2.2.6.2. Injection Sites

For parenteral preparations (IM or SC), residue depletion data from the injection site(s) should be included. Samples should be collected from the last injection site. 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. Sampling 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.

The collection technique should be such that the needle track, the area of drug release and any area of tissue reaction are included, whenever possible.

Collection of an additional ring or surrounding injection site sample during the conduct of tissue residue depletion studies is required in the EU, but generally not required in other regions.

2.2.6.3. 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) or skin plus muscle (fish) in natural proportions, it is unnecessary to assay separate samples of skin and fat or of skin and muscle.

- Muscle samples can be obtained from skeletal (striated) muscles that include intramuscular fat in natural proportion.

- Collection of only a single fat sample type (omental) is recommended (cattle and sheep) or skin with fat (swine and poultry).

2.2.6.4. Special Considerations for Fish Sampling

For fish, edible tissue samples will be represented with muscle and skin in natural proportions. The recommended standard procedure is to homogenize the entire fillet on one side of a fish (with adhering skin) with an aliquot of this homogenate used for analysis. This approach reduces sample variability and helps to assure that the drug concentration in the analyzed sample is representative of the entire edible tissue, which contains both white and red muscle.

For large fish, in order to keep sample size to a manageable level, samples should be obtained from different parts of the body and combined for analysis. For fish species that are too small to obtain individual samples, it is recommended that muscle/skin samples in natural proportions from more than one fish be combined to generate the necessary composite sample. The Sponsor is advised to consult with the appropriate regional authorities to determine if any additional tissues should be harvested for analysis. National/regional differences in what constitutes a major or minor species may also affect data collection recommendations.

2.2.6.5. Milk Sampling

Milk samples should be obtained from all animals at evenly spaced milking times (*ca* 12 hr) 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 may 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 guideline, the Sponsor may 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).

2.2.6.6. Egg Sampling

Egg samples are to 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 may be combined for analysis.

2.2.7. 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 is 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 guideline for conduct of the "*Metabolism Study to Determine the Quantity and Identify the Nature of the Residues.*" If such data are available, then a single time point study conducted with the specified minimum number of animals is recommended to confirm acceptability of 0-day withdrawal.

- Poultry: 12 birds
- Large Animals: 6 animals
- Milk: 10 animals

The sacrifice time chosen for this study should be consistent with the peak concentrations observed during the total residue depletion study, a minimum transit time (*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 0 is justified for the single time point. However, for dairy animals, a minimum of 10 animals is recommended as this is sufficient 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) would be considered for the 0-day withdrawal designation.

While a 0-day milk withdrawal designation may 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 require terminal sacrifice for sample collection, compliance with this recommendation is straightforward.

For fish, there is generally no standardized transit time between the end of medication and arrival at the slaughter facility. As such, samples should be collected while fish remain on treatment. Suggested intervals include 0-6 hours following final treatment. The appropriate regional authorities should be consulted for additional advice on sampling times.

2.3. Analytical Method for Assay of Marker Residue

The Sponsor is responsible for submitting 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, eggs and honey. 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 the VICH document *"Guidelines for Validation of Analytical Methods Used in Residue Depletion Studies.*