International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

#257

Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Species: Marker Residue Depletion Studies to Establish Product Withdrawal Periods in Aquatic Species

Guidance for Industry

VICH GL57

Submit comments on this guidance at any time. Submit electronic comments to https://www.regulations.gov/. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number FDA-2016-D-4461.

For further information regarding this document, contact AskCVM@fda.hhs.gov.

Additional copies of this guidance document may be requested from the Policy and Regulations Staff (HFV-6), Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either https://www.fda.gov/AnimalVeterinary/default.htm or https://www.regulations.gov/.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
August 2019

VICH GL 57 (MRK) –RESIDUES IN FISH
For implementation at Step 7

Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Species: Marker Residue Depletion Studies to Establish Product Withdrawal Periods in Aquatic Species

Adopted at Step 7 of the VICH Process by the VICH Steering Committee
in March 2019
for implementation by February 2020

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 USA.

Table of Contents

Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Species: Marker Residue Depletion Studies to Establish Product Withdrawal Periods in Aquatic Species

Guidance for Industry

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

1. INTRODUCTION

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 by national/regional regulators. This guidance was prepared after consideration of the current national/regional requirements and recommendations for evaluating veterinary drug residues in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) regions.

The objective of this guidance is to provide study design recommendations that will facilitate the universal acceptance of the generated residue depletion data to fulfill the national/regional requirements.

This document is an extension to the parent residue guidance: CVM Guidance for Industry (GFI) #207/VICH GL48, "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, VICH GL57, provides recommendations on what should be included in a marker residue depletion study design for aquatic food-producing species.

Metabolism studies based on CVM GFI #205/VICH GL46, "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," can be used in aquatic food-producing species to identify a marker residue.

In general, FDA's guidance documents 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 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.

2.2. Scope

The intent is that a residue depletion study conducted according to the recommendations described in this guidance would satisfy the data requirements or recommendations for establishment of appropriate withdrawal periods in all VICH regions. Conducting a depletion study under worst-case conditions provides data for calculating the withdrawal period. It may be desirable to conduct an additional study or provide additional information to further define the withdrawal period under alternate management conditions or to adjust the withdrawal period based on the concept of degree days.

The guidance encompasses food-producing aquatic species. The principles of this guidance are also applicable to eggs from aquatic species for human consumption. Studies should be conducted in conformity with the applicable principles of Good Laboratory Practice (GLP). The in vivo phase should be conducted under GLP conditions; if this is not practical, a justification for all deviations from GLP and the impact on the study results should be provided.

2.3. Test Article

The test article used for the study should be representative of the commercial formulation. Use of final Good Manufacturing Practice (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.

2.4. Study Design

2.4.1. Animals

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, antimicrobials). In the latter case, an appropriate wash-out time should be observed for the animals prior to enrollment in the actual study.

Study animals should be representative of the commercial species and representative of the target animal population that will be treated. The source of the animals, health status, age/development stage, and body weights should be reported. The bodyweight ranges should be consistent with the intended product label for the proposed use. If the product is intended to be used at various stages of development, then the study should be conducted in animals

representing the highest development stage (the stage that has a metabolic state that is representative of market size).

2.4.2. Critical Study Design Parameters

Critical residue depletion design parameters to address include water temperature, housing, and salinity. The body temperature and hence absorption, metabolism, and excretion of aquatic species is driven by the surrounding water temperature. Generally, the lower the water temperature the slower the depletion, but higher temperatures may result in higher absorption of drug. Table 1 shows examples of critical design parameters. The sponsor should investigate the effects of the critical design parameters and provide a study design that would result in the worst-case scenario for residues. Selection of the final design parameters should be justified and should be consistent with the proposed use of the product. If more than one option from Table 1 is consistent with the proposed use, then the conditions that result in the worst-case scenario should be chosen.

Table 1. Critical Study Design Parameters

Critical Parameter	Options	Choice
Water Temperature	High or Low within the test animal's recommended water temperature range	Choose the temperature that results in the worst-case scenario for residues
Salinity	Salt or Fresh Water	If applicable choose the one that results in the worst-case scenario for residues
Housing	Recirculation or flow-through or net pens	If applicable choose the one that results in the worst-case scenario for residues

2.4.3. Animal Husbandry

Adequate environmental conditions should be ensured to be consistent with animal welfare, in accordance with applicable national and regional regulations. Additionally, endemic pathogens or parasites should be controlled or eliminated so as to maintain the health of the test animals. Animals should be allowed adequate time to acclimatize to surroundings, procedures, and stocking density. Normal husbandry practices should be applied to the extent possible.

2.4.3.1. Housing

The study should be conducted under commercial growing conditions or the housing should mimic that used in commercial growing conditions.

Examples of possible housing are flow-through cages (free-swimming), racks (attached, e.g., oysters), net pens, recirculating water systems, and ponds. The holding conditions should be suitable so as to prevent escape of test animals or entry of predators. If more than one housing condition is used commercially, then the housing condition that potentially results in maximum tissue residues should be selected.

2.4.3.2. Feeding

Animals should be given feed appropriate, in both quality and quantity, to their development stage and to ensure adequate nutrition and growth, as per commercial conditions. An adequate number of animals (stocking density) should be present in the enclosure to ensure proper feeding behavior. The feed supplied to the animals should be free from other drugs and/or contaminants.

2.4.3.3. Water Temperature

Water temperature is critical to the residue depletion rate in animals whose body temperature is dictated by their environment. However, it is recognized that deviations from the recommended water temperature ranges may occur during study conduct, because studies conducted under commercial conditions or over an extended duration are subject to natural fluctuations in water temperature.

Water temperature should be recorded at least daily until the last animals are euthanized.

2.4.3.4. Water Quality Parameters

Animals should be raised in water that has quality and quantity appropriate for their development stage as per commercial conditions.

Water quality parameters that may be critical to study outcome should be monitored at a frequency appropriate to the study. Contaminants known to be capable of interfering with the study should be monitored. Water should be exchanged at a rate suitable to maintain health and welfare.

2.4.3.5. Animal Anesthesia

Chemical anesthesia or sedation can be used for finfish in order to handle them for group allocation, treatment, and euthanasia. Chemicals used for these processes should cause no interference in the assay for the marker residue.

2.4.4. Single Species Claim

Selection of the final design parameters for the worst-case scenario should be justified (see section 2.4.2 *Critical Study Design Parameters*).

2.4.4.1. In-feed Treatment

A claim for a single species can be supported by conducting a study in that species. In order to be accepted in VICH regions, studies should be conducted within the lowest range of temperatures in which in-feed treatment is administered under commercial settings.

2.4.4.2. Injectable Treatment

A claim for a single species can be supported by conducting a study in that species. In order to be accepted in VICH regions, studies should be conducted within the lowest range of

temperatures in which the injection is administered under commercial settings unless a higher temperature is justified (see section 2.4.2. *Critical Study Design Parameters*).

2.4.4.3. Immersion

A claim for a single species can be supported by conducting a study in that species in consideration of worst-case scenario parameters (see section 2.4.2. *Critical Study Design Parameters*). Immersion treatments may result in differential drug absorption at different water temperatures. Selection of the appropriate water temperature should be investigated and subsequently justified. Water quality parameters should be selected to ensure lack of interference with drug treatment.

2.4.5. Single Order Claim

A claim for an order can be supported by conducting a study in a representative species. The resulting withdrawal period can then be applied to other species of the same order. However, residue data in a second species to confirm the withdrawal period are recommended. The representative species listed in Table 2 are the species that can be reared at recommended temperatures so that the data can be accepted by all regions or countries. However, the second species need not come from Table 2.

Treatment parameters should be the same as described for a single species claim (see section 2.4.4. *Single Species Claim*).

The choice of representative species generally depends on critical residue depletion design parameters. Critical parameters include water temperature, salinity, and housing conditions. Selection of the final design parameters for the worst-case scenario should be justified.

Table 2 shows recommended target water temperature ranges for the residue depletion studies using representative species for different orders of finfish and shrimp. Representative species are chosen based on: 1) the species being either widely cultured in a certain region (or a country) or closely related to such a species, 2) residue depletion studies being able to be carried out at recommended water temperature range at which the species are cultured, and 3) the assumption that the representative species have similar metabolism to other species in the same order. For immersion treatments the effect of temperature on residues should be considered (see section 2.4.4.3. *Immersion*).

Table 2. Representative Species and Recommended Water Temperature Range for Residue Depletion Study

Order	Representative Species	Recommended Water Temperature Range (°C)
Salmoniformes[1]	Atlantic salmon (*Salmo salar*) Coho salmon (*Oncorhynchus kisutch*) Rainbow trout (*Oncorhynchus mykiss*)	5-10
Cypriniformes	Carp (*Cyprinus carpio*) Common bream (*Abramis brama*)	15-20
Perciformes[1]	European seabass (*Dicentrarchus labrax*) Hybrid striped bass (*Morone saxaltilis X Morone chrysops*) Red sea bream (*Pagrus major*) Yellowtail (*Seriola quinqueradiata*) Walleye (*Sander vitreus*)	15-20
Scorpaeniformes	Mebaru (*Sebastes inermis/Sebastes cheni/Sebastes ventricosus*)	10-15
Silurformes	Channel catfish (*Ictalurus punctatus*) Mudfish (*Clarias anguillaris*)	16-21
Osmeriformes	Ayu (*Plecoglossus altivelis*)	13-18
Anguilliformes	Eel (*Anguilla japonica*) European eel (*Anguilla anguilla*)	20-25
Pleuronectiformes	Bastard halibut (*Paralichthus olivarceus*) Summer flounder (*Paralichthys dentatus*)	15-20
Tetraodontiformes	Japanese pufferfish (*Takifugu rubripes*)	13-18
Acipenseriformes	Siberian sturgeon (*Acipenser baerii*)	14-19
Gadiformes	Atlantic cod (*Gadus mohrua*)	5-10
Shrimp or prawns in the order of *Decapoda*	Japanese tiger prawn (*Penaeus japonicus*) Whiteleg shrimp *(Penaeus vannamei)*	18-23

[1] Order contains fresh and salt water representative species that have different salinity requirements based on their life stages.

2.5. Number of Animals for the Study

The number of animals used should be large enough to allow a meaningful assessment of the data. Residue data from a minimum of 10 animals per time point are recommended. For small finfish, shrimp, or mollusks, a composite sample of multiple animals may be used. In cases where a composite is critical, a sufficient number of animals should be collected in order to facilitate assessment of the marker residue. It is recommended that composite residue data from a minimum of 10 pools per time point be assessed. It is recommended that animals should be euthanized at a minimum of four appropriately distributed time intervals. 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 material for related analytical method testing.

2.6. Dosing and Route of Administration

2.6.1. General guidance

Animal treatment should be consistent with the intended product label.

At least the highest intended treatment dose should be administered for the maximum intended duration for the proposed product. 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 CVM GFI #205/VICH GL46.

2.6.2. Immersion Treatment

Animals can be treated with the test article dissolved or suspended in water.

2.6.3. In-feed Treatment

Animals may be treated by incorporation of the test article into the feed to deliver on a group basis standardized mg/kg body weight dose. Generally individual medication of aquatic species is not possible as they will not eat if confined singly, so dosing should be conducted on a group basis. Ideally animals should consume the medicated feed within a short period of feeding so that the test article does not leach into the water. During the acclimation period tests should be conducted to determine the group feeding rate and body weights to ensure the target dose is administered. If feed remains and if it is possible, the uneaten feed should be collected and used to adjust the administered dose calculation.

2.6.4. Injectable Treatment

Animals should be treated with an injectable product, by the intended route (such as intramuscular, intravenous, intraperitoneal, or intracardial) in accordance with the proposed label. The dose injected should be the maximum amount as per the proposed label. Animals may require anesthesia in order to be handled for the treatment. Individual animal weights should be reported.

2.7. Animal Euthanasia

Animals should be euthanized using commercially applicable procedures, observing appropriate exsanguination times. Chemical euthanasia can be used unless it will interfere with the analysis of the marker residue.

2.8. Sampling

2.8.1. General Considerations

Following euthanasia, edible tissue samples in sufficient amounts should be collected, trimmed of extraneous material, weighed, and divided into aliquots (if appropriate). 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 generally bears the responsibility for demonstrating residue stability through to the time of assay.

2.8.2. Tissue Sampling

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

Table 3 indicates the recommended samples for collection for all VICH regions. Table 4 indicates the additional tissues that should be sampled to address specific national/regional consumption habits and/or legal concerns.

In principle, for finfish, muscle including skin in natural proportions should be sampled for a single order claim. For a single species claim for finfish, skin can be eliminated from samples if the skin of the particular species is not consumed in any VICH region.

Table 3. Sample Collection from Animals in the Marker Residue Depletion Study (All VICH Regions)

Aquaculture Species	Edible Tissue Samples
Finfish with edible skin	Muscle including skin in natural proportions, which is the entire fillet with the overlying skin from one or both sides of the fish (scales can be included or excluded based on consumption and practicality of removal).
Finfish with inedible skin (Example: Channel catfish, threadsail filefish)	Muscle, which is the entire fillet from one or both sides of the fish.
Mollusks	Soft tissue excluding shell.
Shrimp or prawns with hard (inedible) shell	Soft tissue including mid-intestinal gland, excluding shell.
Shrimp or prawns (during molting) with soft (edible) shell	The entire animal including the shell is considered as the edible tissue. The edible tissue for shrimp includes the mid-intestinal gland and shell.

The entire sample as defined above should be collected, homogenized, and then subsamples (if appropriate) taken from the homogenate.

Table 4. Additional Tissues that can be Collected to Address Specific National/Regional Consumption and/or Legal Concerns in the Marker Residue Depletion Study

Order	Edible Tissue Type
Any orders of finfish	Either one additional tissue that has been shown to have the highest concentration or slowest depletion of residue among the tissues of visceral organs by previous residue studies, or the offal mixture of available liver, kidney, spleen, stomach, intestine, heart, ovary and testis.

Samples in Table 3 and Table 4 should be collected separately from individual animals, but if the amount of samples collected from one animal is not sufficient for the assay of marker residue, composite samples from multiple animals may be appropriate. For composite samples at least 10 composite samples should be prepared at each sampling period.

2.8.3. Sampling of Eggs for Human Consumption from Treated Aquatic Species

Eggs should be collected from a minimum of 10 sexually mature individuals of the aquatic species. Ten composite samples with an equal amount of eggs from each individual (collect sufficient sample for analysis) should be prepared for residue analysis.

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

For products administered as one treatment or as several treatments (for example daily for 3-5 days), or for continuous use products in which residues have reached steady state, a single time point study may qualify for 0-day withdrawal, provided that the absorption and depletion characteristics of the drug have been described, for example, as indicated in CVM GFI #205/VICH GL46, "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)." If such data are available, then a single time point study conducted with the specified minimum number of animals is recommended to demonstrate 0-day withdrawal.

Number of animals recommended: a minimum of 15 individuals or 15 composites.

The sampling time chosen for this study should be consistent with the peak concentrations.

Higher numbers from those recommended in Section 2.5 Number of Animals for the Study are generally appropriate for single time point determinations.

2.10. 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. The method(s) should be capable of reliably determining concentrations of marker residue which encompass the appropriate reference point (i.e., MRL (Maximum Residue Limit) / Tolerance) for the respective tissues or products.

The parameters to be included in the method validation are fully discussed in CVM GFI #208/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."

3. GLOSSARY

The following definitions are applied for purposes of this document.

Aquatic species include finfish, crustaceans, and mollusks.

Degree days means an expression of the withdrawal period where it is assumed that time multiplied by water temperature is constant.

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.

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

Shrimps and **prawns** belong to the family of *Penaeidae*. This includes most of the shrimps or prawns cultured worldwide but exclude crabs, *machrobrachium*, lobsters, and crayfishes. Some regions use the term shrimp and some use the term prawns and these terms can be used interchangeably.