Contains Non-Binding Recommendations

206

Guidance for Industry

Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Comparative Metabolism Studies in Laboratory Animals

VICH GL47

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-8229.

For further information regarding this document, contact Julia Oriani, Center for Veterinary Medicine, (HFV-151), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 240-276-8204, e-mail:julia.oriani@fda.hhs.gov

Additional copies of this draft guidance document may be requested from the Communications Staff (HFV-12), Center for Veterinary Medicine, Food and Drug Administration, 7519 Standish Place, Rockville, MD 20855, and may be viewed on the Internet at either http://www.fda.gov/AnimalVeterinary/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
September 15, 2011

VICH GL 47 (MRK) – METABOLISM AND RESIDUE KINETICS

February 2011

For Implementation at Step 7 - Final

STUDIES TO EVALUATE THE METABOLISM AND RESIDUE KINETICS OF VETERINARY DRUGS IN FOOD-PRODUCING ANIMALS: LABORATORY ANIMAL COMPARATIVE METABOLISM STUDIES

Adopted at Step 7 of the VICH Process

by the VICH Steering Committee

in February 2011

for implementation in February 2012

Table of Contents

STUDIES TO EVALUATE THE METABOLISM AND RESIDUE KINETICS OF VETERINARY DRUGS IN FOOD-PRODUCING ANIMALS: COMPARATIVE METABOLISM STUDIES IN LABORATORY ANIMALS (VICH GL47)

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

1. INTRODUCTION

1.1. Objective of the guidance

The objective of this guidance is to provide recommendations for internationally harmonized procedures to identify the metabolites of veterinary drugs produced by laboratory animals. The purpose of the comparative metabolism studies is to compare the metabolites of the laboratory animals used for toxicological testing to the residues of the veterinary drugs in edible tissues of food-producing animals, in order to determine if the laboratory animals used for toxicological testing have been exposed to the metabolites that humans can be exposed to as residues in products of food-producing animal origin.

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

The human food safety evaluation of veterinary drug residues helps ensure that food derived from treated food-producing animals is safe for human consumption. As part of the data collection process, studies should be conducted to characterize the metabolites to which laboratory animals are auto-exposed during the toxicological testing of the veterinary drug. The purpose of these studies is to determine whether the metabolites that people will consume from tissues of target food-producing animals are also produced by metabolism in the laboratory animals used for the safety testing. It is understood that, if the laboratory animals produce substantially similar metabolites as those produced by the food-producing animal, the laboratory animals will have been auto-exposed to the metabolites that humans will consume from tissues of treated food-producing animals. Auto-exposure of metabolites will ordinarily be taken as evidence that the safety of metabolites has been adequately assessed in the toxicology studies.

2.2. Scope

Demonstration of metabolites from the laboratory animal can be generally accomplished in one or more *in vitro* studies or in an *in vivo* study.

Use of one or more *in vitro* laboratory animal metabolism studies (e.g., laboratory animal liver slice metabolism) for comparison to the metabolism in the food-producing animal can be conducted to demonstrate that the relevant laboratory animal produces the metabolites that are found as residues in the edible tissues of the target food-producing animal. Conducting i*n vitro* studies can avoid the use of *in vivo* laboratory animal studies, can reduce the number of animals that are euthanized, and can reduce the cost of comparative metabolism studies. If the *in vitro* or *in vivo* studies do not demonstrate the metabolites produced by the target food-producing animal, the sponsor should address by other means the relevance to consumer safety of the food-producing animal metabolites.

Laboratory animal *in vitro* and *in vivo* metabolism studies are most often accomplished using radiolabeled drugs. These studies are capable of monitoring all of the drug-derived residues resulting from the administration of test material (note: generally only the major metabolites should be identified). This guidance, therefore, recommends procedures for metabolism studies conducted with radiolabeled drugs. However, alternative approaches (i.e., not using radiolabeled drugs) to characterize the metabolites in laboratory animals can be suitable when the metabolites produced by the target food-producing animal as residues in edible tissues are readily identified in urine or tissues of the laboratory animals by chemical means.

Generally auto-exposure has been adequately demonstrated if laboratory animals produce each of the major metabolites of the residue that people will consume from edible tissues of treated food-producing animals. Qualitative information on the metabolites in laboratory animals should be reported. Quantification of the metabolites found in urine, fluids or tissues of laboratory animals is not generally an objective of the comparative metabolism studies. Generally only the major metabolites found as residues in the food-producing animal should be identified in the laboratory animals. Metabolites observed in laboratory animals that are

not observed in the food-producing animal are not relevant to the objective of assuring that the laboratory animals are auto-exposed to the residue metabolites that humans will consume.

Comparative metabolism studies should be conducted in compliance with applicable Good Laboratory Practice (GLP).

2.3. Comparative Metabolism Studies in Laboratory Animals

2.3.1. Test Materials

2.3.1.1. Drug

The chemical identity (including, for example, the common name, chemical name, CAS-number, structure, stereochemistry and molecular weight) and purity of the drug substance should be described. The test drug should be representative of the active ingredient to be used in the commercial formulation.

2.3.1.2. Radiolabeled Drug

The position(s) of the radiolabel should be indicated. The characteristics of the radiolabeled drug used in comparative metabolism studies should meet the specifications identified in the guidance 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" in the target food-producing animal regarding: a) the nature of the radiolabel, b) the site of the label in the test molecule, and c) the purity and specific activity of the radiolabeled drug.

2.3.1.3. Analytical Standards

Analytical standards should be available for the parent drug and, if possible, for metabolites known or expected to exist, for use in the chromatographic comparison of drug metabolites. The metabolites can be isolated from tissues generated in the target food-producing animal metabolism study (VICH GL 46).

2.3.2. *In vitro* Test Systems

Single or multiple in vitro metabolism test studies can be used as an alternative for the *in vivo* comparative metabolism studies.

The laboratory animal species used in the comparative metabolism study should preferably be the same species (and for rodents the same strain) as was used in the pivotal study for determining the toxicological acceptable daily intake (ADI) of the veterinary drug. In case another species is used, the choice of species should be justified in terms of relevance. The source of the animals, their weights, health status, ages and gender should be reported.

Various test systems have been published and are widely used. *In vitro* systems for comparative metabolism studies include primary hepatocytes, liver microsomes, the S9 sub-cellular fraction, cytosol, liver slices and whole cell lines. Protocols for these *in vitro* studies

have not yet been standardized (e.g., OECD), therefore some strengths and weaknesses of each of these systems are discussed below:

- Primary (fresh or cryopreserved) hepatocytes: Primary hepatocytes are liver cells that are useful in evaluating phase I and phase II metabolism and moreover have the added advantage of taking membrane transport effects into account. These hepatocytes can be prepared in suspension, monolayer culture or sandwich cultures. The sandwich cultures have the advantage of maintenance of enzyme activities for a longer duration of time. If the food-producing animal residue metabolites are demonstrated in a primary hepatocytes system, then comparative metabolism has generally been demonstrated. Use of a primary hepatocytes-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

- Liver microsomes: Liver microsomes include most of activities of cytochrome P450 (CYP) and flavin-containing monooxygenase (FMO) systems for evaluating phase I metabolism, along with uridine diphosphate-glucuronosyl-transferase (UDPGT) for phase II glucuronidation. If the food-producing animal residue metabolites are demonstrated in a liver microsome system, then comparative metabolism has generally been demonstrated. Use of a liver microsome-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

- S9 sub-cellular fraction: The S9 sub-cellular fraction contains the same phase I and phase II enzymes present in liver microsomes as well as additional systems such as sulfotranferases and N-acetyltransferases. The S9 sub-cellular fraction is suitable for evaluating phase I and II metabolism or phase I metabolism followed by phase II conjugation. If the food-producing animal residue metabolites are demonstrated in a S9 sub-cellular fraction system, then comparative metabolism has generally been demonstrated. Use of a S9 sub-cellular fraction-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

- Cytosol: This represents the supernatant fraction remaining following microsomal centrifugation. It contains some of the phase II conjugation systems but otherwise represents a relatively incomplete matrix for metabolic work. In general, the use of cytosolic systems alone is unlikely to provide a complete comparative metabolism profile, but if the food-producing animal residue metabolites are demonstrated in a cytosol system, then comparative metabolism has generally been demonstrated. Use of a cytosol-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

- Liver slices: the use of whole liver slices for metabolism research is possible, however, the liver cell viability and corresponding enzyme activities decrease rather rapidly compared with the other alternatives. The conduct of comparative metabolism studies using liver slice methodology should not be used unless cell viability and enzyme activity can be demonstrated. However, if the food-producing animal residue metabolites are demonstrated in a liver-slice system, then comparative metabolism has generally been demonstrated. Use of a liver-slice-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

- Whole cell lines: Use of whole cell lines is not currently recommended because the enzymatic activity is generally low. However, if the food-producing animal residue metabolites are demonstrated in a whole cell line system, then comparative metabolism has generally been demonstrated. Use of a whole cell line-based system can be complimentary to one or more of the other *in vitro* systems to demonstrate the metabolism for a laboratory animal species.

It is generally possible that only one of these specific *in vitro* options could be used for demonstration of comparative metabolism. However, if the target-species metabolic profile includes evidence of both phase I and phase II biotransformation, the Sponsor should consider investigating multiple options (*e.g.* microsomes and S9) to reproduce the complete metabolic profile.

Although many variations in test conditions have been reported in the literature, the following represents some general guidance for conduct of *in vitro* comparative metabolism studies:

- Test molecules are usually incubated in the *in vitro* system at 37 ^{o}C.
- The concentrations of target molecules are typically lower than 100µM.
- The incubation time is dependent upon the rate of metabolism of the target molecules and should be adjusted accordingly.
- Cofactors of phase I and II metabolism are scientifically necessary for incubation of liver microsomes and S9, such as NADPH (NADPH regeneration system) for phase I metabolism, UDPGA for glucuronidation, PAPS for sulfation.

When more standardized *in vitro* system metabolism study protocols become available, the general guidance above can be replaced according to the standardized protocols.

2.3.3. *In Vivo* Test Systems

2.3.3.1. Animals

The laboratory animal species used in the comparative metabolism study should preferably be the same species (and for rodents the same strain) as was used in the pivotal study for determining the toxicological acceptable daily intake (ADI) of the veterinary drug. In case another species is used, the choice of species should be justified in terms of relevance. The source of the animals, their weights, health status, ages and gender should be provided.

2.3.3.2. Animal Handling

Animals should be allowed adequate time to acclimatize. Normal laboratory animal caretaking practices should be applied. (Note: metabolism cage housing can be used).

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. An appropriate wash-out time should be observed for the animals prior to enrollment in the actual trial. Animals should have a known history of medication.

Animal caretaking practices and disposal of animals and tissues from animals should be in compliance with all applicable national and regional laws and regulations.

2.3.3.3. Number of Animals

Enough animals should be treated with the drug in the comparative metabolism study to provide enough composited tissue or excreta for analysis. The samples of like material from different animals can be composited for a single analysis. There is no minimum number of animals for a comparative metabolism study; however, four animals of each gender are often used (but less can be used) to assure there is enough sample material. Demonstration of comparative metabolism is not generally conducted in each gender; therefore, the samples of like material can be pooled (without regard to gender) to increase the likelihood of demonstrating the metabolites of interest when gender differences in metabolic ratios might exist.

2.3.3.4. Drug Formulation

The drug formulation, method of dose preparation, and stability of the drug in the formulation during the treatment period should be described. It is not critical that the formulation used in the comparative metabolism studies is the same as the commercial product.

2.3.3.5. Route of Administration

The drug should be administered orally. Gavage or bolus dosing can be used to ensure that animals receive the complete dose and to minimize environmental concerns.

2.3.3.6. Dosing

The dose should be high enough to result in concentrations of metabolites in excreta or tissues for comparison. The dose should be administered daily for enough time that the drug undergoes all relevant metabolic events, including those associated with enzyme induction. Normally, administration for five days is used unless there are data to show a longer time of administration can better demonstrate the formation of the metabolites of interest. Doses near the minimum toxic dose can be used to generate high concentrations of the metabolites of interest in tissues and urine but lower doses can be used.

2.3.3.7. Animal Euthanasia

Animals should be humanely euthanatized. Chemical euthanasia can be used unless it will interfere with analysis of the metabolites of interest.

Animals should be euthanized for metabolite analysis at a single time point, usually 2-4 hours after the last dose of the test substance. Multiple days of dosing provides the presence of metabolites resulting from sequential metabolism of the parent drug over time, and therefore additional euthanasia time points are not called for.

2.3.3.8. Sample Collection

Before euthanasia, urine, feces, and blood can be collected for analysis. The samples should be analyzed immediately or stored frozen (unless freezing causes a stability problem for the metabolites of interest) until analysis. Freezing of the samples is to reduce microbial metabolism from altering the metabolic profile. If the samples are stored after collection, the sponsor should ensure that the radiolabeled compound remains intact throughout the storage period.

Following euthanasia, samples of tissues can be collected. The tissue samples should be analyzed immediately or stored frozen (unless freezing causes a stability problem for the metabolites of interest) until analysis. Freezing of the samples is to reduce microbial metabolism from altering the metabolic profile. If the samples are stored after collection, the sponsor should ensure that the radiolabeled compound remains intact throughout the storage period.

Comparative metabolism can be demonstrated with one or more excreta or tissues. Samples that are typically taken for qualitative metabolite analysis can include blood/blood fractions, excreta, liver, bile, kidney, fat or other tissues. Enough tissue of each type should be taken from each animal for analysis or for pooling from more than one animal for analysis.

2.3.3.9. Determination of Total Radioactivity

Determination of total radioactivity in samples and accounting for the mass balance of the radioactivity are not normally conducted for the *in vivo* comparative metabolism studies. When total radioactivity is to be determined, the procedures presented in VICH GL 46 should be followed.

2.3.4. Separation and Comparison of Metabolites

Commonly available analytical technology, including, for example, high performance liquid chromatography, thin layer chromatography, gas chromatography, and mass spectrometry, are typically used for the separation of the total residue into its components and comparison of the drug-derived residues.

2.3.4.1. Analytical Methods

Similar procedures for chromatography and chemical characterization as those employed in VICH GL 46 should be used in the *in vivo* comparative metabolism studies in laboratory animals. Those methods can also be useful for *in vitro* investigations, although the sample preparation would be different. A description of the analytical methods should be provided as described in VICH GL 46. The repeatability of retention times for the analytical method should be demonstrated.

2.3.4.2. Extent of Characterization/Major Metabolites

Characterization and structural identification of the metabolites and demonstration of the tissue extraction efficiency during the comparative metabolism study are not normally conducted when the

comparison of the chromatographic retention time(s) demonstrate the presence of the metabolites of interest in the laboratory animal.

2.3.4.3. Nonextractable Metabolites

Characterization of nonextractable metabolites in comparative metabolism studies in laboratory animals is normally not performed. Characterization of the covalently bound metabolites of a veterinary drug in laboratory animals should be performed only when the nonextractable residue contains a metabolite of interest that is not present in enough quantity for characterization in the easily extractable portion. In this case, the procedures identified in VICH GL 46 should be followed.

3. GLOSSARY

The following definitions apply for the 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.

Major Metabolites are those comprising ≥ 100 µg/kg or ≥ 10% of the total residue in a sample collected from the target animal species in the metabolism study (VICH GL 46).

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

Metabolites of interest refers to the veterinary drug (parent) and its metabolites that were demonstrated in the edible tissues of the food-producing animal and have relevance to the toxicological ADI established for the veterinary drug.

Nonextractable residues are residues that are not readily extractable from tissues using mild aqueous or organic extraction conditions. 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 or (c) physical encapsulation or integration of radioactive residues into tissue matrices.

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