European Medicines Agency
Veterinary Medicines and Inspections

London, 14 December 2009
Doc. Ref. EMEA/CVMP/VICH/463104/2009-CONSULTATION

VICH Topic GL47

at step 4

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

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

VICH GL 47 (MRK) – COMPARATIVE METABOLISM 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: COMPARATIVE METABOLISM STUDIES IN LABORATORY ANIMAS

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

The objective of this guidance is to provide recommendations for internationally harmonized procedures to identify the metabolites of veterinary food animal drugs in laboratory animals used for toxicological testing for the purpose of comparison to the residues of the drugs in food animals. The purpose of comparative metabolism studies is to determine if laboratory animals used for toxicological testing have been exposed to the metabolites that humans will be exposed to as residues in products of food animal origin.

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

The human food safety evaluation of veterinary drug residues assures that food derived from treated animals is safe for human consumption. As part of the data collection process, studies are 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 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 animal, the laboratory animals will have been auto-exposed to the metabolites that humans will consume from tissues of treated food animals. Autoexposure of metabolites ordinarily will be taken as evidence that the safety of metabolites has been adequately assessed.

2.2. Scope

Metabolism studies in laboratory animals 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 (see previous paragraph regarding the need to identify only the metabolites observed as food animal residues). This guidance, therefore, recommends procedures for metabolism studies conducted with radiolabeled drugs. However, alternative approaches (i.e., not using radiolabeled drug) to characterize the metabolites in laboratory animals may be suitable when the metabolites of toxicological interest are readily identified in urine or tissues of the laboratory animals by chemical means.

Use of an *in vitro* laboratory animal metabolism study for comparison to the metabolism in the food animal (e.g., laboratory animal liver slice metabolism) may be used to replace the *in vivo* laboratory animal study to demonstrate that the relevant laboratory animal produces the metabolites of interest that are produced by the target food animal. *In vitro* studies may reduce the number of animals that must be sacrificed and may reduce the cost of comparative metabolism.

If the *in vitro* studies do not demonstrate the metabolites of interest, the sponsor needs to address the relevance of these metabolites to consumer safety by other means

Auto-exposure provides an adequate test of the safety of the veterinary drug if laboratory animals produce each of the major metabolites (those comprising ≥100 µg/kg or ≥10% of the total residue) of the residue that people will consume from tissues of treated food animals. Qualitative information on the metabolites in laboratory animals is sufficient. Quantification of the metabolites found in urine, fluids or tissues of laboratory animals is not required. Only those metabolites found as residues in the food animal need to be identified as metabolites in the laboratory animals. Metabolites observed in laboratory animals that are not observed in the food 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 according to international standards in compliance with, or equivalent to, applicable Good Laboratory Practice (GLP) regulations.

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, including all of the factors for active ingredients composed of more than one related chemical structure.

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 in the guidance "Metabolism Study to Determine the Quantity and Identify the Nature of Residues" in the target food animal regarding: a) the nature of the radiolabel, b) 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 parent drug and, if possible, for metabolites known or expected to exist, for use in the chromatographic comparison of drug metabolites. The metabolites may be isolated from the target food animal metabolism study or may be an extract of the target food animal tissue.

2.3.2. In vivo Test Systems

2.3.2.1. 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, it is required to justify the choice of species in terms of relevance. The source of the animals, their weights, health status, ages and gender should be provided.

2.3.2.2. Animal Handling

Animals should be allowed adequate time to acclimatize. Normal husbandry practices (including vaccinations, standard treatment for parasites, antibiotic therapy, etc.) should be applied as needed (with note that metabolism cage housing may be used as needed). Animals should be healthy and an appropriate washout period should be observed following normal husbandry medications or following treatment with compounds related to the test substance.

2.3.2.3. Number of Animals

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

2.3.2.4. Drug Formulation

The drug formulation, method of dose preparation, and stability of the drug in the formulation during the treatment period should be described. The formulation used in the comparative metabolism studies does not need to be the same as the commercial product. Using the formulation that is used in the toxicology testing and that resulted in toxic effects, may increase the likelihood that the metabolites of interest are generated in the laboratory animal.

2.3.2.5. Route of Administration

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

2.3.2.6. Dosing

The dose should be high enough to result in concentrations of metabolites in urine or tissues sufficient for comparison. The dose should be administered daily for a sufficient period of time with the intent that the drug undergoes all relevant metabolic events, including those associated with enzyme induction. Normally, administration for five days is sufficient unless there are data to show a longer time of administration is needed in order to demonstrate the formation of the metabolites of interest. Doses near the minimum toxic dose are not required but are often used to generate high concentrations of the metabolites of interest in tissues and urine.

2.3.2.7. Animal Euthanasia

Animals should be humanely euthanatized. Chemical euthanasia should be avoided if it will interfere with analysis for the metabolites of interest.

Animals are euthanized for metabolite analysis at a single time point, usually 2-4 hours after the last dose of the test substance. Multiple daily dosing provides the presence of metabolites at various metabolic time points and therefore more euthanasia time points are not required.

2.3.2.8. Sample Collection

Before euthanasia, urine, feces, and blood may 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 bears the responsibility for demonstrating that the radiolabeled compound remains intact throughout the storage period.

Following euthanasia, samples of tissues may 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 bears the responsibility for demonstrating that the radiolabeled compound remains intact throughout the storage period.

Samples that are typically taken for qualitative metabolite analysis may include blood/blood fractions, excreta, liver, bile, kidney, and fat. Other tissues may also be used to demonstrate metabolism. Sufficient tissue of each type is taken from each animal for analysis or for pooling from more than one animal for analysis. Only excreta, blood or tissues needed to demonstrate the metabolites of interest need to be harvested or analyzed for comparison.

2.3.2.9. Determination of Total Radioactivity

Determination of total radioactivity in samples and accounting for the mass balance of the radioactivity are not required for the in vivo comparative metabolism studies. When total radioactivity is to be determined, the procedures presented in the gduidance "Metabolism Study to Determine the Quantity and Identify the Nature of Residues" should be followed.

2.3.3. In vitro test methods

In vitro metabolism studies may be used as an alternative for the *in vivo* comparative metabolism studies. Various test systems have been published and are widely used. *In vitro* systems for comparative metabolism studies include liver microsomes, the S9 sub-cellular fraction, cytosol, primary hepatocytes, liver slices and whole cell lines. Because there are no standardized (e.g. OECD) protocols for these *in vitro* studies, some strengths and weaknesses of each of these systems are discussed below:

- 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.

- 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.

- 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. Although potential metabolism is clearly compound specific, in general, the use of cytosolic systems alone are unlikely to provide a complete comparative metabolism profile.

- Primary (fresh or cryopreserved) hepatocytes: Primary hepatocytes are liver cells that are useful in evaluating Phase I and Phase II metabolism but 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.

- 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 is usually not recommended.

- Whole cell lines: Use of whole cell lines is not recommended because the enzymatic activity is generally low.

Use of only one of these specific in vitro options may be sufficient to satisfy the requirement for demonstration of comparative metabolism. However, if the target-species metabolic profile includes evidence of both phase I and phase II biotransformation, it may be necessary to investigate 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 will need to be adjusted accordingly.
- Cofactors of phase I and II metabolism are needed for incubation of liver microsomes and S9, such as NADPH (NADPH regeneration system) for phase I metabolism, UDPGA for glucuronidation, PAPS for sulfation.

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 the guidance “Metabolism Study to Determine the Quantity and Identify the Nature of Residues” should be used in the in vivo comparative metabolism studies in laboratory animals. Those methods may 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 the guidance “Metabolism Study to Determine the Quantity and Identify the Nature of Residues”. 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 required 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

Normally there is no need to characterize nonextractable metabolites in comparative metabolism studies in laboratory animals. Characterization of the covalently bound metabolites of a veterinary drug in laboratory animals may be appropriate only when the nonextractable residue contains a metabolite of interest that is not present in sufficient quantity for characterization in the easily extractable portion. In this case, the same guidelines identified in the guidance for identifying the nature and quantity of residues in the target food animal should be followed.

3. 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 of the chemical per kilogram (μg/kg) of body weight.

Major Metabolites are those comprising ≥100 μg/kg or ≥10% of the total residue.

Minor Metabolites are those comprising <100 μg/kg or <10% of the total residue.

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.

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.

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.

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

Residue of toxicological concern refers to the residues that have relevance to the toxicological ADI established for the veterinary drug.