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

STUDIES TO EVALUATE THE METABOLISM AND RESIDUE KINETICS OF VETERINARY DRUGS IN FOOD-PRODUCING SPECIES: STUDY DESIGN RECOMMENDATIONS FOR RESIDUE STUDIES IN HONEY FOR ESTABLISHING MRLS AND WITHDRAWAL PERIODS

Recommended for Consultation at Step 4 of the VICH Process
in January 2017
by the VICH Steering Committee

This Guideline has been developed by the appropriate VICH Expert Working Group and will be 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

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 the national/regional requirements in order to establish appropriate Maximum Residue Limits (MRLs) or other safe limits in honey following the treatment of honeybees with veterinary drug products, or to justify withdrawal periods in honey for registration purposes when an MRL already exists.

Use of veterinary drug products in honeybee production is considered as a minor use in minor species in most jurisdictions.

1.2. Background

This guidance is one of a series developed to facilitate the mutual acceptance of residue chemistry data for veterinary drug products 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 VICH regions.

2. GUIDANCE

2.1. Purpose

Residue studies in honey are recommended for registration or approval, as applicable, of a veterinary drug product for use in honeybees.

These studies may be used to:

- measure the residues in honey
- generate data suitable for establishment of appropriate maximum residue limits.
- justify the withdrawal period for a veterinary drug product in accordance with an existing MRL and/or generate data suitable for the establishment of risk-management measures (*e.g.* use restrictions) in order to address consumer safety concerns. It is generally accepted that the most practical withdrawal period in honey is a zero-day withdrawal, meaning that all harvested honey should be safe for human consumption. (Honey is harvested when at least 75% of the honeycells within a frame are filled and capped.) However, additional measures (*e.g.* the time interval between end of treatment and start of honey flow) may need to be followed.

Design elements for residue studies in honey differ in many respects from those in commodities from other food producing species because honey is a unique food of animal origin. There is minimal pharmacokinetic depletion of residues in honeybees following treatment. When present in honey, residue concentrations are reduced mainly by dilution as more honey is produced during the honey flow. Residue concentrations might also be influenced by thermal degradation (as temperature inside the hive reaches 32-36 ºC), acidic hydrolysis (honey pH ranges 3.4 – 6.1) or other chemical reactions with honey matrix

components. Honey production rate depends on factors such as temperature, rain, season of the year, climatic zone, food source/type and honeybee species/subspecies.

2.2. Scope

The intention is that one set of residue studies, conducted in multiple locations within one or more regions, would satisfy the data recommendations for establishment of appropriate safe limits for a veterinary drug (*i.e.*, the specific active substance)/veterinary drug product in honey.

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

2.3. Residue Studies

2.3.1. General considerations

When conducting residue studies for honey, treatments are typically applied to honeybee colonies in accordance with Good Beekeeping Practice. Treatments are generally applied once per year after honey harvesting and should be completed before honey flow commences.

2.3.2. Test Article

The test article (the veterinary drug product) used for the study should be representative of the commercial formulation. Final Good Manufacturing Practices (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.

2.3.3. Residues to monitor

Metabolic or total residue studies using radiolabelled drugs are not requested for MRL assessment/approval of veterinary drug products used in honeybees. It is anticipated that in most cases the residue to monitor would be the parent drug. However, data on physicochemical properties of the active drug substance and other scientific information might be useful to reveal the identity of putative transformation and/or degradation products. If data indicate transformation or degradation of parent drug, an alternative residue or combination of residues may need to be monitored. For substances that are prone to transformation/degradation, prior to conducting residue studies, additional (*in-vitro*) studies may be used to determine their stability in honey (during its production and up to its harvest). Variables to be tested include pH, temperature, time and exposure to (UV)-light. The selected conditions should be sufficiently justified.

2.3.4. Bees and beekeeping conditions

Healthy and strong colonies should be used (See Glossary for ‘colony strength’). The honeybee species/subspecies should be recorded. The colonies per site (See 2.3.6.) should be uniform in adult honeybee population. The hives per site should be uniform in the number of frames and box size and should be uniquely identified. Hive construction should be adequately described. The hives should consist of one brood box only. A super box with frames should be added at the start of honey flow. The number of frames in each box should be recorded.

Neither the colonies, boxes, nor frames should have a history of exposure to the veterinary drug.

The study should be conducted at locations that mimic the conditions found at the time of the year when apiarists would normally treat colonies with the particular veterinary drug product.

2.3.5. Dosing and method of Administration

The design should cover the maximum treatment regimen. The method of applying the veterinary drug product in the study should be representative of the intended commercial use. The route of administration should be described in detail.

If the veterinary drug product is intended to be applied in more than one method, a separate residue study for each method of administration is recommended. Alternatively, a single study representing the worst-case scenario can be conducted with the resulting safety parameters (*i.e.*, proposed MRLs, use restrictions) being applicable to all methods. Full justification of the worst-case scenario should be provided.

2.3.6. Study design

Residue studies should be conducted in four sites of differing agro-ecological areas within one or more regions. If the residue studies are intended to support an application for a national license, then depending on the country of application (size, variety of landforms and climatic conditions), two to three sites (of differing agro-ecological areas) may be considered sufficient. In such cases the national authorities could be consulted. For the duration of the studies, information on climate (temperature, rainfall and any other parameter considered relevant for the performance of the veterinary drug product) and beekeeping management practices should be provided. In addition, data on the plants in the area in which honeybees forage during the study as well as data on any supplemental feed given to the honeybees should be reported.

The time of treatment, the approximate time of the start of honey flow and the time of sample collection/colony should be provided.

2.3.6.1. Colonies

Six colonies per site should be treated, resulting in 24 colonies per residue study. Depending on the type of application fewer treatment sites may be sufficient (See 2.3.6 referring to application for a national license). A single sampling timepoint per colony is considered appropriate. This is the first timepoint when honey from each colony is ready to be harvested for human consumption (super honey from one or more frames). Honey harvest refers to the collection of honey from the honeycombs once they are filled with capped honey; at least 75% of the honeycells of the selected honeycombs should be filled and capped. Figure 1 outlines a theoretical scheme (example) of sample collection per site.

2.3.6.2. Residues in Comb Honey for Lipophilic Drug Substances Only

Residue studies should be conducted as described in 2.3.6.1. In addition, for the last colony harvested per site, a pooled wax sample (all available from the single colony) should be collected and analysed following honey extraction (Figure 1). To determine residues in comb

honey from the separate concentrations in honey and wax, consider that 1 kg of comb honey consists of 22/23 kg honey and 1/23 kg wax[1].

2.3.6.3. Treatment during Honey Flow

In cases of veterinary drug products that could be used during the honey flow, the basic study design should be followed. The sponsor should provide justification for modifications to the basic study design. Points to consider are transfer of the veterinary drug to existing and newly produced honey.

2.3.7. Sampling

2.3.7.1. Sample Preparation

Super honey

The honey from all honeycombs in the collected frames from each colony should be harvested. The honey should be extracted, filtered and thoroughly mixed to produce a pooled sample to represent that particular colony. Extraction of honey from a honeycomb may be facilitated by centrifugation. Sample processing (all activities after sampling and up to analysis) should take into account the stability properties of the residues. The amount of the pooled honey produced should be provided. The pH and moisture content of all pooled honey samples should be measured and reported.

Wax

For wax samples, the combs should be homogenized after honey extraction. The amount of the bulk wax sampled should be provided.

2.3.7.2. Sample storage

If the chemical analysis cannot be completed immediately following sample collection, the samples should be stored appropriately. If samples are stored after collection, the Sponsor bears the responsibility for demonstrating residue stability through to the time of assay. The parameters and recommendations to assess sample storage are discussed in the VICH GL 49, "Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies"[2].

2.4. Analytical Method for Assay of Residues

The Sponsor should submit a validated analytical method for the determination of the residues in the samples generated. The method(s) should be capable of reliably determining concentrations of the residues that encompass the appropriate proposed reference point for honey (*i.e.,* MRL).

The parameters to be included in the method validation are fully discussed in the VICH GL 49, "Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies" (See Footnote 2).

[1]Beeswax Production, Harvesting, Processing and Products, Coggshall and Morse.Wicwas Press. 1984-06-01. p. 41. ISBN 1878075063.
[2] VICH GL 49: Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food Producing Animals: Validation of Analytical Methods Used in Residue Depletion Studies, EMA/CVMP/VICH/463202/2009.

3. GLOSSARY

The following definitions are applied for purposes of this document.

Beehive is a place used for housing a colony of bees, commonly stackable wooden boxes consisting of a bottom board, brood box/es and super box/es containing movable frames.

Bee colony is the aggregate of worker bees, drones, queen, and developing brood living together as a family unit in a hive or other dwelling.

Brood box (brood chamber) is a box in which the queen is confined and brood is reared. Brood refers to eggs, embryo's larval and pupal stages of bees.

Colony strength is evaluated by estimating the adult honey bee population in the hive and depends on the time of the year and colony management practices.

Comb honey is honey stored by bees in the cells of freshly built broodless combs or thin comb foundation sheets made solely of beeswax and sold in sealed whole combs or sections of such combs (Revised Codex Standard for honey, 2001).

Frame is a rectangular wooden support designed to hold combs, usually 10 to each (brood or super) box.In frames, a wax foundation is usually installed.

Good Beekeeping Practice refers to best practice recommendations found in numerous references, for example: Hygiene in the apiary (A manual for hygienic beekeeping) Ed. Dalibor Titera, BRI Dol 2009; National best management practice for beekeeping in the Australian environment, The Australian honey bee industry council, 2007.

Honey is 'the natural sweet substance produced by honeybees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature' according to the Codex definition (Revised Codex Standard for honey, 2001)[3].

Honey flow is a period of time when one or more nectar or honeydew sources are in abundance such that honeybees can store a surplus of honey.

Honey harvest refers to the collection of honey from the honeycombs once they are filled with capped honey; at least 75% of the honeycells in a frame should be filled and capped.

Honeybees are a subset of bees in the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax. Although there are 7 species of honeybees (*A. andreniformis, A. cerana,A. dorsata, A. florea, A. koschevnikovi, A. mellifera, A. nigrocincta*) only two of them *A. mellifera* (Western or European honeybee) and *A. cerana* are maintained by beekeepers, with the former being the most commonly domesticated species. *A. mellifera* is native to Europe, Asia and Africa and was introduced to North America in the early 1600s. Since then it has spread worldwide. There are many subspecies that have adapted to the local geographic and climatic environments, and in addition hybrid strains have been bred [*e.g.* the Africanized bee (*A. mellifera lingustica* X *A. m. scutellata*)].

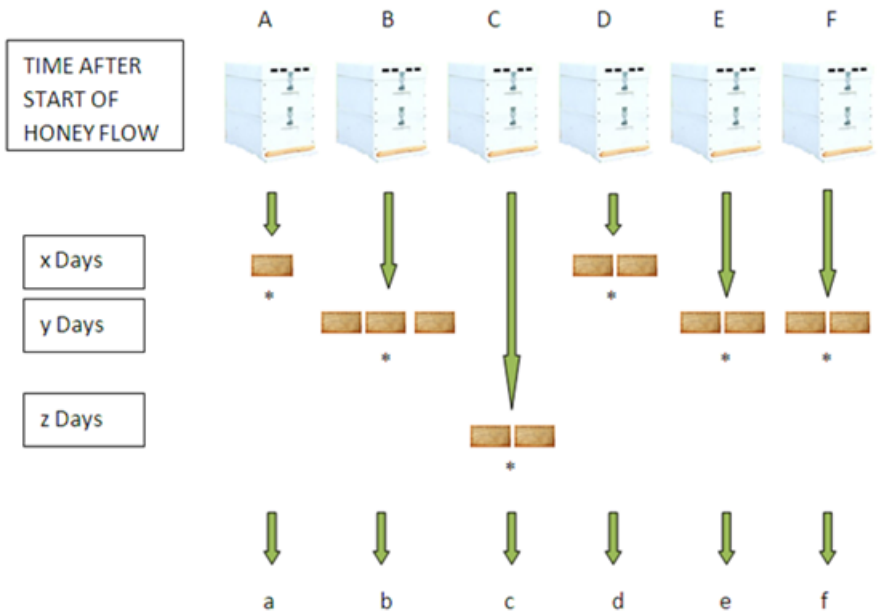
[3] Revised Codex Standard for honey (2001). CODEX STAN 12-1981, Rev.1 (1987), Rev.2 (2001).

Honeycomb is a mass of hexagonal wax cells built by honeybees to contain their stores of honey and pollen (honeycomb in the super box) or for raising brood (broodcomb in the brood box).

Lipophilic substance refers to a chemical substance having high (log $K_{ow} \geq 3$) octanol/water partition coefficient.

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. In the case of honey this may include transformation and degradation products.
Super box is a box in which the honeybees store honey (super honey) and that is placed above a queen excluder and the brood chamber.
Wax (or comb) foundation is a plate made of wax with the base of the honeycomb on which honeybees will construct a complete comb.
Withdrawal period is the period necessary between the last administration of a veterinary drug product to animals and the production of foodstuffs from such animals, in order to protect public health by ensuring that such foodstuffs do not contain residues in quantities in excess of the maximum residue limits established.

Zero-day withdrawal refers to a label indication that allows entry of edible tissues/animal products into the food chain without regard to the time of last drug administration.



x days < y days < z days

*: Number of frames collected per hive

a-f: Pooled honey samples from the collected frames of each hive

Under 2.3.6.2. (lipophilic compounds) a pooled wax sample should be collected following honey extraction of c honey sample, as in this figure C colony is the last colony harvested

Figure 1. A theoretical scheme of sample collection per site.

The figure outlines a theoretical example of sample collection per site. A single sampling timepoint per colony is considered to be appropriate. This is the first timepoint when honey from each colony can be harvested for human consumption (only super honey from one or more frames). Honey harvest refers to the collection of honey from the honeycombs which are filled with capped honey. The figure illustrates that the time points when honey is mature (at least 75% of the honeycells in one or more frames are filled and capped) and the number of mature honeycombs per colony usually varies from hive to hive. The first honey harvest is considered the worst case in terms of residues.