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Guidance for Industry

Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims

FINAL GUIDANCE

Comments and suggestions regarding this guidance should be sent to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. Comments may be submitted electronically on the Internet at http://www.regulations.gov. All comments should be identified with Docket Number 2006D-0138.

For questions regarding this document, contact Michelle L. Stull, Center for Veterinary Medicine (HFV-133), Food & Drug Administration, 7500 Standish Place, Rockville, MD 20855, 240-276-8351, E-mail: michelle.stull@fda.hhs.gov.

Additional copies of this final 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 http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
October 1, 2007

TABLE OF CONTENTS

Guidance for Industry

Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Claims[1]

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INTRODUCTION

This guidance provides recommendations to industry relating to study design and describes the criteria that the Center for Veterinary Medicine (CVM) intends to use to evaluate effectiveness studies for swine respiratory disease (SRD) claims.

Section 1 of the guidance suggests designs for studies intended to demonstrate the effectiveness of a new animal drug intended for the treatment or control of SRD. CVM uses the term SRD to refer to the component of acute respiratory disease in swine associated with bacterial pathogens such as *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pasteurella multocida,* or *Streptococcus suis*.

For the purposes of this guidance, SRD is not considered synonymous with "Enzootic Pneumonia," "Porcine Respiratory Disease Complex" (PRDC), or any other complex or syndrome. Enzootic Pneumonia is a common term used to describe *Mycoplasma hyopneumoniae* co-infection with other bacterial respiratory pathogens (such as *P. multocida*). PRDC is mixed viral and bacterial infection of two or more respiratory pathogens, such as *M. hyopneumoniae, A. pleuropneumoniae*, *S. suis, P. multocida*, Pseudorabies virus, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and/or Swine Influenza Virus (SIV).

Section 2 of the guidance describes study design recommendations and criteria CVM intends to use to determine the effectiveness of a new animal drug intended for 1) treatment or

[1] This final guidance has been prepared by the Office of New Animal Drug Evaluation in the Center for Veterinary Medicine.

control of SRD associated with *Mycoplasma hyopneumoniae* and other bacterial pathogens; or 2) reduction in severity of effects of respiratory disease associated with *Mycoplasma hyopneumoniae*.

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 guidances means that something is suggested or recommended, but not required.

SECTION 1: Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease

Effectiveness must be demonstrated by substantial evidence consisting of one or more adequate and well-controlled studies [21 USC 360b(d)(1)(E) and (d)(3); 21 CFR 514.1(b)(8)(ii); 21 CFR 514.4(a); and 21 CFR 514.117]. CVM recommends use of an adequate and well-controlled field study to demonstrate effectiveness of a new animal drug intended for use in the treatment or control of SRD. A sponsor may propose an induced infection or other model study that mimics field conditions. The protocol should include justification for the choice of study design.

CVM recommends that sponsors submit protocols for review and concurrence prior to beginning studies that CVM and the sponsor agree are essential to CVM's decision to approve or deny an application. Although sponsors are not required to submit study protocols for review, CVM's concurrence with a protocol represents a fundamental agreement between CVM and the sponsor that we agree with the design, execution, and analyses proposed in the protocol we reviewed, and that we will not later alter our perspectives on these issues unless public or animal health concerns are evident that we did not recognize at the time we reviewed the protocol. (See Animal Drug User Fee Act Performance Goals and Procedures, http://www.fda.gov/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/ucm042936.htm. Protocol concurrence does not guarantee that the results of the study will support a particular finding or approval of the new animal drug.

CVM recommends that sponsors follow closely the format for writing protocols that is presented in CVM Guidance for Industry No. 85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01, section 6, available on the CVM website. The following information does not cover every aspect of a protocol. The information that follows supplements Guidance 85. It provides CVM's recommendations specific to studies designed to evaluate effectiveness against swine respiratory disease.

1. Recommended Study Design

The overall design of the study should be thoroughly described in the protocol. A reasonable design should provide a valid and precise estimate of the effectiveness of the investigational drug. The study design should also be reasonably robust in the face of circumstances that may arise during the course of a study, such as loss of animals at a field site.

a. Site Selection

CVM recommends a multi-site field study to demonstrate effectiveness. CVM recommends that sponsors select the number and geographic location of sites that will represent the target population of animals that may be exposed to the new animal drug after approval. The protocol should include a rationale for the number and geographic locations of the sites chosen for the study. The production system type and facility location(s) should be specified in the protocol.

A well-designed field study may include sites that have multiple facilities, multiple rooms within a facility, multiple pens within a room, and multiple animals within a pen.

The organization of the facility is very important because there is a potential for common influences to result in statistical dependencies among the observed responses that should be included in the statistical model. For example, pigs within a pen may influence each other's response to treatment by reinfecting each other. Pens within the same room may share some common influences in response to treatment.

The sponsor should select site(s) for a field study based on a recent history of SRD in the herd or on the farm. CVM recommends that the sponsor confirm that the target pathogen(s) are present in the study pig population prior to including a site. The target swine respiratory pathogen(s) should usually be identified through pre-treatment samples. Pre-treatment samples should be obtained by sacrifice and culture of lung tissues, serology, polymerase chain reaction (PCR), or other appropriate diagnostic tests. Nasal swabs are not recommended for providing a definitive identification of organisms causing acute SRD.

b. Experimental Unit

The experimental unit is the smallest unit of organization in the study design to which the experimental groups (treatments) are randomized, independent of how the individual pigs are assigned. For example, if pigs are individually randomized to experimental groups and pigs from all groups are commingled within each pen, then the individual pig is the experimental unit. If a pen of pigs is assigned at random to an experimental group, then the pen is the experimental unit, even though the pigs have been assigned at random to the pen. Likewise, if a room with multiple pens is

randomized to an experimental group, then the room is the experimental unit, even though a randomization process has been used to assign pigs to pens within the room.

For products administered in feed or water, the pen should be the experimental unit. For injectable products, the individual pig should be the experimental unit if pigs from all experimental groups are commingled within each pen.

The sponsor should select an appropriate experimental unit and provide appropriate replication of the experimental unit for each of the experimental groups in the study. For example, if the pen is the experimental unit and pens are grouped into rooms, then it may be possible to have several pens in a room assigned to each experimental group.

The pen is considered an important grouping factor, even if it is not considered the experimental unit. We recommend that pens mimic commercial conditions (as closely as practical) in terms of square feet per pig, pen size, number of pigs per pen, and number of pens per house.

c. Number of Animals

We recommend that sponsors provide a rationale for the number of pigs they plan to use in the study, the number of pigs per experimental treatment, the number of sites, the number of experimental units and the number of pigs per experimental unit, and the numbers associated with any other organizational levels in the study.

There may be numerous considerations in deciding the number of pens and the number of pigs per pen at each site. Practical limitations and the need to mimic commercial conditions may be constraints. Within these constraints, we recommend the following:

- Estimate the amount of variation in the primary response variables at each level of organization in the study. Factors such as variation among animals in the response to treatment, variation among sites in the severity of disease, and variation in response among breeds under different environmental conditions, may all tend to influence the number of pigs that should be used in a study. Attention to these points should aid in the statistical calculations used to allocate resources in the study.

- Aim for balance. For example, we recommend using approximately the same number of pigs per pen within and among sites. If experimental groups are commingled within each pen, then we recommend using the same allocation ratio of pigs per experimental group for each pen, such as a 1:1 ratio for test article to placebo control in each pen. For a control claim, we recommend that the ratio of pigs with the disease or condition to healthy pigs be as similar as possible among pens and among experimental groups.

- Include an adequate number of pigs per pen to accommodate losses during the study and to provide adequate precision for pen-based outcomes, such as the percentage of pigs that are "cured" or "improved" in a pen. In situations where experimental groups are commingled in a pen, this applies to the number of pigs per experimental group per pen.

Thus, we encourage the sponsor to provide a statistical justification for their study numbers based on assumptions from prior knowledge of the factors listed above and other factors deemed critical.

d. Experimental Groups

CVM recommends that the experimental groups (treatments) include the "treated" group(s) (receiving the test article at the proposed label dose(s) for the proposed frequency and duration) and a "control" group. CVM recommends that the sponsor use a placebo concurrent control (referred to as "placebo control" in the remainder of this guidance) so that the "control" group pigs receive the same handling and procedures as the "treated" group pigs. Using a placebo may also help maintain the masking of the pigs as to experimental group.

For SRD field studies, CVM recommends a superiority comparison of the test article to a placebo control group. If a sponsor would like to propose a field study with a non-inferiority comparison to an active treatment concurrent control group (referred to as "active control" in the remainder of this guidance), they are encouraged to discuss this further with CVM. Issues that the sponsor should be prepared to address include, but are not limited to: (1) the selection of an appropriate active control article; (2) the statistical justification of study numbers, including number of sites, pens, pigs and other organizational units; and (3) the use of a valid statistical model for a non-inferiority evaluation that incorporates important elements of the study design such as dependencies previously discussed and the establishment of a delta, the maximum allowable difference between test article and active control.

e. Randomization

Randomization is recommended to reduce bias in the process of selecting animals and assigning them to experimental units. The experimental unit may be an organizational level that has subunits, for example, pigs as subunits within the experimental unit of pen. In this case, it is also recommended to reduce bias in the allocation of subunits to that unit, for example, allocating pigs to the pens.

Randomization in a study design with multiple levels of organization usually includes at least two recommended steps. For example, if the pen is the experimental unit (all pigs within the pen will be assigned to the same treatment), then the first step should be to randomly assign pigs to pens. The second step should be to randomly assign each pen to an experimental group. If the pig is the experimental unit and all

experimental groups are represented in each pen, then the first step should be the random allocation of pigs to experimental groups. The second step should be the random allocation of pigs within each experimental group to pens.

Blocking and stratification may be used to maintain balance in the study design. Blocking is recommended as a means to remove variation associated with variables other than the experimental treatment so that the error variation is less. The success of the blocking procedure is measured by the percent of the overall variation that is accounted for. Typical blocking variables are location in a pig house, severity of disease or condition, or weight. Stratification is recommended when one pre-sets classes and one wants to be sure that pigs are included in the study from each pre-set class in a specified ratio. For example, one might pre-set weight classes and specify the percent of pigs that should be enrolled in each class. Blocking is more commonly recommended in SRD studies.

CVM recommends that the sponsor include the following information in the protocol concerning the process of randomization:

- Identify all of the steps to be used in the process of randomization.
- Describe the method(s) to be used to generate random assignments.
- Describe the relationship between the experimental unit, the process of randomization, and other organizational levels of the study design.
- Identify any blocking variables, restrictions to randomization, and stratification variables that are part of the randomization process.

f. Masking

Masking is recommended to decrease bias in the management and the evaluation of the animals in the study. We recommend that the masking methods planned for the study, including which personnel will have access to treatment assignments, be thoroughly described in the protocol.

- Treatment assignments. We recommend that each pig and each pen be assigned a unique identification (ID) number. A code for pen, room, and facility may be included as part of the pig ID number. However, the ID numbers should **not** include a number or letter code that references the pig's treatment assignment. For example, "A" and "B" should not be used as part of the ID numbers to reference the treatment and the placebo control groups. Similarly, color or other grouping codes should not be used to reference the pig's or the pen's treatment assignment. Personnel who are not involved in caring for the pigs or conducting clinical evaluations should maintain the list that matches the pig ID code or pen ID to the experimental group assignment. By following this practice, evaluators may be less likely to guess the experimental group assignment based on the responses of several pigs. Also, if one treatment assignment is accidentally

unmasked, the masking assignments of all the other experimental units in that same group are less likely to be revealed.

- Clinical evaluations and necropsies. We recommend that a separate sheet be used to record observations for each evaluation period (for example, day) so that observations/scores will not be influenced by the previous evaluations. Within the same evaluation period, it is acceptable to list data for multiple animals on the same sheet. Personnel involved in caring for the pigs and conducting clinical evaluations of the pigs should not be aware of treatment assignments. Personnel conducting necropsies and making observations that are intended to be used as decision variables (scoring lung lesions or evaluating extent of lung involvement, for example) should not be aware of treatment assignments.

- Evaluation of histopathological data. If histopathological data will be examined, appropriate techniques to minimize the introduction of bias should be used. Appropriate methods may include, but are not limited to, informal peer review, targeted masked review, or review by a pathology working group. We recommend that personnel evaluating the histopathological data not be aware of treatment assignments.

2. Selection of Study Animals

For SRD studies, we recommend that study pigs be representative of U.S. commercial production and the intended use population in terms of production class, breed, and sex, as follows:

a. Class/size: Weaned to finishing pigs with weight range being representative of the production class.

b. Breeds: Representative of U.S. production, including large white or other commercial crossbred animals.

c. Sex: Both sexes should be represented. If the drug is intended for use in replacement breeding animals the sponsor should contact CVM for guidance on additional target animal safety (TAS) studies.

d. Other Information: Pig source, treatment and vaccination history, herd health status, and other pertinent information should be described.

3. Inclusion/Exclusion Criteria

The sponsor should propose and state in the protocol the specific minimum criteria used to enroll a pig in the study, and specific criteria for exclusion.

a. Inclusion Criteria:

Eligible pigs should be enrolled in the study when they meet the minimum criteria specified in the protocol. For a treatment claim, *all* pigs enrolled should meet the criteria. In general, for a control claim, at least 15% of pigs in an experimental unit should meet the criteria. The minimum disease threshold may vary based on the dosage form or other drug properties. Use of a threshold other than 15% should be justified in the protocol. In addition, CVM recommends that for a control claim, the proportion of pigs meeting entrance criteria be as similar as possible among pens and among experimental groups.

The scoring system to identify pigs for inclusion should contain comprehensive, non-overlapping descriptors that allow a uniform evaluation among multiple observers and multiple sites.

CVM suggests the following minimum criteria:

- Body temperature ≥ 104°F, and
- Respiratory score ≥ 2 (0 = Rate and pattern normal, no abnormal nasal discharge; 1 = Mild – Slightly increased respiratory rate, some roughness in breathing; 2 = Moderate – Increased respiratory rate, some abdominal breathing; 3 = Severe – increased respiratory rate with abnormal effort - open mouth breathing, grunting, dog sitting), and
- Depression score ≥ 2 (0 = Normal - Alert, active, normal appetite, well-hydrated, coat normal; 1 = Mild – moves slower than normal, slightly rough coat, may appear lethargic but upon stimulation appears normal; 2 = Moderate – inactive, may be recumbent but is able to stand, gaunt, may be dehydrated; 3 = Severe – down or reluctant to get up, gauntness evident, dehydrated).

b. Exclusion Criteria:

The sponsor should state in the protocol the criteria to be used to exclude pigs from enrollment in the study. Examples of exclusion criteria include pre-existing conditions or diseases unrelated to SRD (such as enteric disease, lameness, neurological disease, septicemia, etc.), outside weight range, unthrifty appearance, abnormal conformation, or history of treatment with any antimicrobial, including antimicrobials in feed, typically within 14 days of the start of the study.

4. Post-inclusion Removal of Sites, Pens, or Pigs from the Study

The protocol should state the circumstances that would cause a site, pen, or pig to be removed from the study after the study has started. CVM suggests that the primary investigator decide if a site, pen, or pig should be removed from the study.

a. An "exit" examination should be conducted on any pig removed from the study. The results of the examination may serve as documentation of the reason for removal. Pigs removed from the study for undocumented reasons should be considered treatment failures.

b. The following are examples of events that might lead to removal of pigs after the study has started.

- *While the study is in progress but prior to the final evaluation, a pig is determined to be a treatment failure.* Pigs showing severe clinical signs of SRD may be removed from the study for humane reasons and treated with appropriate therapy. These pigs should be counted as treatment failures and be included in the effectiveness evaluation.

- *While the study is in progress but prior to the final evaluation, a pig develops signs of other diseases or conditions unrelated to SRD*. Pigs exhibiting signs of other diseases or conditions unrelated to SRD that are considered confounding to the study may be removed. Documentation (for instance, results of microbiology, serology, necropsy, histopathology, and other diagnostic tests) and a rationale for removal should be provided. If any pigs are excluded from the effectiveness evaluation, justification for the exclusion of these pigs should be provided.

- *While the study is in progress, a pig experiences a test article-related adverse event.* All adverse events should be documented, regardless of severity or cause. If a pig experiences a test article-related adverse event that is severe enough to result in removal from the study, the pig should generally be analyzed as a treatment failure. CVM will evaluate the circumstances of such withdrawals on a case-by-case basis to consider other options with regard to the effectiveness evaluation.

- *While the study is in progress, a pig dies or needs to be euthanized.* All pigs that die or are euthanized during the study should be necropsied and representative lung tissues from all pigs should be cultured. Positive cultures should be identified and minimum inhibitory concentrations (MICs) should be determined from isolates. A pig that dies or is euthanized that has gross lesions of pneumonia, with or without a positive culture of an organism associated with SRD should be considered a treatment failure and should be included in the effectiveness evaluation.

c. The protocol should state whether a pig should stay in the study pen after the pig is removed from the study. Pigs removed from the study pens for treatment of severe SRD, adverse events, or other diseases should not be added back to the pen during the study.

d. The reason for dropping any site, pen, or pig from the analysis should be explained in the final study report.

5. Assessment of Effectiveness

a. Measurements and Observations:

- The sponsor should define in the protocol the primary and secondary variables and describe when and how they should be measured. The measured variables should have clinical relevance. There should be a logical relationship between the variables intended to be measured, the timing and frequency of study observations and measurements, and the proposed claim. The protocol should state how each variable contributes to the study conclusion.

- CVM considers success/failure determination as the primary variable for SRD studies.

  We recommend that all pigs be classified as a success or failure on the predetermined evaluation day(s). CVM recommends using the following criteria to classify a pig as a success or failure.

Table 1. Success/failure criteria for SRD studies.

SUCCESS	FAILURE
Alive, and	All pigs that do not meet the definition of success on the evaluation day(s).
T <104°F, and	
Respiratory score = 1 or 0, and	
Depression score = 1 or 0	

- Mortality may be a secondary variable.

- The time points for measuring variables in a field study should be carefully considered. The most appropriate time points depend on the proposed claim, product characteristics or formulation, pharmacokinetics, and treatment regimen.

- The timing and frequency of study observations should be specified in the protocol. CVM suggests that repeated observations and other measurements be done at the same time of day to decrease variation.

- The sponsor should describe in the protocol any additional procedures and/or tests they plan to use for assessment, including the time of sampling and the interval between sampling, storage of samples, and the analysis or testing method.

b. Microbiological Endpoints:

(1) Pathogens Included in the Label Claim (Indications): Only pathogen(s) obtained from the study animals should be included in the proposed claim. Isolates may be obtained by necropsying study animals, identifying lung lesions, and culturing SRD pathogens. The number of isolates needed in order to consider the pathogen(s) for inclusion in the label claim should be stated in the protocol. For each pathogen species, CVM recommends that at least 30 isolates be collected from a minimum of 30 pigs. If a sponsor wishes to add pathogens to the claim in a later supplement, then CVM may request that additional effectiveness data be generated with isolation of the additional pathogen(s) from field studies.

(2) *In Vitro* Minimum Inhibitory Concentration (MIC) Data for the Microbiology Section of the Label: The microbiology section should only include MIC data for the pathogen species included in the indication. MIC data should be obtained from field isolates from pivotal effectiveness studies. CVM recommends that MIC data proposed to be included on the label (microbiology section) be obtained from at least 30 isolates from a minimum of 30 pigs for each pathogen species included in the indication. Susceptibility testing should be conducted using validated methods such as those described by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards). Susceptibility testing should include quality control strains to verify that the test method is valid.

c. Basis for Study Conclusion:

To have CVM consider a drug and dosage regimen effective, CVM recommends that the sponsor demonstrate:

- that the target pathogen(s) were present and contributed to an acute respiratory disease outbreak in the study animals, and
- the proportion of successes is statistically significantly different (at an α level of 0.05) in the treated group compared with the placebo control group, and
- there was clinically relevant improvement in the treated group compared with the placebo control group for the decision variable(s), as stated in the protocol.

The day(s) selected to evaluate success or failure may vary depending on the pathogen, the drug, disease pathophysiology, and supporting arguments, which may include data such as pharmacokinetic/pharmacodynamic relationships and the existence of a post-antibiotic effect. CVM generally recommends classifying each pig as either a success or a failure 48 hours after drug concentrations drop below therapeutic blood levels for the target pathogens. For drugs with poor correlation between drug concentrations in the blood and at the site of infection, the evaluation should be made 48 hours after the drug concentrations at the site of infection have dropped below the MIC of the target pathogens.

6. Statistical Methods

For SRD effectiveness studies, the primary response variable should typically be the classification of each pig as either a "success" or a "failure." This variable should be measured for each pig in the study. The sponsor should tabulate the number of pigs in each pen that were successes and the number that were failures. For a design with commingled treatments, the summary should separate the responses of pigs from each pen into experimental groups.

The sponsor should conduct a statistical comparison between the investigational drug and the placebo control in terms of the proportion of successes in each group. The null hypothesis should be that each group has the same population proportion of successes. This should be tested against a two-tailed alternative hypothesis. The appropriate statistical model depends on what the experimental unit is and how it is replicated in the study design. Linear mixed models or generalized linear mixed models may be appropriate for the primary response variable as well as secondary variables depending on the nature of the response variable.

Site is considered an important contributor to the inferential value of the study. The sponsor and CVM should agree at the protocol stage on the number and location of sites and how information from these sites should be evaluated and combined.

We suggest that the sponsor work with CVM at the protocol stage to develop an acceptable approach to the analysis of data in support of the proposed claim for the investigational drug.

SECTION 2: Recommended Study Design and Evaluation of Effectiveness Studies for Swine Respiratory Disease Associated with *Mycoplasma hyopneumoniae*.

Mycoplasma hyopneumoniae is rarely the only pathogen involved in SRD. The presence of *M. hyopneumoniae* in the lung tends to decrease the immune defenses in the lung, which allows other pathogens to cause clinical signs of respiratory disease. For this reason, CVM suggests several possible claims regarding *M. hyopneumoniae* – 1) reduction in severity of effects of respiratory disease associated with *M. hyopneumoniae*, or 2) a treatment (or control) of SRD claim in which *M. hyopneumoniae* is included in the list of associated target pathogens. Sponsors may also propose (with justification and supporting data) other appropriate claims and claim wording.

The study design concepts and considerations discussed in Section 1 (Swine Respiratory Disease) provide guidance for designing field studies to evaluate the effectiveness of a new animal drug against SRD associated with bacterial pathogens such as *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pasteurella multocida,* or *Streptococcus suis*. Many of these concepts are also appropriate for studies conducted to generate effectiveness data for *M. hyopneumoniae*-associated claims. Section 2 provides additional guidance for designing studies and evaluating the effectiveness of a new animal drug for claims associated with *M. hyopneumoniae*.

Due to the complexity of *M. hyopneumoniae* infection in pigs, CVM recommends an appropriately validated model study in addition to an adequate and well-controlled field study. The model study should show that the drug has a therapeutic effect specifically against *M. hyopneumoniae* in swine, and the field study should show that the drug is effective under field use conditions in an SRD outbreak where *M. hyopneumoniae* is present.

1. Model Study Considerations

An induced infection model study mimicking natural infection should show that the drug is effective against *M. hyopneumoniae,* for example, by providing evidence that organism numbers were reduced in the pigs after the drug was given. The model study may also show that the drug provided a clinical effect (such as decreased lung lesions) in the treated animals.

The sponsor should provide data to support that the inoculating strain used in the model study is representative of current (within three years of the start of the study) North American field isolates. The dynamics of infection should have biological relevance for field infection in pigs.

21 CFR 514.4(b)(3)(ii) requires that studies using models be validated to establish an adequate relationship of parameters measured and effects observed in the model with one

or more significant effects of treatment. We recommend that the sponsor provide information showing that:

- the model study method can be reproduced by different investigators, and
- the model study methods and endpoints would be acceptable to the scientific community. For example, evidence that the model study has been published in a peer-reviewed scientific journal may be provided.

2. Field Study Considerations

A field study should demonstrate that the drug has a therapeutic (clinical) effect under field use conditions where *M. hyopneumoniae* is present. Serology may be used to support the presence of *M. hyopneumoniae* but should not be used alone as verification. The following standards are recommended to verify that *M. hyopneumoniae* is present in the study group:

- Live animal diagnostics: PCR (nasal swab or tracheal wash) – at least 20% of the study pigs should be positive for *M. hyopneumoniae*; or
- Necropsy (lung tissue): fluorescent antibody, immunohistochemistry, PCR, or culture of lung tissue – 5-10% of the study pigs should be positive for *M. hyopneumoniae.*

The information below summarizes the additional recommended considerations for a field study protocol to test the effectiveness of a new animal drug intended for one of the following claims.

a. Treatment or Control of SRD associated with *M. hyopneumoniae*

A claim for either the treatment or control of SRD where *M. hyopneumoniae* is present may be based on a well designed SRD field study that demonstrates that the drug was effective against *M. hyopneumoniae* in the presence of other bacterial pathogens in an acute SRD outbreak. Design considerations are the same as those recommended in Section 1, with the additional recommended step of verification of the presence of *M. hyopneumoniae* as described above.

b. Reduction in Severity of Effects of Respiratory Disease associated with *M. hyopneumoniae*

A "reduction in severity" claim should be based on the evaluation of the chronic effects of *M. hyopneumoniae*.

(1) Study Design

A well designed field study for a "reduction in severity" claim may follow a group of pigs from nursery to slaughter to demonstrate that the drug was effective

in reducing the chronic adverse (harmful) effects of *M. hyopneumoniae* (such as fewer acute SRD outbreaks with fewer pigs needing treatment, less prevalent/less severe lung lesions, or maintenance and uniformity of weight gains). The sponsor may suggest an alternate study length, based on the intended dosing regimen and study endpoints.

(2) Experimental Unit

In field studies associated with *M. hyopneumoniae*, there is no recommendation at the present time for a "typical" experimental unit. The experimental unit may be the pig, the pen, the room, the facility, or some other organizational level. Therefore, we recommend that the sponsor justify the appropriateness of the choice of experimental unit in terms of the field use of the product.

(3) Enrollment Criteria

When there is 1) documented evidence of respiratory disease associated with *M. hyopneumoniae* in the herd(s) of origin; and 2) a positive identification of *M. hyopneumoniae* in the group of pigs currently targeted for the study as described above, the entire group of study pigs should be enrolled.

(4) Assessment of Effectiveness

a) The time points for measuring variables in a field study for a "reduction in severity" claim should be carefully considered. A field study for this claim may be considerably longer than a study for a disease of acute onset and short duration. In addition, clinical signs and lung lesions may vary considerably among pigs at different stages of disease.

b) Serial necropsies should be conducted to compare lung lesions at specific points (for example, 4 weeks after therapy begins) relative to the treatment period(s). The number of pigs requiring alternate or additional therapy for acute respiratory disease should be recorded. Physical health parameters such as average daily gain (ADG) and uniformity (weight variability) data should also be recorded.

c) Basis for conclusion of study:

- The decision variables may include dichotomous "success"/"failure" classifications, a count of the number of pigs requiring additional or alternate therapy, a continuously distributed variable such as weight gain; and scores of lung lesions.

- The choice of statistical methodology should consider points covered in Section 1. Linear mixed models or generalized linear mixed models may be appropriate depending on the nature of the response variable.

SECTION 2: Swine Respiratory Disease associated with *Mycoplasma hyopneumoniae*

- For studies with multiple variables (such as mortality, clinical signs and lung lesions), the protocol should specifically state how all of the variables will be considered in determining effectiveness. A decision tree or flow chart may assist in determining effectiveness if some, but not all, variables show significant differences between treated and placebo control groups.

- Data on physical health variables, such as weight gain, feed intake, and feed efficiency may also be analyzed. Improvement in physical health variables without statistically significant improvement in other primary decision variables should not be used to demonstrate effectiveness for *Mycoplasma* claims.

d) To have CVM consider a drug and dosage regimen effective, CVM recommends that the sponsor demonstrate:

- that *M. hyopneumoniae* was present and contributed to respiratory disease in the study animals, and
- there was a statistically significant improvement (at an α level of 0.05) in the treated group compared with the placebo control group for the decision variable(s), as stated in the protocol, and
- there was clinically relevant improvement in the treated group compared with the placebo control group for the decision variable(s), as stated in the protocol.