217

Guidance for Industry

Evaluating the Effectiveness of Anticoccidial Drugs in Food-Producing Animals

(This guidance #217 will supersede the CVM draft Guidance for Industry #40, entitled "Draft Guideline for the Evaluation of The Efficacy of Anticoccidial Drugs and Anticoccidial Drug Combinations in Poultry," dated April 1992.)

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-2011-D-0784.

For further information regarding this document, contact Emily R. Smith, (HFV-135), Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 240-276-8344, e-mail: emily.smith2@fda.hhs.gov.

Additional copies of this 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/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm or http://www.regulations.gov.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Veterinary Medicine
November 2012

Table of Contents

Introduction 4
I. General Considerations 5
A. Protocol Development 5
B. Animal Welfare Considerations 6
C. Substantial Evidence of Effectiveness 6
D. Masking 7
E. Personnel Training and Experience 7
F. Record Keeping 7
G. Nutritional Content and Preparation of Experimental Diets 7
1. Feed Form 8
2. Demonstration of Nutritional Adequacy 9
3. Nutrient Assays Falling Outside the Specification Limits 9
H. Drug Assays 9
1. Assay Limits for Drugs 10
2. Feed Sampling for Drug Assays 10
I. Combination Approvals 10
II. Study Considerations for Poultry 11
A. General Recommendations 11
1. Study Animals 11
2. Concomitant Drug Therapy and Vaccination 11
3. Bird Placement/Tracking 11
4. Housing/Management 12
5. Weighing equipment 12
B. Battery Studies 12
1. Test animals 12
2. Housing 13
3. Feed management 13
4. Inoculum 13
Table 1. Maximum acceptable age of commonly recognized field isolates of *Eimeria* spp. in chickens 14
Table 2. Maximum acceptable age of commonly recognized field isolates of *Eimeria* spp. in turkeys 14
5. Predicting the virulence of the inoculum 14
6. Battery Study Design 15
7. Variables for Evaluation of Efficacy 16
8. Statistical Analysis 20
9. Basis of Study Conclusion 21
10. Anticoccidial Sensitivity Tests 21
C. Commercial Field Study 21
1. Housing 22
2. Test Animals 22
3. Bird accountability 22
4. Feed accountability 22
5. Experimental Design 22

6. Variables for Evaluation of Effectiveness 23
7. Descriptive Statistics 23
III. Effectiveness of Anticoccidial Drugs for Minor Uses and for Minor Species 24
IV. Effectiveness of Anticoccidial Drugs in Food-Producing Mammals 25
A. Study requirements 25
B. Method of Infection 25
C. Experimental Design 25
D. Variables for Evaluation of Effectiveness 26
GLOSSARY 27
APPENDIX 1 28
APPENDIX 2 30
REFERENCES 31

EVALUATING THE EFFECTIVENESS OF ANTICOCCIDIAL DRUGS IN FOOD-PRODUCING ANIMALS

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.

Introduction

This guidance provides recommendations to industry relating to study design and describes criteria that the Center for Veterinary Medicine (CVM) thinks are the most appropriate for the evaluation of the effectiveness of anticoccidial drugs in poultry and other food-producing animals.

Section I discusses general considerations regarding the development of protocols, study conduct, animal welfare, substantial evidence of effectiveness, feed preparation, nutritional content of experimental diets, and the assessment of drug concentrations in experimental diets.

Section II discusses the studies CVM recommends to substantiate effectiveness of an anticoccidial drug in poultry.

Sections III and IV provide information regarding the development of studies to demonstrate the effectiveness of anticoccidials for minor uses and minor species and the effectiveness of anticoccidials in food-producing mammals, respectively.

The guidance is not a comprehensive source of information on conducting clinical effectiveness studies. Alternative study designs for providing substantial evidence of effectiveness may be acceptable. Sponsors should contact CVM to discuss their development plan prior to initiating any studies. Sponsors and clinical investigators should consult the Code of Federal Regulations (21 CFR Parts 511 and 514) for information on the proper shipment, use, and disposition of investigational new animal drugs, as well as submission of the results of clinical investigations.

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.

I. GENERAL CONSIDERATIONS

We consider the indication, "For the control of coccidiosis caused by (specific *Eimeria* spp.)" appropriate for anticoccidial drugs; however, a treatment indication may be considered. You should design your protocol to support your intended indication.

A. Protocol Development

A protocol should be developed to specifically describe the plan for conducting an effectiveness study. The protocol must include a clear statement of the study objective(s), state the hypothesis, describe the experimental design in detail, and include success, entrance, and exclusion criteria (21 CFR §§ 514.117(b)(2), (4)-(6)). Your protocol should be based upon sound scientific principles and processes. The characteristics of an adequate and well-controlled study are described in 21 CFR § 514.117. Sponsors should follow the format for writing protocols that is recommended in CVM Guidance for Industry No. 85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01, section 6 (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052417.pdf).

CVM recommends that sponsors submit protocols for review and obtain a Protocol Concurrence Letter from CVM before beginning essential studies[1]. CVM's concurrence with a study protocol represents a fundamental agreement with the design, execution, and analyses proposed in the protocol. CVM concurrence represents a commitment that we will not later alter our perspectives on these issues unless public or animal health concerns appear that we did not recognize at the time of the protocol assessment. Because this concurrence does not extend to any subsequent changes a sponsor may make to the protocol, sponsors may want to seek concurrence on the revised protocol if they make changes. Protocol concurrence does not guarantee that the results of the study will support a particular finding or approval of the new animal drug. [2]

Sponsors may choose to submit either a master or a site-specific protocol. Sponsors should identify which type of protocol they are submitting. A master protocol provides general information on principles that apply to all study sites. Master protocols allow any clinical investigator to have all the details needed to conduct the entire study, including, but not limited to the following: test and control article specifications, blocking and randomization schemes, a description of the animal model, inclusion/exclusion criteria, variables of interest, statistical analysis, treatment groups, schedule, and success criteria. Additionally, a master protocol may be used as the basis from which more detailed, site-specific protocols are developed and written.

A site-specific protocol should contain information present in a master protocol plus any

[1] See Guidance for Industry #215: Target Animal Safety and Effectiveness Protocol Development and Submission, Final Guidance

[2] Animal Drug User Fee Act Performance Goals and Procedures, http://www.fda.gov/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/ucm042936.htm

detailed information pertaining to the study site, including, but not limited to the following: location of the study/studies, personnel involved, diet(s) to be fed, detailed facility diagrams showing cage or house locations, location of feeders and waters, environmental conditions, and standard operating procedures.

If you are submitting a protocol to CVM, we suggest planning your schedule so that there is sufficient time for us to review it before the proposed start date of any studies.

B. Animal Welfare Considerations

All studies using live animals that are conducted in the United States must conform to the requirements of the Animal Welfare Act (AWA), which is administered by the United States Department of Agriculture (USDA). The USDA has issued policies and regulations on how to comply with the requirements of the AWA. In addition, many research institutions that conduct research studies using live animals are also accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Farm animals used for biomedical research (such as drug studies) fall under the purview of the AWA and USDA regulations. Two publications: the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching," published by the Federation of Animal Science Societies (FASS), and the "Guide for the Care and Use of Laboratory Animals," published by the Institute for Laboratory Animal Research (ILAR), may assist researchers in the implementation of the USDA regulations. These guides provide information on the appropriate handling, housing, care, treatment, and transportation of farm animals for nonagricultural purposes and should be referenced when designing studies to demonstrate the effectiveness of anticoccidial drugs in food-producing animals.

C. Substantial Evidence of Effectiveness

Effectiveness must be demonstrated by substantial evidence consisting of one or more adequate and well-controlled studies [21 U.S.C. §§ 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 battery studies and a multi-location commercial-scale field study to establish the effectiveness of anticoccidial drugs in poultry. Battery studies are conducted to evaluate the efficacy of investigational new animal drugs against individual and mixed *Eimeria* species in the target animal under controlled laboratory conditions. Commercial field studies are necessary to confirm effectiveness under conditions of use. You should conduct a minimum of two battery studies for each *Eimeria* species being tested and at least two battery studies with the mixture of *Eimeria* species. Power calculations can help you determine the number of experimental units needed at a site. For independent substantiation, studies should be conducted by at least two different investigators using different recent United States field isolates. Your protocol should specify the number of individual species and mixed species studies that you will conduct to demonstrate the efficacy of your anticoccidial drug. A multi-location commercial field study should be conducted in the appropriate number of sites to ensure that the two principle objectives for the demonstration of effectiveness, independent substantiation and inferential value, are met. You should

specify in the protocol the number of study sites that you will use. See Sections III and IV for information on the recommended studies to establish the effectiveness of anticoccidials for minor uses and minor species and the effectiveness of anticoccidials in food-producing mammals, respectively.

You are required to demonstrate by substantial evidence that the new animal drug is effective at the dose or over the dose range selected (see 21 U.S.C § 360b(d)(1)(E)). For the purposes of dose characterization, you should submit sufficient information to characterize the critical aspects of the dose-response relationship. You may derive dosage characterization from dose titration studies, pilot studies, foreign studies, or scientific literature.

D. Masking

In order to minimize bias, you should mask all personnel responsible for day-to-day management of the animals, including those making and recording observations. You must describe masking procedures in the protocol and final study report (21 CFR § 514.117(b)(7)). Masking is an important design technique for avoiding bias in clinical trials (see CVM Guidance for Industry (GFI) #85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01).

E. Personnel Training and Experience

As specified in 21 CFR § 511.1(b)(7)(i), all personnel involved with the investigation must have adequate scientific training and experience with the target animal species and disease models used in the studies.

F. Record Keeping

Good record keeping is a critical component in determining the effectiveness of a drug when it is being tested. The integrity and accuracy of the data collected are critical to the acceptance of a study as substantial evidence of effectiveness. CVM's recommended standard for record keeping and data management is contained in CVM GFI #85: Good Clinical Practices: VICH GL9, Final Guidance, 05/09/01.

G. Nutritional Content and Preparation of Experimental Diets

You should feed animals nutritionally adequate diets so that observed responses can be attributed to the drug, rather than a possible nutritional effect. You should formulate diets to meet predominant commercial practices for the species and class of animal being fed, and for the geographic region where you conduct the study. You may use agricultural survey data, e.g., Agrimetrics, Agri-Tech, etc., to support information on predominant commercial practices.

Nutrient recommendations for different food-producing animals, published by the National Research Council (NRC), may serve as a reference for formulating diets. You should specify feed additives, such as antioxidants, pellet binders, copper sulfate, etc., in the diet formulation and ensure that any feed additives do not confound the effects of the test article.

You should prepare experimental feeds from a uniform basal diet. A uniform basal diet is consistent with regard to nutrient densities and ingredients. If you derive the uniform basal diet from several mixer batches, you should ensure that the diet corresponding to each treatment group is composed of equal aliquots from each mixer batch. In addition, you should describe the procedures used to prepare a uniform basal diet and collect feed samples in your protocol. We realize that there may be alternative methods for preparing a uniform basal diet. Before initiating any studies, we suggest that you discuss and obtain protocol concurrence with us regarding the procedure you wish to use.

You should divide each batch equally among all treatment groups. When a batch of feed runs out for one or more treatment groups, you should discontinue feeding that batch of feed to all remaining treatment groups and begin feeding the new batch of feed to all treatment groups at the same time. Feed from the previous batch should be weighed and properly accounted for in the final study report.

A NOTE ABOUT MIXING FEEDS

Properly mixed feed is critical for the success of your study. Proper mixing of feedstuffs and feed additives will ensure the uniform dispersal of nutrients and drug(s) in finished feeds. Personnel responsible for mixing feed should be aware of the performance and capabilities of the feed mixer(s) that will be used to prepare experimental diets. Mix medicated feeds according to 21 CFR Part 225 - Current Good Manufacturing Practice for Medicated Feeds, to ensure adequate homogeneity. All standard operating procedures used for feed mixing should be consistent with applicable GMP regulations for the manufacture of medicated feeds. Your protocol should state that the medicated feed will be mixed at a commercial feed mill and shipped to the study site, or that the medicated feed will be mixed using a validated mixer. Your final report should include all relevant records.

1. Feed Form

The feed form of the experimental diet should reflect predominant commercial practices and take the following considerations into account: the species and class of animal being fed, the geographic region where the study is conducted, and the recommendations of the supplier of the genetic stock.

For example, for poultry battery studies, feed is typically mixed and fed as a mash, and for poultry commercial field studies the feed is mixed into a mash, pelleted, and crumbled. You should be aware that the milling process may affect the effectiveness of the drug(s). For example, poultry feed that is pelleted is typically subjected to temperatures ranging from 170 to 200 °F. Exposure of the feed to high temperatures may affect the stability of the drug(s).

2. Demonstration of Nutritional Adequacy

Test animals should be fed a nutritionally adequate diet in order to minimize confounding effects of nutrition on the study results. To demonstrate the nutritional adequacy of the experimental diets, you should include in your protocol a complete and detailed list of the amounts of all feed ingredients and vitamin/mineral premixes that will be used in the uniform basal diet. You should use ingredients that are representative of feedstuffs commonly used in each geographic location. You should state the standard to which the nutrient levels are being compared (e.g., NRC, literature, broiler management guide). For a uniform basal poultry diet, you should report the calculated nutrient levels of crude protein, methionine, cystine, lysine, calcium, phosphorus (total and available), and metabolizable energy.

To ensure that experimental animals receive proper nutrient densities in the diet, you should conduct proximate and chemical analyses on a composite sample from the uniform basal diet. You should include a description of the analyses that you intend to conduct in your protocol and specify the number of assay replicates. You should indicate whether the analyses are reported on an as-fed or dry-matter basis.

The purpose of nutrient analysis is to provide additional assurance that the diets are within the calculated values specified in the protocol. To determine the nutritional adequacy of the diet, we recommend using a 90% specification limit (SL) approach to calculate upper and lower specification limits for nutrient concentrations. If you re-assay the composite sample, you should adjust your specification limits (See Appendix 1); however, the specification limits should be within those found in predominant commercial practice.

3. Nutrient Assays Falling Outside the Specification Limits

Nutrient concentrations that fall outside the SL are not necessarily deemed unsatisfactory. We will consider the magnitude of the deviation from the SL, the nutritional circumstances, and the clinical consequences, when the adequacy of the diet is determined. When you document a deviation, you should provide a written justification of the reason, and possible clinical relevance of the deviation.

H. Drug Assays

The purpose of conducting drug assays is to verify that the drug mixed in the feed and used in the study is present at the appropriate concentration. Before approval of a new animal drug, feed assay method transfer studies are conducted to determine the percent coefficient of variation[3] (CV%) for fortified feed samples assayed by the analytical method. The CV% is then used to calculate the permissible analytical variation (PAV), which accounts for the inherent variability in the analytical method, as well as in the medicated feed manufacturing and sampling procedures, e.g. [(2*CV%) + 5%]. Assay limits are determined by applying the ± PAV to the average percent recovery value of fortified feed samples, giving a range of assay values that would be acceptable for a medicated feed manufactured at the intended

[3] The CV% is defined as the standard deviation divided by the mean, multiplied by 100.

concentration. For example, if the CV% was 7.5%, and the PAV for this feed was (2*7.5%) + 5% = 20%, then the assay limits (assuming 100% recovery) would be 80 to 120%.

1. Assay Limits for Drugs

Assay limits for approved drugs in experimental feeds are codified in 21 CFR Part 558.4. Concentrations of approved drugs in experimental feeds should fall within these assay limits regardless of where and when a feed sample is collected.

For investigational new animal drugs for which assay limits have not been codified, levels should conform to the investigational assay limits derived through the feed assay method transfer studies. If the assay limits are not established before conducting studies, the assay method used to determine drug concentration(s) should be no more variable than the method that will be subjected to the method validation process.

Note that a PAV relates to a single application of the assay method. Under the proper mixing conditions using the correct quantity of the drug, a single assay of a feed sample should fall within the assay limits. If a drug assay(s) falls outside of the assay limits, the feed should not be used in the study and the reason for the out-of-assay-limit result(s) should be investigated and discussed in your final study report.

2. Feed Sampling for Drug Assays

In your protocol, you should propose a feed sampling method for the study drug assay. The method used should provide for a representative sample for the drug assay. The most relevant feed sampling point is where and when feed is offered to the animals, with the representative composite samples collected within 24 hours of feeding the animals. If you choose to also confirm the drug concentration at the feed mill, please include the assay results with your raw study data.

In addition, you should assay every batch of medicated and control feed for the presence of the last non-study drug (based on your mixing records), unless all of the equipment used in the study is dedicated and isolated to avoid cross-contamination.

I. Combination Approvals

The Animal Drug Availability Act of 1996 (ADAA) amended the Federal Food, Drug, and Cosmetic Act (the Act) and changed the requirements for the approval of certain combinations of new animal drugs that have been previously separately approved. There are important criteria to consider before you submit a combination new animal drug application. For example, under section 512(d)(4) of the Act, a sponsor seeking approval of a combination of two or more previously approved new animal drugs may not need to conduct additional effectiveness studies, if each new animal drug in the combination has at least one unique non-overlapping indication. However, among other things, all of the following criteria must be met: the new animal drugs must provide for appropriate concurrent use,

must be physically compatible, and not have disparate dosing regimens (21 U.S.C. §§ 360b(d)(4)(C) & (D)). If you intend to submit a combination new animal drug application, we recommend you contact CVM for specific requirements.

II. STUDY CONSIDERATIONS FOR POULTRY

General recommendations for all types of poultry studies are provided first, followed by specific recommendations for battery studies and the commercial scale field study.

A. General Recommendations

1. Study Animals

In support of substantial evidence of effectiveness, studies should be conducted using the target species and class for which the drug is intended. You should use healthy chicks or poults from contemporary, commercial genotypes. You should also document the source of the chicks or poults and describe the age and health status of the breeder flocks.

You should minimize stresses outside the scope of the study and handle birds gently at all times. You should only remove birds from a study to alleviate suffering due to a clinically diagnosed disease or injury. Humanely euthanize any birds that you remove. You should describe the removal criteria in your protocol.

2. Concomitant Drug Therapy and Vaccination

You should not use concomitant drug therapy during the study because the use of concomitant drug therapy can influence the test drug response and/or mask adverse drug reactions.

You may follow a vaccination program to protect the birds against prevalent infectious disease(s), other than coccidiosis. The vaccination program should not debilitate the birds or otherwise compromise your study. If vaccinations are used, please address in your protocol any possible effects on the outcome of the study. In addition, the study protocol should provide information concerning any vaccination program, including: 1) method (eye, spray or water); 2) date of vaccination; 3) age of the bird at vaccination; 4) source; 5) vaccine lot number and expiration date; 6) type of vaccine (live, modified live, or killed organisms); 7) handling of vaccine; and 8) any other information pertaining to the vaccine(s).

3. Bird Placement/Tracking

Your protocol should include a description of the method you intend to use to ensure the accuracy of the number of birds placed in each cage or house at the start of the study. If early chick/poult mortality is a concern, you may stock birds more densely, but within the range of commercial stocking density. You should not replace birds during the study.

Your protocol should include a method that provides for: 1) accounting for the birds;

2) verifying the randomization of birds to cages, and treatments to cages or houses; 3) detecting migration or misplacement of birds; and 4) preserving the identification of the original sex.

4. Housing/Management

To maximize the inferential value of the studies, the available square footage for the housing of study birds should reflect commercial practices in the geographical location of the study and take into account variation in seasonal temperatures and the final weight of the birds. You should use a number of birds per feeder or waterer that is consistent with commercial practices.

You should observe birds at least twice a day to appropriately manage the study and to collect dead birds for necropsy. Frequent visits to the facility will allow you to obtain clinical and management observations, such as feathering characteristics, abnormal excreta, or behavior. You should adopt sanitation and bio-security programs to prevent the inadvertent introduction or spread of pathogens.

5. Weighing equipment

All scales/balances used for weighing birds and feed should be calibrated on a regular basis to ensure accurate and consistent measurements. You should include a description of the calibration method or a copy of the standard operating procedures in your protocol and provide the documentation in the final study report.

B. Battery Studies

Battery studies are conducted to evaluate the efficacy of investigational new animal drugs against individual and mixed coccidial species in the target animal under controlled laboratory conditions.

1. Test animals

You should use day-old birds (chicks or poults) representative of genetic stock used in contemporary poultry production. You should also acclimate the birds for 10 to 14 days and individually identify them (*via* wing band, leg band, etc.) before randomly assigning them to cages and provide non-medicated feed *ad libitum* during this acclimation period. You should house the birds in a way to prevent inadvertent coccidial infection during the acclimatization period. In addition, as protocols are generally designed to support anticoccidial control claims, you should start the birds on the experimental diets at least 48 hours prior to the inoculation of oocysts.

You should identify the method of determining the sex of birds at hatch (i.e., feather or vent sexing) in your protocol. On the final weigh day, you should verify the sex of each

bird phenotypically. If the sex is in doubt or conflicts with the original sex determination, you should determine the sex by postmortem examination.

2. Housing

You should give special consideration to the placement of birds within the battery. You should place non-inoculated, non-medicated control groups in the uppermost battery tier to prevent coccidial contamination of these birds. We prefer that male and female birds be separately housed. You should randomly assign males and females to separate cages so that each cage has the same number of birds and the numbers of male and female cages are the same. You should use uniform lighting within a block, utilizing typical commercial photoperiods as recommended by the breeder (e.g., 24 hours lighting, or 23 hours lighting with one hour off, etc.). In this type of study, a block is defined as a row of cages containing at least one cage for every treatment by sex combination.

3. Feed management

You should measure feed consumption concurrently with body weight gain to document drug intake. Each time feed is added, you should record the date, the type of feed, and the feed weight. You should design and manage feed containers in such a way as to minimize feed wastage and spillage into other feeders [i](Williams, 1996). You should record the weight of the remaining feed, per cage, at the end of the study.

4. Inoculum

The inoculum is a critical factor in the validation of the disease model used in battery studies. The inoculum should represent field strains of coccidia that have been exposed to currently approved anticoccidial drugs. You should use sporulated oocysts from recent field isolates of commonly recognized poultry coccidial species (Table 1 and Table 2) that have been exposed to contemporary anticoccidial drugs to propagate the inocula. Recent field isolates reflect a state of anticoccidial susceptibility likely to be seen once the product is in use. The isolates should not come from houses using anticoccidial vaccinations. Laboratory strains are not representative of field strains of coccidia and are not acceptable.

Table 1. Maximum acceptable age of commonly recognized field isolates of *Eimeria* spp. in chickens

SPECIES	MAXIMUM ACCEPTABLE AGE OF THE ISOLATE (Years)
E. maxima	3
E. acervulina	3
E. tenella	3
E. brunetti	5
E. mitis	3
E. necatrix	5

Table 2. Maximum acceptable age of commonly recognized field isolates of *Eimeria* spp. in turkeys

SPECIES	MAXIMUM ACCEPTABLE AGE OF THE ISOLATE (Years)
E. meleagrimitis	3
E. adenoeides	3
E. gallopavonis	3

You should use coccidia species that originate from North America, and are representative of contemporary poultry production trends. You should report from where and when they were isolated, the identity of the drug(s) in the feed at the time of the outbreak, and predominant species involved. Your isolate should be as pure as possible; however, single cell isolations are not necessary. The isolates should be passaged through susceptible birds, oocysts collected at appropriate times and sporulated to produce the inoculum. Limit the passages to preserve the identity of the isolate. The mixed inoculum should be comprised of individual species in proportions adequate to provide the appropriate virulence. Recombine as necessary to achieve the desired concentration of each individual species for the mixed inoculum. You should administer the inoculum directly into the crop using a gavage technique. The non-inoculated, non-medicated control group should receive an equal volume of water by the same method as the treated birds.

5. Predicting the virulence of the inoculum

Virulence titration studies allow you to predict the number of oocysts that should be given in the subsequent battery studies in order to induce an acceptable coccidial infection. You should conduct a virulence titration study(ies) for each *Eimeria* species. You do not have to conduct studies for each species concurrently. Virulence titration studies should include birds in a non-infected, non-medicated control group, and multiple groups given incrementally increasing numbers of oocysts.

You should conduct virulence titration studies no more than two to three weeks before the battery study to maintain the integrity of the isolate. You should submit the results of the virulence titration study as part of the final effectiveness study report. If you want CVM's concurrence on your virulence titration protocol, you should include it along with the effectiveness study protocol.

For chickens, poultry pathologists and parasitologists have developed methodology to evaluate the virulence of coccidia based on intestinal lesion scores, body weight gain, and mortality.[ii] A clinically relevant level of virulence is characterized by 30% lower body weights and a 3.0 unit increase in lesion scores[iii] in the inoculated non-medicated birds when compared to the non-inoculated non-medicated birds. This level of virulence should result in lesion scores and body weight differences in the battery study that will provide an adequate model for assessing drug efficacy. For battery studies using species of coccidia that typically result in mortality, the mortality in the infected, non-medicated birds should be around 25% to adequately model a naturally occurring outbreak. We expect that the mortality will be most severe when *E. tenella* or *E. necatrix* are the inoculated species.

Standardized lesion scoring systems for turkeys have not been established; however, the presence of intestinal lesions can be an indicator of virulence.[iv] We recommend obtaining CVM concurrence for any new virulence scoring systems before using them in a battery study. The most reliable clinical predictor of coccidial virulence in turkeys is body weight. At the conclusion of a battery study, the body weights of the infected non-medicated birds should be 30% less than the body weights of non-infected non-medicated birds to represent a clinically relevant level of virulence.

For some species of coccidia in turkeys, mortality is also important for determining virulence. In species of coccidia that typically result in mortality, 25% mortality in the infected, non-medicated birds should be expected if the disease challenge adequately mimics naturally occurring infection. We expect that the mortality will be most severe when *E. adenoeides* is the inoculated species.

6. Battery Study Design

We recommend a randomized complete block design for battery studies to account for heterogeneous environmental effects, such as temperature or humidity, which may exist within a facility. Efforts should be made to ensure homogeneous distribution of cages within each block; however, you should place the non-inoculated, non-medicated control groups in the top cages to prevent cross contamination. The cages used in the study should be identical in size. Male and female birds should be caged separately.

In battery studies, rows of cages usually form homogeneous blocks. Each cage (replication of a treatment group) is an experimental unit. You should block the battery horizontally to avoid the confounding factor of environmental differences among the rows of treated cages. You should describe the randomization procedures used to assign birds to cages, cages to blocks, and treatments to cages within a block in the protocol and final study report.

To allow for individual bird variation, we recommend replication of cages within treatments. You should consider the number of replicates per treatment relative to the virulence to ensure statistically significant differences. To accommodate the number of replicates required batteries can be connected together. You should use an adequate sample size to ensure that the study has sufficient power to detect differences among treatments. For determination of clinical significance, however, we recommend a minimum of three or four cages of each sex per treatment, with a minimum of ten to twelve birds per cage.

You should use a minimum of three treatment groups:

- non-inoculated, non-medicated control (NNC);
- inoculated, non-medicated control (INC); and
- inoculated, medicated (investigational new animal drug).

7. Variables for Evaluation of Efficacy

a. Lesion scores

In chickens, evaluate lesion scores 6 or 7 days post-inoculation in all birds. Only one person should score lesions at each study location. The lesion scoring details will differ depending on whether single or mixed *Eimeria* species are being studied. See Johnson and Reid (1970) [iii] for examples of single species and mixed species lesion scoring methodology for chickens. For deaths attributable to coccidiosis, assign the highest lesion score to the most severely affected region of the intestine.

Alternative methods of lesion scoring may be acceptable, but we recommend that you discuss them with us before conducting the battery studies. Regardless of the method chosen, you should provide a detailed description of the pathology associated with each lesion score grade in your protocol and final study report.

b. Fecal scores (dropping scores)

For turkeys, visual inspection of infected intestines (lesion scoring) for evaluating anticoccidial efficacy can prove challenging because the pathology associated with turkey *Eimeria* spp. can be subtle and is less conducive to standardized scoring. Therefore, fecal scoring is an option for use as a primary variable for evaluation of anticoccidial efficacy in turkeys in lieu of lesion scores.

Fecal scores allow for a clinically relevant qualitative assessment of the severity of the coccidial infection through an examination of the gross appearance of the feces in each battery cage dropping tray. You should assign fecal scores to indicate the severity of diarrhea using a well defined numerical scoring system (e.g., 0, 1, 2, and 3). Your protocol should include a detailed description for each numerical score to ensure consistent scoring between investigators and a description of the time post-inoculation when scoring will occur.

c. Mortality

Some species of coccidia cause significant mortality in the field. Therefore, it is important to measure mortality as an indicator of drug efficacy. You should maintain a daily record of mortality by cage and include the following information in the final report: date, cage identification, bird identification, sex, body weight, and necropsy diagnosis. You should necropsy all birds that die or are removed from a study and classify as coccidiosis or non-coccidiosis related mortalities. Perform wet mount examinations from scrapings of intestinal lesions to identify the species of coccidia. If circumstances prohibit a timely necropsy of the bird, you may freeze the bird and necropsy at a later date.

Include only coccidiosis mortalities in the statistical analysis for the evaluation of efficacy. For deaths attributable to coccidiosis, you should assign the highest lesion score to the most severely affected region of the intestine.

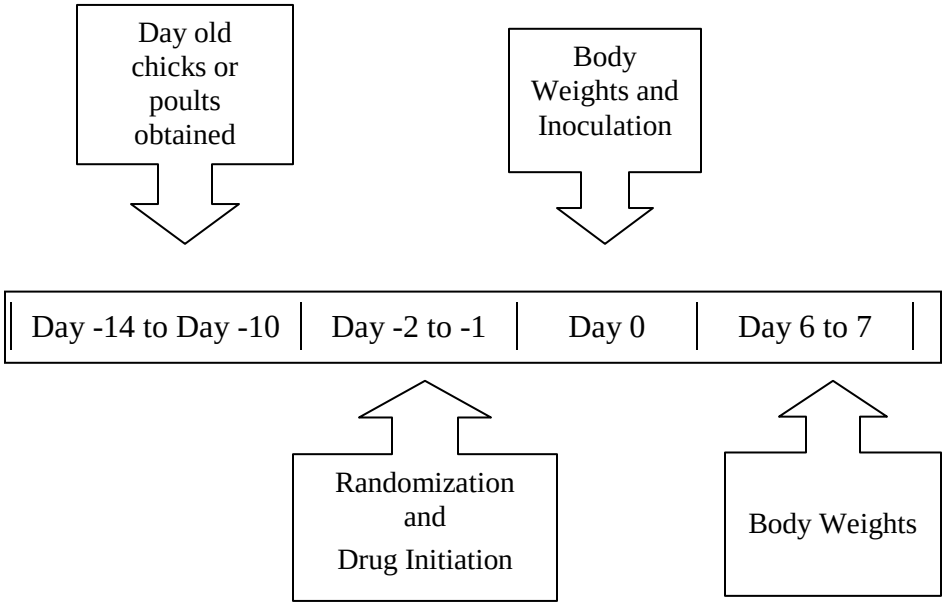
d. Body weight

Body weight change is used in battery studies as a clinical measure of the short-term effects of the drug against coccidiosis following inoculation of the target coccidial pathogen.

You should record body weights of individual birds on the following days (see Diagram 1):

1) Day 0, Inoculation (10 to 14 days of age)
2) Day 6 or 7, Lesion scoring (20 or 21 days of age)

Diagram 1. Example of timing of body weight measurements by Study Day



Body weights (initial and final) of birds that died or were culled for non-coccidiosis reasons should not be used in the analysis of the change in weight for each cage. You should calculate the change in weight for live and dead birds (coccidiosis related mortality) from the time of inoculation until the time of lesion scoring. This measurement assesses the efficacy of the anticoccidial drug on coccidial infection throughout the study period.

The average weight gain (AWG) per bird in a particular cage is the total cage gain divided by the number of birds used in calculating the total final cage weight.

$$\textbf{AWG per bird in cage} = \frac{\textbf{Total cage gain}}{\textbf{\# birds}^{1}}$$

Total cage gain = Total final cage weight[2] - Total initial cage weight[3]

[1] Number of live birds at the end of the study plus the number of birds that died of coccidiosis during the study.

[2]Total final cage weight: Sum of body weights at the end of the study plus the body weights at death of birds dying from coccidiosis.

[3]Total initial cage weight: Sum of body weights for birds that start the study minus the body weights of birds that died of non-coccidiosis related causes during the study.

The average daily weight gain (ADWG) for the treatment group equals the sum of the ADWG values for each cage divided by the number of cages in the treatment group.

$$\textbf{ADWG for treatment group} = \frac{\textbf{Sum of ADWG per cage for the treatment group}}{\textbf{\# cages}}$$

ADWG per cage = [(Final weight of bird 1 - initial weight of bird 1) + (final weight of bird 2 - initial weight of bird 2)...+ (final weight of bird N - initial weight of bird N)] ÷ [(days survived for bird 1) + (days survived for bird 2) ...+ (days survived for bird N)].

We ask that you outline the procedures used to minimize bias in the measurement of body weight in the protocol. For example, weigh treatment groups within a block in a random order to minimize any potential bias. After Day 0, you should not handle the birds until the end of the study (except for birds that die during the study).

Secondary (supportive) variables

e. Feed efficiency

Coccidiosis causes intestinal pathology that may lead to a reduction in the utilization of nutrients. Feed efficiency is used as a measure of the utilization of nutrients. Feed efficiency measured in anticoccidial drug studies is an indirect measure of overall animal health. If you use feed efficiency as a supportive variable, you should outline the procedures used in the measurement of feed consumption in your protocol. In order to minimize a potential bias,

you should weigh feed from treatment groups within a block in a random order. The removal of feeders from each cage at equal intervals is one acceptable method to weigh back feed.

Calculate feed efficiency (FE) as the ratio of the total feed consumed (FC) to the change in weight (CW) of all the birds in the entire study as follows:

$$\mathbf{FE = FC \div CW}$$

Total feed consumed is defined as the sum of all feed additions to a cage minus the sum of all feed weighed back from that cage. The change in weight (CW) during the study period is defined as the sum of final weights of all birds, including those that are alive at the end of the study, those that died of coccidiosis during the study, and those that died of non-coccidiosis causes during the study, minus the sum of the initial weights of all birds at the start of the study as follows:

CW during study = (Sum of final weights of all birds) – (Sum of initial weights of all birds)

Calculate the overall feed efficiency (OFE) of a treatment group as the mean feed efficiency of the cages within a treatment group as follows:

$$\textbf{OFE of a treatment group} = \frac{\textbf{FE cage 1 + FE cage 2 + ... FE cage N}}{\textbf{\# cages}}$$

f. Oocyst counts[4]

You may use differences in oocyst counts between treatment groups as a supportive variable. Oocyst counts can be difficult to interpret for reasons such as the following:

- Characteristics unique to a specific *Eimeria* species may affect intestinal peristalsis;
- The day of maximum oocyst shedding may vary between *Eimeria* species;
- Oocyst concentration in the excreta is not a linear function of oocyst concentration in the inoculum; and
- Excreta volume varies with many factors, including the *Eimeria* species inoculated, the severity of the resulting infection, and feed intake during the infectious period.

You should describe the methodology used to collect (including timing of collection), process, and analyze the oocysts in your protocol and final study report.

[4] Upon agreement with CVM (through protocol concurrence or a pre-submission conference agreement), oocyst counts may be considered a primary variable in minor poultry species.

8. Statistical Analysis

The final report should contain:

- Raw data in its original form;
- Raw data in an electronic format (Excel, SAS or text file);
- Program code to read raw data and create calculated variables;
- Statistical programs with documentation; and
- All statistical output (e.g., analysis results).

You should also provide a document identifying the purpose or content of each file and a document that describes variable names, abbreviations, formats, and how variables are used in the analysis.

a. Evaluation of an acceptable level of virulence

You should compare the inoculated, non-medicated control group to the non-inoculated, non-medicated control group to verify an acceptable level of virulence. The differences between these groups should be clinically relevant and statistically significant at $\alpha = 0.05$ using a two-sided test. (See section II.B.5 for a discussion of differences CVM considers clinically relevant).

b. Evaluation of efficacy

You should compare the medicated group(s) to the inoculated, non-medicated control group to establish efficacy. The differences between these groups should be clinically relevant and statistically significant at $\alpha=0.05$, two sided. The non-inoculated, non-medicated control group should not be included in the statistical analysis comparing the inoculated, non-medicated control group and the investigational treated group(s).

c. Analysis

The protocol should describe the statistical model used to analyze each primary and secondary variable. The statistical model used to analyze the data should reflect the experimental design and the level of measurement. The type of model should be based on the response variable. For example, response variables that are at the interval/ratio level of measurement can be described using a linear model; for those that are nominal/ordinal, a generalized linear model is appropriate. If a randomized complete block design is used, as recommended, then the data should be analyzed using a mixed model analysis of variance (ANOVA). The model should include block as a random effect, the main effects for treatment and sex (if sexes are included and separated by cage) and the appropriate interactions as fixed effects.

You should conduct a separate statistical analysis for each area of the intestine that is lesion scored. For all birds that die or are removed from the study due to coccidiosis, you should assign the highest lesion score to the most severely affected region of the intestine.

9. Basis of Study Conclusion

You should define the basis of your study conclusion in your protocol, prior to the start of the study using the following success criteria (See Appendix 2).

a. In order for a study to be considered in the overall determination of efficacy, the study should demonstrate an acceptable level of virulence, as described in Section B.8.a. above.

b. In order to conclude that the drug is effective, the study should meet the following success criteria:

1) For chickens and turkeys, a statistically significant (α=0.05, two sided) and clinically relevant difference in body weight gain, and mortality (if appropriate) between the group(s) treated with the investigational new animal drug and the inoculated, non-medicated control group, and;

2) For chickens, a statistically significant (α=0.05, two sided) and clinically relevant difference of at least one lesion score unit between the group(s) treated with the investigational new animal drug and the inoculated, non-medicated control group.

3) For turkeys, a statistically significant (α=0.05, two sided) and clinically relevant difference in fecal scores between the group(s) treated with the investigational new animal drug and the inoculated, non-medicated control group.

If a study does not meet the predefined success criteria, the conclusion will be that the drug is ineffective. All studies that include an acceptable level of virulence, regardless of the outcome, will be used in the overall determination of efficacy.

10. Anticoccidial Sensitivity Tests

Anticoccidial sensitivity tests (AST) are studies conducted with birds in battery cages to evaluate the effectiveness of a variety of anticoccidial drugs against a particular field isolate. Because of their study design and objectives, ASTs do not provide substantial evidence of effectiveness. However, if conducted, the results should be submitted.

C. COMMERCIAL FIELD STUDY

You should conduct a multi-location commercial-scale field study to confirm that the feeding level you have selected is effective under commercial production conditions. You should conduct this study under actual conditions of use in commercial-scale facilities, under a variety of production systems and conditions. You should use an appropriate number of geographic locations representing different environmental conditions, using a genetic line of

birds commonly used in that region, and using a ration that is representative of local commercial practices.

1. Housing

You should conduct the commercial-scale field study in housing typical for the species and class being tested. We recommend using paired houses, preferably identical and facing in the same direction. If facilities permit appropriate separation, you should divide each house into equal parts (split house design) to allow for more replicates at a study location.

You should not use artificial infection in the commercial-scale field study. Coccidia present in or on older litter should provide sufficient challenge to accurately assess the effectiveness in a commercial situation.

2. Test Animals

You should use at least two different genetic lines of birds to provide independent substantiation of effectiveness among typically used commercial birds. The birds in each location should originate from the same breeder flock source. Typically, birds are one day of age at the beginning of the study. You should rear the birds to an age that represents a marketable weight (by sex). You should consider the indication and target class when selecting the type of birds you will use in the studies.

3. Bird accountability

Bird accountability includes the number placed in each house, the number that died or were culled during the study, and the number of live birds sent to market at study end. The protocol should describe the timing, location, and procedures associated with bird accountability.

4. Feed accountability

You should document feed weights each time you add feed, make a diet change, and at the end of the study. The protocol should describe procedures for weighing the feed and appropriate procedures to minimize feed wastage and spillage.

5. Experimental Design

Because these studies are conducted under commercial conditions using a large number of birds, two treatment groups are recommended: 1) the investigational new animal drug and 2) a group treated with a currently approved anticoccidial drug or vaccine. The latter group also serves as a standard for comparison. The length of study should be consistent with the targeted birds' age and class (e.g., broilers, replacements).

6. Variables for Evaluation of Effectiveness

a. Mortality

You should report historical mortality data for the facility. You should record the number of mortalities and birds culled daily. You should diagnose the cause of mortalities whenever possible. When necessary, perform detailed necropsies to obtain accurate diagnoses. If you diagnose or suspect coccidiosis, you should select a pre-determined number of birds for lesion scoring and oocyst speciation.

If circumstances warrant ending the study prematurely (excessive mortality, natural disaster, etc), you should attempt to contact CVM as soon as possible to discuss the impact these events might have on the development plan. Adverse reactions associated with the investigational use of a new animal drug must be reported in accordance with 21 CFR § 511.1(b)(8)(ii).

b. Bird weights

You should record the final bird weights as soon after harvest as possible to avoid artifacts introduced during transit to the processing plant. Alternatively, a random sample of birds, representative of the flock, may be weighed before any pre-harvest fasting. Pre-harvest fasting includes the removal of feed and water before catching birds for transport to the processing plant. If you choose the pre-harvest weight collection method, you should provide a detailed description of the procedure in the protocol.

c. Condemnation numbers

You should collect condemnation data from all birds on each treatment. You should separate the number of condemned carcasses attributable to the farm (i.e., tuberculosis, leukosis, septicemia, toxemia, inflammatory processes, synovitis, tumors, bruises, and air sacculitis) from the number of condemned carcasses attributable to the processing plant (i.e., overscald, contamination, no viscera, and plant rejects).

d. Feed consumption

You should calculate feed consumption by subtracting the sum of the feed weighbacks from the sum of the feed additions to each house.

7. Descriptive Statistics

The following results may be used to evaluate the effectiveness of the investigational new animal drug in comparison to the group treated with the approved anticoccidial drug or vaccine: percent mortality, mean final live weight per bird, feed consumption per bird, and the feed conversion. If the split house design is used, the calculations should be made on a per-treatment basis; otherwise, the calculations should be made on a per-house basis since the entire house receives the same treatment.

% Mortality =

$$100 \times \frac{\text{(\# of birds placed at beginning of study – \# of live birds at end of study)}}{\text{(\# of birds placed at beginning of study)}}$$

$$\text{Mean final live weight per bird} = \frac{\text{Total weight of live birds at end of study}}{\text{Total number of live birds at end of study}}$$

Feed Consumption = Sum of feed additions - Sum of feed weighbacks

$$\text{Estimated feed consumption per bird} = \frac{\text{Total feed consumed}}{\text{\# of live birds at end of study}}$$

$$\text{Estimated feed conversion} = \frac{\text{Total feed consumed}}{\text{Total live weight}}$$

Means, medians, standard deviations, ranges, minimums, and maximums, where appropriate, should be calculated for the variables listed above. These should be presented in table format by treatment and location and by treatment combined across locations, where appropriate. These results will be used in the evaluation of the clinical relevance of the study. Because of the small number of experimental units, statistical testing is not appropriate for this design.

In addition, we recommend for descriptive purposes only that 95% confidence intervals on mortality be calculated assuming the binomial distribution. The binomial confidence intervals treat bird as the experimental unit and thus fail to capture the dependence among birds within houses. However, these intervals do provide a useful general summary of the precision of the estimate of mortality.

III. EFFECTIVENESS OF ANTICOCCIDIAL DRUGS FOR MINOR USES AND FOR MINOR SPECIES

The Minor Use and Minor Species Animal Health Act of 2004 (MUMS Act) amended the Act to establish new regulatory procedures that provide incentives intended to make more new animal drugs legally available to veterinarians and animal owners for the treatment of minor animal species and uncommon diseases in major animal species. Minor animal drug use includes any new animal drug for use in a minor species or a new animal drug for use in any animal species for control of an infrequently occurring or geographically limited disease.

Minor species include any species other than cattle, horses, swine, chickens, turkeys, dogs, and cats (21 U.S.C. §§ 201(nn) and (oo)). Minor avian species include game birds, semi-domestic waterfowl, and ratites. CVM's Office of Minor Use and Minor Species determines whether your application qualifies for the incentives provided under the MUMS provisions of the Act. For additional information on effectiveness requirements for anticoccidial drugs

used in minor species, see CVM's Guidance for Industry (GFI) #61: FDA Approval of New Animal Drugs for Minor Uses and for Minor Species, Final Guidance (1999).

IV. EFFECTIVENESS OF ANTICOCCIDIAL DRUGS IN FOOD-PRODUCING MAMMALS

We strongly recommend that sponsors contact CVM early in the development process, before conducting any studies in food-producing mammals. Study designs are difficult to standardize because coccidiosis is prevalent only under certain management conditions and because of differences between species and classes in the prevalence and presentation of clinical disease. Decisions on the specific study requirements, including the experimental design, appropriate effectiveness variables, and basis of study conclusions including the appropriate statistical analysis, made in consultation with CVM should greatly facilitate the development process.

A. Study requirements

Substantial evidence of effectiveness may be satisfied with dose confirmation studies analogous to battery studies, without a commercial scale field study. Commercial scale field studies are generally not necessary in mammals because dose confirmation studies are already conducted under field conditions. In addition, commercial management practices for food-producing mammals are more variable than in poultry production systems and would be difficult to standardize.

Effectiveness should be demonstrated for all *Eimeria* species included in the label indications. The species of coccidia used in the studies should be representative of the coccidial species that cause clinical disease. The test animal should be representative of the age and class of animal susceptible to coccidiosis. Justification of the coccidia species and the target animal class should be provided in the protocol. We consider mixed species infections acceptable.

B. Method of Infection

CVM prefers natural infections because they more closely reflect current field isolate characteristics. We may consider induced infections if you provide acceptable justification in your protocol for not using natural infections. If you use induced infections, the recently isolated and propagated inoculum should represent the exposure of field strains of coccidia to current anticoccidial drugs. Laboratory strains are not acceptable. You should verify coccidial infection in all test animals by an accepted method before initiating the study.

C. Experimental Design

You should use a minimum of two treatment groups: 1) infected, non-medicated control, and 2) infected, medicated (investigational new animal drug). You should include enough

replicates to make a meaningful statistical comparison. Use the standard attributes of an adequate and well-controlled study, as described in 21 CFR § 514.117.

D. Variables for Evaluation of Effectiveness

Appropriate primary effectiveness variables may include: fecal oocyst counts, fecal scores, average daily weight gain, and mortality. Clinical observations such as body condition, fecal consistency, attitude, and hydration may supplement the primary variables. CVM will consider a literature-based justification of the clinical relevance of any variables.

GLOSSARY

The following definitions are presented for use with this guidance document.

Block: A homogeneous, but extraneous (not of interest in the study) grouping of experimental units designed to account for variance related to the extraneous grouping, thus (if successful) reducing the error variance used in hypothesis testing. In a statistical model, blocks are considered a random effect.

Composite sample: A pooled sample representing samples collected from each batch of feed. The number of samples from each batch of feed included in the pooled sample should increase with increasing batch size.

Confidence interval for a population parameter: A range of values, calculated from the sample observations, which are believed, with a chosen degree of confidence, to contain the true parameter value. For a 95% confidence interval, one is 95% confident that the true value of the parameter lies within the given range. However, for a particular interval the true value of the parameter is either in the given range or not. For example, if one took repeated samples of size 100, calculated 95% confidence intervals from each sample, one expects that on the average 95 out of the 100 calculated confidence intervals would contain the true parameter.

Descriptive statistics: The mathematical calculations used to characterize the data collected in a study; at this step no statistical inferences are examined. Some common descriptive statistics are means, medians, standard deviations, ranges, minimums, maximums, and confidence intervals.

Experimental unit: An entity to which a specific treatment is applied. Each unit has an equal chance of being selected or assigned to a given treatment and units are treated and observed independently.

Masking: A procedure to reduce potential experimenter and observer bias, both conscious and unconscious, in which designated study personnel are kept uninformed of the treatment assignments.

Randomization: A procedure to reduce potential assignment bias by assigning study animals to treatment or control groups where each animal has an equally likely chance of being chosen.

Randomized complete block design: A design in which the experimental units to which the treatments are applied are separated into homogeneous groups of k experimental units, where k represents the number of treatments. Within each group of k experimental units, the units are randomly assigned so that each unit in the group receives a different treatment. The groups are called blocks and the design is called a randomized complete block design.

APPENDIX 1: CALCULATING UPPER AND LOWER SPECIFICATION LIMITS FOR NUTRIENTS

You should select a laboratory that will conduct the chemical analyses of your uniform basal diet. Once a laboratory has been selected, you should obtain the coefficient of variation (CV)[5] associated with each laboratory assay procedure. The CV, its justification, and a reference to the methodologies for each assay should be stated in the protocol.

The specification limits (lower = LSL, upper = USL) for a diet formulated to contain a theoretical (calculated) nutrient concentration is calculated as follows:

$$LSL = X - t_{(\alpha/2,\infty)} * (CV * X)$$

$$USL = X + t_{(\alpha/2,\infty)} * (CV * X)$$

where,

$t_{(\alpha/2, \infty)} = t_{(.10/2, \infty)} = 1.645$,

CV = coefficient of variation associated with the chemical assay, and

X = theoretical (calculated) nutrient concentration.

For example, the 90% specification limit for a diet formulated to contain 0.50 % dietary methionine, given a hypothetical CV of .12, would be calculated as follows:

$$LSL = 0.50\% - 1.645 * (0.12 * 0.50\%)$$

$$USL = 0.50\% + 1.645 * (0.12 * 0.50\%),$$

$$LSL = 0.50\% - 0.10\%$$

$$USL = 0.50\% + 0.10\%$$

Therefore, the calculated lower and upper specification limits for the nutrient concentrations for methionine may range from 0.40% to 0.60% .

Because the t is equal to $t_{(.10/2, \infty)}$, the CV used should be based on a large number of samples (greater than 30 samples). This CV should be obtained from the analytical laboratory that will conduct the nutrient assays. Analytical laboratories that routinely conduct nutrient analyses of feedstuffs should be able to provide you with CVs for each assay used.

For products labeled for a specific dietary nutrient concentration, the chemically determined

[5] The CV is defined as the standard deviation divided by the mean.

nutrient concentration should fall within the specification limits. When a single assay is conducted, the assay value should fall within the specification limits, as given above. Because multiple assays of a single feed sample provide additional information on the nutrient concentration, the assay values should be averaged and the specification limit should be adjusted. In this situation, you can apply the adjusted specification limit method to the data. This procedure will verify that the population from which the assay values are collected is similar with respect to the mean and variance as the population used to establish the assay coefficient of variation.

If there are multiple assays conducted on a composite sample, the specification limit should be adjusted as follows:

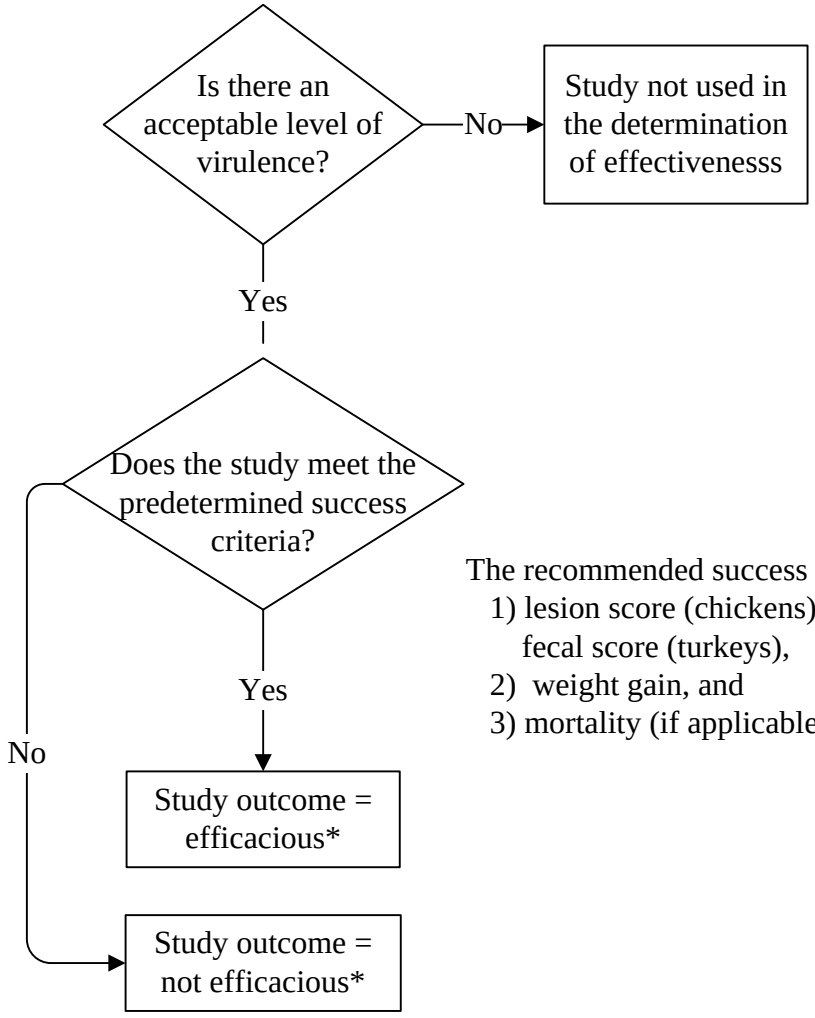
$$LSL_{adj} = X - t_{(\alpha/2,\infty)} * (CV / \sqrt{n} * X),$$
$$USL_{adj} = X + t_{(\alpha/2,\infty)} * (CV / \sqrt{n} * X),$$

where

CV = the coefficient of variation, and

$\sqrt{n}$ = the square root of the number of assays conducted on a single feed sample.

APPENDIX 2: STUDY CONCLUSION DECISION TREE



The recommended success criteria are:
1) lesion score (chickens) or fecal score (turkeys),
2) weight gain, and
3) mortality (if applicable).

*Both outcomes will be evaluated in the overall determination of effectiveness.

REFERENCES

[i] Williams, R.B., 1996. The ratio of water and food consumption of chickens and its significance in the chemotherapy of coccidiosis. *Vet. Res. Commun*. 20, 437- 447

[ii] Waletzky, E., 1970. Laboratory Anticoccidial Evaluation Trials: Review of Designs, Variables, Criteria, and Predictive Value for Field Use. *Experimental Parasitology*. 28:42-62.

[iii] Johnson, J., and W.M. Reid, 1970. Anticoccidial drugs: Lesion Scoring Techniques in Battery and Floor-Pen Experiments with Chickens. *Experimental Parasitology*. 28:30-36.

[iv] Holdsworth PA, Conway DP, McKenzie ME, Dayton AD, Chapman HD, Mathis GF et al. World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens and turkeys. *Vet Parasitol* 2004; 121(3-4):189-212.