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DRAFT GUIDELINE FOR THE EVALUATION OF THE EFFICACY OF ANTICOCCIDIAL DRUGS AND ANTICOCCIDIAL DRUG COMBINATIONS IN POULTRY

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Prepared by:
Center for Veterinary Medicine
Food and Drug Administration
7500 Standish Place
Rockville, Maryland 20855

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I. Introduction

This draft guideline addresses the conduct of studies required by 21 U.S.C. 360b(d) and 21 CFR 514.1 to demonstrate the effectiveness of new animal drugs. The draft guideline reflects principles commonly recognized by the scientific community as appropriate and necessary to collecting scientific data. A person may follow the draft guideline or may choose to follow alternate procedures. The person choosing to use alternate procedures may wish to discuss the matter further with the agency to prevent an expenditure of money and effort on activities that may later be determined to be unacceptable to FDA.

This draft guideline does not bind the agency, and it does not create or confer any rights, privileges, or benefits for or on any person. Where the guideline states a requirement imposed by statute or regulation, however, the requirement is law and its force effect are not changed in any way by virtue of its inclusion in the guideline.

The objective of this draft guideline is to provide drug sponsors and clinical investigators with guidance on how to design and execute experiments, and collect the effectiveness data necessary to obtain approval of an anticoccidial or an anticoccidial in combination with an antibiotic and/or arsenical for use in poultry feeds. The draft guideline delineates the types of studies, experimental designs and procedures that can be used to demonstrate that an anticoccidial is effective in preventing coccidial infection. The draft guideline also describes the types of studies, experimental designs and procedures that can be used to demonstrate the effectiveness of each active ingredient in an anticoccidial, antibiotic and/or arsenical combination.

The draft guideline is organized into three sections. The first section describes experimental procedures that should be implemented in studies designed to demonstrate the efficacy of an anticoccidial drug, and anticoccidial drug(s) in combination with antibiotic(s) and/or arsenical(s). This section provides direction on the development of protocols, acceptable record-keeping practices, feed preparation, nutritional content of experimental diets, assessment of drug concentration in experimental diets, and experimental procedures and design. The second section of the draft guideline discusses specific considerations for evaluating the effectiveness of anticoccidial drugs. The third section of the draft guideline describes specific considerations for evaluating the effectiveness of an anticoccidial in combination with antibiotic(s) and/or arsenical(s).

The draft guideline is not a comprehensive source of information on conducting clinical efficacy studies with poultry drugs. Drug sponsors are encouraged to consult the Federal Food, Drug and Cosmetic Act and the Code of Federal Regulations 21 CFR § 511 (investigational new animal drugs) and 21 CFR § 514 (new animal drug applications) for information on the proper shipment, use and disposition of investigational new animal drugs, and appropriate reporting of the results of clinical investigations. Drug sponsors are also encouraged to consult other CVM guidelines, e.g., *Guideline for Drug Combinations for Use in Animals*, 1983; *Preclearance Guidelines for Production Drugs*, 1975; *Guidelines for Evaluation of Effectiveness of New Animal Drugs for Use in Poultry Feeds for Pigmentation*, 1984; and *Guideline on the Conduct of Clinical Investigation: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Trials*, 1992; for additional technical guidance.

II. General Considerations

A. Protocol Development

The logical beginning of any experiment should be a clear understanding of the questions that need to be answered from the experiment. Moreover, the Center for Veterinary Medicine (CVM) believes that the time and effort put into planning an experiment should be the most rigorous and exacting component of the experimental process. Although, there is no regulatory requirement that animal drug sponsors submit protocols, the CVM strongly encourages the sponsor to submit all protocols for comment. The sponsor should allot sufficient time so that all issues concerning the experiment can be resolved, well in advance of conducting studies.

The CVM recognizes that no two products are alike. Consequently, protocols may reflect design idiosyncrasies unique to a particular drug. Regardless of the drug's uniqueness, drug efficacy should be demonstrated through a combination of battery, floor, and field trials. The sponsors are strongly urged to review this draft guideline and to incorporate these recommendations when developing protocols.

B. Record-Keeping

The integrity of the data collected and reported is a critical component in determining efficacy of the drug(s) being tested. The data should be collected and managed such that

they are valid. Valid data should have the following characteristics: 1) the data should be signed and dated by the person making the observation entry, 2) the data should be original, i.e., that it should be the first recording of the observations, 3) the data should be legible, 4) the data should be contemporaneous, and 5) the data should be accurate. Should more than one person record data, the data entries should be attributable to the person(s) collecting the information. The collection of data and maintenance of records should be done so that the CVM's and other agency personnel are able to trace data presented in the NADA to the original data, also referred to as "source" data in clinical studies.

The Center believes that the data should be collected in the following manner to maintain the characteristics of valid data. Data should only be recorded in a bound laboratory notebook, where appropriate, or to forms designated specifically for recording of a particular observation(s), i.e., not on pad or scrap paper for future transcription to an official record sheet. All data should be recorded in a permanent medium. Each original data sheet should be signed by the person making the observation and/or recording the data. Should more than one person make the observation and/or record the data, entries should be properly attributable to each person. If a mistake occurs during the actual collection or post collection period, the mistake should be corrected by a single strike-through and the new data recorded. The changes to the source data should be initialed and dated by the person making the change(s) and a statement provided as to why the change(s) were made. Should, for any reason, an original data sheet need to be recopied, all the data should be transferred (including the properly noted changes) and the reason for the recopying explained in a memorandum signed by the investigator. The original record and recopied data sheets should be retained and submitted to the NADA with a copy of the investigator's memorandum. If data are captured electronically, proper system controls should be employed to ensure that the data are valid. Additional information on investigator record-keeping and record-retention can be found in the *Guideline on the Conduct of Clinical Investigation: Responsibilities of Clinical Investigators and Monitors for Investigational New Animal Drug Trials* (1992).

C. Drug Assays

The concentrations of approved drugs in experimental feeds are to conform to the assay limits set forth in 21 CFR § 558.4. For approved drugs for which assay limits have not been codified in 21 CFR § 558.4, refer to the *Federal Register* notice of approval for assay limits. For unapproved new drugs for which assay limits have not been codified, levels should

conform to a permissible analytical variation (PAV) derived through the method validation process. Copies of all original analytical assay records should be submitted to the NADA. The "Certificate of Analysis" (COA) for each lot of the Type A medicated article used in the study should be submitted to the NADA.

A permissible analytical variation relates to a single drug assay value. Under the proper mixing conditions, using the correct quantity of the Type A medicated article, a single assay of a feed sample should fall within the limits of the PAV. If an assay value does not fall within the limits of the PAV, the sponsor should scientifically justify the omission of the assay value in determining the drug concentration in the feed.

The sponsor should indicate in the protocol the number of assays that will be conducted on each feed sample. If multiple assays of a single feed sample are performed, the CVM will allow one assay value outside the PAV limit. Because multiple assays of a single feed sample provide additional information on the drug concentration, all assay values should be averaged and the PAV adjusted. The adjusted PAV should be calculated as follows:

$$Adjusted\ PAV = \frac{PAV - 5\%}{\sqrt{n}} + 5\%$$

where,

PAV = permitted assay variation established through the method validation process (assay limits for approved drugs are codified in 21 CFR § 558.4), and

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

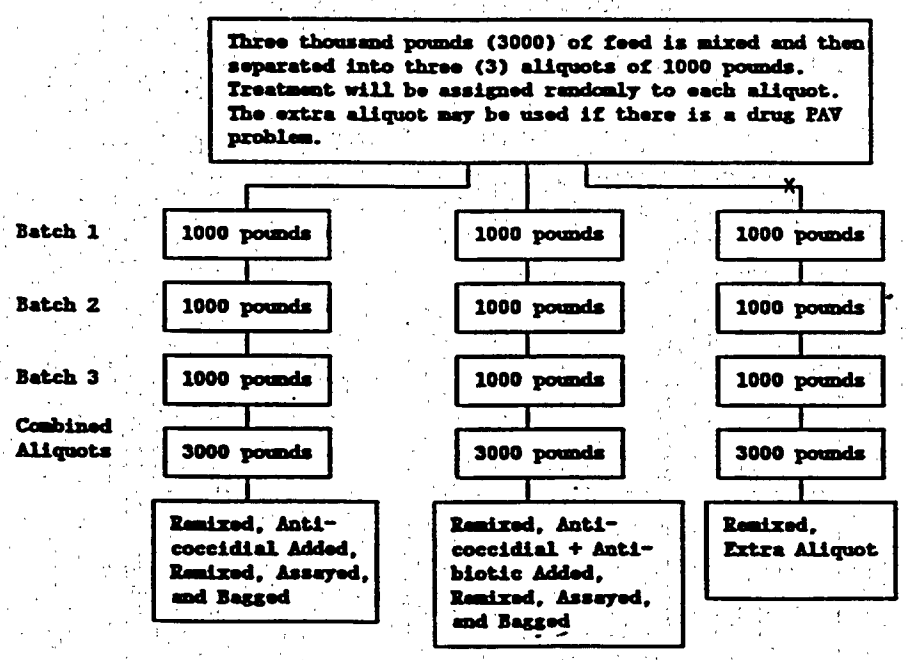
The sponsor should calculate the adjusted PAV and construct new assay limits. The average of the assay values should fall within the new assay limits. This is necessary because the PAV concept is predicated on a single assay value. This procedure will verify that the population from which the assay values are collected is similar with respect to the mean and variance as the population used to establish the assay coefficient of variation.

D. Nutritional Content and Preparation of Experimental Diets

The CVM desires that the experimental data be collected on animals consuming nutritionally adequate diets so that the observed responses are attributable to the drug(s) rather than a possible drug-nutrient interaction. Nutrient recommendations for poultry, published by the National Research Council (1984), can serve as a reference for formulating diets. As an alternative, diets may be formulated to meet predominant commercial practices for the species and class of animal being fed. These practices should be supported by agricultural survey data, e.g., Agrimetrics and Agri-Tech.

Experimental rations should be prepared from an uniform basal diet. The uniform basal diet is consistent with regard to nutrient densities and ingredients. If the uniform basal diet is derived from several mixer batches, the diet corresponding to each treatment group should be composed of aliquots from each mixer batch. For example, to prepare 3000 pounds of an uniform basal diet for an anticoccidial and anticoccidial + antibiotic drug combination study, a mixer with a minimum capacity of 3000 pounds is needed (Figure 1).

Figure 1. Procedures for Construction of an Uniform Basal Ration for a Two Drug Combination Study



When individual batches are to be fed separately then each batch should be equally divided among treatment groups. All treatments should be switched from batch 1 to batch 2 feed, from batch 2 to batch 3 feed, etc., at the same time. Also, the experimental diets should be switched at the same time across treatment groups, e.g., from starter to grower feeds. Additionally, diets used in related battery and floor pen studies should contain similar nutrient densities. The procedures that will be used to prepare an uniform basal diet and collect feed samples should be described in the protocol.

Proper mixing of feedstuffs and additives will ensure the uniform dispersal of nutrients and drug(s) in finished feeds. The sponsor and clinical investigator should be cognizant of the performance and capabilities of the feed mixer used to prepare the experimental diets. One method that can be used to determine mixer performance is to measure the time needed for mixer performance assays to be either the lesser of a ten percent (or less) coefficient of variation, or two times the analytical variation of the selected assay (Behnke, 1991; Wicker and Poole, 1991). Each investigator should develop a Standard Operating Procedure (S.O.P.) to prevent contamination of experimental feeds.

The feed form (pellet, crumble, or mash) of the experimental diet should reflect the predominant conditions of use. It is important to conduct these studies using the predominant feed form because the milling process may affect the efficacy of the drug(s). Pelleted feeds are subjected to temperatures ranging from 170 to 200 °F, which may affect the stability of the drug(s) and microbiological profile of the feed.

To demonstrate the nutritional adequacy of the experimental diet(s), the sponsor should include in the protocol a complete and detailed list, including amounts therein, of all feed ingredients and vitamin/mineral premixes used in the uniform basal diet. Ingredients should be representative of feedstuffs commonly used in each geographical location. At a minimum, the calculated nutrient levels of the uniform basal diet should be reported for crude protein, methionine, cystine, lysine, calcium, phosphorus (total and available), and metabolizable energy.

To ensure that the experimental animals receive the proper nutrient densities in the diet, chemical analysis should be performed on the uniform basal diet. A composite sample from the uniform basal diet, which is representative of the diet, should be assayed for the following: methionine, cystine, lysine, calcium, and phosphorus. The exact number of assay replicates should be specified in the protocol. Proximate analysis should be conducted

on the composite sample. The sponsor should indicate whether analyses are reported on an as-fed or dry-matter basis.

The purpose of conducting chemical analyses is to provide additional assurance that the diets are within the formulated values specified in the protocol. The CVM recommends using a 90% confidence interval approach about the nutrient's calculated value to determine the adequacy of the diet. The CVM considers the confidence interval approach to be a means by which the sponsor and investigator can be assured that the diets are nutritionally adequate and acceptable to the CVM for the nutrients assayed. Nutrient concentrations that fall outside the confidence interval are <u>not</u> necessarily unsatisfactory to the CVM. The CVM will consider the magnitude of the deviation from the confidence interval and nutritional circumstances when the adequacy of the diet is determined.

The confidence interval approach can be applied in the following manner. The sponsor should select a laboratory that will conduct the chemical analyses of the uniform basal diet. Once a laboratory has been selected, the sponsor should obtain the coefficient of variation associated with each laboratory assay procedure. The coefficient of variation and a reference to the methodologies for each assay should be stated in the protocol.

The 90% confidence interval (CI) for a diet formulated to contain a theoretical (calculated) nutrient concentration is calculated as follows:

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

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, a CI for a diet formulated to contain 0.50% dietary methionine, given a hypothetical coefficient of variation of 12%, would be calculated as follows:

$$CI = 0.50\% \text{ Methionine} \pm 1.645 * (0.12 * 0.50) \text{ and}$$
$$CI = 0.50\% \text{ Methionine} \pm 0.10\%.$$

Therefore, nutrient concentrations for methionine may range from 0.40 to 0.60% and will be acceptable to the CVM. Because the t is equal to $t_{(.10/2,\infty)}$, the coefficient of variation used should be based on a large number of samples (greater than 120 samples).

For products labeled for a specific dietary nutrient concentration, the chemically determined nutrient concentration should fall within the confidence interval. When a single assay is conducted, the assay value should fall within the confidence interval, 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 CI should be adjusted. This is necessary because the CI concept is predicated on a single assay value. In this situation, the sponsor can apply the adjusted CI 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. The coefficient of variation used in the confidence interval should be adjusted as follows:

$$\text{Adjusted CV} = \text{CV} / \sqrt{n},$$

where,

CV = the coefficient of variation, and

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

E. General Experimental Procedures

All personnel involved with the investigation should have adequate scientific training and experience with the species used in the experiments. The personnel responsible for the day-to-day management of the animals and for making and recording observations should be blinded to the experimental treatments. The blinding procedures should be described in the protocol.

Studies should be conducted using the target animal for which the drug is intended. The birds used should be from contemporary, commercial genotypes. Healthy chicks or poults should be used in the experiments. The source of the chicks or poults should be documented. The age and health status of the breeder flocks should also be described.

Stresses outside the scope of the experiment should be minimized. Birds should be handled gently at all times. Birds may be removed from an experiment only to alleviate suffering due to a diagnosed disease or injury. Necropsy results should be reported on birds removed from the study. No concomitant drug therapy should be used on any bird or pen during the experiment. Use of concomitant drug therapy can influence the test drug response and/or mask adverse drug reactions.

Vaccination programs, if warranted, should be designed to protect the birds against prevalent infectious disease(s), yet not debilitate the birds or otherwise compromise the experiment. Information concerning any vaccination program should be provided, i.e., method (eye, spray or water), date of vaccination, age of the bird at vaccination, source, lot number, type of vaccine (live, modified live or killed organisms), handling of vaccine, expiration date and any other information pertaining to the vaccine(s).

Water and feed should be provided ad libitum. Feeders should be maintained in such a way as to minimize feed wastage. Final disposition of dead birds, live birds at the end of the study, and remaining medicated feed and premix should be reported in the NADA.

The protocol should include a description of a method that ensures the accuracy of the number of birds placed in each pen/cage at the start of the experiment. Extra birds should not be placed within each pen/cage for removal during the study. Also, birds should not be replaced during the study. The method of sexing chicks or poults should be identified, i.e., feather or vent sexing. If on the final weigh day the gender of a bird cannot be determined, the bird's gender should be determined by postmortem examination. The protocol should include a method that provides for: 1) accountability of the birds, 2) verifying the randomization of birds to pens, 3) controlling bird/pen mixups, 4) detecting migration or misplacement of birds, 5) preserving the identification of the original gender, and 6) allowing pre-selection and predesignation of birds for scoring of lesions. The CVM believes that wing-banding is a method that accomplishes all the objectives listed above; however, the CVM will consider alternative methods of identification.

The available pen square footage for experimental birds should reflect commercial practices in the geographical location of the study, variation in seasonal temperatures and the final weight of the birds. The number of birds per feeder or waterer should be indicative of commercial practices. In a field trial or non-challenge study, the birds should be reared to a constant age that represents a marketable weight.

Under commercial grow-out, turkey poults are reared sex-separately and managed accordingly. Thus, in order to simulate commercial management conditions, toms and hens should be reared in separate pens.

Birds should be observed with adequate frequency to appropriately manage the experiment and to collect dead birds for necropsy prior to decomposition. Frequent visits to the facility will allow the investigator to obtain clinical and management observations, e.g., lesion scores, feathering characteristics, abnormal excreta, or behavior.

Procedures used in the measurement of body weight and feed consumption should be developed to minimize bias. In order to minimize a potential bias, treatment groups within a block should be weighed in a random order. For example, if a single treatment group is the first to be weighed in each block, then a possible artificial treatment difference (bias) between treated and control birds can occur. One acceptable procedure used to weighback feed would be to remove feeders from each pen at equal intervals. Other weighing procedures can adequately address this issue. In any event, the weighing procedure should be outlined in the protocol. The CVM does not condone the collection of intermediate body weights, as this procedure may also introduce bias.

Drug combinations are considered as a single entity and assigned a single withdrawal period. Because the withdrawal period is established for the combination, all drugs within the combination should be removed from the feed for the labeled withdrawal period. All studies should be conducted with the labeled withdrawal period.

F. Experimental Design

Randomized complete block designs are typically utilized when conditions suggest that heterogenous environmental effects may exist within a facility. The block should be homogeneous with respect to its environment. In battery facilities, tiers typically form the blocks. In floor pen studies, contiguous pens typically form the blocks. The use of other designs may be acceptable if the sponsor can document their appropriateness. The randomization procedures used to assign birds to pens/cages, and treatments to pens/cages within a block, should be described in the protocol.

The protocols should include diagram(s) of the buildings and identify the blocking and treatment allocation schemes used. These diagram(s) should be specific for each location and include the following: orientation of the building, type of building, ventilation and lighting systems, pen/cage dimensions, and placement of the feeder and waterer within the pen/cage.

The number of studies that will be conducted should be specified in the protocol prior to initiation of any experimentation. For field trials and non-challenged floor pen studies, a minimum of three replicates (representing three different geographical locations) should be conducted. If additional studies are conducted because of a lack of statistical significance in efficacy determination, the probability levels should be adjusted appropriately using methods such as those used by O'Brien and Fleming (1979). When more than three studies have been specified in a protocol, then the declared number of studies should be conducted prior to evaluating the data. Data should not be evaluated until all proposed efficacy studies are completed unless an adverse reaction(s) is observed.

G. Investigator Reports for Clinical Study(ies) and Field Trial(s)

Safety Report

An investigator should immediately report to the sponsor any adverse effect that is observed during the study. In this context, adverse effects are not limited to those directly related to human, animal, or environmental safety, but also include any deviations from expected performance or behavior patterns of the animals used in the study.

Communication Report

An investigator should also maintain an accurate record of all visitors to the study and of all telephone conversations concerning the study. This record should include, but not be limited to, visitations or teleconferences with the study monitor or other representatives of the sponsor and with officers and employees of FDA. This record should also include the time and date of the visit or telephone conversation and its purpose; the name, title, and organizational affiliation of the individual(s); and, a detailed and factual summary of the visit or telephone conversation. An investigator should also maintain a copy of all written correspondence, guidance, and instructions concerning the study.

Study Report

An investigator should prepare and submit to the sponsor upon completion of each of the investigator's study(s) a signed and dated, detailed, independent report that evaluates all observations made during the study. The first report submitted to the sponsor is the investigator's "final" report. Any and all subsequent changes to the report should be considered as amendments. The final report should include, but not be limited to, the following.

1) The name, physical location, and mailing address of the investigator and the specific facility(ies) where each study was performed.

2) The dates on which the study was initiated and completed.

3) The objectives and procedures stated in the study protocol, which should include any changes from the original protocol. A description of all the management practices used, which should include a record of all observations. Sufficient detail should be provided to allow reconstruction or replication of the study.

4) The number, species, breed or stock, source of supply, sex, size, age, physiological state, and disease state (if any) or other pertinent pathological findings of the animals used should be specified. The identification procedures and disposition records for each animal should be provided.

5) The name, identity, strength, purity, composition, quantity, and batch or code mark should be provided for the new animal drug. A description of the dosage, dosage regimen, route of administration, and duration of the treatment should be given. Records of the disposition of the unused new animal drug or animal feeds bearing or containing the new animal drug.

6) Records of all mixing or further dilution of the drug, and results of the drug assays in the feed.

7) All adverse reactions observed during the study.

8) A description of all circumstances that may have affected the quality or integrity of the data. This should include specifying the time frame and the extent of their occurrences.

9) The name of the study monitor, associates, colleagues, and employees involved, and the nature and extent of their participation.

10) The location of all original copies of source data, specimens, samples, and study records should be reported.

11) A statement attesting to the accuracy and completeness of the data, which should include a statement acknowledging that the data were collected in compliance with the Federal Food, Drug, and Cosmetic Act.

12) A copy of all source data.

Report Amendments

Any corrections or additions to the study report should be in the form of an amendment by the investigator. The amendment should clearly identify that part of the study report that is being added, deleted, or corrected and the reasons for the addition, deletion, or correction. The report should be signed and dated by the investigator and by any other individual who requested the amendment.

Retention of Reports

The investigator should maintain in the study records an accurate and complete copy of any verbal or written report generated during or after the study which include, but should not be limited to, the final report and all amendments.

III. Specific Considerations for Evaluating the Efficacy of Anticoccidial Drugs

A. Overview

The objective of clinical investigations with anticoccidial drugs is to identify a dose or dose range of the drug which prevents the infection by the species of coccidia identified/listed on the label. To accomplish this objective, the effectiveness of the anticoccidial drug should be evaluated in a specific sequence of experiments that progresses from well-controlled dose determination studies to commercial-scale field trials.

The information obtained from the initial phase of efficacy evaluation is used to quantify an effective dose or dose range that prevents an infection by each of the species of coccidia specified on the label. The effective dose(s) should be established by evaluating the efficacy of the drug at several levels for each coccidia species. The virulence of each species used in the studies should be characterized to ensure the pathogenicity of the coccidia species. The experimental subjects should be challenged with sufficient quantities of each coccidia species to ensure that the disease is manifested. Dose determination studies are typically conducted in battery cages so that the experimental subjects are exposed to an appropriate level of the disease agent and to provide adequate replication.

The objective of the second phase of efficacy evaluation is to confirm the effective dose or dose range under conditions simulating natural infection. Dose confirmation studies (floor-pen challenge studies) are typically conducted in floor-pens. The adequacy of information gathered during the dose confirmation phase is predicated upon acceptable dose determination studies. The predicted effective dose level(s) should prevent coccidiosis under a simulated natural infection. Lack of confirmation of the effective dose(s) would require reassessment of the predicted dose or dose range. The successful confirmation of the effective dose(s) would permit progression to the final stage of efficacy evaluation.

The final stage of efficacy evaluation is to determine whether the drug level(s) is(are) effective under naturally occurring field infections. These studies should be conducted under actual use conditions in commercial facilities. Studies should be conducted under a variety of production systems and conditions.

B. Anticoccidials for Prophylactic Use

1. Battery Studies

Experimental Design

Chicks and poults should be inoculated at an age when birds are susceptible to coccidia challenge. The CVM believes that two weeks is an appropriate age to inoculate broiler chicks and poults. Birds should be individually identified, e.g., wing-banded. Battery studies should be conducted using both genders unless specific evidence is provided that demonstrates that a gender-drug interaction does not exist. Caging male and female birds separately is preferred.

The administration of medicated feed and oocyst inoculation may be initiated concurrently. However, if the drug exerts its anticoccidial action during the initial stages of coccidial infection, the medicated feed may be administered no more than two days prior to oocyst inoculation. Special consideration should be given to prevent cross-contamination of different medicated feeds. The CVM prefers that the experimental diets be fed in crumble form; however, mash feeds may be acceptable.

Special consideration should be given to the allotment of treatments within the battery. Depending upon the physical design of the battery cages, placing non-infected, non-medicated control groups in the uppermost battery tier may be necessary to prevent coccidial contamination of these experiment subjects. A sanitation and bio-security program should be adopted to prevent the inadvertent introduction of pathogens. Uniform lighting should be established within a block, utilizing typical commercial photoperiods.

Inoculum

The inoculum is a critical factor in the validation of the disease model used in these studies. The use of contemporary inocula will reflect the susceptibility or tolerance of the oocysts to current therapy conventions. Drug tolerance and resistance are well documented occurrences and are a serious limitation on the effectiveness of the anticoccidials. Acquired drug resistance of coccidia strains dictates that the following inoculum procedures be used.

Sporulated oocysts from recent field isolates that are fewer than three years old and that have been exposed to contemporary anticoccidial drugs should be used to propagate the inoculums. Laboratory strains are not acceptable. The strains should originate from different geographical areas. The history of the isolates, e.g., where and when they were isolated, the identity of the drug(s) in the feed at the time of the outbreak, and the predominant species involved, should be reported. Single cell isolations are not required. The isolates should be passed into susceptible birds, cultured, and oocysts collected at appropriate times to produce the inoculum. Virulence studies should be conducted for each Eimeria species in a separate battery. Studies for each species do not have to be conducted concurrently. The use of inoculums over 6 months of age is not recommended. Inoculums of E. mitis over 3 months of age should not be used experimentally without recent titrations. A complete description of the sporulation method should be included. The inoculum should be administered via oral gavage.

Virulence studies should be performed prior to conducting the dose determination studies. Virulence titration studies should include a non-infected control group and three groups given non-zero doses of oocysts. This will allow prediction of the number of oocysts that should be given in the subsequent studies in order to induce an acceptable coccidial infection.

The virulence of the coccidia can be characterized by increase intestinal lesion scores, depression in rate of weight gain and an increase in mortality (Waletzky, 1970). The importance of each criteria depends on the species of coccidia being evaluated. For E. tenella and E. necatrix, an acceptable level of virulence can be characterized by a significant (α=.05) 25% increase in mortality, a significant (α=.05) 20% reduction in rate of weight gain, and a significant (α=.05) 2.5 unit increase in lesion scores over the non-infected control. For E. brunetti and E. maxima, an acceptable level of virulence can be characterized by a significant (α=.05) 10% increase in mortality, a significant (α=.05) 20% reduction in rate of weight gain, and a significant (α=.05) 2.5 unit increase in lesion scores over the non-infected control. For E. mitis and E. acervulina, an acceptable level of virulence will be characterized by a significant (α=.05) 20% reduction in weight gain and a significant (α=.05) 2.5 unit increase in lesion scores over the non-infected control.

For turkeys, the most appropriate predictor of strain virulence is body weight gain. A significant (α=.05) 30 to 40% reduction in body weight gain in infected groups, relative to the non-infected group, will be considered evidence of virulence (E. meleagrimitis, E. gallopavonis, and E. adenoeides). Lesion scores are considered poor predictors of strain virulence.

Dose Determination Studies

Dose determination battery studies should be conducted using each Eimeria species, separately. The objective of these studies is to determine the effective dose or dose range for preventing infection by each Eimeria species. Studies for each species do not have to be conducted concurrently. Following dose determination studies using each Eimeria species, the predicted dose or dose range should be used in a battery study with a mixture of the Eimeria species specified on the label. The objective is to evaluate the dose(s) under maximum challenge. If the predicted dose(s) adequately prevents coccidiosis then the evaluation of the drug may proceed to the floor pen stage. If the dose(s) does not adequately prevent coccidiosis then the dose or dose range should be reassessed.

Two types of dose determination studies should be conducted with each inoculum, i.e., with drug doses that are geometrically and arithmetically spaced. The studies that utilize geometrically spaced doses are conducted to identify a narrower dose range that will be characterized further in studies that utilize arithmetically spaced doses. The studies utilizing arithmetically spaced doses are conducted to identify the optimum dose or dose range. In each type of study, there should be two non-medicated control groups: 1) a non-infected group should be used to verify that no other pathogens have biased the experimental results, and 2) an infected group should serve as the true experimental control. The studies utilizing geometrically spaced doses should contain a minimum of four infected, medicated groups. The studies that utilize arithmetically spaced doses should contain a minimum of three infected, medicated groups. Within 6 to 7 days post-inoculation, apparent coccidiosis infection should be demonstrated in the infected control group while the therapeutic effect of an anticoccidial drug should be demonstrated in the medicated groups. All variables, e.g., mortality and morbidity, lesion scores, feed consumption, and body weight, should be measured at 6 to 7 days post-inoculation.

All mortality and morbidity, whether resulting from coccidiosis or other causes, should be diagnosed by appropriate tests. Necropsy results should be reported. For diagnosed coccidiosis, wet mount examinations of specific gut regions should be made and coccidia speciated. Body weight, date of death, and gender of dead birds should be recorded.

Body weight of individual birds should be recorded on the following days of age:

1) day 12 of age (drug initiation day),

2) day 14 of age (inoculation day; may also be drug initiation day), and

3) day 20 or day 21 of age (6 or 7 days post inoculation; depending on the date that lesions are scored).

Weight gain should be calculated for live and dead birds for the following periods:

1) from days 12 to 14 of age (this measurement identifies the effects of the anticoccidial drug alone),

2) from days 14 to 20 or 21 of age (this measurement assesses the effectiveness of the anticoccidial drug on infection), and

3) from days 12 to 20 or 21 of age (this measurement assesses the effectiveness of the anticoccidial drug on infection throughout the experimental period).

Feed consumption should be measured concurrently with body weight gain.

In chickens, lesion scores should be evaluated 6 or 7 days post-inoculation in pre-selected birds using the method of Johnson and Reid (1970). A difference of one lesion score unit between infected treatment groups will ordinarily be deemed biologically significant. The CVM does not believe that lesion scores are an acceptable measure of coccidial damage in turkeys. However, the CVM will review the merits of any protocol that can adequately justify lesion scoring as an effective measure of coccidiosis in turkeys.

Oocyst counts can be difficult to interpret for a number of reasons. Intestinal peristalsis can be affected by characteristics unique to a specific Eimeria species. For example, E. necatrix inhibits gut motility, delaying oocyst appearance in the excreta. The day of maximum oocyst shedding varies between Eimeria species. Oocyst concentration in the excreta is not a linear function of oocyst concentration in the inoculum. Excreta volume varies with many factors, including the Eimeria species inoculated, the severity of the resulting infection, and feed intake during the infectious period. If oocyst concentrations in the excreta are used, the CVM prefers that the investigator measure the total oocyst counts over a several day collection period. In addition, relatively low oocyst numbers should be inoculated to prevent an asymptotic dose-response to increasing numbers of oocysts. If the entire intestinal tract is homogenized to extract oocysts, then the method should be described in the protocol.

2. Design for Floor Pen Challenge Studies

As stated previously, the objective of the second phase of efficacy evaluation is to confirm the effective dose or dose range under simulated use conditions. For chickens, equal proportions of male and female birds should be reared together. Turkey poults are commercially reared sex-separate; therefore, sex-separate pens are recommended. Birds should be reared on new litter because the used litter from previous studies may bias the current study.

Inoculum and Oocyst Exposure

Refer to **Section III.**, ***Specific Considerations for Evaluating the Efficacy of Anticoccidial Drugs***, under **B., Battery Studies**, for a description of age and strain virulence of the inocula that should be used. Mixed-species inoculation experiments should be conducted. Prior to conducting the floor pen study, virulence titration studies need to be conducted with the mixed inoculum. For chickens, the inoculum should contain all Eimeria species that are on the label. For turkeys, the mixed inoculum should contain Eimeria meleagrimitis, E. gallopavonis, and E. adenoeides.

Chicks and poults should be exposed to a sufficient number of oocysts at two weeks of age to induce coccidiosis. Numerous techniques are available to uniformly expose chicks or poults to oocysts. Birds can be infected by inoculation of the feed and water, by broadcasting the oocysts into the litter, or by utilizing seeder birds. When using seeder birds, special attention should be given to ensure that oocyst shedding from E. acervulina does not dominate the infection. The medicated feed and oocyst inoculation can be administered concurrently. However, if the drug exerts its anticoccidial action during the initial stages of coccidial infection, the medicated feed should not be administered more than two days prior to oocyst inoculation. The method of exposure should be indicated in the protocol.

Experimental Groups

A minimum of two experimental groups should be represented in the floor pen study: 1) an infected, non-medicated group and 2) an infected, medicated group. If the product is to be labeled with a dose range, then a minimum of two infected, medicated groups should be used in the floor pen study, i.e., the lowest and highest dose from the dose range.

Dependent Variables

All mortality and morbidity, whether resulting from coccidiosis or other causes, should be diagnosed for cause by appropriate tests. For diagnosed coccidiosis, wet mount examinations should be made and coccidia speciated. The body weight, date of removal, and gender of dead birds should be recorded.

Birds in a pen should be assigned randomly a priority number for lesion scoring at placement. A minimum of 10% of each gender, from each pen, should be lesion scored. Birds with the highest priority numbers should be weighed, euthanized, and scored for lesions.

Body weights should be recorded on the following days of age:

1) day 1 of age (day of placement),

2) day 12 of age (drug initiation day),

3) day 14 of age (inoculation day; may also be drug initiation day),

4) day 20 or day 23 of age (6 or 9 days post inoculation), and

5) study termination (marketable condition).

Weight gain should be calculated for the following periods:

1) from day 1 of age (day of placement) to drug initiation day and/or drug initiation/inoculation day,

2) from day of inoculation and drug initiation to day of lesion scoring, and

3) from day 1 of age (day of placement) to study termination, and

4) from drug initiation day and/or drug initiation/inoculation day to study termination.

Individual body weights should be taken on all birds removed from the study. Feed consumption should be measured concurrently with body weight. Feed efficiency adjusted

for dead and/or cull birds should be calculated. The adjustment should be calculated by adding the weight of the dead and/or culled birds to that of the live birds.

3. Design of Field Trials

Field trials should be conducted in identical, paired houses. Birds in each house should originate from the same breeder flocks. Field trials should be conducted in at least three different geographical locations. The CVM recommends that these trials utilize at least two different stocks of birds and be conducted in different environmental conditions, e.g., high relative humidity and ambient temperature, among the three locations. Artificial oocyst infection should not be used in field trials. Typically, two treatment groups are used in these trials: 1) the experimental drug dose and 2) a positive control that is a currently approved anticoccidial. Because field trials are conducted on a commercial scale, they are generally exempted from the requirements that apply to battery and floor pen studies with the exceptions of record keeping, randomization procedures, drug assays and the investigator report.

All mortality should be recorded. Historical mortality data for the facility should be reported. Birds from each house should be slaughtered to enable condemnation data (Condemnation Certificates) to be collected on each treatment and ultimately submitted to the NADA. Adverse reactions are to be reported in accordance with 21 CFR § 511.1(b)(8)(ii).

C. Anticoccidials for Therapeutic Use

Anticoccidials proposed for approval with a therapeutic claim, i.e., treatment of an existing coccidial outbreak, should be evaluated in a manner similar to prophylactic use from the point of view of experimental design, the major difference is that to evaluate a therapeutic claim, the drug should be administered no sooner than 72 hours following the inoculation with coccidia. The sponsor may conduct time titration studies.

IV. Specific Consideration for Evaluating the Efficacy of Combinations of Anticoccidial with Antibiotics and/or Arsenicals

A. Overview

Anticoccidials are typically fed in combination with antibiotics and/or arsenicals. The claims associated with antibiotics can be classified as production (growth promoting) or therapeutic in nature. In either case, the efficacy of each drug should be demonstrated in the presence of the other. Pursuant to 21 CFR § 514.1(b)(8)(v), "each ingredient designated as active in any new animal drug combination must make a contribution to the effect in the manner claimed or suggested in the labeling, and, if in the absence of express labeling claims of advantages for the combination such a product purports to be better than either component alone, it must be established that the new animal drug has that purported effectiveness."

The efficacy of an anticoccidial with growth promoting drug(s) is established in battery and floor pen studies. Battery studies are conducted to demonstrate the efficacy of the anticoccidial in the presence of the growth promoting drug(s). The efficacy of growth promoting drug(s), in the presence of the anticoccidial, is established in floor pen studies. For a combination of an anticoccidial and an antibiotic with therapeutic claims, the efficacy of each drug is established in battery studies.

B. Battery Studies

An Anticoccidial in Combination with an Antibiotic with a Therapeutic Claim

In general, the procedures used to evaluate the combination of an anticoccidial and antibiotic(s) with a therapeutic claim are similar to those outlined in Section III., *Specific Considerations for Evaluating the Efficacy of Anticoccidial Drugs*, under B., Battery Studies. The sponsor should be aware that there are differences in the procedures used, the treatment groups required, and the statistical comparisons. These differences are outlined below.

Virulence studies are not required for the disease agent, e.g., E. coli and C. perfringens, for which the antibiotic is labeled. However, the validation of the disease model is determined within the context of the battery study. The validity of the disease model is predicated upon fulfilling the following three criteria: 1) positive cultures of the disease organism, 2) multiple organ involvement, and 3) a significant (α=.05) increase in mortality inherent to the disease.

For the combination of an anticoccidial with prevention of coccidiosis claims and an antibiotic with a therapeutic claim, the efficacy of the anticoccidial should be conducted in the presence of the antibiotic. Battery studies should be conducted for each Eimeria species and the mixed species inoculum. The treatment groups should be the following:

1) non-infected, non-medicated control,

2) infected coccidia, non-medicated control,

3) infected coccidia, anticoccidial (lowest approved dose),

4) infected coccidia, antibiotic (highest approved dose), and

5) infected coccidia, anticoccidial (lowest approved dose) + antibiotic (highest approved dose).

The following comparisons should be performed among the treatment groups.

Comparisons	Purpose
non-infected, non-medicated control vs. infected coccidia, non-medicated control	This comparison determines whether an infection has occurred. Once this has been established the non-infected, non-medicated control group should be excluded from any subsequent analysis.
infected coccidia, non-medicated control vs. infected coccidia, anticoccidial + antibiotic	This comparison demonstrates whether the combination (anticoccidial + antibiotic) is efficacious in preventing coccidiosis.
infected coccidia, anticoccidial vs. infected coccidia, anticoccidial + antibiotic	This test determines whether the effectiveness of the anticoccidial is diminished in the presence of the antibiotic. If the effectiveness of the anticoccidial is reduced a label caution is warranted.
infected coccidia, antibiotic vs. infected coccidia, anticoccidial + antibiotic	This comparison is used to determine if the anticoccidial is effective in the presence of the antibiotic.

For the combination of an anticoccidial with prevention of coccidiosis claims and an antibiotic with a therapeutic claim, the efficacy of the antibiotic should be evaluated in the presence of the anticoccidial. A battery study should be conducted to establish the efficacy of the antibiotic in the presence of the anticoccidial. The infective agent, e.g., E. coli or C. perfringens, used in this battery study should be the organism(s) for which the therapeutic antibiotic is labeled. The treatment groups should be the following:

1) non-infected, non-medicated control,

2) infected bacteria, non-medicated control,

3) infected bacteria, antibiotic (lowest approved dose), and

4) infected bacteria, anticoccidial (highest approved dose),

5) infected bacteria, anticoccidial (highest approved dose) + antibiotic (lowest approved dose).

The following comparisons should be performed among the treatment groups.

Comparisons	Purpose
non-infected, non-medicated control vs. infected bacteria, non-medicated control	This comparison determines whether an infection has occurred. Once this has been established the non-infected, non-medicated control group should be excluded from any subsequent analysis.
infected bacteria, non-medicated control vs. infected bacteria, anticoccidial + antibiotic	This comparison demonstrates whether the combination (anticoccidial + antibiotic) is efficacious in treating a bacterial disease.
infected bacteria, antibiotic vs. infected bacteria, anticoccidial + antibiotic	This test determines if the effectiveness of the antibiotic is diminished in the presence of the anticoccidial. If the effectiveness of the antibiotic is reduced a label caution is warranted.
infected bacteria, anticoccidial vs. infected bacteria, anticoccidial + antibiotic	This comparison is used to determine whether the antibiotic is effective in the presence of the anticoccidial.

For combination(s) of an anticoccidial with an antibiotic where both drugs have a therapeutic claim for their respective diseases, appropriate controls should be present to determine that the disease models are valid, and the evaluation of the effectiveness should be demonstrated in the presence of both diseases together. Furthermore, the effectiveness of each drug should be demonstrated in the presence of the other drug.

An Anticoccidial in Combination with Growth Promotants

The treatment groups for an anticoccidial and a growth promotant should be the following:

1) non-infected, non-medicated control,
2) infected coccidia, non-medicated control,
3) infected coccidia, growth promotant (highest approved dose),
4) infected coccidia, anticoccidial (lowest approved dose), and
5) infected coccidia, anticoccidial (lowest approved dose) + growth promotant (highest approved dose).

The following comparisons should be performed among the treatment groups for a two-way combination.

Comparisons	Purpose
non-infected, non-medicated control vs. infected coccidia, non-medicated control	This comparison determines whether an infection has occurred. Once this has been established the non-infected, non-medicated control group should be excluded from any subsequent analysis.
infected coccidia, non-medicated control vs. infected coccidia, anticoccidial + growth promotant	This comparison demonstrates whether the combination (anticoccidial + growth promotant) is efficacious in preventing coccidiosis for each Eimeria species.
infected coccidia, anticoccidial vs. infected coccidia, anticoccidial + growth promotant	This test determines if the effectiveness of the anticoccidial is diminished in the presence of the growth promotant. If the effectiveness of the anticoccidial is reduced a label caution is warranted.
infected coccidia, growth promotant vs. infected coccidia, anticoccidial + growth promotant	This comparison is used to determine whether the anticoccidial is effective in the presence of the growth promotant.

An Anticoccidial in Combination with an Antibiotic and an Arsenical with Growth Promoting Claim

The treatment groups for the combination of an anticoccidial with an antibiotic and an arsenical (growth promoting claims) should consist of the following:

1) non-infected, non-medicated control,

2) infected coccidia, non-medicated control,

3) infected coccidia, anticoccidial (lowest approved dose),

4) infected coccidia, antibiotic (highest approved dose) + arsenical (highest approved dose), and

5) infected coccidia, anticoccidial (lowest approved dose) + antibiotic (highest approved dose) + arsenical (highest approved dose).

The following comparisons should be performed among the treatment groups for this combination.

Comparisons	Purpose
non-infected, non-medicated control vs. infected coccidia, non-medicated control	This comparison determines whether an infection has occurred. Once this has been established the non-infected, non-medicated control group should be excluded from any subsequent analysis.
infected coccidia, non-medicated control vs. infected coccidia, anticoccidial + antibiotic + arsenical	This comparison demonstrates whether the combination (anticoccidial + antibiotic + arsenical) is efficacious in preventing coccidiosis for each Eimeria species.
infected coccidia, anticoccidial vs. infected coccidia, anticoccidial + antibiotic + arsenical	This test determines if the effectiveness of the anticoccidial is diminished in the presence of the growth promotants. If the effectiveness of the anticoccidial is reduced a label caution is warranted.
infected coccidia, antibiotic + arsenical vs. infected coccidia, anticoccidial + antibiotic + arsenical	This comparison is used to determine whether the anticoccidial is effective in the presence of the antibiotic and arsenical.

An Anticoccidial in Combination with an Antibiotic (growth promoting claims) and an Arsenical (growth promoting and E. tenella claims)

Arsenicals have been approved in combination with other anticoccidials and production drugs for the prevention of coccidiosis due to some field strains of E. tenella that are more susceptible to an arsenical combined with an anticoccidial rather than the anticoccidial alone.

To test an anticoccidial in combination with and an antibiotic and an arsenical the treatment groups should consist of the following:

1) non-infected, non-medicated control,

2) infected coccidia, non-medicated control,

3) infected coccidia, anticoccidial (lowest approved dose),

4) infected coccidia, anticoccidial (lowest approved dose) + antibiotic (highest approved dose),

5) infected coccidia, antibiotic (highest approved dose) + arsenical (highest approved dose), and

6) infected coccidia, anticoccidial (lowest approved dose) + antibiotic (highest approved dose) + arsenical (highest approved dose).

The following comparisons should be performed among the treatment groups for a three-way combination.

Comparisons	Purpose
non-infected, non-medicated control vs. infected coccidia, non-medicated control	This comparison determines whether an infection has occurred. Once this has been established the non-infected, non-medicated control group should be excluded from any subsequent analysis.
infected coccidia, non-medicated control vs. infected coccidia, anticoccidial + antibiotic + arsenical	This comparison demonstrates whether the combination (anticoccidial + antibiotic + arsenical) is efficacious in preventing coccidiosis for each Eimeria species.
infected coccidia, anticoccidial vs. infected coccidia, anticoccidial + antibiotic + arsenical	This test determines if the effectiveness of the anticoccidial is diminished in the presence of the growth promotants. If the effectiveness of the anticoccidial is reduced a label caution is warranted.
infected coccidia, antibiotic + arsenical vs. infected coccidia, anticoccidial + antibiotic + arsenical	This comparison is used to determine whether the anticoccidial is effective in the presence of the antibiotic and arsenical.
infected coccidia, antibiotic + anticoccidial vs. infected coccidia, anticoccidial + antibiotic + arsenical	This comparison is used to determine whether the arsenical is effective in the presence of the anticoccidial and antibiotic.

Prior to conducting combination studies with these or other claims, the sponsor should consult with CVM. The treatment groups necessary to establish efficacy may be different, depending on the claims for each drug.

C. Non-Challenged Floor Pen Studies

The objective of these studies is to demonstrate the efficacy of the growth promotant(s) in the presence of the anticoccidial. Floor pen non-challenge studies should be conducted at a minimum of three geographical locations. Further, the CVM recommends that these studies be conducted in different environmental conditions, e.g., high relative humidity and ambient temperature, and use different stocks across the three geographical locations. Photoperiod regimes and management should simulate prevalent commercial practices in each geographical location. Because challenge and non-challenge studies may be conducted within the facility, an effort should be made to ensure that the litter is uniform across all pens. Artificial infections should not be used in non-challenge floor pen studies.

Statistical Considerations

In broiler floor pen studies, male and female birds (equal proportions of each gender) are reared together to simulate commercial conditions. There are biological differences between the growth and feed efficiency of male and female birds. Because of possible differences in mortality between the genders and errors in initial gender identification, there can be differences in the gender ratio at the conclusion of the study. Body weight and feed efficiency data should be adjusted for these differences.

Upon conclusion of the study, differences can exist in the bird density (pen square foot/number of birds). A change in bird density can influence the environmental stresses and feeder and waterer space per bird, all of which can affect body weight and feed efficiency. Therefore, body weight and feed efficiency should be adjusted for differences in bird density.

A two-way and three-way combination study can be conducted within the same experiment. However, if a three-way combination approval is sought, the sponsor should include both two-way combinations within the experiment. Because the anticoccidial drug group is used in making several comparisons between the two-way and three-way combinations, the sponsor should consider utilizing more anticoccidial drug groups within each block. Unrelated combinations, e.g., anticoccidial + arsenical + antibiotic A and anticoccidial + arsenical + antibiotic B, should not be tested within the same study.

If the sponsor desires to obtain approval for the three- and two-way combinations, the three data sets should be structured in the following manner. The first data set should include only the data from the anticoccidial and the anticoccidial + antibiotic treatment groups. The second data set should include only the data for the anticoccidial, and the anticoccidial + arsenical treatment groups. The third data set should include all the data collected from all four treatment groups.

Each of the following comparisons should be performed to determine the efficacy of each two-way combination:

1) Anticoccidial + Arsenical vs. Anticoccidial, and

2) Anticoccidial + Antibiotic vs. Anticoccidial.

Each two-way combination should demonstrate efficacy over the anticoccidial alone. This comparison determines whether the antibiotic contributes to the effect claimed or purported on the label in the presence of the anticoccidial. Each of the following comparisons should be performed to determine the efficacy of the three-way combination:

1) Anticoccidial + Antibiotic + Arsenical vs. Anticoccidial,

2) Anticoccidial + Antibiotic + Arsenical vs. Anticoccidial + Antibiotic, and

3) Anticoccidial + Antibiotic + Arsenical vs. Anticoccidial + Arsenical.

The CVM interprets 21 CFR § 514.1(b)(8)(v) to mean that the sponsor should demonstrate that the combination of drugs provides a benefit that cannot be obtained by the use of each drug individually, i.e., each drug has to make a contribution to the claimed effect (*Guideline for Drug Combinations for Use in Animals*; October, 1983). Thus, in the case in which three drugs are used in combination, the resulting benefit from the use of the three-way combination must be a benefit that cannot be obtained from combinations involving two drugs, i.e., a three-way combination should demonstrate efficacy over all possible two-way combinations of the same three drugs. The three-way combination should also be shown to be more effective than the anticoccidial alone.

Dependent Variables

All mortality, whether resulting from coccidiosis or other causes, should be diagnosed. Necropsy results should be reported. All information and data, e.g., date, weight, gender of bird, treatment group, diagnosis, and necropsy, associated with these birds should be recorded and submitted to the CVM in the NADA for its review.

Pen or individual body weights should be reported at time of drug treatment (day of placement) and at the conclusion of the study. If the lengths of the studies are different, rate of weight gain (final body weight/length of study) should be calculated and used in the statistical analysis, instead of final body weight. Feed intake should be recorded throughout the study period. Unadjusted feed efficiency (FE) and feed efficiency adjusted (AFE) for dead and cull birds should be calculated.

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