



I-011236-P-0033-EF

MAY 01 2008

U.S. Department of the Interior  
Fish and Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Attention: David Erdahl, Ph.D.  
Branch Chief  
4050 Bridger Canyon Road  
Bozeman, MT 59715

Re: Review of the Effectiveness technical section for 17 $\alpha$ -methyltestosterone

Dear Dr. Erdahl:

The Effectiveness technical section is incomplete. In your Effectiveness technical section submitted November 5, 2007, to the Investigational New Animal Drug (INAD) file for 17 $\alpha$ -methyltestosterone medicated feed, you included two final study reports for field effectiveness trials (Study MT-05-EFF.3-02 and Study MT-05-EFF.3-04); a multi-site summary report incorporating the results of a previously submitted final study report (Study MT-05-EFF.3-01, I-011236-P-0025) with the trials included in the current submission; a draft Freedom of Information (FOI) Summary; draft label language; and All Other Information related to effectiveness. The proposed use for 17 $\alpha$ -methyltestosterone medicated feed is to produce predominantly male populations of tilapia.

The data provided is insufficient to complete the Effectiveness technical section. By submitting additional raw data, explaining the method used to categorize fish as treatment successes or failures, providing a white paper argument regarding the revised success criteria, and reanalyzing the study results, the data may be acceptable to complete the technical section. We have the following comments.

STUDY MT-05-EFF.3-04

#### GENERAL COMMENTS

1. The raw data includes subjective histological assessments of the gonads considered ovotestes (OT). These subjective assessments include counts of oocytes (i.e., ~30, <20, <10, <15, <100) and the location of the oocytes. The examiner did not state if the counts were from a single field, from an average of multiple fields, or from the entire section. Some assessments use phrases such as "numerous oocytes," "bottom of gonad ovarian," or "infiltrate of oocytes at tip," rather than providing oocyte counts. Please explain if the observations recorded were from the examination of a single field; multiple fields; or the entire, or multiple, sections of each ovotestis.
2. In the raw data, two of the gonad sections examined were recorded as OT for Fish 401 from treated tank number 3. The OT sections were not further scored as successes or

failures based on the proportions of testicular tissue present. Please indicate whether this fish was considered a treatment success or a treatment failure.

3. Following collection of the gonads, the gonads of 47 MT-treated fish and 40 control fish slipped out of the tissue cassettes in which the gonads were placed. Because the gonadal tissues were placed in containers that included only gonadal tissues from a single tank, the gonads that slipped out of the cassettes, with a histopathological evaluation, can be incorporated in the results. Please see the Biometrics Comments below for additional information. We contacted you on April 21, 2008, and you stated that the gonads had been processed and evaluated. Please explain the methods used to prepare the gonads for histopathological evaluation and the methods used during the histopathological evaluation. In addition, please submit the raw data associated with these 87 fish.
4. Appendix M in the final study report is entitled, "Presence of Leukocytic Infiltrate and Phagocytizing Cells in Testes." You observed an infiltrate of macrophages in some of the testes examined. From the information provided, approximately 20% of the treated fish and approximately 12% of the control fish were affected. The information in Appendix M indicates that the infiltrate may have been associated with a parasitic infection. These histopathological findings are not discussed in the final study report. Please provide a discussion or information regarding the effects of the infiltration with regard to the etiology and the possible effects on the study fish, including both fish health and the histopathological evaluation of the gonads for sex determination.
5. Appendix N of the final study report is entitled, "White Paper Argument for Intersex Fish." The appendix contains an expert opinion letter, a copy of a survey sent to experts in the field, three replies to the survey, copies of email correspondence, and copies of references from the published literature. The information in this appendix is used to support a statement in your final study report that "the probable growth characteristics of intersex fish detected in this study would have been that of males because of the inability of gonads comprised of immature oocytes scattered throughout testicular tissue to produce estrogen levels sufficient to compromise growth," and the basis of your assertion that intersex fish with  $\geq 75\%$  testicular tissue have male growth characteristics. This criterion was used to reclassify some fish found to have ovotestes from treatment failures to treatment successes. We agree that the study fish with  $> 75\%$  testicular tissue may have male growth characteristics.

Because the information included in Appendix N of Study Number MT-05-EFF.3-04 contains information that is applicable to all of the trials conducted at the three study sites, it should be presented as a separate report. Please provide a "white paper" argument incorporating all of the information included in Appendix N of Study Number MT-05-EFF.3-04 together with the expert opinion paper, the survey results, personal communications, and the published literature, to support the statement that intersex fish with  $\geq 75\%$  testicular tissue have male growth characteristics. Please contact us if you have questions about the information this argument should include.

6. Additionally, please explain the criteria applied to the histopathological results of the gonad examination to determine that the gonads contain either  $\geq 75\%$  testicular tissue or  $>25\%$  ovarian tissue.

#### MULTI-SITE SUMMARY REPORT

#### GENERAL COMMENT

The results regarding the mean percent males in the MT-treated and control groups included in the multi-site summary report for Study MT-05-EFF.3-01 (I-011236-P-0025) and Study MT-05-EFF.3-02 are different from the results included in the final study reports for each individual study report. It appears that the results in the multi-site summary report include reclassified intersex fish as treatment successes (males). Please provide the raw data for the observations used to reclassify intersex fish from treatment failures to treatment successes.

#### BIOMETRICS COMMENTS

1. The protocol stipulated that the treatments would be assigned to the tanks using a completely randomized design. However, in Study MT-05-EFF.3-02, treatments were assigned to tanks using a randomized complete block design, with each block consisting of a pair of side-by-side tanks. The multi-site analysis should be adjusted to reflect this design. Please re-analyze the data with the random effect block nested within site added to the model. Here, block should be defined as follows: 1) the tanks in Study MT-05-EFF.3-02 should be assigned to blocks according to their pairs, and 2) the tanks in studies MT-05-EFF.3-01 and MT-05-EFF.3-04 should be assigned to a single block. Note that treatment, site, and treatment\*site should remain in the model as before. Please contact CVM if you have any questions regarding this analysis.
2. In Study MT-05-EFF.3-02, the gonads of 87 fish were lost because they slipped out of their cassettes. Many of these gonads were recovered and histologically evaluated for sex, but they could not be matched up with the individual fish from which they came. However, it is known from which tank these gonads came. The results from these gonads should be included in the analysis as follows. Please estimate for each tank the numbers of these gonads that were evaluated as male and female. For each tank, define the following quantities:

$m$  = the number of unevaluated fish;

$g_m$  = the number of these gonads that were male;

$g_f$  = the number of these gonads that were female; and

$k$  = the number of fish that had only one gonad examined (as noted in the raw data).

For each treated tank, the number of unevaluated fish included in the analysis as males should be:

$$m - \lambda_2 \quad \text{if } \lambda_1 + k \geq \lambda_2, \text{ and}$$

$$0 \quad \text{if } \lambda_1 + k < \lambda_2,$$

where  $\lambda_1 = \max(\min(g_m, 2m - g_f - k), 0)$ ,  $\lambda_2 = \min(\max(g_f, 2m - g_m - k), 2m - k)$  and  $\min(a, b)$  and  $\max(a, b)$  are the minimum and maximum of  $a$  and  $b$ , respectively. This

number is the minimum possible number of unevaluated fish that could be considered males, given the values of  $m$ ,  $g_m$ ,  $g_f$ , and  $k$ .

For each control tank, the number of unevaluated fish included in the analysis as males should be:

$$m - \text{int}\left(\frac{\lambda_2 - z + 1}{2}\right) - z,$$

where  $z = \max(k - \lambda_1, 0)$ ,  $\lambda_1 = \min(\max(g_m, 2m - g_f - k), 2m - k)$ ,  $\lambda_2 = \max(\min(g_f, 2m - g_m - k), 0)$ , and  $\text{int}(a)$  is the largest integer less than or equal to  $a$ . This number is the maximum possible number of unevaluated fish that could be considered males, given the values of  $m$ ,  $g_m$ ,  $g_f$ , and  $k$ .

Please contact CVM if you have any questions regarding the analysis.

#### FOI SUMMARY COMMENT

We appreciate your cooperation by including the relevant portions of the FOI Summary with this submission. At this time, the effectiveness section of the FOI Summary was not reviewed. Please resubmit the Effectiveness section of your FOI Summary when you resubmit your Effectiveness technical section. The effectiveness section of the FOI Summary will be reviewed once you provide the requested information and the study results are confirmed.

#### LABELING COMMENTS

We appreciate your cooperation by including the relevant portions of the draft label language with this submission. Please resubmit the effectiveness section of your draft labeling when you resubmit your Effectiveness technical section.

#### ALL OTHER INFORMATION

The submitted information is adequate at this time. However, please submit any additional information from the date of your submission, November 5, 2007, when you resubmit your Effectiveness technical section.

#### ADDITIONAL COMMENTS

We offer the following recommendations for future final study reports. We believe that incorporating these recommendations will improve the quality of your final study reports.

1. According to the study protocol, general fish behavior, fish appetite, water temperature, and water dissolved oxygen concentration were to be recorded at least once daily throughout the trials. These data were not collected consistently during either Study MT-05-EFF.3-02 or Study MT-05-EFF.3-04. Either the Study Director or the Study Monitor should have identified the lack of data collection at some point during the studies. Even if the Study Director and the Study Monitor were unable to make on-site inspections, either or both should have been in contact with the On-site Investigator to determine if all the procedures required by the study protocol were being conducted. The

final study report does not include a report from the Study Monitor. For future final study reports, information about the Study Director and Study Monitor contacts with the Investigator should be recorded in the form of a report and included in the raw data. Please refer to section 5 of Guidance for Industry Number 85 – Good Clinical Practice, available on the CVM website.

2. The power outage and pump failure that occurred during Study MT-05-EFF.3-02 were well-described, but not identified as an adverse event. The protocol states that adverse reactions will include any observations that are unfavorable or unintended, whether or not drug-related. By the definition in the protocol, the power outage and pump failure are adverse reactions. Generally, this type of event is considered an adverse event and, in the future, should be clearly identified as an adverse event. Because the mortality was relatively equivalent in all the study tanks, and the cause of the mortality was unlikely to cause death at different rates among MT-treated fish, control fish, male fish, or female fish more frequently, and that about 1000 fish remained in each tank after the adverse event; we believe that the adverse event did not bias the results of the study. However, mortality of the degree that occurred during the study and associated with the power outage and pump failure warrants more than a brief discussion. In the future, please include in the final study report a complete explanation of the impact of any adverse event on the study results and on any analyses.

If you submit correspondence relating to this letter, you should reference this letter by date and the principal submission identifier found at the top of this letter. If you have any questions about this letter, please contact me at 240-276-8341 or Dr. Donald Prater, Leader, Aquaculture Drugs Team at 240-276-8343.

Sincerely,



Cindy L. Burnsteel, DVM  
Acting Director, Division of Therapeutic  
Drugs for Food Animals  
Office of New Animal Drug Evaluation  
Center for Veterinary Medicine