



NOV 13 2007

I-011669-E-0001-EF

U.S. Fish & Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Attention: David Erdahl  
Branch Chief  
4050 Bridger Canyon Road  
Bozeman, MT 59715

Re: Review of effectiveness protocol H202-07-EFF

Dear Dr. Erdahl:

We do not concur with the protocol you submitted on September 26, 2007. This protocol was entitled "The efficacy of 35% PEROX-AID to control mortality due to bacterial gill disease or external columnaris in cool and warmwater fish." You submitted this protocol to the investigational new animal drug (INAD) file I-011669 for hydrogen peroxide. Hydrogen peroxide is proposed for the control of mortality in freshwater-reared finfish due to bacterial gill disease and external columnaris. We found the protocol unacceptable for the following reasons:

GENERAL COMMENTS FOR PROTOCOL CONCURRENCE

1. In many places (Sections 1.1, 5.6 (table), and 7.3, and on Form 2), the protocol indicates that hydrogen peroxide will be administered on alternate days. However, Section 3.2.3 of the protocol says that the hydrogen peroxide will be administered on consecutive days. The dosing regimen should be consistent throughout the protocol. Please correct the protocol and forms where necessary.
2. Section 2.1.8 says that treatments will be administered for up to 60 minutes, whereas the dosing regimen table in Section 5.6 identifies the treatment duration as 60 minutes. Please change Section 2.1.8 so that the treatment duration is 60 minutes. If you are considering evaluating a different treatment duration in some studies, then the exact duration should be specified in both places in the protocol (do not give a range).
3. Please specify that the person(s) conducting the necropsies will be masked to treatment group (Section 5.5).
4. Please remove references to ectoparasites in Section 3.2.4. The protocol discusses secondary disease infections, which includes presence of ectoparasites, in Section 5.3.1.
5. Section 5.4.3 indicates that either a copy or transcription of facility records capturing mortality data for the days leading up to the study should be submitted in the FSR. A

photocopy is acceptable but transcription is not. Please remove the words "or transcription" from this section.

#### BIOMETRICS COMMENTS

1. Sections 4.2 and 4.3 indicate that, while a completely randomized design primarily will be used to assign treatments to tanks, other designs may be used when appropriate. However, in Sections 3.2.1 and 4.4 it is stated that a completely randomized design will be used, and there is no mention of the possibility of other designs. CVM requests that the protocol state that other appropriate designs may be used wherever the study design is discussed. For example, the third sentence of Section 3.2.1 can be reworded as (changes in italics), "Allocation of fish-to-tanks will be done following a completely randomized design *or other appropriate design.*"
2. Section 5.5.1 states that sample counting may be done by masked or unmasked personnel. This is acceptable for those doing pre-treatment sample counting. However, those counting fish at the end of post-treatment, regardless of what counting method they use, should be masked to treatment.
3. Section 6.1.4.1 states that on rare occasions a sample count may be done at the end of post-treatment. However, it does not indicate from which tank(s) the sample of fish will be selected to do the count. CVM recommends that, if this procedure is used, a sample be selected from each tank and its weight applied to only that tank when estimating the number of fish in the tank.
4. The last sentence in the "Actual counts at the end of the study" part of Section 6.1.4.2 states, "Results from hand-counting fish from experimental units will be added to the total mortality in each of the test tanks, resulting in an accurate number of fish/test tank at the start of the study." Please explain the purpose of this statement. It implies that when live fish are hand-counted at the end of the study, the estimated total fish count at the start of the study will be replaced with the sum of the mortality count and the live fish hand count.
5. Assumption 2 in Section 7.3.3 states that the equality of the variances between the two treatment groups is known. The analysis will be done on proportions, and the variance of the mean changes according to the proportion. Therefore, this assumption is not correct. Please remove this assumption from Section 7.3.3.
6. The protocol indicates several times that the tank will be treated as the experimental unit. However, the protocol does not indicate that this will be done in the analysis. We recommend that in Section 7.3.4 the protocol state that tank will be treated as the experimental unit in the analysis. Remember that tank can be treated as the experimental unit in the generalized linear model analysis, for example, in SAS Proc GLIMMIX. To do so, enter the response in the model statement as the ratio of the number of mortalities divided by the total number of fish in the tank.
7. In the generalized linear model analysis, the mean square error should be multiplied by the overdispersion parameter. This can be done, for example, in SAS Proc GLIMMIX by

including a "random residual" statement. Please indicate in Section 7.3.4 that in the analysis the mean square error will be multiplied by the overdispersion parameter.

#### ADDITIONAL COMMENTS

We offer the following recommendations for revision of your protocol and points for consideration. While not required for concurrence of this protocol, we believe that incorporating these recommendations will improve the quality of this study protocol and future protocols.

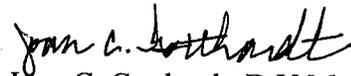
1. In Section 3.3, the protocol provides a table with the number of studies to be conducted. We agree that this reflects the minimum number of studies needed to supplement data from the United States Geological Survey Upper Midwest Environmental Sciences Center (UMESC) to complete the effectiveness technical sections for control of mortality in coolwater species of freshwater-reared finfish due to bacterial gill disease and in warmwater species of freshwater-reared finfish due to external columnaris. The table in the protocol indicates that you will conduct one pivotal and one supportive study in any coolwater species; you should use a different species in each study. For the warmwater species, CVM encouraged UMESC to select a species that is commonly raised in aquaculture facilities in the United States such as hybrid striped bass. Depending on doses used for certain species or lifestages, additional target animal safety data may be required.
2. Your protocol defines the causative agent of bacterial gill disease to genus (*Flavobacterium spp.*) If you wish for the indication to match that which is currently approved for freshwater-reared salmonids, "control of mortality... due to bacterial gill disease associated with *Flavobacterium branchiophilum*," then your studies will need to confirm the presence of this species of bacteria.
3. The proposed method of disease diagnosis is acceptable for this protocol, but definitive diagnosis (for instance, by bacterial culture) using at least a subset of samples is preferable.
4. Section 1.2 of the protocol says that the manufacturing sponsor would ultimately like the label indication for 35% PEROX-AID to be "Use as an external microbicide for all freshwater-reared finfish eggs and freshwater-reared finfish." We remind you that CVM approves claims for specific diseases and etiologic agents for which there is substantial evidence that the drug is effective in controlling or treating the disease or controlling mortality due to the disease. For a claim as an "external microbicide," a sponsor would need to demonstrate that the drug is effective against all microorganisms that can potentially cause disease in fish.
5. Fourteen days is an acceptable length for a post-treatment period without re-infection. In certain circumstances, a post-treatment period between 10 and 14 days may be acceptable. If you analyze data at 10 days post-treatment as described in Section 3.2.4, you will need to provide an adequate and acceptable justification for the decision to shorten the post-treatment in the final study report.

6. Section 4.4 states that all completely randomized design procedures will follow SOP MISC 327 in Appendix E. We believe that you intended to reference SOP MISC 237. Please correct this typographical error.
7. In Section 5.1.2.2, to avoid confusion, please indicate who will calculate the loading rate. Currently, the protocol says "we will mathematically calculate..." and is therefore ambiguous.
8. Section 5.7 indicates that subjects will be removed from experimental units using dip nets that are sanitized before and after use with disinfectant. Please provide more instruction on the disinfection step to ensure that disinfectant does not contaminate the water in the experimental units and contribute to target animal toxicity. For instance, an acceptable explanation would be that the nets will be rinsed with water before use.
9. In Section 5.9.3, the protocol states that if it is not possible to measure hardness, alkalinity, or pH, that it will be acceptable to report historical data. Water quality parameters should be measured during the study.
10. There is no place on Form 1 or other forms to record the water temperature and DO of the reference population tank. Since the protocol calls for this measurement, there should be a place on one of the forms for this information, unless it will be documented in facility records that will be copied for inclusion in the final study report.
11. It would also be appropriate to have a place on Form 1 to report the frequency and number of treatment administrations.
12. In your correspondence to us dated October 9, 2007, you indicated that the use of 35% PEROX-AID under INAD 011669 for the control of mortality caused by BGD and external columnaris would be limited to use at a single facility (Richloam State Hatchery, FL) on a limited number of species. The current protocol is not specific for Richloam State Hatchery and the species listed in your correspondence dated October 9, 2007. Should you wish to conduct studies at other facilities on additional species, please request a categorical exclusion for these facilities and be sure that the species fall under your authorization. We are in the process of evaluating your request for a slaughter authorization dated September 6, 2007, and amendment dated October 9, 2007.
13. Section 13 of the protocol says that unused drug remaining at the end of a study can be kept on-site for future use according to the Service's Study Protocol for Compassionate Aquaculture INAD Exemption under INAD 011669. We have not received a compassionate protocol for BGD and columnaris from you for this INAD. You indicated in your correspondence dated October 9, 2007, that use of the drug under this INAD for control of mortality due to BGD and columnaris would be pivotal in nature. Additionally, your previous correspondence indicates that bacterial and ectoparasite trials will be done at separate facilities. This section of the protocol should not say that the drug will be used in compassionate studies if there is to be no such use of this drug in that manner. Similarly, please remove reference to compassionate protocol use in Section 5.2.1.

We recommend that you submit a revised protocol for our review to obtain our concurrence before you begin this study. Our concurrence with your protocol would mean we fundamentally agree with the design, execution, and analyses proposed in your protocol, and that we commit 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. However, even with our concurrence, we could make no commitment that the data obtained from a study implementing your protocol will support an approval.

If you submit correspondence relating to this letter, your correspondence should reference the date and the principal submission identifier found at the top of this letter. If you have any questions or comments, please contact me at 301-827-7571 or Dr. Donald Prater, Aquaculture Drugs Team Leader, at 301-827-7567.

Sincerely,



Joan C. Gotthardt, D.V.M.  
Director, Division of Therapeutic  
Drugs for Food Animals  
Office of New Animal Drug Evaluation  
Center for Veterinary Medicine



# United States Department of the Interior

U.S. FISH & WILDLIFE SERVICE  
AQUATIC ANIMAL DRUG APPROVAL PARTNERSHIP PROGRAM  
4050 BRIDGER CANYON ROAD  
BOZEMAN, MT 59715  
PHONE 406-994-9905/FAX 406-582-0242



September 26, 2007

Dr. Joan Gotthardt  
Director, Division of Therapeutic Drugs  
for Food Animals  
Document Control Unit, HFV-199  
Center for Veterinary Medicine  
7500 Standish Place, MPN-2  
Rockville, MD 20855

Dear Dr. Gotthardt:

The purpose of this submission is to request a formal review of the enclosed pivotal efficacy Study Protocol titled "The efficacy of 35% PEROX AID<sup>®</sup> to control mortality due to bacterial gill disease or external columnaris in cool and warmwater finfish." The Study Protocol is identified by Study Protocol Number H2O2-07-EFF. Please note that this protocol is nearly identical to Study Protocol CHLT-07-EFF, which received protocol concurrence from CVM on July 26, 2007 (I-009321-E-0086-OT). Use of 35% PEROX AID<sup>®</sup> in pivotal field efficacy trials will be conducted under the U. S. Fish and Wildlife Service's 35% PEROX AID<sup>®</sup> (hydrogen peroxide) compassionate INAD 11-669. Efficacy data generated from these studies will be used to support an expansion of the FDA-approved label claim for this aquaculture drug. We refer to your file number 11-669 dated September 11, 2007.

The current sponsor of INAD 11-669 is Dr. David Erdahl, Branch Chief, U. S. Fish and Wildlife Service, AADAP Program, 4050 Bridger Canyon Road, Bozeman, MT 59715. We would like to thank you in advance for your time and consideration with respect to the above described request. If you have questions, please contact Dr. Erdahl at (406)-994-9904.

Sincerely,

Dr. David Erdahl  
Branch Chief, AADAP Program

Enclosure: 3 copies of Study Protocol H2O2-07-EFF

