

JAN 10 2008

I-010541-P-0147-TS

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

Re: Isoeugenol (AQUI-S) target animal safety study on cutthroat trout

Dear Dr. Erdahl:

The target animal safety section for the use of isoeugenol (AQUI-S) to sedate freshwater-reared finfish to handleable remains incomplete. We reviewed your submission dated July 10, 2007, as amended on August 7, 2007, and find these data to be acceptable. This study demonstrates that there is an adequate margin of safety above 40 mg/L AQUI-S for sedation of cutthroat trout to handleable. To complete the target animal safety technical section for all freshwater-reared salmonids, you will need acceptance of the validation of the dose verification method used in pivotal studies. To complete the technical sections for coolwater and warmwater species of freshwater-reared finfish, you will need acceptable studies in two representative species from each temperature group. The target animal safety technical section for the use of isoeugenol (AQUI-S) to sedate all species of freshwater-reared finfish will be complete upon acceptance of studies in two species from each temperature group, as agreed in our meeting on February 7, 2006, with AQUI-S, New Zealand, Ltd.

ADDITIONAL COMMENTS

During our review of your final study report, we noted the following items. While, ultimately, these items did not impact our ability to accept your data and conclusions, you should address them in future investigations.

1. We noted that the light cycle in the containment building is different on the weekends than during the week. We recommend that in future studies when testing is performed through the weekend, the light cycle should be no different than it is on weekdays.
2. We noted that during your determination of ET80s, you used nine fish from reference tank 1 and six fish from reference tank 2, rather than similar numbers from each tank, i.e. eight and seven. Please use an equal number of fish from each reference tank in the future, or explain the discrepancy in your final study report.

3. As previously discussed in our letter dated, December 8, 2006 (P-0124), we noted that a standard concentration above the highest test concentration was not used to generate the calibration curve for dose verification. In future studies, you should include a standard whose concentration is higher than the highest test exposure concentration, or provide an explanation in your final study report.
4. You note that in Deviation #3 that a fish jumped from Tank 11 into Tank 12. In future studies, you should make modifications to ensure that fish cannot escape from the test tanks.
5. In the tables that summarize the histology scores (Appendix K4a-d), only mild, moderate, and severe scores were reported (2, 3, 4). No normal (1) or absent (0) scores were provided. In future tables, please provide a statement that blank boxes indicate normal findings and please report all absent scores. You state that in some instances the spleen was not examined because of the small size; this is acceptable but the number not examined should still be reported, and a reason provided in the final study report.
6. In future dose verification studies, please document the exact time between when test article solutions are made and when they are analyzed.
7. In studies in which fish were exposed to 0 mg/L and were not 'sedated' when moved from exposure to recovery containers, you collected all the fish by pouring them through a net and collecting a small sample to determine dissolved oxygen (DO) and pH. The agitation of the water during the pouring could have artifactually caused an increase in DO. Please collect DO measurements before agitating the water in future studies.
8. As previously discussed in our letter dated, December 8, 2006 (P-0124), the study procedure deviated from the study protocol in that only histopathological lesions that were moderate or severe and that appeared to be test article-induced prompted microscopic examination of the same tissue at the next longest exposure-duration of the same concentration. In future studies, please adhere to the protocol by examining tissues in the next lower dose group when any lesions are found. You may seek guidance from CVM on how to revise the protocol if you believe greater clarity is needed with respect to when tissues should be evaluated in order to distinguish potential test-article induced lesions from spontaneous disease and/or background lesions.
9. The study procedure deviated from the study protocol during the selection of exposure durations for each exposure concentration. The study protocol states that specific multiples of the ET80<sub>HEC</sub> will be used to determine the duration. Different durations, other than those multiples in the protocol, were used based on testing of fish. Although the durations are aimed to generate certain survival data, please indicate why those particular multiples were selected for evaluation.

10. We noted that the protocol included with the final study report was AQUIS-06-TAS-FISH.2, but we concurred on protocol AQUIS-06-TAS-FISH.1 in a letter dated October 11, 2006 (T-0132). When a new version of a concurred protocol is used for the study, please summarize the differences between the old and new versions of the protocol.
11. The study procedure deviated from the study protocol with regard to the tissues examined for histology. The protocol states that "at 96 hours post-exposure, gill, eye, brain, and skin will be examined, as well as tissues in which lesions were observed in fish sampled from the 24-hour post-exposure period will be examined." In the FSR, the only tissues examined at 96 hours were gill and/or posterior kidney. In future studies please adhere to the protocol and indicate why the deviation occurred.

#### FOI SUMMARY COMMENTS

A revised FOI Summary section is enclosed. Minor changes were made including the addition of a paragraph on secondary variable results.

#### DRAFT LABEL COMMENTS

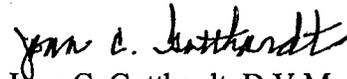
Several comments are provided regarding the draft label submitted.

1. As requested in our letter, dated November 28, 2006 (G-0115, G-0116, G-0018), please provide a spreadsheet that contains the data used to calculate the median times to handleable to assist us in assessing whether these times are valid. An alternative way to present the data would be to replace the median times and ranges with the 80<sup>th</sup> percentile times; this approach has been used to illustrate time to handleable and time to recover in the FOI summaries drafted for effectiveness studies. Other ways to illustrate the approximate times to effect and recovery can be considered as well. Regardless of the final presentation of expected times to handleable, please provide us with a spreadsheet that illustrates how you derived the numbers you intend to include on the label.
2. A conversion factor between grams and milliliters and vice versa was provided on the right panel to help users more readily determine the correct dose required for their specific treatment. Please place this information on the middle panel-back where the rest of the calculations are located.
3. As requested in our letter, dated November 28, 2006, under Precautions, please place a statement on the label that a recovery vessel should always be available; not having water available once sedation or anesthetization is initiated could jeopardize the animal's life if overdose occurs.
4. In the following label statement a graph is referred to, but is not provided "Ensure treatment groups are of a size as to be safely handled within the confines of the operable window (see graph)." Please either provide a graph or rewrite the part "...confines of the operable window."

5. There are two instances in which the word fish remains, instead of finfish. Please replace the word fish with finfish in the two sentences that begin with "Safety testing has been done on small fingerling fish" and "Do not induce anesthesia in more fish..."
6. Please change the statement "Precautions should be taking when using this product on male and female broodfish" to "Safety testing has not been conducted on male and female broodfish."

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 240-276-8342, or Dr. Donald Prater, Leader, Aquaculture Drugs Team at 240-276-8343.

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

Enclosure: FOI Summary

### III. TARGET ANIMAL SAFETY:

#### Toxicity Study

Title: "The Safety of AQUI-S as an Anesthetic on Cutthroat Trout *Oncorhynchus clarki*."

Study Director: James D. Bowker, MS

Study Location: U.S. Fish and Wildlife Service  
Bozeman Fish Technology Center  
4050 Bridger Canyon Road  
Bozeman, MT 59715

#### General Study Design:

1. Purpose: To demonstrate the safety of a 40 mg/L dose of isoeugenol (AQUI-S) administered as a static bath to small fingerling cutthroat trout. This study was conducted in accordance with Good Laboratory Practice (21 CFR 58) regulations.
2. Animals: Small fingerling cutthroat trout; total length ranged from 4.0 to 6.1 cm
3. Test article: 50% isoeugenol (AQUI-S)
4. Study Design: Groups of 20 test fish were exposed to 0, 40 (1X), or 80 (2X) mg/L AQUI-S<sup>®</sup> for one of four exposure durations. Durations were selected to approximate 50 to 100% survival at each concentration (see Table 1). Each of the twelve combinations of AQUI-S concentration and exposure duration was replicated four times.

From a reference population, healthy fish were collected and examined to establish a baseline for fish health. The examinations included a gross morphologic and microscopic evaluation. A preliminary study was conducted on fish from the reference population to establish an estimated time for sedation to handleable at the 80<sup>th</sup> percentile, ET80. The ET80 values were then used to determine the exposure durations for each test concentration (Table 1). The exposure duration for the control group (0 mg/L) was set at the longest duration (40 mg/L).

Fish were randomly allocated to test tanks prior to exposure to isoeugenol (AQUI-S). Following exposure, four fish (up to two dead fish and up to four live fish) per replicate were collected for gross morphologic and microscopic evaluation upon death, at 24 hours post-exposure (for live fish), and at 96 hours post-exposure (for live fish).

Table 1. Exposure durations for each concentration.

<i>Concentration</i>	<i>T1 (minutes)</i>	<i>T2 (minutes)</i>	<i>T3 (minutes)</i>	<i>T4 (minutes)</i>
0 mg/L	8.0	9.75	10.50	12.00
40 mg/L	8.0	9.75	10.50	12.00
80 mg/L	2.5	3.00	4.00	4.75

5. Measurements and Observations: Mortality, gross morphologic, and microscopic lesions were the primary variables. Fish behavior, AQUI-S concentration, and water quality parameters were evaluated as secondary variables. Microscopic evaluation included the following tissues: skin, eye, gill, muscle, brain, heart, spleen, liver, anterior kidney, posterior kidney, stomach, pyloric intestine, and rectal intestine.

Results: Table 2 summarizes the mean survival for each concentration at the exposure durations listed in Table 1. Maximum exposure durations at 40 and 80 mg/L AQUI-S, where survival was acceptable (i.e.,  $\geq 95\%$ ), were approximately 8 and 3 minutes, respectively (see Table 2). Safety breakpoint intervals (the exposure-duration range during which mean survival of test fish dropped below 95%) for 40 and 80 mg/L AQUI-S were 8 to 9.8 and 3 to 4 minutes, respectively. And margins of safety (the time difference between the maximum safe exposure duration and the time it takes to sedate 80% of fish to handleable) for each concentration tested were 5.8 and 1.9 minutes, respectively (see Table 3). Microscopic findings in treated fish included gill epithelial separation, gill hypertrophy, and posterior kidney hydropic degeneration, but were not considered clinically significant.

Temperatures ranged from 12.8 to 14.2 °C. Dissolved oxygen ranged from 7.6 to 9.0 mg/L; pH ranged from 7.76 to 7.95; the mean alkalinity was 172 mg/L CaCO<sub>3</sub>; and the mean hardness was 264 mg/L CaCO<sub>3</sub>. AQUI-S concentrations were within acceptable ranges (-0.4 to +10.5% of the target concentrations). The only abnormal fish behavior noted was headshaking, but it did not have an effect on fish health or survival. At 40 mg/L, headshaking behavior occurred in 75 to 100% of the fish in all experimental units upon immersion for no more than 15 to 45 seconds; behavior during recovery was normal. At 80 mg/L, headshaking occurred in 100% of the fish in all experimental units except one; behavior during recovery was normal.

Table 2. Mean survival. Exposure durations T1, T2, T3, and T4 are specified in Table 1.

<i>Survival</i>	<i>T1</i>	<i>T2</i>	<i>T3</i>	<i>T4</i>
0 mg/L	100 %	100 %	100 %	100 %
40 mg/L	100 %	85 %	86 %	80 %
80 mg/L	99 %	99 %	83 %	48 %

Table 3. Margin of safety based on longest exposure time and determined ET80, where the ET80 is the 80<sup>th</sup> percentile time-to-handleable.

<i>AQUI-S</i> concentration (mg/L)	<i>Longest safe exposure</i> <i>duration (minutes)</i>	<i>ET80</i> (minutes)	<i>Safety Margin</i> (minutes)
40	8.0	2.18	5.82
80	3.0	1.13	1.87

Conclusions: Isoeugenol (AQUI-S) is safe when administered to cutthroat trout at a dose of 40 mg/L for sedation to handleable. There is an adequate margin of safety above 40 mg/L based on concentration and duration of exposure. The label for isoeugenol (AQUI-S) should include a statement indicating the drug should be tested on a small number of fish before administration to large groups and the lowest label dose should be used to achieve the desired level of sedation for a given population of fish. The label should also include a precaution that salmonids may exhibit temporary headshaking upon immersion.



## 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 406-994-9904/FAX 406-582-0242

August 07, 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 append an additional (second) copy of the AQUI-S<sup>®</sup> Target Animal Safety Study Protocol Number AQUIS-06-TAS-FISH.2 to the Final Study Report submitted to CVM on July 10, 2007. This second copy of the Study Protocol titled "The Safety of AQUI-S<sup>®</sup> as an Anesthetic to Freshwater Fishes" is being submitted based upon a specific request from CVM.

The current sponsor of INAD #10-541 is Dr. David Erdahl, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, U.S. Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, MT. We would like to thank you in advance for your time and consideration. If you have any questions, please contact Dr. Erdahl at (406) 994-9904.

Sincerely,

Dr. David Erdahl  
Branch Chief, AADAP Program

Enclosure: additional copy of Study Protocol AQUIS-06-TAS-FISH.2





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July 10, 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 Final Study Report (FSR) titled "The Safety of AQUI-S<sup>®</sup> as an Anesthetic on Cutthroat Trout *Oncorhynchus clarki*." The FSR is identified by Study Number AQUIS-06-TAS-FISH.2-01. Please note that we also request that the FSR be included in the AQUI-S<sup>®</sup> target animal safety technical section in support of a New Animal Drug Approval for AQUI-S<sup>®</sup>, and that the FSR be filed in the Service's Investigational New Animal Drug (INAD) file #10-541. We refer to your file number INAD 10-541 P-0084 dated July 01, 2005.

The enclosed FSR summarizes results from a study in which the maximum safe exposure durations (survival  $\geq$  98%), safety breakpoint intervals, and the margins of safety were established for exposure of cutthroat trout at 40 and 80 mg/L AQUI-S<sup>®</sup> under the study conditions specified in the FSR. Specifically, the FSR demonstrated the following: 1) the maximum safe exposure durations for 40 and 80 mg/L AQUI-S<sup>®</sup> were approximately 8.0 and 3.0 min respectively; 2) the safety breakpoint intervals for 40 and 80 mg/L AQUI-S<sup>®</sup> were approximately 8.0 – 9.8 and 3.0 – 4.0 min, respectively; 3) the margins of safety (maximum safe exposure duration minus the ET80 for that dose) for 40 and 80 mg/L AQUI-S<sup>®</sup> were 5.8 and 1.9 min, respectively; and 4) the only post-exposure pathologies of note that were observed were gill epithelial separation and gill hypertrophy. Although it could be argued that the noted pathologies may have been test-article induced and potential safety concerns, based on documentation in the literature we contend that it is more likely that these morphological changes in gill tissue were in fact procedural artifacts of histological preparation. It is also important to note that severe pathologies were only noted at relatively low prevalence, and without an easily discernible "pattern of effect" between treatments. Hence, it is our contention that at "safe" AQUI-S<sup>®</sup>

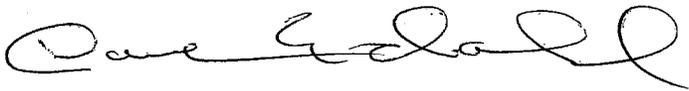


concentrations these histological changes were not pathologic, nor did they contribute to test fish mortality. Furthermore, we speculate that test fish that died during the study did so because they simply became too deeply sedated (via a combination of dose and duration) to recover. This result/conclusion was certainly not unexpected.

It is our opinion that the target animal safety data generated in this study support the approval of AQUI-S<sup>®</sup> for use at a concentration of 20 - 40 mg/L to sedate all freshwater salmonid fishes for a variety of husbandry and management practices. Please note that a draft Freedom of Information (FOI) summary of this study and draft label claim for use of AQUI-S<sup>®</sup> on all freshwater salmonids are appended to the FSR.

The current sponsor of INAD #10-541 is Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief - 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

- Enclosures:
- FSR titled "The Safety of AQUI-S<sup>®</sup> as an Anesthetic to Cutthroat Trout *Oncorhynchus clarki*", AQUIS-06-TAS-FISH.2-01 (3 copies)
  - Draft TAS Freedom of Information Summary (3 copies)
  - Research protocol titled "The Safety of AQUI-S<sup>®</sup> as an Anesthetic on Freshwater Fishes", AQUIS-06-TAS-FISH.2 (1 copy)
  - References, copies of all references cited in the FSR (1 copy)
  - Draft label claim for the use of AQUI-S<sup>®</sup> on all freshwater salmonids (3 copies)