DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

I-010541-P-0136
I-010541-P-0137
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JUL 10 2007

U.S. Department of 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: Request for review of study summaries for inclusion in the Freedom of Information (FOI) Summary

Dear Dr. Erdahl:

Your study summaries submitted January 4, 2007 (P-0136), January 5, 2007 (P-0137), January 8, 2007 (P-0138), January 9, 2007 (P-0139), and January 10, 2007 (P-0140) to be included in the substantial evidence section of the effectiveness section of the Freedom of Information Summary for AQUI-S (50% isoeugenol) have been revised. The study summaries included in your submissions describe data previously submitted to CVM on March 31, 2003 (P-0031), May 8, 2003 (P-0034), May 9, 2003 (P-0035), May 14, 2003 (P-0036), and May 28, 2003 (P-0037). A copy of the revised study summaries has been included.

The effectiveness technical section is not yet complete. To obtain an effectiveness technical section complete, validation of the dose verification method used in pivotal effectiveness studies is required as discussed in a pre-submission conference between CVM and AQUI-S New Zealand, Ltd. (AQNZ) on February 7, 2006. Also, draft language for the dosage characterization part of the effectiveness FOI Summary section should be submitted.

If you submit correspondence relating to this letter, your correspondence should reference the date and the principal submission identifiers 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, Leader, Aquaculture Drugs Team, at (301) 827-7567.

Sincerely,

Joan C. Gotthardt

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:
Effectiveness section of the FOI Summary

II. EFFECTIVENESS:

A. Dosage Characterization:

(To be provided at a later time)

B. Substantial Evidence:

1. Field Study

a. "The Efficacy of AQUI-S as an Anesthetic for use on Adult Steelhead Trout *Oncorhyncus mykiss*" (Study Number AQUIS-01-EFF-01)

b. Study Director: James D. Bowker

Investigator: Daniel Carty

Study Site: U.S. Fish and Wildlife Service
Dworshak National Fish Hatchery
Ahsahka, ID

c. Study Design:

1) Objective: To evaluate the effectiveness of AQUI-S to sedate to handleable individual sub-adult steelhead trout exposed to concentrations of 10, 20, 40, and 60 mg AQUI-S/L of water at a water temperature of approximately 6 °C.

2) Study Animals: 75 adult steelhead trout *Oncorhyncus mykiss*. The fish had recently returned to the hatchery to spawn.

3) Treatment Groups: The study included 4 treatment groups receiving 10, 20, 40, and 60 mg AQUI-S/L of water and one treatment group receiving a positive control, TRICAINE-S (MS-222/tricaine methanesulfonate) at a dose of 80 mg/L of water. The experimental unit was individual fish. Each treatment group was tested separately. Each test solution was prepared in three to five 75-gallon tanks containing 50 gallons of the test solutions. The four test article concentrations and the control article were administered in a completely random order. A total of 15 fish were included in each treatment group, with fish tested individually and a relatively equivalent number of fish exposed in each of the three to five tanks. The fish were arbitrarily selected from the reference population by crowding and using a dip-net to capture fish.

4) Drug Administration: AQUI-S (50% isoeugenol) or the positive control was administered by immersion. Fish were exposed for no longer than 60 minutes. Fish were recovered in a communal tank, with the recovery period lasting no longer than 30 minutes.

5) Measurements and Observations: The two primary variables were the time for each fish to become sedated to the handleable stage and the time for each fish to recover. A fish was identified as sedated to handleable when the fish was initially unresponsive to external stimuli except strong pressure, lost equilibrium, and did not avoid obstacles. A fish was identified as recovered when the fish initially regained equilibrium and could swim away from objects inserted in the tank. Also recorded were the length of the fish, the presence of *Saprolegnia* sp., the general and respiratory behavior, and the water temperature and dissolved oxygen concentration of the test solutions. Water hardness, alkalinity, and pH of the source water were measured.

d. Results: Median times to handleable and recovery are included in the following table.

Table 1. Median times to handleable and recovery for sub-adult steelhead trout at approximately 6 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	10	9.83	0.9
	20	5.38	2.13
	40	3.50	5.55
	60	2.22	2.62
MS-222	80	2.02	2.18

The fish were approximately 84 cm in length. *Saprolegnia* sp. was found on fish at equivalent, low levels in all treatment groups. General and respiratory behaviors of all fish were normal.

Mean water temperature ranged from 5.7 to 6.0 °C. Mean dissolved oxygen concentrations ranged from 10.0 to 11.6 mg/L. Water hardness, alkalinity, and pH were 10 mg/L, 14 mg/L, and 8.0, respectively.

e. Adverse Reactions: There were no mortalities during the study. No adverse reactions were reported in this study.

f. Conclusion: AQUI-S concentrations of 10, 20, 40, and 60 mg/L administered as an immersion are effective for sedating sub-adult steelhead trout, at a water temperature of about 6 °C, to handleable within 10 minutes with fish recovering within 10 minutes.

2. Field Study

a. "The efficacy of AQUI-S as an anesthetic for use on sub-adult rainbow trout, lake trout, and mountain whitefish" (Study Number AQUIS-01-SUPP-EFF-01)

b. Investigator: James D. Bowker

Investigator: Daniel Carty

Study Site: U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

c. Study Design:

1) Objective: To evaluate the effectiveness of AQUI-S to sedate to handleable individual sub-adult rainbow trout, lake trout, and mountain whitefish exposed to concentrations of 20, 40, and 60 mg AQUI-S/L of water at a water temperature of approximately 10 °C.

2) Study Animals: 168 rainbow trout *Oncorhyncus mykiss*, 96 lake trout *Salvelinus namaycush*, and 84 mountain whitefish *Prosopium williamsoni*. The mountain whitefish all had deformed heads.

3) Treatment Groups: The study included 3 treatment groups receiving 20, 40, and 60 mg AQUI-S/L of water and one treatment group receiving a positive control, TRICAINE-S (MS-222/tricaine methanesulfonate) at a dose of 80 mg/L of water. Each concentration of the test article and the control article was tested in triplicate. The experimental unit was a group of fish. Each group contained 8 lake trout, 7 mountain whitefish, or 14 rainbow trout. The fish were arbitrarily selected from the reference population by crowding and using a dip-net to capture fish. The test article was administered in a systematic order from lowest to highest concentration followed by the control article. Each treatment group was tested separately. Each test solution was prepared in a 75-gallon tank containing 20 gallons of the test solutions.

4) Drug Administration: AQUI-S (50% isoeugenol) or the positive control was administered by immersion. Fish were exposed for no longer than 60 minutes. Fish were recovered in a communal tank with the recovery period lasting no longer than 30 minutes.

5) Measurements and Observations: The time for each fish to become sedated to the handleable stage and the time for each fish to recover were measured as the two primary variables. A fish was identified as sedated to handleable when the fish was initially unresponsive to external stimuli except strong pressure, lost equilibrium, and did not avoid obstacles. A fish was identified as recovered when the fish initially regained equilibrium and could swim away from objects inserted in the tank. Also recorded were the length of the fish, the general and respiratory behavior, and the water temperature and dissolved oxygen concentration of the test solutions. Survival of fish was monitored for 24 hours post-exposure. Water hardness, alkalinity, and pH of the source water were measured.

d. Results: Median times to handleable and recovery for each fish species are included in the following tables.

Table 2. Median times to handleable and recovery for sub-adult rainbow trout at approximately 10 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	20	4.0	3.3
	40	2.4	3.9
	60	1.8	4.8
MS-222	80	1.1	1.8

Table 3. Median times to handleable and recovery for sub-adult lake trout at approximately 10 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	20	7.7	6.0
	40	2.9	5.9
	60	1.9	6.5
MS-222	80	1.7	1.6

Table 4. Median times to handleable and recovery for sub-adult mountain whitefish at approximately 10 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	20	5.8	4.9
	40	3.7	7.0
	60	2.9	8.5
MS-222	80	1.1	1.4

The mean length of the rainbow trout, lake trout, and mountain whitefish were 14.2 cm, 20.1 cm, and 23.4 cm, respectively. General and respiratory behaviors of all fish were normal in the fish exposed to AQUI-S at a concentration of 20 mg/L and in the fish exposed to MS-222.

Water hardness, alkalinity, and pH were 201 mg/L, 164 mg/L, and 7.9, respectively. Mean water temperatures ranged from 9.1 to 9.9 °C. Mean dissolved oxygen concentrations ranged from 9.1 to 9.7 mg/L.

e. Adverse Reactions: There were no mortalities during the study. Head shaking was observed in 11% of the fish exposed to 40 mg AQUI-S/L and 78% of the fish exposed to 60 mg AQUI-S/L for the first 10 seconds of the exposure.

f. Conclusion: AQUI-S concentrations of 20, 40, and 60 mg/L administered as an immersion are effective for sedating sub-adult rainbow trout, lake trout, and mountain whitefish, at a water temperature of about 10 °C, to handleable within 10 minutes with fish recovering within 10 minutes.

3. Field Study

a. "The efficacy of AQUI-S as an anesthetic for use on adult cutthroat trout" (Study Number AQUIS-01-SUPP-EFF-02)

b. Study Director: James D. Bowker

Investigator: Damon Keen

Study Site: Idaho Fish and Game
Henry's Lake State Fish Hatchery
Island Park, ID

c. Study Design:

1) Objective: To evaluate the effectiveness of AQUI-S to sedate to handleable individual adult Yellowstone cutthroat trout exposed to concentrations of 10, 20, 25, 30, and 34 mg AQUI-S/L of water at a water temperature of approximately 6 °C.

2) Study Animals: 90 adult Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri*. The fish had recently returned to the hatchery to spawn.

3) Treatment Groups: The study included 5 treatment groups receiving 10, 20, 25, 30, and 34 mg AQUI-S/L of water and one treatment group receiving a positive control, TRICAINE-S (MS-222/tricaine methanesulfonate) at a dose of 80 mg/L of water. The experimental unit was a group of five fish. Each treatment group included three replicates. The fish were arbitrarily selected from the reference population by crowding and using a dip-net to capture fish. The test article was administered in a systematic order from lowest to highest concentration followed by the control article. Each treatment group was tested separately. Each test solution was prepared in one or two 75-gallon tanks containing 50 gallons of the test solutions.

4) Drug Administration: AQUI-S (50% isoeugenol) or the positive control was administered by immersion. Fish were exposed for no longer than 10 minutes with the recovery period lasting no longer than 30 minutes. Each group of 5 exposed fish was recovered separately.

5) Measurements and Observations: The two primary variables were the time for each fish to become sedated to the handleable stage and the time for each fish to recover. A fish was identified as sedated to handleable when the fish was initially unresponsive to external stimuli except strong

pressure, lost equilibrium, and did not avoid obstacles. A fish was identified as recovered when the fish initially regained equilibrium and could swim away from objects inserted in the tank. Survival of fish was monitored for 24 hours post-exposure. Also recorded were the length of the fish, the general and respiratory behavior, and the water temperature and dissolved oxygen concentration of the test solutions. Water hardness, alkalinity, and pH of the source water were measured.

d. Results: Median times to handleable and recovery are included in the following table.

Table 5. Median times to handleable and recovery for adult Yellowstone cutthroat trout at 6 to 9 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	10	8.1	7.6
	20	4.7	5.6
	25	4.2	4.0
	30	4.5	3.4
	34	3.2	8.1
MS-222	80	1.7	5.0

The mean length of the fish was 47.0 cm. General and respiratory behaviors of all fish were normal.

Water hardness, alkalinity, and pH were 80 mg/L, 98 mg/L, and 8.82, respectively. Water temperatures ranged from 6.8 to 8.8 °C. Mean dissolved oxygen concentrations ranged from 9.1 to 9.8 mg/L.

e. Adverse Reactions: There were no mortalities during the study. No adverse reactions were reported in this study.

f. Conclusion: AQUI-S concentrations of 10, 20, 25, 30, and 34 mg/L administered as an immersion are effective for sedating adult Yellowstone cutthroat trout, at water temperatures of 6 to 9 °C, to handleable within 10 minutes with fish recovering within 10 minutes.

4. Field Study

a. "The efficacy of AQUI-S as an anesthetic for use on sub-adult bull trout *Salvelinus confluentus*" (Study Number AQUIS-01-SUPP-EFF-03)

b. Investigator: James D. Bowker

Investigator: Daniel Carty

Study Site: U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

c. Study Design:

1) Objective: To evaluate the effectiveness of AQUI-S to sedate to handleable individual sub-adult bull trout exposed to concentrations of 10, 20, 40, 60, and 120 mg AQUI-S/L of water at a water temperature of approximately 9 °C.

2) Study Animals: 30 sub-adult bull trout *Salvelinus confluentus*

3) Treatment Groups: The study included 4 treatment groups receiving 10, 20, 40, 60, and 120 mg AQUI-S/L of water, and one treatment group receiving a positive control, TRICAINE-S (MS-222/tricaine methanesulfonate) at a dose of 80 mg/L of water. The experimental unit was individual fish. Each treatment group included five replicates. The fish were arbitrarily selected from the reference population by using a dip-net to capture fish. The test article was administered in a systematic order from lowest to highest concentration followed by the control article. Each treatment group was tested separately. Each test solution was prepared in five 5-gallon buckets each containing 2 gallons of the test solution.

4) Drug Administration: AQUI-S (50% isoeugenol) or the positive control was administered by immersion. Fish were exposed for no longer than 60 minutes with the recovery period lasting no longer than 30 minutes. Fish were recovered in individual buckets containing fresh water.

5) Measurements and Observations: The two primary variables were the time for each fish to become sedated to the handleable stage and the time for each fish to recover. A fish was identified as sedated to handleable when the fish was initially unresponsive to external stimuli except strong pressure, lost equilibrium, and did not avoid obstacles. A fish was identified as recovered when the fish initially regained equilibrium and could swim away from objects inserted in the tank. Survival of fish was monitored for 24 hours post-exposure. Also recorded were the length of the fish, the general and respiratory behavior, and the water temperature and dissolved oxygen concentration of the test solutions.

d. Results: Median times to handleable and recovery are included in the following table.

Table 6. Median times to handleable and recovery for sub-adult bull trout at 9 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	10	28.8	6.4
	20	9.4	7.4
	40	3.3	5.9
	60	2.3	6.2
	120	1.3	5.2
MS-222	80	1.6	3.7

The mean length of the fish was 10.8 cm. General and respiratory behaviors of all fish were normal in the fish exposed to AQUI-S at concentrations of 10, 20, and 40 mg/L and in the fish exposed to MS-222.

Mean water temperatures ranged from 8.9 to 9.1 °C. Mean dissolved oxygen concentrations ranged from 9.4 to 9.7 mg/L.

e. Adverse Reactions: There were no mortalities during the study. Head shaking was observed in all of the fish exposed to AQUI-S at concentrations of 60 and 120 mg/L for the first 10 seconds of the exposure.

f. Conclusion: AQUI-S concentrations of 20, 40, 60, and 120 mg/L administered as an immersion are effective for sedating sub-adult bull trout, at water temperatures of 9 °C, to handleable within 10 minutes with fish recovering within 10 minutes.

5. Field Study

a. "The efficacy of AQUI-S as an anesthetic for use on sub-adult hybrid striped bass *Morone saxatilis* x *M. americana*" (Study Report Number AQUIS-01-SUPP-EFF-04)

b. Investigator: James D. Bowker

Investigator: Daniel Carty

Study Site: Kent SeaTech Corporation
Mecca, CA

c. Study Design:

1) Objective: To evaluate the effectiveness of AQUI-S to sedate to handleable individual sub-adult hybrid striped bass exposed to concentrations of 20, 40, and 60 mg AQUI-S/L of water at a water temperature of approximately 27 °C.

3) Treatment Groups: The study included 4 treatment groups receiving 20, 40, and 60 mg AQUI-S/L of water and one treatment group receiving a positive control, TRICAINE-S (MS-222/tricaine methanesulfonate) at a dose of 80 mg/L of water. The experimental unit was individual fish. Each treatment group included 5 replicates. The fish were arbitrarily selected from the reference population by using a dip-net to capture fish. The test article was administered in a systematic order from lowest to highest concentration followed by the control article. Each treatment group was tested separately. Each test solution was prepared in multiple 1-gallon buckets each containing 0.75 gallons of the test solution.

4) Drug Administration: AQUI-S (50% isoeugenol) or the positive control was administered by immersion. Fish were exposed for no longer than 60 minutes with the recovery period lasting no longer than 30 minutes. Fish were individually recovered in 1-gallon buckets containing fresh water.

5) Measurements and Observations: The two primary variables were the time for each fish to become sedated to the handleable stage and the time for each fish to recover. A fish was identified as sedated to handleable when the fish was initially unresponsive to external stimuli except strong pressure, lost equilibrium, and did not avoid obstacles. A fish was identified as recovered when the fish initially regained equilibrium and could swim away from objects inserted in the tank. Survival of fish was monitored for 24 hours post-exposure. Also recorded were the length of the fish, the general and respiratory behavior, and the water temperature and dissolved oxygen concentration of the test solutions.

d. Results: Median times to handleable and recovery are included in the following table.

Table 7. Median times to handleable and recovery for sub-adult hybrid striped bass at approximately 27 °C

Drug	Concentration (mg/L)	Time to Handleable (min)	Time to Recovery (min)
AQUI-S	20	6.3	2.0
	40	4.4	3.7
	60	3.2	4.1
MS-222	80	3.8	1.7

The mean length of the fish was 19.1 cm. General and respiratory behaviors of all fish were normal.

Mean water temperatures ranged from 27.0 to 27.8 °C. Mean dissolved oxygen concentrations ranged from 9.4 to 11.3 mg/L.

e. Adverse Reactions: There were no mortalities during the study. No adverse reactions were reported in this study.

f. Conclusion: AQUI-S concentrations of 20, 40, and 60 mg/L administered as an immersion are effective for sedating sub-adult hybrid striped bass, at water temperatures of 27 °C, to handleable within 10 minutes with fish recovering within 10 minutes.