

AQUI-S[®]E as an Anesthetic Clinical Field Trials - INAD 11-741

Year 2010 Annual Summary Report on the Use of AQUI-S[®]E as a Fish Anesthetic in Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

AQUI-S[®]E has been used experimentally in the U. S. under the U. S. Fish and Wildlife Service's (Service) compassionate INAD Exemption #11-741 by fish culturists and fisheries managers to sedate or anesthetize a variety of fish species for various purposes. In calendar year 2010 the efficacy of AQUI-S[®]E was evaluated in 39 trials involving 36,591 fish to induce different sedation levels in a variety of test fish. Trials were conducted at 18 fish culture facilities, including one Service fish hatchery, 16 state fish hatcheries, and one tribal hatchery. Overall, efficacy of AQUI-S[®]E used under INAD #11-741 showed that 97% of the trials appeared efficacious as a fish anesthetic because fish were anesthetized to the desired level of anesthesia within a "reasonable" amount of time and recovered from anesthesia; and were ineffective in 3% of the trials.

Introduction

Anesthetics are physical or chemical agents that act on an animal by initially inducing a calming effect and subsequently inducing loss of equilibrium, mobility,

consciousness, and reflex action (Summerfelt and Smith 1990). Anesthetics are widely used on hatchery-reared and wild fishes, usually to reduce stress caused by handling or transporting. As such, fish anesthetic research has been conducted on many compounds, including carbonic acid (Gelwicks et al. 1998), sodium bicarbonate (Peake 1998), quinaldine, benzocaine, and 2-phenoxyethanol (Gilderhus and Marking 1987; Iwama et al. 1989; Munday and Wilson 1997). Although several of these are effective fish anesthetics, the only compounds currently approved by the U.S. Food and Drug Administration (FDA) for use as anesthetics on fish are two 3-aminobenzoic acid ethyl ester methanesulfonate products: (1) FINQUEL[®], which is registered by Fort Dodge Laboratories, Fort Dodge, IA, and sold by Argent Chemical Laboratories, Redmond, WA, and (2) Tricaine-S[®] (i.e., MS-222), which is manufactured and sold by Western Chemical, Inc., Ferndale, WA. Both FINQUEL[®] and Tricaine-S[®] are effective fish anesthetics (Schoettger and Julin 1967; Schoettger et al. 1967); however, use of either is restricted to four fish families and requires a 21-d post-treatment “withdrawal” period before harvestable-size fish can be slaughtered for market, released to be caught and consumed by humans, or rendered in any way. Ultimately, the 21-d withdrawal period severely limits the conditions under which FINQUEL[®] and Tricaine-S[®] can be used.

Fish culturists and fisheries managers in the U.S. have long needed an FDA-approved fish anesthetic for which no withdrawal period is required. A zero-withdrawal (or immediate release) anesthetic would allow food fish to be released, stocked, or slaughtered “immediately” following treatment. In numerous fisheries management programs, and particularly those involving wildstock population assessment and

evaluation, there is a critical need for such an anesthetic. AQUI-S®E has been developed in New Zealand as an anesthetic for use on food-fish with no withdrawal period. The active ingredient in AQUI-S®E, eugenol, is used in perfumeries, flavorings, essential oils, and in medicine as a local antiseptic and anesthetic.

In the U.S., multi-partner efforts are underway to generate the efficacy, target animal safety, human food safety, and environmental fate data needed to support a New Animal Drug Approval for the use of AQUI-S®E as a zero-withdrawal fish anesthetic. As part of those efforts, the U. S. Fish and Wildlife Service (Service) has coordinated collection of pivotal and supplemental effectiveness data under compassionate INAD Exemption #11-741 to determine whether AQUI-S®E can effectively sedate or anesthetize a variety of coldwater, coolwater, and warmwater fishes.

Purpose

The purpose of this report is to summarize the results of calendar year 2010 (CY10) AQUI-S®E field efficacy trials. Furthermore, it is expected that these data will be used to establish an AQUI-S®E database for the purpose of developing an appropriate label claim for the legal use of this new animal drug in aquaculture.

Facilities, Materials, and Methods

1. Facilities

Eighteen fish culture facilities used AQUI-S®E during CY10, including one Service fish hatchery, 16 state fish hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 40.0 - 68.0 °F, with a mean treatment temperature of 52.2°F.

2. AQUI-S®E used in trials

All AQUI-S®E used in CY10 trials was supplied by AQUI-S New Zealand, LTD, Lower Hutt, New Zealand. The current AQUI-S®E formulation consists of 50% eugenol (active ingredient) and 50% adjuvant to help dissolve eugenol in water.

3. Drug dosages

As described in INAD Study Protocol #11-741, AQUI-S®E was administered as a single static bath for up to 15 minutes at dosages ranging from 10 to 75 mg eugenol/L.

Fish Species

1. Species and size of fish treated

The following 10 fish, which included seven salmonid and three non-salmonid species, were treated during CY10. Treated fish ranged in length from 2.0 - 46.0 in. with a mean length of 12.6 in.

Salmonids:

brook trout *Salvelinus fontinalis*

lake trout *S. namaycush*

brown trout *Salmo trutta*

land locked Atlantic salmon *S. salar*

cutthroat trout *Oncorhynchus clarki*

fall chinook salmon *O. tshawytscha*

rainbow trout *O. mykiss*

Non-Salmonids:

muskellunge *Esox masquinongy*

bluehead sucker *Catostomus discobolus*

Utah sucker *C. ardens*

2. Level of Anesthesia

Fish were exposed to AQUI-S®E for a duration sufficient to reach the specified level of anaesthesia (e.g., sedation for handling purposes or anesthetized for surgical procedures). After fish reached the desired level of sedation/anesthesia, they were removed from AQUI-S®E solutions, returned to freshwater, and allowed to recover.

Data Collected

Treatment duration or time to desired level of anesthesia

Efficacy was measured by documenting the time required for test fish to reach a specified level of anesthesia and to recover from that specified level of anesthesia. A trial was considered effective if treated fish became sedated or anesthetized and recovered when returned to freshwater, regardless of the time required for that event to occur. Note that Investigators used the terms “sedation” or “sedated” and “anesthesia” or “anesthetized” interchangeably.

Sedation should be the term used when fish are anesthetized for handleable, fish husbandry, or fisheries management practices; and anesthetized should be the term used when fish are anesthetized for surgical procedures.

Discussion of Study Results

- 1. Summary results on the efficacy of AQUI-S[®]E for anesthetizing fish** (Note: summary data regarding specific trials are described in Tables 1 - 4; summary of the number of trials conducted, test fish treated, treatment regimens used, and fish species tested with AQUI-S[®]E in CY10 are listed in Table 5; and a summary of the individual AQUI-S[®]E trials conducted during CY10 under INAD #11-741 are listed in Table 6.)

A. Efficacy of AQUI-S[®]E on salmonids - handleable

Eleven trials involving fall chinook salmon, cutthroat trout, and rainbow trout were treated with 10 - 45 mg eugenol/L AQUI-S[®]E. In these trials, the desired level of sedation was to the handleable stage of anesthesia (Table 1). The test fish became

handleable between 1.0 - 10.0 min and recovered within 0.5 - 15.0 min. All trial results appeared to be effective.

B. Efficacy of AQUI-S[®]E on salmonids to anesthetized

In 24 trials, brook trout, brown trout, cutthroat trout, lake trout, rainbow trout, and land locked Atlantic salmon were treated with 20 - 75 mg eugenol/L AQUI-S[®]E and the time for test fish to reach the anesthetized stage of anesthesia was measured (Table 2). The test fish became anesthetized between 0.5 - 10.0 min and recovered within 0.0 - 15.0 min; all of these trials appeared effective.

C. Efficacy of AQUI-S[®]E on non-salmonids - handleable

One trial involving adult muskellunge were exposed to 20 mg eugenol/L AQUI-S[®]E and fish were left in the treatment bath for 11.3 min (Table 3). Test fish did not become handleable within the investigators allotted time frame; this trial was not effective.

D. Efficacy of AQUI-S[®]E on non-salmonids to anesthetized

In three trials, bluehead sucker, muskellunge, and Utah sucker, were treated with 20 - 25 mg eugenol/L AQUI-S[®]E and the time for test fish to reach the anesthetized stage of anesthesia was measured (Table 4). In all trials, test fish became anesthetized within 5.0 - 10.0 min and recovered within 2.75 - 20.0 min.; all trial results appeared effective.

2. Observed Evidence of Toxicity or Adverse Reactions

No toxicity or adverse effects relating to AQUI-S®E treatment were reported in 36 of the trials. In the remaining three trials adverse effects were noted: 1) the investigator noted in one trial that one fish died after being left in the treatment bath too long; 2) in another trial the investigator noted that one rainbow trout (out of 30) was agitated when it was placed in the treatment bath; 3) and finally in one rainbow trout trial the investigator noted that four fish died during the treatment; it was also noted that 208 mortalities occurred with MS222 and 2 mortalities with the control fish.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for AQUI-S®E INAD #11-741

Please see the attached current study protocol for AQUI-S®E INAD #11-741. Note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 7. Facilities and Names of Investigators” for facilities that signed-up to participate in the AQUI-S®E INAD #11-741 during CY10. Facilities not listed in Appendix III-a of the current AQUI-S®E INAD #11-741 study protocol have been

highlighted. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facilities received AQUI-S®E during CY10 but never used the drug:

1. Eagle Fish Health Lab
2. Fort Richardson SFH

Correspondence sent to AQUI-S®E Participants

Please see the attached correspondence that was sent to all AQUI-S®E participants after the AADAP Office received their sign-up form for CY10.

Number of Treated Fish under Slaughter Authorization

Total number of fish treated during CY10 was 36,591. The total number of treated fish to count against the slaughter authorization dated September 9, 2009 is 36,591. No changes have occurred to the current AQUI-S®E INAD #11-741 study protocol.

Summary of Study Results

During CY10, 39 AQUI-S®E efficacy trials were conducted in which test fish were sedated or anesthetized with AQUI-S®E in a static bath for durations ranging from 0.5 to 11.3 min at concentrations ranging from 10 to 75 mg eugenol/L. During this period, seven salmonid and three non-salmonid fish species involving 36,591 fish, were treated with AQUI-S®E. Test fish were sedated to either the handleable stage

of anesthesia or to the anesthetized stage. Treated fish ranged in size from 2.0 - 46.0 inches. Water temperature during treatment ranged from 40.0 - 68.0°F. Overall, efficacy of AQUI-S®E used under INAD #11-741 showed that 97% of the trials appeared efficacious as a fish anesthetic because fish were anesthetized to the desired level of anesthesia within a “reasonable” amount of time and recovered from anesthesia; and were ineffective in 3% of the trials. Furthermore, Investigators reported no evidence of toxicity or adverse effects related to AQUI-S®E treatment in 92% of the trials. Data from trials generated under the compassionate INAD (i.e., supplemental data) should be considered ancillary efficacy data and should provide useful corroborative data to support a future label claim for AQUI-S®E.

References

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- Munday, P. L., and S. K. Wilson. 1997. Comparative effects of clove oil and other chemicals in anesthetization of *Pomacentrus amboinensis*, a coral reef fish. *Journal of Fish Biology* 51:931-938.
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- Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213 - 272 *in* C. B. Schreck and P. B. Moyle, editors. *Methods for fish biology*. American Fisheries Society, Bethesda, Maryland.

Table 1. Summary of AQUI-S®E to Anesthetize Salmonids to the Handleable Stage - CY10

Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
CUT	Clark's Fork SFH	1	Juvenile	20	1.50	4.00	100	51.0
CUT	Dubois SFH	1	Fingerling	20	3.00	1.50	10	58.0
CUT	Lahontan NFH	1	Adult	20	5 - 10	5 - 10	354	55.0
FCS	Nez Perce Tribal Hatchery	1	Adult	30	2.0 - 3.0	5.0 - 7.0	893	41.9
RBT	Boulder Rearing Station	1	Adult	30	6.00	5.00	1,600	52.0
RBT	Jim Hinkle Spring River SFH	1	Fingerling	20	2.75 - 3.25	2.0 - 2.25	519	68.0
RBT	Leaburg SFH	1	Juvenile	30	1.0 - 5.0	0.75	8,026	59.2
RBT	Oregon Hatchery Research Center	1	Fingerling	10	1.25 - 4.25	0.5 - 4.5	30	53.8
RBT	Roaring River SFH	2	Adult	40 - 45	1.0 - 2.0	5.0 - 15.0	9,060	44.0
RBT	William H Donham SFH	1	Fingerling	20	1.25	1.75	250	66.0

Table 2. Summary of AQUI-S®E to Anesthetize Salmonids to Anesthetized Stage - CY10

Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Anesthetized (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
BKT	Manchester SFH	2	Adult	25 - 50	8 - 10	5 - 10	86	50.0
BNT	Dubois SFH	2	Fingerling	20 - 30	2.25 - 2.75	1.75 - 2.75	20	58.0
BNT	Ed Weed FCS	1	Juvenile	30	1.5 - 2.75	1.75 - 3.5	674	49.5
CUT	Dubois SFH	1	Fingerling	30	2.50	1.75	10	58.0
CUT	Nevada Fisheries Division	1	Juvenile	25	3.50	12.75	1	40.0
LAS	Ed Weed FCS	1	Adult	30	2.25 - 3.25	4.5 - 7.0	132	48.0
LAT	Ed Weed FCS	1	Juvenile	30	1.25 - 1.75	2.25 - 4.5	701	49.5
RBT	Boulder Rearing Station	4	Adult	30 - 50	4 - 6	5 - 7	557	52.0
RBT	Dubois SFH	2	Fingerling	20 - 30	1.25 - 2.25	1.5 - 2.25	40	58.0
RBT	McNenny SFH	3	Adult	50 - 75	0.5 - 3.75	0 - 7.75	152	52.0
RBT	Nevada Fisheries Division	1	Adult	20	2.0 - 2.5	2.50	520	40.0
RBT	Nevada Fisheries Division	2	Juvenile	20 - 25	1.75 - 2.0	3.25 - 5.0	2,002	40.0 - 56.0
RBT	Roaring River SFH	1	Adult	45	2.00	10.0 - 15.0	2,710	44.0
RBT	Springfield Office	1	Adult	30	0.75 - 1.75	1.5 - 3.75	7,990	59.2
RBT	Wolf Lake SFH	1	Fingerling	25	1.00	2.00	45	52.0

Table 3. Summary of AQUI-S®E to Anesthetize Non-Salmonids to the Handleable Stage - CY10

Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
MUE	Spirit Lake SFH	1	Adult	20	11.28 - did not become handleable	-	3	52.0

Table 4. Summary of AQUI-S®E to Anesthetize Non-Salmonids to the Anesthetized Stage - CY10

Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Anesthesia (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
BHS	Jackson Regional Office	1	Adult	20	10.00	15.0 - 20.0	56	50.0
MUE	Wolf Lake SFH	1	Fingerling	25	5.00	2.75	45	58.6
UTS	Jackson Regional Office	1	Adult	20	10.00	15.00	5	50.0

Table 5. Number of Trials Conducted, Fish Treated, Treatment Regimens Used, and Fish Species Evaluated with AQUI-S®E in CY10

Number of Treatment Trials:	39
Number of Treated Fish :	36,591
Treatment Regimes Used:	
Salmonids to handleable 10 - 45mg/L	11 trials
Non-salmonids to handleable 20mg/L	1 trial
Salmonids to anesthetized 20 - 75mg/L	24 trials
Non-salmonids to anesthetized 20 - 25mg/L	3 trials
Treatment Water Temperature (°F):	40.0 - 68.0
Size of Treated Fish:	2.0 - 46.0

Species Treated:

Salmonids:

brook trout *Salvelinus fontinalis*
lake trout *S. namaycush*
brown trout *Salmo trutta*
land locked Atlantic salmon *S. salar*
cutthroat trout *Oncorhynchus clarki*
fall chinook salmon *O. tshawytscha*
rainbow trout *O. mykiss*

Non-Salmonids:

muskellunge *Esox masquinongy*
bluehead sucker *Catostomus discobolus*
Utah sucker *C. ardens*