

AQUI-S®E/20E as an Anesthetic Clinical Field Trials - INAD 11-741

Year 2011 Annual Summary Report on the Use of AQUI-S®E/20E as a Fish Anesthetic in Field Efficacy Trials

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Summary

AQUI-S®E/20E 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 2011 the efficacy of AQUI-S®E/20E was evaluated in 67 trials involving 115,900 fish to induce different sedation levels in a variety of test fish. Trials were conducted at 16 fish culture facilities, including one Service fish hatchery, one United States Geological Survey (USGS) research facility, 12 state fish hatcheries, and two private facilities. Overall, efficacy of AQUI-S®E/20E used under INAD #11-741 showed that 93% 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; ineffective in 6% of the trials; and characterized as inconclusive in 1% 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/20E 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/20E, 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/20E 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/20E 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 2011 (CY11) AQUI-S[®]E/20E field efficacy trials. Furthermore, it is expected that these data will be used to enhance data in the existing AQUI-S[®]E/20E database established from previous years, and will be considered in the “body of evidence” for the purpose of developing an appropriate label claim for the use of AQUI-S[®]E/20E in aquaculture.

Facilities, Materials, and Methods

1. Facilities

Sixteen fish culture facilities used AQUI-S[®]E/20E during CY11, including one Service fish hatchery, one USGS research facility, 12 state fish hatcheries, and two private facilities. Water temperature during treatments at the various testing facilities ranged from 33.8 - 72.5 °F, with a mean treatment temperature of 51.0°F.

2. AQUI-S[®]E/20E used in trials

All AQUI-S[®]E/20E used in CY11 trials was supplied by AQUI-S New Zealand, LTD, Lower Hutt, New Zealand. The AQUI-S[®]E formulation consists of 50% eugenol (active ingredient) and 50% adjuvant to help dissolve eugenol in water; however, this formula was replaced with AQUI-S[®]20E during CY11. The current AQUI-S[®]20E formulation is 10% eugenol (active ingredient).

3. Drug dosages

As described in INAD Study Protocol #11-741, AQUI-S[®]E/20E was administered as a single static bath in 64 trials for up to 15 minutes at dosages ranging from 7 to 100 mg eugenol/L. Study protocol deviations occurred when fish were treated for longer than 15 minutes or over 100 mg eugenol/L in three trials. The following is an explanation for each deviation:

1) Pallid sturgeon were treated for 21.5 minutes in one trial with the treatment dose of 15.3 mg eugenol/L. Half of the tested fish became handleable at 21.5 minutes. Fish are a T&E species and will not be available for human consumption.

2) Pacific lamprey were treated for 16.25 - 21.5 minutes in one trial with the treatment dose of 30 mg eugenol/L. The investigator noted that all test fish would be euthanized following the study.

3) Pacific lamprey were treated for 4.0 - 6.25 minutes in one trial with the treatment dose of 200 mg eugenol/L. The investigator noted that all test fish would be euthanized following the study.

Fish Species

1. Species and size of fish treated

The following 20 fish, which included 11 salmonid and nine non-salmonid species, were treated during CY11. Treated fish ranged in length from 1.0 - 32.6 in. with a mean length of 11.5 in.

Salmonids:

brook trout *Salvelinus fontinalis*

Arctic char *S. alpinus*

lake trout *S. namaycush*

brown trout *Salmo trutta*

Atlantic Salmon *S. salar*

land locked Atlantic salmon *S. salar*

cutthroat trout *Oncorhynchus clarki*

chinook salmon *O. tshawytscha*

coho salmon *O. kisutch*

rainbow trout *O. mykiss*

Steelhead trout *O. mykiss*

Non-Salmonids:

bluehead sucker *Catostomus discobolus*

channel catfish *Ictalurus punctatus*

common carp *Cyprinus carpio*

gizzard shad *Dorosoma cepedianum*

Klamath River lamprey *Lampetra similis*

Pacific lamprey *L. tridentata*

Lamprey spp. *Entoshenos* spp.

pallid sturgeon *Scaphirhynchus albus*

roundtail chub *Gila robusta*

2. Level of Anesthesia

Fish were exposed to AQUI-S®E/20E 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/20E solutions, returned to freshwater, and allowed to recover. Please note in 2011 the AADAP Office changed the level of anaesthesia reported in several studies from anesthetized to handleable as fish were sedated for non surgical purposes. A note was made in each of the studies reporting this change made by the AADAP Office.

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/20E for anesthetizing fish (Note:

summary data regarding specific trials are described in Tables 1 - 5; summary of the number of trials conducted, test fish treated, treatment regimens used, and fish species tested with AQUI-S[®]E/20E in CY11 are listed in Table 6; and a summary of the individual AQUI-S[®]E/20E trials conducted during CY11 under INAD #11-741 are listed in Table 7.)

A. Efficacy of AQUI-S[®]E/20E on salmonids - handleable

Forty-three trials involving Arctic char, Atlantic salmon, brook trout, brown trout, chinook salmon, coho salmon, cutthroat trout, lake trout, landlocked Atlantic salmon, rainbow trout, and steelhead trout were treated with 13.4 - 85 mg eugenol/L AQUI-S[®]E/20E. In these trials, the desired level of sedation was to the handleable stage of anesthesia (Table 1). The test fish became handleable between 0.25 - 10.0 min and recovered within 0.25 - 20.0 min when timed. Treatments appeared to be effective in 40 trials while three trials were ineffective.

B. Efficacy of AQUI-S[®]E/20E on salmonids to anesthetized

One trial was conducted on chinook salmon that were treated with 16.5 - 19.8 mg eugenol/L AQUI-S[®]E/20E and the time for test fish to reach the anesthetized stage of anesthesia was measured (Table 2). The test fish became anesthetized between 2.75 - 4.75 min and recovered within 3.0 - 7.75 min. This trial appeared to be effective.

C. Efficacy of AQUI-S[®]E/20E on non-salmonids - handleable

Eighteen trials involving Klamath river lamprey, lamprey spp., Pacific lamprey, and pallid sturgeon were treated with 7 - 200 mg eugenol/L AQUI-S[®]E/20E. In these trials, the desired level of sedation was to the handleable stage of anesthesia (Table 3). The test fish became handleable between 0.5 - 21.5 min (note: pallid sturgeon did not become handleable at the 7 mg eugenol/L AQUI-S[®]E/20E dose) and recovered within 0.25 - 17.5 min when timed. Treatments appeared to be effective in 16 trials, ineffective in one trial, and inconclusive in one trial.

D. Efficacy of AQUI-S[®]E/20E on non-salmonids to anesthetized

In one trial bluehead suckers were treated with 20 mg eugenol/L AQUI-S[®]E/20E and the time for test fish to reach the anesthetized stage of anesthesia was measured (Table 4). Test fish became anesthetized within 10.0 - 11.0 min and recovered within 17.0 - 18.0 min. Treatment appeared to be effective in this trial.

E. Efficacy of AQUI-S[®]E/20E on non-salmonids to euthanized

Four trials involving channel catfish, common carp, gizzard shad, and roundtail chub were treated with 30 mg eugenol/L AQUI-S[®]E/20E. In these trials, the desired purpose of AQUI-S[®]E/20E was to euthanize the fish (Table 5). The test fish were euthanized between 2.0 - 9.0 min. Treatments appeared to be effective in all four trials.

2. Observed Evidence of Toxicity or Adverse Reactions

No toxicity or adverse effects relating to AQUI-S[®]E/20E treatment were reported in 61 of the trials. In the remaining six trials adverse effects were noted in trials involving Pacific lamprey treated between 30 - 200 mg eugenol/L AQUI-S[®]E/20E. The investigator noted in these trials that the fish were extremely agitated in the initial exposure to the anesthetic and they would try to climb out of the treatment tank.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for AQUI-S[®]E/20E INAD #11-741

No changes have occurred to the current study protocol for AQUI-S[®]E/20E INAD #11-741.

Facility Sign-up List

Please see “Table 8. Facilities and Names of Investigators” for facilities that signed-up to participate in the AQUI-S[®]E/20E INAD #11-741 during CY11. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facilities received AQUI-S®E/20E during CY11 but never used the drug:

1. Port Angeles
2. Black River Hatchery
3. Rathbun SFH (submitted data under pivotal protocol)
4. Marine Finfish Reproduction Center
5. Eagle Fish Health Lab
6. Kooskia NFH

Correspondence sent to AQUI-S®E/20E Participants

Please see the attached correspondence that was sent to all AQUI-S®E/20E participants after the AADAP Office received their sign-up form for CY11.

Number of Treated Fish under Slaughter Authorization

Total number of fish treated during CY11 was 115,900. The total number of treated fish to count against the slaughter authorization dated September 9, 2009 is 152,491.

Summary of Study Results

During CY11, 67 AQUI-S®E/20E efficacy trials were conducted in which test fish were sedated, anesthetized, or euthanized with AQUI-S®E/20E in a static bath for durations ranging from 0.25 to 21.5 min at concentrations ranging from 7 to 200 mg

eugenol/L. During this period, 11 salmonid and nine non-salmonid fish species involving 115,900 fish, were treated with AQUI-S®E/20E. Test fish were sedated to the handleable stage of anesthesia, the anesthetized stage, or were euthanized. Treated fish ranged in size from 1.0 - 32.6 inches. Water temperature during treatment ranged from 33.8 - 72.5°F. Overall, efficacy of AQUI-S®E/20E used under INAD #11-741 showed that 93% 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; while 6% of the trials were ineffective, and 1% of the trials were characterized as inconclusive. Furthermore, Investigators reported no evidence of toxicity or adverse effects related to AQUI-S®E/20E treatment in 91% 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/20E.

References

- Gelwicks, K. R., D. J. Zafft, and J. P. Bobbitt. 1998. Efficacy of carbonic acid as an anesthetic for rainbow trout. *North American Journal of Fisheries Management* 18:432-438.
- Gilderhus, P. A., and L. L. Marking. 1987. Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management* 7:288-292.
- Iwama, G. K., J. C. McGeer, and M. P. Pawluk. 1989. The effects of five anesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. *Canadian Journal of Zoology* 67:2065-2073.
- 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.
- Peake, S. 1998. Sodium bicarbonate and clove oil as potential anesthetics for nonsalmonid fishes. *North American Journal of Fisheries Management* 18:919-924.
- Schoettger, R. A., and A. M. Julin. 1967. Efficacy of MS-222 as an anesthetic on four salmonids. United States Department of Interior Resource Publication 19, Bureau of Sport Fisheries and Wildlife, Washington, D.C.
- Schoettger, R. A., C. R. Walker, L. L. Marking, and A. M. Julin. 1967. MS-222 as an anesthetic for channel catfish: its toxicity, efficacy, and muscle residues. United States Department of Interior Resource Publication 19, Bureau of Sport Fisheries and Wildlife, Washington, D.C.
- 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/20E to Anesthetize Salmonids to the Handleable Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
efficacious	ARC	Fort Richardson SFH	1	adult	20	4.5 - 8.5	5.5 - 12.5	30	33.8
efficacious	ATS	Rich Pass	2	adult	13.4 - 38.5	6.0 - 10.0	8.0 - 10.0	305	48.0
efficacious	ATS	Scatter Creek Hatchery	2	fingerling	20	2.0 - 5.0	5.00	22,700	50.0
efficacious	BKT	Ed Weed FCS	1	juvenile	30	3.0 - 3.5	3.5 - 4.0	126	34.0
ineffective	BKT	Kentucky Dept Fish Wildlife	2	adult	30 - 40	5.00	6.0 - 7.0	6,020	43.7
efficacious	BKT	Kentucky Dept Fish Wildlife	1	adult	60	2.5 - 3.5	7.0 - 7.5	5,600	43.7
efficacious	BNT	Bennington FCS	1	adult	30	1.5 - 2.5	3.25 - 4.0	7,600	50.0
efficacious	BNT	Ed Weed FCS	2	juvenile	30	2.0 - 3.0	3.0 - 4.5	813	34.0 - 50.2
efficacious	COS	Arcata FWO	2	juvenile	22.5 - 30	0.5 - 2.5	0.25 - 5.5	235	45.2 - 55.4
efficacious	CSA	Arcata FWO	3	juvenile	15 - 30	0.5 - 5.25	0.25 - 2.0	1,240	45.7 - 54.0
efficacious	CUT	Clarks Fork SFH	1	fingerling	25	0.5 - 1.0	0.5 - 1.0	5,400	53.2
efficacious	CUT	Clarks Fork SFH	1	juvenile	25	0.75 - 1.0	1.75 - 2.15	900	50.0
efficacious	LAS	Ed Weed FCS	1	juvenlie	30	3.0 - 5.25	1.5 - 4.0	2,793	34.0
efficacious	LAS	Ed Weed FCS	1	adult	30	3.0 - 4.0	3.0 - 4.0	108	50.2

Table 1. Summary of AQUI-S®E/20E to Anesthetize Salmonids to the Handleable Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
efficacious	LAT	Ed Weed FCS	1	juvenile	30	2.0 - 3.0	3.0 - 3.75	456	50.3
efficacious	RBT	Bennington FCS	1	adult	30	0.75 - 1.0	1.5 - 2.0	4,000	50.0
efficacious	RBT	Boulder Rearing Station	7	adult	50	4.0 - 5.0	6.00	1,105	52.0
efficacious	RBT	Kentucky Dept Fish Wildlife	3	adult	40 - 60	0.5 - 1.25	2.0 - 5.0	43,360	43.7 - 49.3
efficacious	RBT	Reno Headquarters	1	adult	25	2.00	3.00	2,735	54.9
ineffective	RBT	Roaring River SFH	1	adult	50	10.00	15.00	1,075	44.0
efficacious	RBT	Roaring River SFH	3	adult	45 - 85	2.0 - 10.0	5.0 - 20.0	5,920	44.0
efficacious	RBT	Tillett FRS	2	adult	17 - 25	0.75 - 5.0	1.0 - 7.0	2,017	54.0
efficacious	STT	Arcata FWO	2	juvenile	22.5 - 30	0.25 - 3.0	0.25 - 5.0	63	45.7 - 55.4
efficacious	STT	Ed Weed FCS	1	juvenile	30	3.0 - 4.5	2.5 - 5.0	804	34.0

Table 2. Summary of AQUI-S®E/20E to Anesthetize Salmonids to Anesthetized Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Anesthetized (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
efficacious	CSA	ODFW Fish Trap at Willamette Falls	1	Adult	16.5 - 19.8	2.75 - 4.75	3.0 - 7.75	32	54.0

Table 3. Summary of AQUI-S®E/20E to Anesthetize Non-Salmonids to the Handleable Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
efficacious	EAM	Arcata FWO	3	fingerling	15 - 30	1.0 - 4.5	0.25 - 3.5	52	45.2 - 56.9
efficacious	KRL	Arcata FWO	2	juvenile	15 - 30	1.25 - 7.0	0.25 - 5.25	74	45.2 - 56.2
efficacious	PCL	Arcata FWO	2	juvenile	15	0.5 - 5.25	0.5 - 2.25	6	45.2 - 56.5
efficacious	PCL	Columbia River Research Lab	6	juvenile	30 - 200	4.0 - 21.5	2.25 - 17.5	98	53.0 - 54.0
inconclusive	PLS	SIU - Fisheries Illinois Aquaculture Center	1	juvenile	15.3	21.5	15.75	16	72.5
efficacious	PLS	SIU - Fisheries Illinois Aquaculture Center	3	juvenile	36.4 - 77.9	1.5 - 5.5	2.25 - 3.75	80	72.5

Table 3. Summary of AQUI-S®E/20E to Anesthetize Non-Salmonids to the Handleable Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Handleable (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
ineffective	PLS	SIU - Fisheries Illinois Aquaculture Center	1	juvenile	7	did not become handleable	-	16	72.5

Table 4. Summary of AQUI-S®E/20E to Anesthetize Non-Salmonids to the Anesthetized Stage - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Anesthesia (min)	Time to Recovery (min)	Number Treated	Temp. (°F)
efficacious	BHS	Jackson Regional Office	1	Adult	20	10.0 - 11.0	17.0 - 18.0	4	50.0

Table 5. Summary of AQUI-S®E/20E to Euthanize Non-Salmonids - CY11

Efficacy	Fish Species	Facility	Number of Trials	Age	Dose (mg eugenol/L)	Time to Anesthesia (min)	Number Treated	Temp. (°F)
efficacious	CCF	Reno Headquarters	1	adult	30	8.0 - 9.0	20	59.0
efficacious	CAP	Reno Headquarters	1	juvenile/adult	30	4.0 - 5.0	20	59.0
efficacious	GIS	Reno Headquarters	1	juvenile/adult	30	2.0 - 3.0	20	59.0
efficacious	RTC	Reno Headquarters	1	juvenile/adult	30	3.0 - 4.0	57	62.0

Table 6. Number of Trials Conducted, Fish Treated, Treatment Regimens Used, and Fish Species Evaluated with AQUI-S®E/20E in CY11

Number of Treatment Trials:	67
Number of efficacious trials	62
Number of ineffective trials	4
Number of inconclusive trials	1
 Number of Treated Fish :	 115,900
Number of fish treated in efficacious trials	108,773
Number of fish treated in ineffective trials	7,111
Number of fish treated in inconclusive trials	16
 Treatment Regimes Used:	
Salmonids to handleable 13.4 - 85mg/L	43 trials
Non-salmonids to handleable 7 - 200mg/L	18 trials
Salmonids to anesthetized 16.5 - 19.8mg/L	1 trial
Non-salmonids to anesthetized 20mg/L	1 trial
Non-salmonids to euthanized 30mg/L	4 trials
 Treatment Water Temperature (°F):	 33.8 - 72.5
 Size of Treated Fish:	 1.0 - 32.6

Species Treated:

Salmonids:

brook trout *Salvelinus fontinalis*
 Arctic char *S. alpinus*
 lake trout *S. namaycush*
 brown trout *Salmo trutta*
 Atlantic Salmon *S. salar*
 land locked Atlantic salmon *S. salar*
 cutthroat trout *Oncorhynchus clarki*
 chinook salmon *O. tshawytscha*
 coho salmon *O. kisutch*

rainbow trout *O. mykiss*
Steelhead trout *O. mykiss*

Non-Salmonids:

bluehead sucker *Catostomus discobolus*
channel catfish *Ictalurus punctatus*
common carp *Cyprinus carpio*
gizzard shad *Dorosoma cepedianum*
Klamath River lamprey *Lampetra similis*
Pacific lamprey *L. tridentata*
Lamprey spp. *Entoshenos spp.*
pallid sturgeon *Scaphirhynchus albus*
roundtail chub *Gila robusta*