Efficacy and Safety of AQUI-S as an Anesthetic on Freshwater Fish

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• Use of anesthetics is an important tool with broad application to fish culture and management programs.

• Only approved anesthetics are Finquel and Tricaine-S (i.e., MS-222). Both require a 21-d post-treatment withdrawal period before harvestable fish can be released or slaughtered.

• Zero-withdrawal period anesthetic highly desirable, particularly by field biologists.
BACKGROUND

Potential Zero-Withdrawal Anesthetics

- **Clove oil** – active ingredient 90 to 95% eugenol; shown to be an effective anesthetic; however, a “crude product” and no sponsor so zero chance of gaining FDA-approval; (i.e., not legal). See Guidance to Industry Document 150 on status of clove oil and eugenol for anesthesia of fish.

- **AQUI-S** – active ingredient isoeugenol; used abroad to achieve “rested harvesting”; used in U.S. experimentally; sponsored by AQUI-S New Zealand LTD; stands best chance of gaining FDA-approval; INAD established.
AQUI-S isn’t quite comparable to MS-222 when it comes to 1) time to knock out fish and 2) time for fish to recover (MS-222 is faster for both).

AQUI-S is a bit more challenging to use (i.e., more care must be taken to mix properly, and it may foam up).

But . . .

- AQUI-S Will Not Replace MS-222 ! ! !

- need for an anesthetic with fewer human health concerns

- **No Withdrawal Period**
Pivotal Efficacy Protocol

Each fish is an experimental unit; the time required for each fish to become handleable and the time to recover is recorded.

15 replicates per treatment group; two life-stages; two temperatures

Individual fish are exposed separately.

Individual fish are allowed to recover separately.

Dose-verification of all AQUI-S concentrations.

Data survival curves are analyzed using the Kaplan Maier method.
Primary Response Variables

Time (minutes) to:

Handleable
Recovery from Handleable

Multiple: species
life-stages
water temperatures
Determining Handleable

Handleable

- loss of reactivity to external stimuli except strong pressure
- loss of equilibrium; unable to avoid obstacles in it’s path
- suitable for most culture/management activities including spawning, tagging, weighing, measuring, etc.

Similar to Stage 3 – 4 anesthesia described in Summerfelt and Smith (1990).
Determining Recovery

Recovery

A fish is considered to be recovered when it regains normal equilibrium in the water column and exhibits the ability to avoid obstacles in its path.
AQUI-S Anesthetic Studies
Pivotal Efficacy Data

USFWS
Bozeman Fish Technology Center
Bozeman, MT (April 2002)

-Rainbow Trout, fingerling and adult

-20, 40, and 60 mg/L AQUI-S
and 80 mg/L MS-222

-Water 8 and 15ºC
Total number of fish exposed = 15 fish per concentration
AQUI-S Anesthetic Studies
Pivotal Efficacy Data

USFWS
Dworshak National Fish Hatchery
Ahsahka, ID (March 2002)

-Steelhead Trout, returning adults

-20, 40, and 60 mg/L AQUI-S
and 80 mg/L MS-222

-Water 6°C
Total number of fish exposed = 15 fish per concentration

Handleable

6°C

Steelhead trout returning adults

Recovery
AQUI-S Anesthetic Studies
Pivotal Efficacy Data

Montana Fish, Wildlife & Parks
Miles City State Fish Hatchery &
USFWS
Bozeman Fish Technology Center

- Pallid and Shovelnose sturgeon, subadult

-20, 40, 60, and 80 mg/L AQUI-S
and 80 mg/L MS-222

-Water 12°C
Handleable

12°C

Pallid and Shovelnose subadult

Total number of fish exposed = 15 fish per concentration

Recovery
Cold- vs Cool- and Warm-
comparison of species

Appear to be substantial differences in times to induce handleable and times to recover between cold- and cool- and warm-water species
Time to Handle-able for Cold, Cool, and Warm Water Species

Increase in concentration for cool water species

![Median Time to Handleable (min) vs AQUI-S Concentration (mg/L) for different species and temperatures](chart.png)
EFFICACY
SUMMARY

Proposed Label Claim
(based on preliminary results and discussion with the Sponsor)

Use AQUI-S
at concentrations ranging from:

○ 20 - 60 mg/L to induce handle-able for cold water species

○ 60 - 80 mg/L to induce handle-able for cool and warm water species

○ you may stock or harvest fish immediately following exposure to AQUI-S
FDA requires fish to be able to survive . . .

- for a **duration** of at least 3x the time to handleable (i.e., overexposed)

- a **concentration** 2x the target concentration (i.e., overdosed)
SAFETY

rainbow trout
fingerling

Percent Survival

AQUI-S Concentration (mg/L)

RBT  3x duration
**SAFETY**

histology data on rainbow trout

- No pathological changes in tissues of fish exposed to 15 mg/L AQUI-S

- Mild and reversible lesions were seen in gill and kidney tissue of fish exposed to 40, 60, and 120 mg/L AQUI-S

Photos and slide analysis by Charlie Smith, histopathologist
SAFETY

pallid sturgeon
largemouth bass
0 years

Percent Survival

80 mg/L 160 mg/L

AQUI-S Concentration

PLS 3x duration
LMB 3x duration
AQUI-S appears to be ... SAFE

- for salmonids at concentrations <60 mg/L
- for cool and warm water species at concentrations <80 mg/L
SUMMARY

- Increased water temperature decreases time to recovery

- $20\text{ - }60\text{ mg/L}$ to anesthetize cold water species to the handleable stage

- $60\text{ - }80\text{ mg/L}$ AQUI-S to anesthetize cool and warm water species to the handleable stage

- Preliminary work with AQUI-S shows that it is SAFE under the current FDA regulations
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