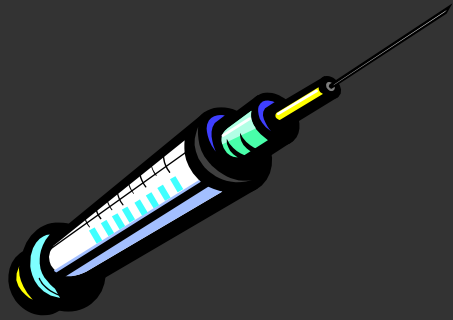


Efficacy and Safety of AQUI-S as an Anesthetic on Freshwater Fish

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**U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana**





BACKGROUND

- **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.



OVERVIEW

AQUI-S vs MS-222

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

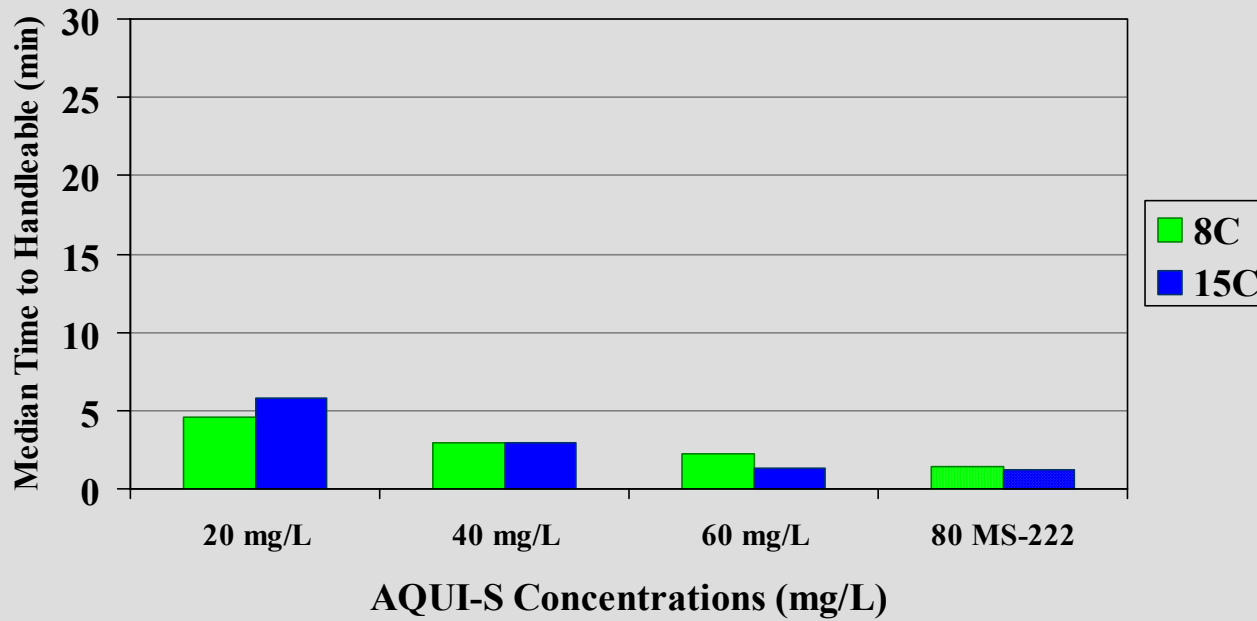
**Bozeman Fish Technology Center
Bozeman, MT (April 2002)**

-Rainbow Trout, fingerling and adult

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

-Water 8 and 15°C



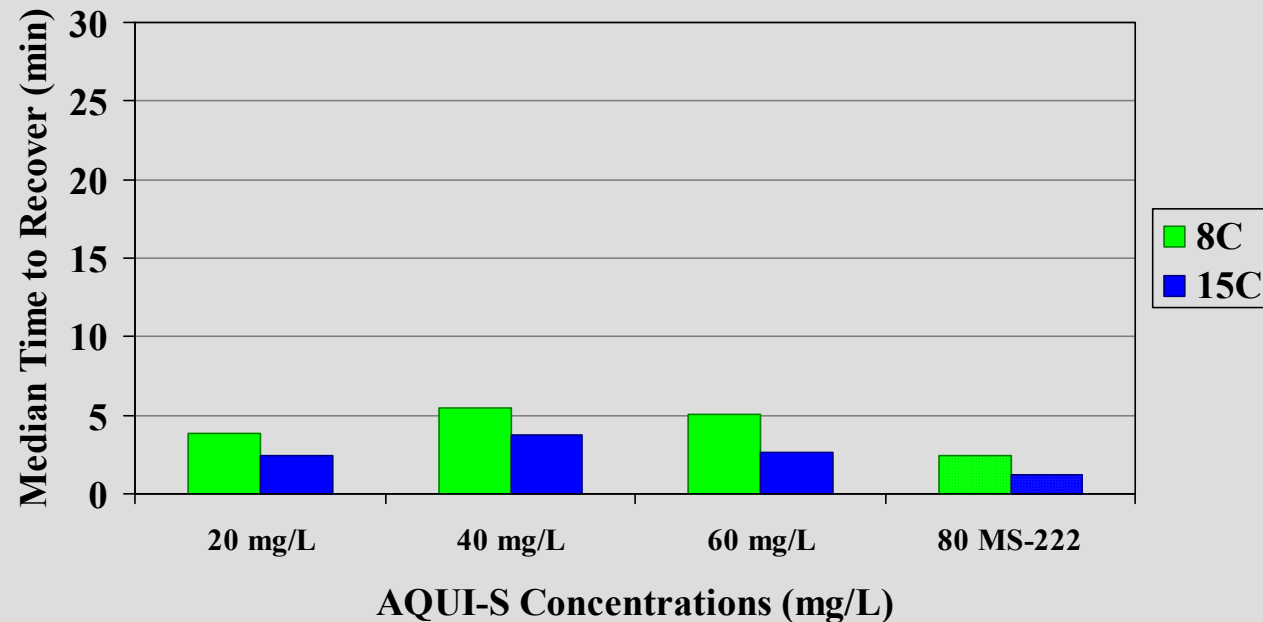


Handleable



**rainbow trout
fingerling & adult**

Total number of fish exposed = 15 fish per concentration



Recovery

AQUI-S Anesthetic Studies

Pivotal Efficacy Data

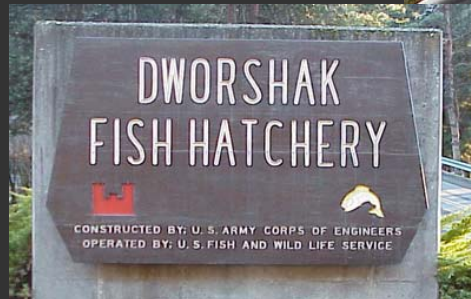
USFWS

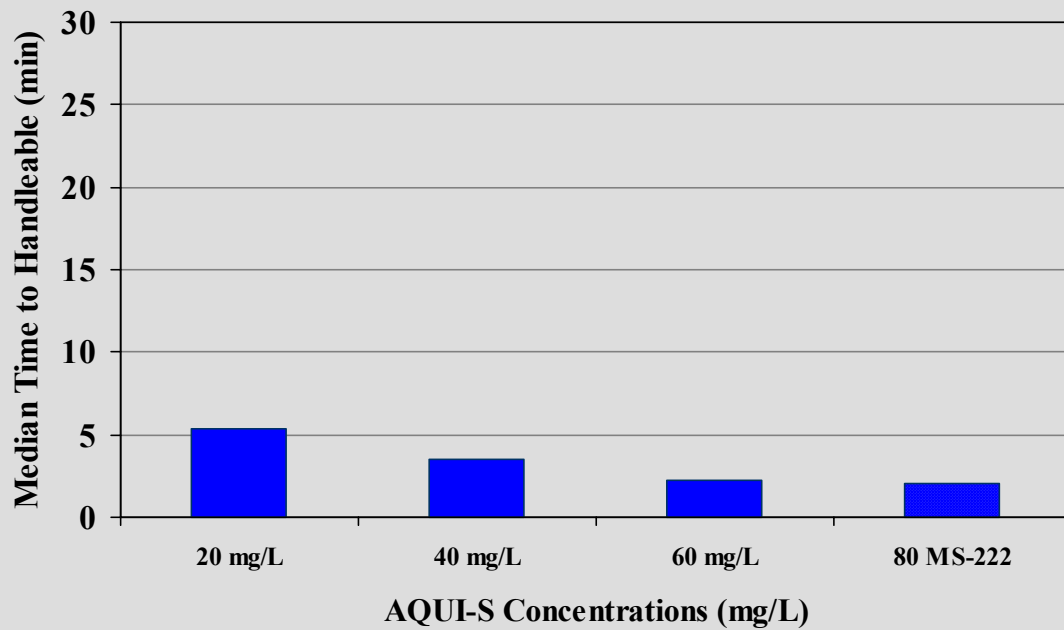
**Dworshak National Fish Hatchery
Ahsahka, ID (March 2002)**

-Steelhead Trout, returning adults

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

-Water 6°C





Handleable

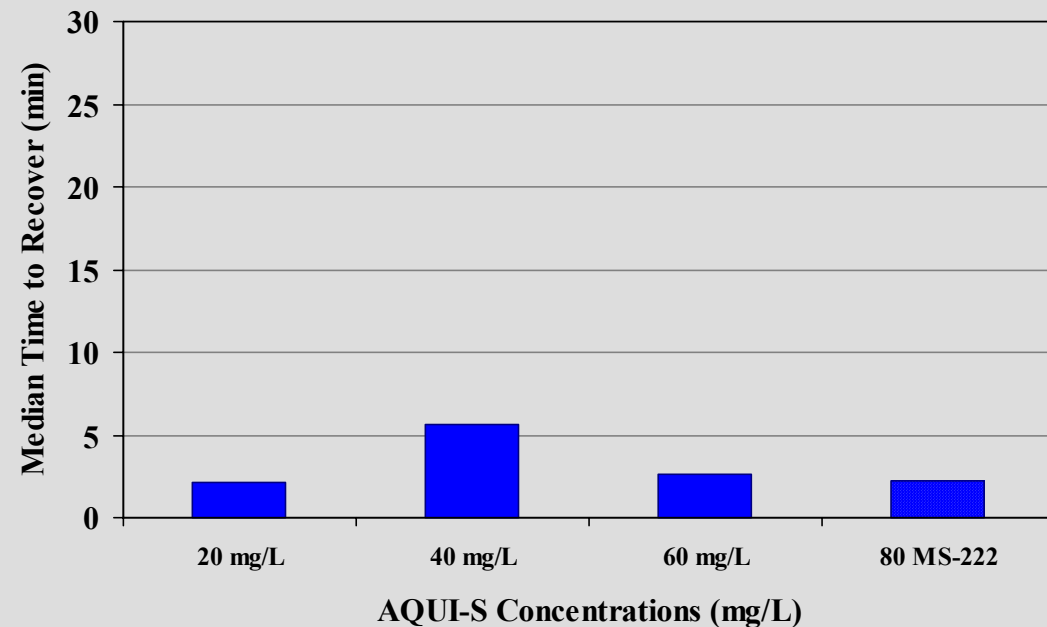
6°C

**Steelhead trout
returning adults**

Total number of fish exposed = 15 fish per concentration



Recovery



AQUI-S Anesthetic Studies

Pivotal Efficacy Data

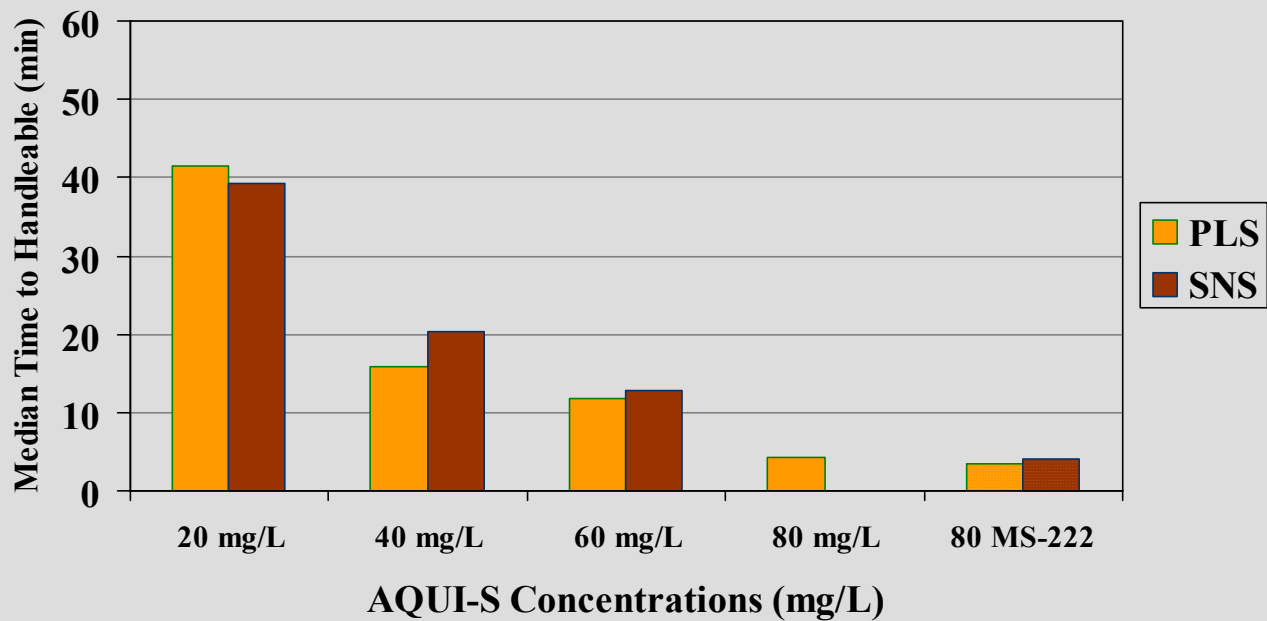
Montana Fish, Wildlife & Parks
Miles City State Fish Hatchery &
USFWS
Bozeman Fish Technology Center

- Pallid and Shovelnose sturgeon, subadult

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

-Water 12°C





Handleable

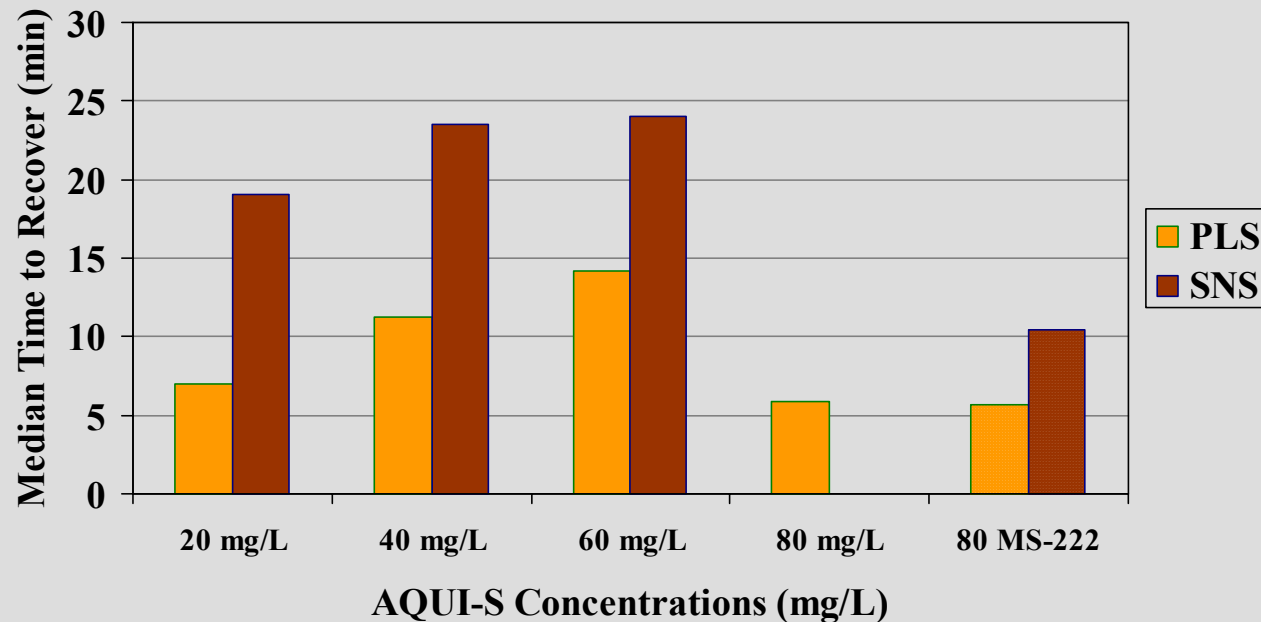
12°C

**Pallid and Shovelnose
subadult**

Total number of fish exposed = 15 fish per concentration



Recovery



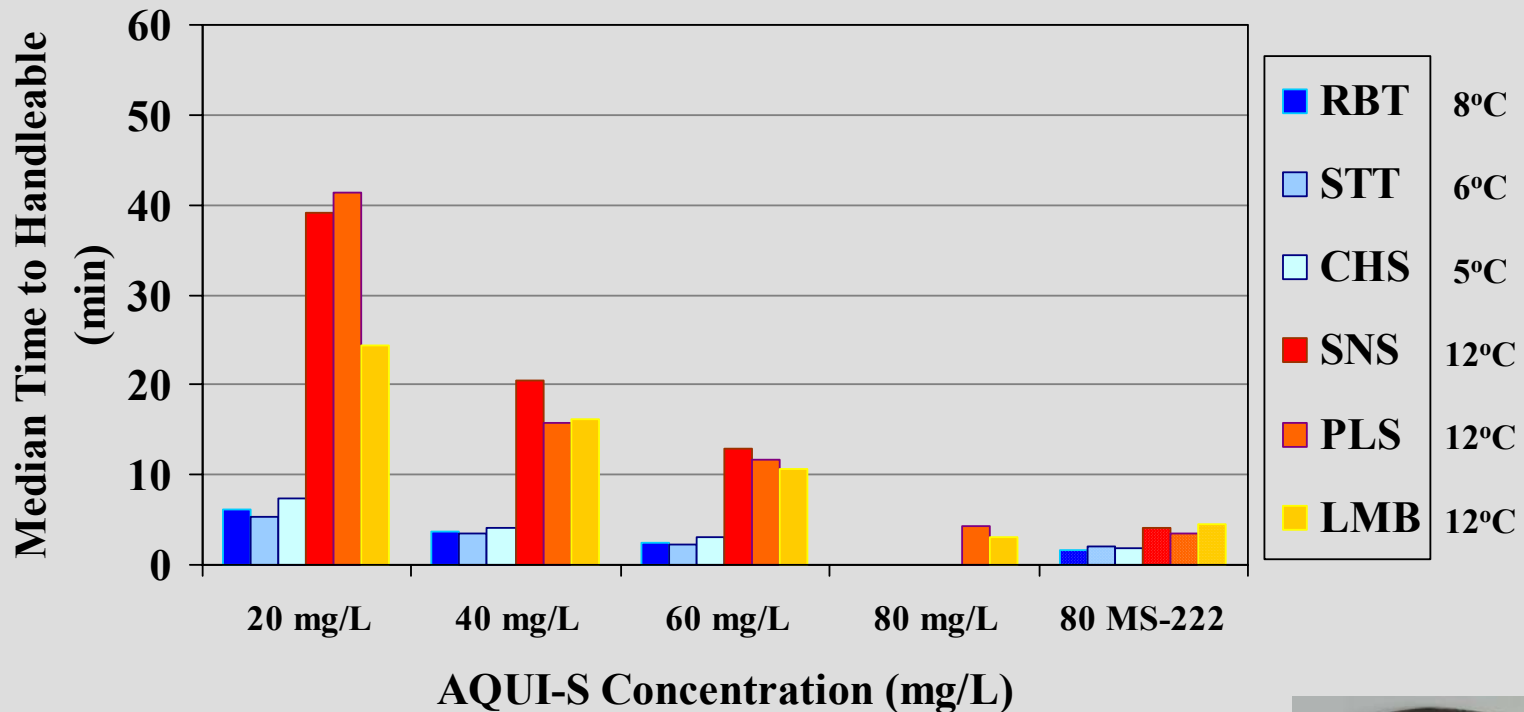
Cold- vs Cool- and Warm- comparison of species

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



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





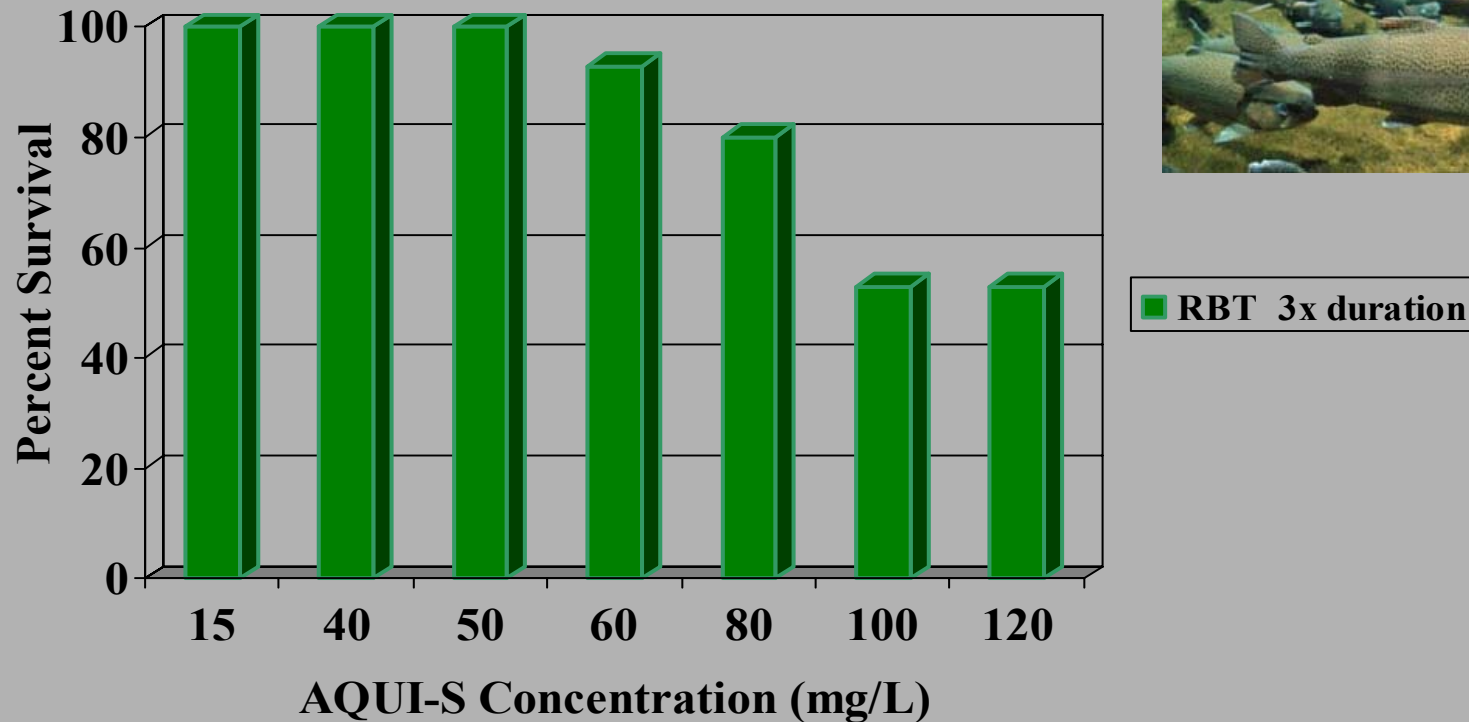
SAFETY

FDA requires fish to be able to survive . . .

- o for a duration of at least 3x the time to handleable (i.e., overexposed)**
- o a concentration 2x the target concentration (i.e., overdosed)**

SAFETY

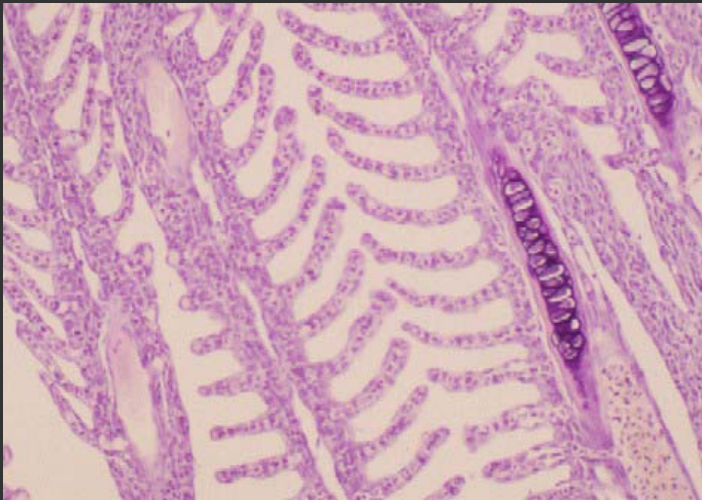
rainbow trout
fingerling



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



normal gill

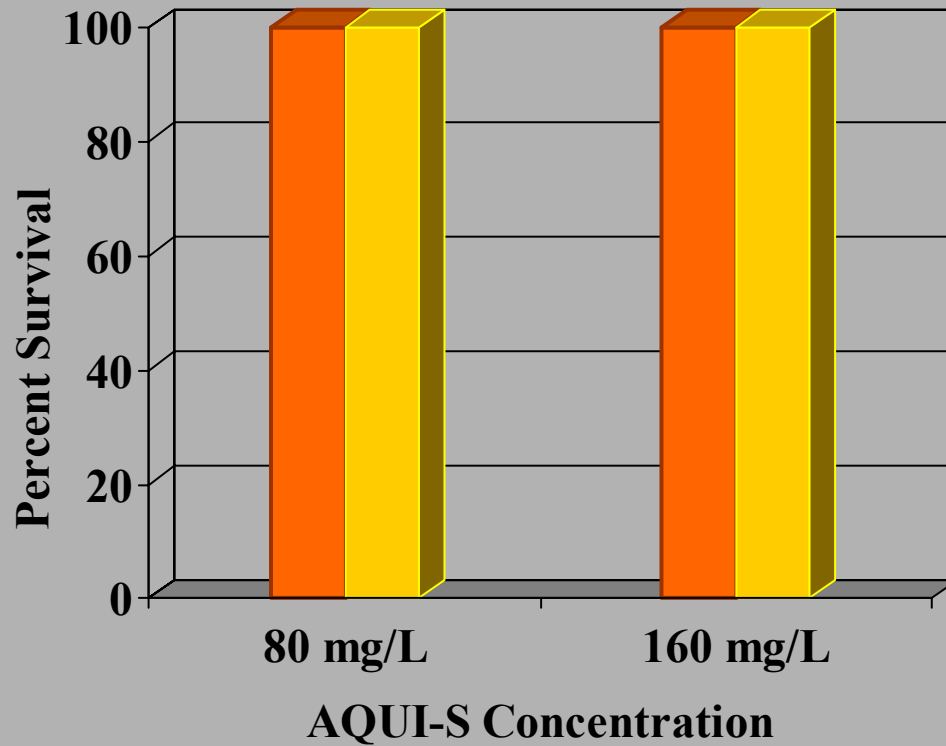


lifting of lamellar epithelium
and mild degeneration of lamellar epithelium

Photos and slide analysis by Charlie Smith, histopathologist

SAFETY

pallid sturgeon
largemouth bass
0 years



■ PLS 3x duration
■ LMB 3x duration



SAFETY

SUMMARY

AQUI-S appears to be . . . SAFE

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





S U M M A R Y

- Increased water temperature decreases time to recovery
- 20 - 60 mg/L to anesthetize cold water species to the handleable stage
- 60 - 80 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

Acknowledgements



We would like to thank the staff at . . .

Bozeman Fish Technology Center (USFWS)

Miles City SFH (MT FWP)

Dworshak NFH (USFWS)

Idaho Fish Health Center (USFWS)

Henry's Lake SFH (ID F&G)

Eagle Fish Health Lab (ID F&G)

McCall Spring Chinook Salmon Hatchery (ID F&G)

. . . for their help in completing AQUI-S efficacy studies.