



SEDATING FISH WITH AQUI-S20E:

WILL MY FISH BE ALIVE IF I GRAB ANOTHER CUP OF COFFEE??

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What is AQUI-S20E?

- Fish sedative/anesthetic
- 10% eugenol
- Sponsor: Aqui-S New Zealand, Ltd
- Available under USFWS INAD 11-741
 - Immediate release for field use
 - 3-d withdrawal period for all other uses
 - \$198/L
- Efforts underway to gain FDA approval
 - Initial claim: To sedate all freshwater finfish to handleable
 - Demonstrate that it's effective at the lowest dose
 - Demonstrate that it's safe at the highest dose



Is it Effective?

- Define effectiveness
 - Sedate fish within 5 min
 - Preferably within 1 – 2 min
 - Lose equilibrium & ability to swim
- Conduct preliminary testing to identify doses
 - Salmonids - 25 mg/L eugenol
 - 12°C
 - Coolwater finfish – 40 mg/L eugenol
 - 18°C
 - Warmwater finfish – 60 mg/L eugenol
 - 24°C



How Effective?

- Conducted 20 trials
 - May – November, 2011
- Tested 12 fish species
 - Rainbow, cutthroat, brown, and lake trout
 - Yellow perch, walleye, common carp, and fathead minnow
 - Sunshine bass, blue and channel catfish, Nile tilapia
- Four locations
 - USFWS Bozeman Fish Technology Center
 - USGS Upper Midwest Environmental Sciences Center
 - Southern Illinois University – Carbondale
 - Iowa DNR Rathbun Fish Culture Research Facility



Effectiveness Results

Target dose	Mean time to sedation	Mean time to recovery
25 mg/L (salmonids)	2.0 min (range, 1.3 – 3.2)	6.2 min (range, 3.6 – 10.8)
40 mg/L (coolwater)	2.1 min (range, 1.4 – 3.1)	8.0 min (range, 5.9 – 10.2)
60 mg/L (warmwater)	1.4 min (range, 0.9 – 1.7)	8.0 min (range, 5.2 – 12.9)

Sufficient data collected to complete effectiveness data requirements

Demonstrate Safety

Target Animal Safety studies

- Test only 3 fish species
 - Rather than 6
- Early life stage fish
 - Rainbow trout - 5.8 cm
 - yellow perch – 6.1 cm
 - channel catfish – 5.3 cm
- Determine
 - Highest efficacious dose
 - 1x dose
 - Overdose dose
 - 1.5x dose
 - ET80's
 - Effective time to sedate 80% of the fish to handleable
 - Exposure durations
 - 4 durations
- Determine safety based on:
 - Mortality
 - Gross & microscopic lesions
 - General behavior



Margin of Safety

- What is an adequate margin of safety?
 - Unlikely that:
 - First time users will kill fish?
 - Fisheries professional will kill fish?
- Higher dose results in faster time to sedation
 - Faster to respiratory arrest
 - Smaller margin of safety
- How long is long enough?
 - 1x dose: 3 – 4 min \geq ET80
 - 1.5x dose: 2 – 3 min \geq ET80
- Acceptable survival?
 - \geq 95%



ET80's and Exposure Durations

- ET80
 - FDA term
 - Sedate 15 fish to handleable –then do the math
- Exposure durations
 - Lots of pilot testing
 - T1 - 100% survival
 - T2 – 100% survival
 - ✓ Longest duration with no mortality
 - T3 – 70 to 80% survival
 - T4 – 50 to 70% survival
 - ✓ Need mortality to better assess dose-related pathologies

Doses and Durations

- **Rainbow trout (25 mg/L)**
 - 40 mg/L (ET80 = 1:01)
 - Durations: 3:30, 4:30, 6:30, and 8:45
 - 60 mg/L: (ET80 = 0:37)
 - Durations: 2:15, 2:45, 3:30, and 4:45
- **Yellow perch (40 mg/L)**
 - 80 mg/L (ET80 = 0:54)
 - Durations: 5:00, 7:00, 9:00, and 10:30
 - 120 mg/L: (ET80 = 0:47)
 - Durations: 4:00, 5:00, 6:00, and 6:45
- **Channel catfish (60 mg/L)**
 - 100 mg/L (ET80 = 0:22)
 - Durations: 3:30, 4:30, 8:00, and 12:00
 - 150 mg/L: (ET80 = 0:18)
 - Durations: 2:30, 4:00, 6:00, and 10:00



Experimental Design

- 3 replicates/exposure group
 - Experimental unit = exposure bucket/recovery tank (15 fish/unit)
 - 3 doses x 4 durations = 12 dose/duration combinations
 - Replicates conducted on three different days
- Expose fish for predetermined time (static bath)
 - T1 – T4
 - Control exposure durations = 1x exposure durations
- Allow fish to recover - monitored for 24 hr (flow thru)
 - Monitor survival and behavior
 - Necropsy dead fish/histology
- Collect 4 fish/unit for histology
 - Trout and catfish only (YEP tissues collected/stored)
 - Gill, posterior kidney, liver

Experimental Procedures

- **Prestudy**
 - Fish health
 - Move fish to recovery tanks
- **Exposures**
 - Prepare bulk tubs of AQUI-20E or control
 - Move fish from recovery tanks to exposure buckets
 - Monitored general behavior
 - Water temp and DO concentration before and after
 - Collect samples for dose verification (UV spec)
- **Recovery**
 - Moved from exposure buckets to recovery tanks
 - Monitored for survival/ behavior at 15 min, 1 hr, 2 hr, 4 hr, and 24 hr
 - Dead fish sampled < 1 h post-exposure for histology
 - Sampled and terminated at 24 hr post-exposure



Results

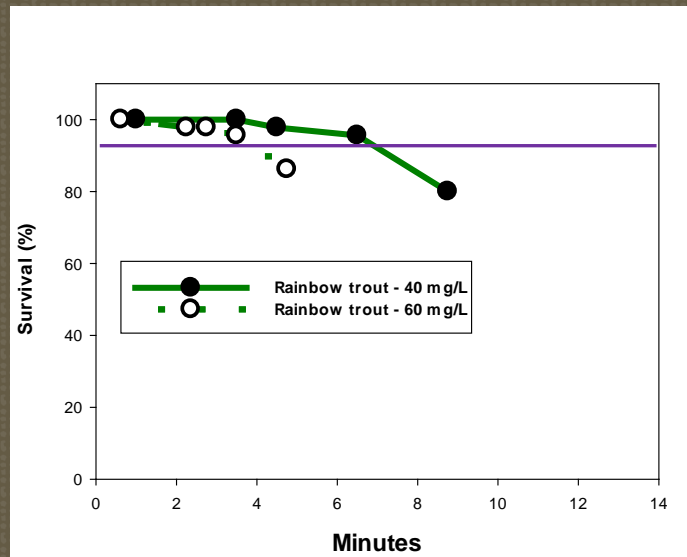
- Mean water temperature
 - Rainbow trout – 15.2°C
 - Yellow perch – 18.8°C
 - Catfish – 26.2°C
- Abnormal behavior during exposure
 - Rainbow trout – head-shaking (10 – 20 sec)
 - Yellow perch – coughing, head-shaking
 - Catfish - none
- Gross lesions/microscopic lesions
 - None/pending
- Dose verification
 - Within 5% of target dose



Rainbow trout survival (%)

40 mg/L – to 6.5 min

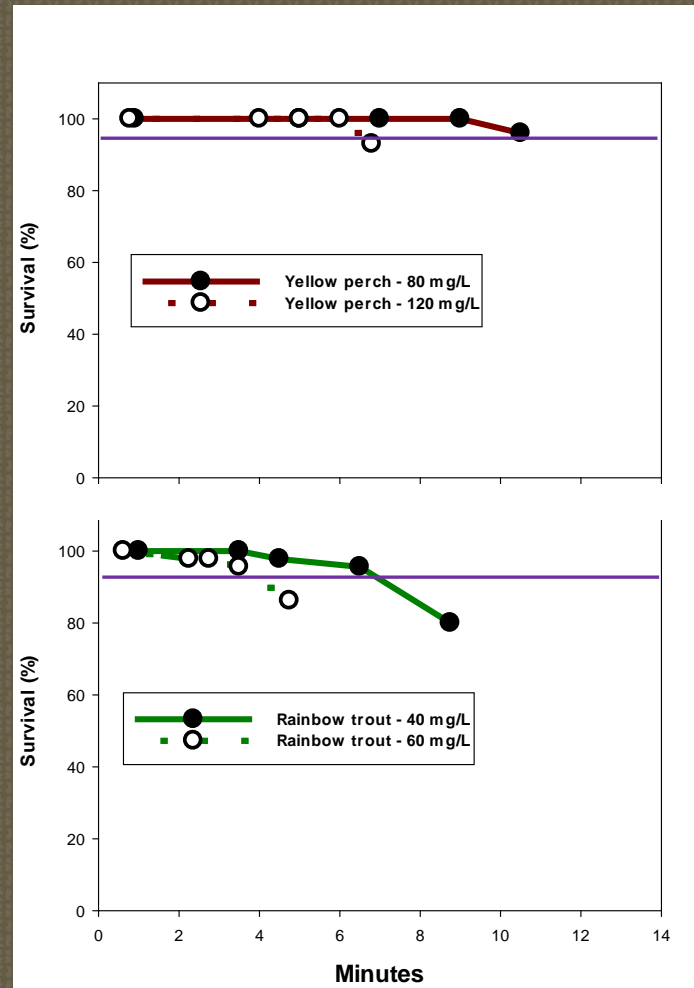
60 mg/L – to 3.5 min



Yellow perch survival (%)

80 mg/L – to at least 10.5 min

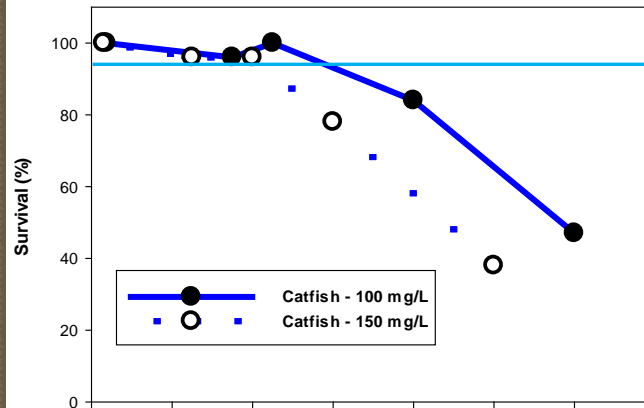
120 mg/L – to 6.0 min



Catfish survival (%)

100 mg/L – to 4.5 min

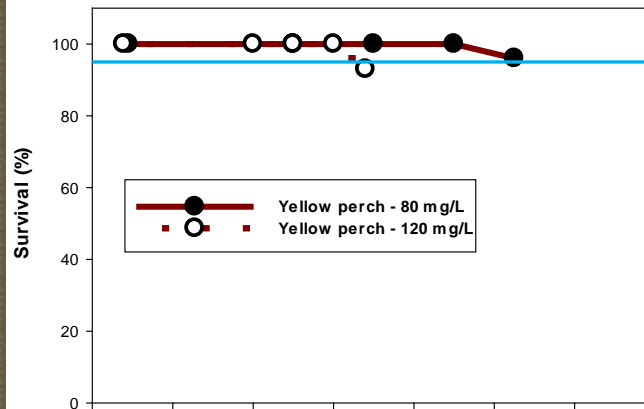
150 mg/L – to 4.0 min



Yellow perch survival (%)

80 mg/L – to at least 10.5 min

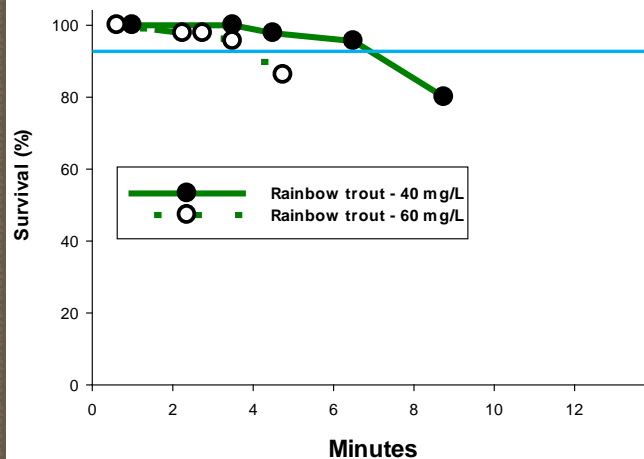
120 mg/L – to 6.0 min



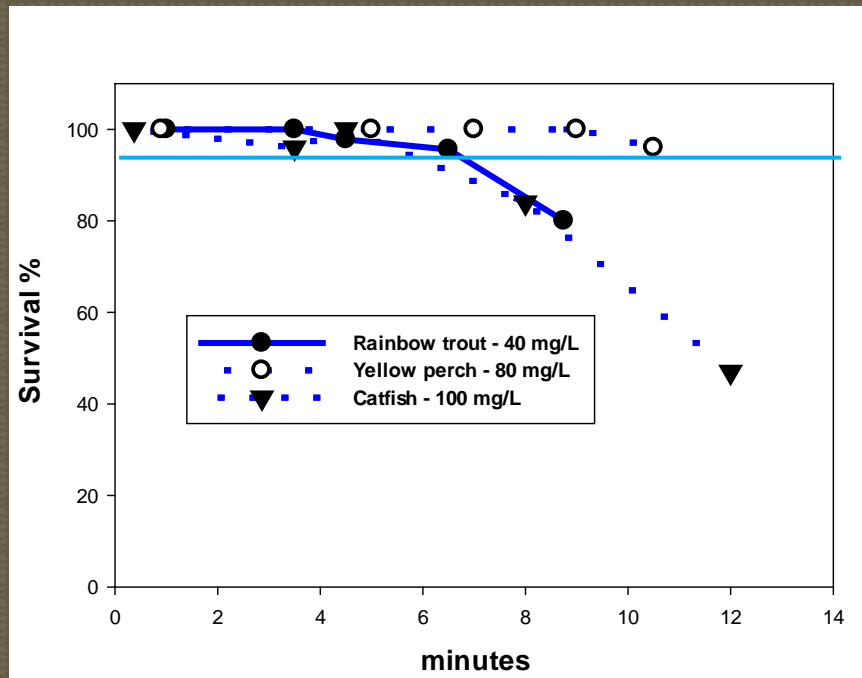
Rainbow trout survival (%)

40 mg/L – to 6.5 min

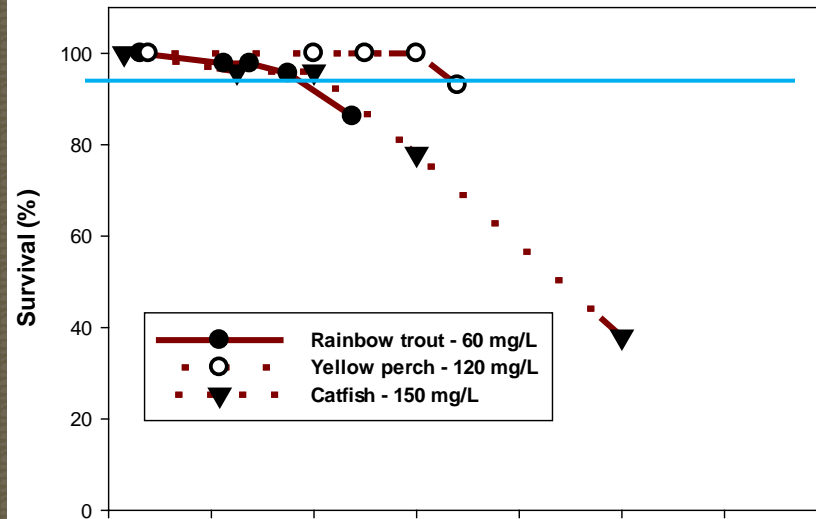
60 mg/L – to 3.5 min



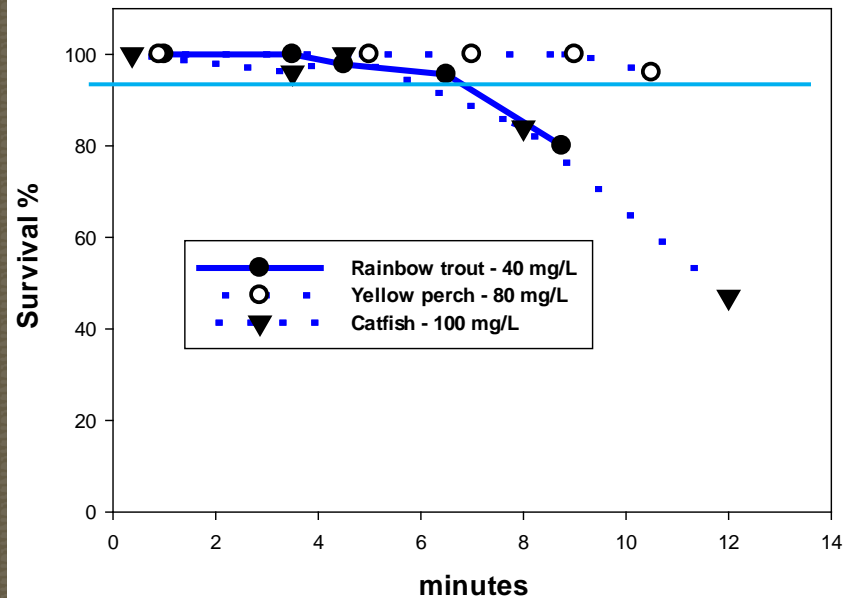
At the highest efficacious dose - to 5.8 min



At the overdose
dose – to 3.8 min



At the highest efficacious
dose - to 5.8 min



Observations / Summary

- Recovery times increased:
 - Rainbow trout – up to 5 min
 - Yellow perch – up to 12 min
 - Catfish – up to 45 min
- Safe durations for 1x dose:
 - Rainbow trout – 4.5 min beyond ET80
 - Yellow perch – at least 9.5 min beyond ET80
 - Catfish – 4.0 min beyond ET80



Conclusion

- Adequate margins of safety to support:
 - Salmonids: 25 – 40 mg/L
 - Coolwater finfish: 40 – 80 mg/L
 - Warmwater finfish: 60 – 100 mg/L
- If you want another cup of coffee...
 - You had better make it real quick



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Questions??