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

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

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 ≥ ET80
  ▪ 1.5x dose: 2 – 3 min ≥ ET80

• Acceptable survival?
  ▪ ≥ 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

Rainbow trout survival (%)  
40 mg/L – to 6.5 min  
60 mg/L – to 3.5 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??