The Effects of Duration and Concentration of Episodic Zinc Exposure to the Fathead Minnow, *P. promelas*.

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National Pollution Discharge & Elimination System (NPDES)

- Prohibits the discharge of any pollutant into navigable waters
- Require periodic testing of effluents using whole effluent toxicity testing
WET Testing

- Plant, vertebrate, invertebrate
- Acute
  - 96 hours or less
  - Endpoint is Mortality
- Chronic
  - 7 day life cycle test
  - Endpoints are growth, reproduction, mortality
WET Testing Limitations

- Surveyed 250 dischargers in 15 states
- Compared results of WET tests to actual stream impairment

- 20.3% there was a false negative
- 30.4% there was a false positive

- 17.5% there was a false negative
- 22.8% there was a false positive

Diamond et al. 2000
WET Testing Limitations

• Emphasis on concentration & continuous exposure
  – No regard to duration, frequency, or potential recovery periods
• Ignores ambient water quality of the receiving stream & spatial and temporal variability of contaminant
• Fails to consider latent effects, multiple routes of exposure, and risk to higher levels of organization
Ambient Water Quality Criteria

• Water quality criteria regulate how much of a chemical can be present in surface waters of the US

• Similar forms of testing (96 Hour LC50’s) are used for development of water quality criteria

• Research has shown that streams that exceed ambient water quality criteria for many chemicals can still support high quality ecosystems
Zinc In The Environment

- Many US waterways exceed the US EPA ambient water quality criteria for zinc

- These watersheds still support robust ecosystems and with high species richness

- Illustrates possible uncertainty in the EPA’s current approach to development of water quality criteria
Zinc is commonly used in industry for many purposes such as electroplating, galvanizing, and insecticides.

Zinc is elevated in the environment during rain events due to leaching from non-point sources such as guard rails.

Effluents from industrial plants where zinc is used in production may serve as point sources.
Zinc In The Environment

- Zinc in stormwater runoff from a drainage basin in Lodi, NJ
- See high levels of zinc in runoff, characteristic of non-point source pollution
  Wilber et al. 1980

- Zinc loading from mine effluents in the Animas watershed, CO, USA
- Contaminated point source effluents decrease during storm events
  Besser et al. 1999
Previous Work

- Episodic zinc exposure in *D. magna* has been characterized previously by Dr. Tham Hoang

- Found higher mortality with increasing duration and concentration

Hoang et al. 2007
Objectives

• Characterization of pulse duration and concentration of zinc, on the survival and growth of the fathead minnow, *P. promelas*

• Examine how pulsed exposures compare to reported LC50 values for acute continuous exposures
Experimental Design

Zinc Concentration:
- 100 µg/L
- 200 µg/L
- 400 µg/L
- 800 µg/L
- 1600 µg/L

Exposure Duration:
- Continuous
- 24 Hour
- 12 Hour
- 8 Hour
- 4 Hour
- 2 Hour
- 1 Hour

Endpoints:
- 10 Fish
- Replicate 1
- Replicate 2
- Replicate 3
- Replicate 4

Growth
Mortality
Methods

- All exposures performed in 600 mL polypropylene cups

- Moderately hard water was produced by adding reagent grade salts (NaHCO₃, CaSO₄·2H₂O, MgSO₄, KCl) to deionized water (Super-Q™; Millipore Corp. Bedford, MA, USA)

- Zinc was added as ZnCl

- < 24 Hour old fathead minnows were exposed for experimental durations then transferred to clean water and monitored for 21 days

- Fed brine shrimp daily followed by a renewal
Methods (cont.)

- Hardness, Alkalinity, Temperature, Dissolved Oxygen, and pH were measured on days 0, 7, 14 and 21.

- Survival was monitored daily and final weight was measured at the experiments’ conclusion.

- All zinc concentrations were verified using atomic absorption spectrometry or inductively coupled plasma spectrometry.

- Statistical significance was determined using one way ANOVA’s (SAS Institute Inc., Cary, NC, USA).
Results
Results

One Hour Exposure

Survival (%) vs. Time (d)

- Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L

Two Hour Exposure

Survival (%) vs. Time (d)

- Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L

Four Hour Exposure

Survival (%) vs. Time (d)

- Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L

Eight Hour Exposure

Survival (%) vs. Time (d)

- Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L
Results

Twelve Hour Exposure

Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L

Ten Hour Exposure

Survival (%)
Survival (%)

Time (d)
Time (d)

Twenty-Four Hour Exposure

Control
- 75.82 µg/L
- 148.0 µg/L
- 293.1 µg/L
- 726.8 µg/L
- 1428 µg/L

Continuous Exposure

Control
- 89.07 µg/L
- 192.2 µg/L
- 365.2 µg/L
- 726.8 µg/L
- 1428 µg/L
Episodic and Continuous Exposure of Fat Head Minnows to Zinc

Survival (%) vs. Zn Concentration (µg/L)

- 24 Hours: \( P < 0.0001 \)
- 12 Hours: \( P < 0.0001 \)
- 8 Hours: \( P < 0.0001 \)
- 4 Hours: \( P < 0.0001 \)
- 2 Hours: \( P < 0.0001 \)
- 1 Hour: \( P = 0.0351 \)
- Continuous: \( P < 0.0001 \)
**P. Promelas** zinc LC$_{50}$ by exposure duration
* = Calculated using linear regression  $R^2 = 0.90$

$P.\ promelas$ LT50 by Exposure Concentration

$R^2 = 0.9798$
The effect of zinc exposure concentration and duration on the final weight of surviving fathead minnows
Discussion
Reported LC$_{50}$’s

- Reported 96 Hour LC$_{50}$’s for the fathead minnow are between 393 and 50,200 µg/L (EPA Ecotox Database, Accessed 10/24/2007)

- Hardness seems to have the largest effect on zinc toxicity

- LC50’s from studies with similar hardness to this study (80-100 mg/L as CaCO$_3$) are between 9,900 – 25,000 µg/L (Ambient Water Quality Criteria, 1987)
Latent Mortality

- 21 day LC50 for lowest pulse (2 hrs) was 1384 µg/L
- 7-18 fold difference in long term test
- Zinc may cause latent mortality in these fish
## Latent Mortality

<table>
<thead>
<tr>
<th>Zinc Concentration</th>
<th>Exposure Time</th>
<th>% Survival at end of exposure</th>
<th>% survival at the end of 21 days</th>
</tr>
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<td>80</td>
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<tr>
<td></td>
<td>2 Hours</td>
<td>92.5</td>
<td>45</td>
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</tbody>
</table>
Current Water Quality Criteria

• Current water quality criteria for Zinc is 120 µg/L for both acute and chronic exposure

• 21 day single pulse exposures produce LC50’s of 402 µg/L (12 Hours)

• 21 day continuous exposures gives an LC50 of 234 µg/L

• Longer exposure test produce LC50’s very close to current water quality criteria

• Longer term pulsed exposure tests indicate that the metrics used determine these criteria may not accurately capture the actual mortality that occurs in the environment
Conclusions

• Mortality increased with increasing concentration and increasing pulse duration

• There was no clear trend for chronic endpoints

• Large differences between reported LC50’s and pulsed exposure LC50’s may indicate latent mortality as a result of zinc exposure

• Longer term testing produces LC50’s that are within a factor of two of the regulatory values for zinc

• Short term, constant exposure bioassays may not predict real world environmental effects
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Questions?