Effects of pH and dissolved organic carbon on the toxicity of silver nanowires to *Daphnia magna*:

Acute toxicity and spICP-MS measurement of silver nanowire uptake

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(Formerly UC Berkeley)
Silver Nanowires (AgNWs)

- Use is increasing!
- Emerging contaminant.
- Distinct toxicological properties.
- Unknown environmental and toxicological impact.

TEM image: 2 µm x 30 nm “short” polyvinyl pyrrolidone (PVP)-coated AgNW. Dr. Benjamin Gilbert, LBL
AgNWs

- Unique physio-chemical properties.
  - Electrons travel a greater distance.
  - Wires require lower temperature during fabrication.

- Printed electronics, Multi-touch sensors.
  - Stewart et al (2017)
  - Large et al (2016)
  - Cann et al (2016)

Ian Stewart, Duke University
Materials Under Study

- Four AgNWs
  - Short: 2 µm x 30 nm, or
  - Long: 20 µm x 60 nm
- Polyvinyl pyrrolidone (PVP), or
- Inorganic amorphous aluminum-doped silica (SiO$_2$)
- L-PVP, S-PVP, L-SiO$_2$, S-SiO$_2$
- Ionic silver (Ag$^+$)

SEM of long (top) and short (bottom) AgNW. Photos by Dr. Benjamin Gilbert.
Daphnia magna

- Freshwater crustacean
- Parthenogenic reproduction
- “Keystone” species
- Ecological indicator

Photo: Dr. Leona Scanlan
Previous Research

- Physiochemical characterization of AgNWs.
- Established acute LC$_{50}$s in two *Daphnia* growth media.
- Analyzed transcriptomic effects (microarray).
  - Toxicity from wires is NOT (only) due to Ag$^+$ in media.
- Showed internalization of AgNW and formation of AgNP *in vivo*.
Hemolymph Assay

- SEM: found uptake into body
- All four AgNWs
- SiO$_2$ coating dissolved
- PVP coating had stringy attachments
Rob Reed, Chris Higgins, James Ranville  
Colorado School of Mines

**spICP-MS**  
Single particle inductively coupled plasma mass spectrometry

Allows assignment of particle size based on pulse intensity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Plasma</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metals in solution</strong></td>
<td><img src="image1" alt="Diagram" /></td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>Sample containing dissolved metals</td>
<td>Dwell time</td>
<td>Intensity counts</td>
</tr>
<tr>
<td><img src="image3" alt="Diagram" /></td>
<td>constant stream of charged ions</td>
<td><img src="image4" alt="Diagram" /></td>
</tr>
</tbody>
</table>

| **Nanoparticulate sample** | ![Diagram](image5) | ![Diagram](image6) |
| Sample containing inorganic nanoparticles, at very low concentration | Dwell time | Intensity counts |
| ![Diagram](image7) | pulses of charged ions | ![Diagram](image8) |

*Constant signal*

*Individual pulses*
AgNWs detected in hemolymph

Left: raw spICP-MS data.  
Right: Binned spICP-MS data shows size distribution of the wires inside the animal.
AgNW Uptake

• Concentration effect
  • Fewer pulses seen in 1/10 LC\(_{50}\) than LC\(_{50}\)

• Pulses seen following Ag\(^+\) exposure in both media and hemolymph.
  • Precipitation and/or sorption of Ag\(^+\) to biomolecules

• Pulses seen in Daphnia post-depuration.
  • AgNWs were from hemolymph, not gut.
Present Questions

• How does environment affect acute toxicity of Ag\(^+\) or AgNW?
  • Changes in pH
  • Addition of different amounts of dissolved organic carbon (DOC)

• Are AgNW internalized if the aquatic environment changes?
### Acute Toxicity

<table>
<thead>
<tr>
<th>µg silver/L</th>
<th>COMBO</th>
<th>pH 6.5</th>
<th>pH 8.8</th>
<th>20 mg/L DOC</th>
<th>2 mg/L DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC50</td>
<td>95% CI</td>
<td>LC50</td>
<td>95% CI</td>
<td>LC50</td>
</tr>
<tr>
<td>L-PVP</td>
<td>234</td>
<td>210-257</td>
<td>959</td>
<td>597-1540</td>
<td>520</td>
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<tr>
<td>S-PVP</td>
<td>421</td>
<td>322-603</td>
<td>149</td>
<td>104-190</td>
<td>248</td>
</tr>
<tr>
<td>L-SiO2</td>
<td>522</td>
<td>404-633</td>
<td>2309</td>
<td>1364-4383</td>
<td>2266</td>
</tr>
<tr>
<td>S-SiO2</td>
<td>155</td>
<td>141-169</td>
<td>102</td>
<td>78-123</td>
<td>158</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>0.8</td>
<td>0.4-1.3</td>
<td>0.26</td>
<td>0.19-0.31</td>
<td>0.11</td>
</tr>
</tbody>
</table>

- Purple: Scanlan et al 2013
- Red: large degree of uncertainty (failed fit test in PROBIT).
- 24-hour exposure with neonate daphnids (< 24 hours old).
- Subsequent uptake analysis with sp-ICP-MS in adult daphnids.
<table>
<thead>
<tr>
<th>Wire</th>
<th>Medium 1</th>
<th>Medium 2</th>
<th>p-value</th>
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<tbody>
<tr>
<td>L-PVP</td>
<td>COMBO</td>
<td>pH 6.5</td>
<td>&lt; 0.05</td>
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<tr>
<td>L-PVP</td>
<td>COMBO</td>
<td>pH 8.8</td>
<td>&lt; 0.1</td>
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LC₅₀: lethal concentration at which 50% of daphnids died; 24-hour; determined with PROBIT software.

Method to determine statistical significance from (Wheeler, Park et al. 2006) based on overlap of 95% CI.
Environmental Effects

- pH 6.5
  - Long wires had decreased toxicity compared to COMBO.
  - Short wires had increased toxicity.
  - Increased the toxicity of Ag⁺.
  - Increased toxicity of short wires in COMBO was partially due to silver dissolution; pH may affect the coating.
Environmental Effects

- pH 8.8
  - Long wires were less toxic at higher pH
  - S-PVP was more toxic, S-SiO$_2$ was not changed.
  - Ag$^+$ was more toxic.
Environmental Effects

- 2 mg/L DOC (low)
  - Long wires were less toxic
  - S-PVP and Ag⁺ were more toxic
  - No changes with S-SiO₂
Environmental Effects

- 20 mg/L (high)
  - All wires were less toxic
  - Ag⁺ had no significant difference
LC\textsubscript{50} Summary

- High levels of DOC (20 mg/L) significantly decreased toxicity of AgNW.

- S-PVP and Ag\textsuperscript{+} had similar patterns (more toxic in pH 6.5 and pH 8.8 and with low DOC).

- S-SiO\textsubscript{2} wires remained the most toxic under all conditions; LC\textsubscript{50} was typically not significantly different from COMBO (except at pH 6.5).

- Lower pH usually increased toxicity; high pH usually decreased toxicity.
Hemolymph Extraction

- Adult daphnids exposed to 1/10 LC$_{50}$ for 24 hours.
- Removed from medium and pierced with needle, hemolymph aspirated with 10 µL pipette.
- Typically removed 2 µL per daphnid, 5 animals per replicate, 5 replicates per sample.
spICP-MS

- Perkin Elmer Nexion 300D
  - 100µs dwell times
- S-SiO₂: all four “new” conditions
- S-PVP: COMBO and low DOC
- L-SiO₂: COMBO and low DOC
- L-PVP: COMBO and pH 8.8

(Hemolymph Samples)

- S-SiO₂-20 mg/L DOC
- S-SiO₂-2 mg/L DOC
- S-SiO₂-pH 8.8
- S-SiO₂-pH 6.6
- S-PVP-COMBO
- S-PVP-2 mg/L DOC
- L-SiO₂-2 mg/L DOC
- L-SiO₂-pH 8.8
- L-PVP-COMBO
- L-PVP-pH 8.8

(Intended to do more, ran out of wires! 😞)
spICP-MS

Particle number per mL

- One-way ANOVA with Tukey post-hoc pairwise comparisons
- No statistically significantly differences
  - Large variability
spICP-MS

Particle number per mL normalized to exposure conc.

- One-way ANOVA with Tukey post-hoc pairwise comparisons
- None are statistically significantly different
  - Test for outliers?

![Graph showing particle number per mL normalized to exposure conc. with different conditions and their respective particle counts.](image-url)
spICP-MS

Average wire length (nm)

- One-way ANOVA with Tukey post-hoc pairwise comparisons
- S-PVP in COMBO is significantly longer than any other
Average wire length (nm) - outlier removed

- Duplicate data set: one is 10x higher than any other measured value (212.5 nm).
- Statistics changed for many comparison sets
  - Warrants further analysis
spICP-MS

Average wire length (nm) - outlier removed, normalized

- One-way ANOVA, Tukey
- Statistics changed for many comparison sets
  - Only 8 significant differences instead of 19
Summary

Acute Toxicity

- Will further analyze raw acute data to correct for multiplicity of comparison

spICP-MS

- Detected AgNWs in hemolymph
- High variability; outlier test?
- Possibly saw degradation of samples
  - 5 years in storage at -80° C
Acknowledgements

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