Silver Nanoparticles in the Aquatic Freshwater Ecosystem

Toxicology

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Uses Of Silver as an Antibacterial

Cyrus – King of Persia 580-529 BCE; Egyptian Silver water vessels.

Images: listverse.com, metmuseum.com, nanosilver.eu, nanoprom.sk.
• The burden and characteristics of AgNP in use and in our aquatic environment remains largely unknown
• Abundance of data but no answers
• Relevance of existing toxicity data questionable
  o Actual Environmental Concentrations (ng/L) vs Toxicity endpoints (µg/L)
  o Suitability of test matrices
• Develop a toolbox for relevant toxicity testing specific to AgNPs in the aquatic ecosystem
Figure 1.1: The number of publications returned from a search using Keyword “Silver Nanoparticles” on ScienceDirect.com (Correct as at June 8th 2018)
Which Forms Are Used Commercially?

- We Don’t Really Know!
- Forms Include: uncoated, coated, nanofibers / nanowires, powdered, colloidal
- Coatings include:
  - PVP, tween, citrate, protein, alkanes, EDTA, silica, proprietary coatings, caffeine and many more.
- Sizes range from 1nm – 100nm.
- Proprietary AgNP’s not well described (even on patents).
- Move to standardised testing with reference materials by OECD & EU.

Environmental Fate

“Where do you go to my lovely?”

Images: simply-science-nbep.blogspot.ie; motmac.com; pubs.usgs.gov
Nanoparticle in ≠ Nanoparticles out!

\[ \text{Ag} \rightarrow \text{AgCl} \]

>450nm Agglomerates (Geranio et al., 2009)

\[ \text{Ag}^+ \]

\[ \text{Ag}_2\text{S} \]

Ion Source used in toxicity testing

The literature review formed part of WP 1 and this review was published.
Experimental Design – Multi-Trophic Test Battery

1. Primary Producer
   - ISO 8692:2012 *Pseudokirchnerriella subcapitata* freshwater algal growth inhibition test (with modifications, novelties and media comparisons)

2. Primary Consumer
   - ISO 6341: 2012 Inhibition of mobility of *Daphnia magna* Acute Toxicity test (with modifications).

3. Secondary Consumer
   - Acute toxicity assay to higher invertebrates such as *Hydra*.

1-3. Biomagnification
   - Detection of Ag uptake at each trophic level following sub-lethal dose
Primary Producers

*Pseudokirchneriella subcapitata*

**Test Media Optimisation**

- **ISO 8692: 2012 – Jaworski’s Medium**
  - Contains chelating agent EDTA
  - Interference with metallic analytes
  - ISO under review and AIT have contributed on *bioavailability* effect of media

- **Comparisons with modified EDTA free Chu #10 media**
  - Toxicity / Sensitivity are very different
  - Growth rates are also different
  - No significant difference between AgNP & AgNO₃

*Image: ccap.ac.uk*
Figure Comparison of Test sensitivity to AgNO₃ and Algal Growth Rates under different media conditions. (n=3, SEM indicated).

A: Cultured & Tested in EDTA-X,
B: Cultured in JM, 3 Passages in EDTA-X, Tested in EDTA-X;
C: Cultured in JM, 2 Passages in EDTA-X, Tested in EDTA-X;
D: Cultured in JM, 1 Passage in EDTA-X, Tested in EDTA-X;
E: Cultured & Tested in JM
Results – *Pseudokirchneriella subcapitata* (Algae)

In preparation for publication in *Science of the Total Environment*
Consumers

*Daphnia pulex & magna*

- Acute toxicity to *D. pulex*
  - Modified from ISO 6341: 2012
  - EPA (US) Moderately hard freshwater
- Fecundity studies with *D. magna*
  - Cumulative no. of offspring over 30 days acute NoEC.

*Tetrahymena thermophila*

- Ciliate protozoan
  - Tested in both Artificial Freshwater (US EPA-Moderately hard) & Distilled water.
  - 24-32 hour Acute Substrate Utilisation
Primary Consumer

**Daphnia pulex**

*IC*$_{50}$ 4.2 µg/L

Concentration response curve showing the acute toxic effect of AgNP on *Daphnia pulex* over 24 hours (SEM indicated, n=3).

*IC*$_{50}$ 9.3 µg/L

Concentration response curve showing the acute toxic effect of AgNO$_3$ on *Daphnia pulex* over 24 hours. (SEM indicated, n=3)
**Consumer**

**Daphnia pulex & magna**

- *Daphnia pulex* Immobilisation
  - AgNP- 24hr – IC$_{50}$ 4.2 µg/L
  - AgNO$_3$ -24hr – IC$_{50}$ 9.3 µg/L

- *Daphnia magna* Fecundity
  - Cultured in 0.1 µg/L (100ng/L) semi static
  - Daily number of neonates reduced by
    - 33% after 8 days
    - 80% after 12 days

**Results**

**Tetrahymena thermophila**

- Substrate Utilisation
  - AgNP- 34hr –
    - IC$_{50}$ 2.8mg/L in Artificial Freshwater
    - IC$_{50}$ 1.9mg/L in ddH$_2$O
    - Not a significant difference

- *Daphnia pulex* is 3 orders of magnitude more sensitive than the protozoan.
Secondary Consumer

- *Gammarus pulex*: Proving difficult to culture.
- *Hydra attenuata*
  - Many diverse endpoints including teratogenicity, regeneration and both sexual and asexual reproduction.
Hydra Morphology Scale

Representative endpoints for the hydra bioassay as graded in the Wilby et al. 1988 method.

10: Extended Tentacles, Reactive
9: Partially Contracted, Slow Reactions
8: Clubbed Tentacles, Slightly Contracted
7: Shortened Tentacles, Slightly Contracted
6: Tentacles and Body Shortened
5: Totally Contracted, Tentacles Visible
4: Totally Contracted, No Tentacles Visible
3: Expanded, Tentacles Visible
2: Expanded, No Tentacles Visible
1: Dead But Intact
0: Disintegrated
Results - *Hydra attenuata*

Control

12.5 µg/L

25 µg/L

50 µg/L

75 µg/L

100 µg/L

100 µg/L

In preparation for publication in *Science of the Total Environment*
Results - *Hydra attenuata* (outlier makes a difference)

**Morphology**

- **Wilby Score** vs **[AgNP] μg/L**
  - EC$_{50}$ (96hr) 95.8 μg/L

- **EC$_{50}$ (96hr) 29 μg/L**
  (with 100μg/L omitted from analysis)

No significant difference between AgNP and AgNO$_3$
Results - *Hydra attenuata* – regeneration

- Basal disc and hypostome excised
- Gastric region exposed for 96 hrs
- Regeneration assessed as per Wilby scale (morphology)
- Significantly more sensitive than morphology endpoint.
- No significant difference between AgNP and AgNO₃
Hydra ssDNA damage Comet Assay

- Single cell electrophoresis – alkaline - using a fluorescent dye (SyberGold).
- DNA damage proportional to migration
- DNA damage assessed by Tail moment
- No more sensitive than Morphology endpoint

<table>
<thead>
<tr>
<th>[AgNP] μg/L</th>
<th>IC50</th>
<th>NoEC</th>
<th>LoEC</th>
<th>MoEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>92.4</td>
<td>12.5</td>
<td>25.0</td>
<td>200.0</td>
</tr>
</tbody>
</table>

Comets formed in Hydra attenuata exposed In-vivo to AgNPs and performed under alkaline conditions, single cell gel electrophoresis. Cells stained with SyberGold nucleic acid stain.

Legend: Lethal Toxicity endpoint. (A) Control (Normal Hydra); (B) 12.5 μg/L (minimal signs of toxicity, i.e. clubbing of tentacles); (C) 25 μg/L (shortened tentacles and contraction of Column); (D) 50 μg/L; (E) 75 μg/L (tuip phase); (F) 100 μg/L (anomaly – No Comets observed; (G) 150 μg/L; (H) 200 μg/L (Disintegration or death of Hydra).
Chronic Toxicity to *Daphnia magna* & *Hydra attenuata*

- No effect on *Daphnia magna* reproduction in first week
- Fecundity reduced by 33% after 8 days
- Fecundity reduced by 80% after 12 days
- *Hydra attenuata* fed with *Daphnia magna* neonates cultured in 0.1 µg/L AgNP exhibited no morphological, regeneration or budding impairments.
Conclusion

Summary of the effects of AgNP and AgNO$_3$ on a multi-trophic test battery including the algae *Pseudokirchneriella subcapitata* and the freshwater invertebrates *Daphnia pulex*, *Daphnia magna* and *Hydra attenuata*.

<table>
<thead>
<tr>
<th>Test</th>
<th>Parameter</th>
<th>AgNP (25 nm PVP coated)</th>
<th>Ag$^+$ from AgNO$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E$<em>{r}$C$</em>{50}$ [µg/L]</td>
<td>95% CI</td>
</tr>
<tr>
<td><em>Pseudokirchneriella subcapitata</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JM$^{(1)}$</td>
<td></td>
<td>6.76</td>
<td>5.28–8.66</td>
</tr>
<tr>
<td>EDTA-X$^{(2)}$</td>
<td></td>
<td>0.70</td>
<td>0.59–0.85</td>
</tr>
<tr>
<td>Combination$^{(3)}$</td>
<td></td>
<td>1.89</td>
<td>1.40–2.55</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>US EPA - Acute</td>
<td>7.85</td>
<td>5.8–10.7</td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>US EPA - Acute</td>
<td>4.2</td>
<td>3.4–5.0</td>
</tr>
<tr>
<td></td>
<td>Fecundity</td>
<td>Reduced by 33% on day 8 and 80% on day 12 cultured in 0.1µg/L AgNP</td>
<td></td>
</tr>
<tr>
<td><em>Hydra attenuata</em></td>
<td>Morphology</td>
<td>29</td>
<td>18–50</td>
</tr>
<tr>
<td></td>
<td>Regeneration</td>
<td>6.98</td>
<td>4.9–9.7</td>
</tr>
<tr>
<td></td>
<td>Comet Assay</td>
<td>Same as morphology</td>
<td></td>
</tr>
</tbody>
</table>

(1) Javorki’s Media (JM), (2) EDTA-X is EDTA free media adapted from Chu #10 and used for all culturing and testing. (3) Combination media is algae cultured in JM, passaged once in EDTA-X and then tested in EDTA-X.
Conclusion

Relative Sensitivities (based on median effective concentrations)

- **Pseudokirchneriella subcapitata** (algae) in EDTA free media is most sensitive acute test.
- **Hydra** regeneration is similar in sensitivity to ISO algae test.
- **Daphnia pulex** acute test similar to ISO algal test
- **Daphnia pulex** chronic test most sensitive
Conclusions

Findings

• “One size fits all” approach not appropriate for the ecotoxicological assessment of AgNPs
• Bioavailability of Ag needs to be addressed e.g. interference with EDTA
• Ionic silver good proxy for AgNPs as similar toxicities reported for AgNO$_3$ and AgNPs
• Chronic testing need to included in toolbox

Recommendations

• New EU databases announced this year are welcome, but don’t go far enough
• An Irish mandatory register of nanomaterials in use is needed urgently
• Urgent need to define AgNPs in use
• New or adapted standardised and validated tests suitable for ENMs urgently needed
References


