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DeTER (WP-3)

Silver Nanoparticles in the Aquatic Freshwater Ecosystem

Toxicology

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One Health Conference 2018

NDP National Development Plan
epa Environmental Protection Agency

Uses Of Silver as an Antibacterial

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

- Phoenicians
- Romans
- Egyptians
- Macedonians
- Hypocrites

Images: *listverse.com, metmuseum.com, nanosilver.eu, nanoprom.sk.*

References: Alexander, J.W., 2009. History of the medical use of silver. Surg. Infect. (Larchmt). 10, 289–292.

- 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
  - Actual Environmental Concentrations (ng/L) v Toxicity endpoints (µg/L)
  - Suitability of test matrices
- Develop a toolbox for relevant toxicity testing specific to AgNPs in the aquatic ecosystem

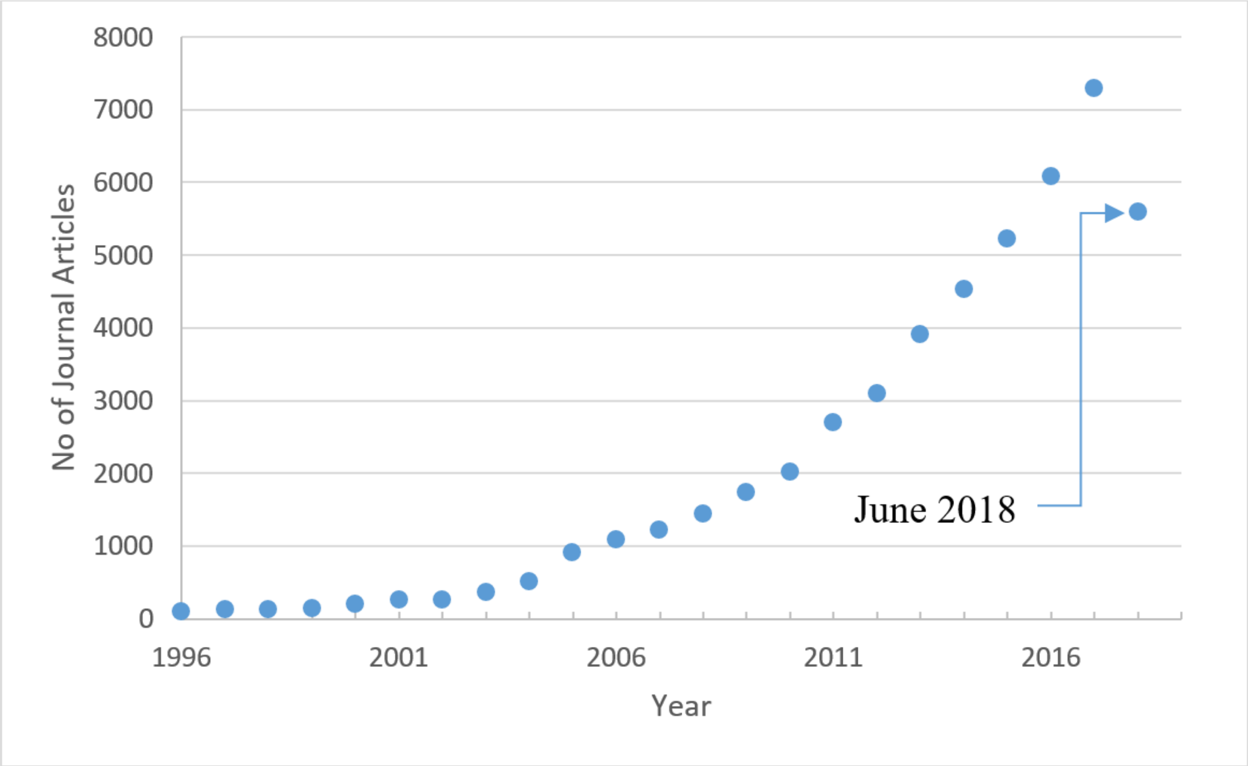


Figure 1.1: The number of publications returned from a search using Keyword “Silver Nanoparticles” on Sciencedirect.com (Correct as at June 8th 2018)

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.

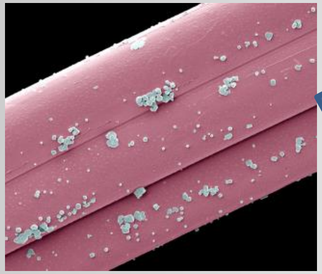
References: Wijnhoven, S. *et al.,* (2009). Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology 3, 109–138.*

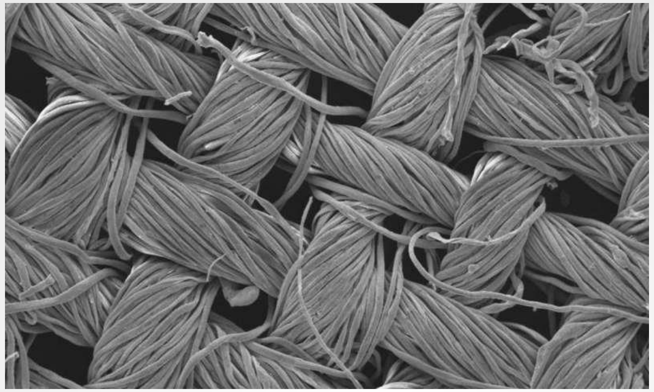
Environmental Fate

"Where do you go to my lovely?"

Images: *simply-science-nbep.blogspot.ie; motmac.com; pubs.usgs.gov*

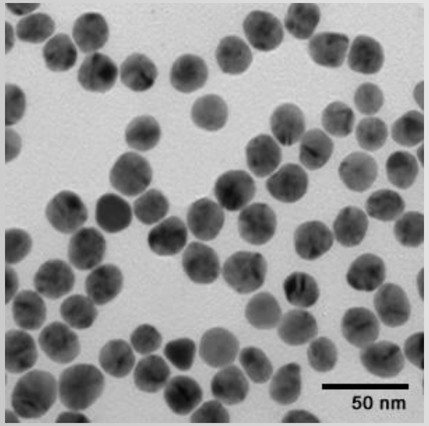
Nanoparticle in ≠ Nanoparticles out!

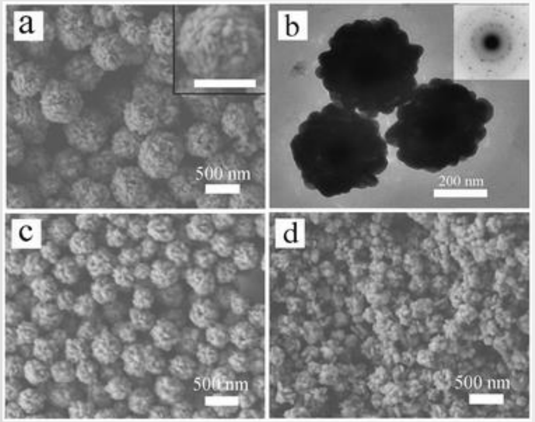




AgCl

Ag^+





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

Ag_2S

$AgNO_3$
Ion Source used in toxicity testing

References: **Kaegi, R**., *et al.,* (2011). Behavior of metallic silver nanoparticles in a pilot wastewater treatment plant. *Environ. Sci. Technol*. 45, 3902–3908;
Lorenz, C., *et al*., (2012). Characterization of silver release from commercially available functional (nano)textiles. Chemosphere 89, 817–824.

The literature review formed part of WP 1 and this review was published

Science of the Total Environment 575 (2017) 231–246

Contents lists available at ScienceDirect

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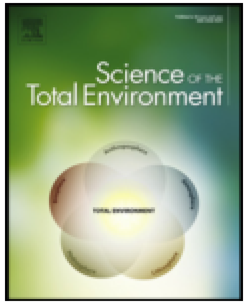
journal homepage: www.elsevier.com/locate/scitotenv



Review

Silver nanoparticles in the environment: Sources, detection and ecotoxicology

E. McGillicuddy [a,b,*], I. Murray [c], S. Kavanagh [a,b,1], L. Morrison [d], A. Fogarty [c,e], M. Cormican [a,b], P. Dockery [f], M. Prendergast [b], N. Rowan [c,e], D. Morris [a,b]

Experimental Design – Multi-Trophic Test Battery

1 - 3

Biomagnification

- Detection of Ag uptake at each trophic level following sub-lethal dose

3

Secondary Consumer

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

2

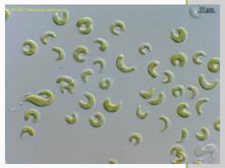
Primary Consumer

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

1

Primary Producer

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



Primary Producers

Pseudokirchneriella subcapitata

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 & $AgNO_3$

Image: *ccap.ac.uk*

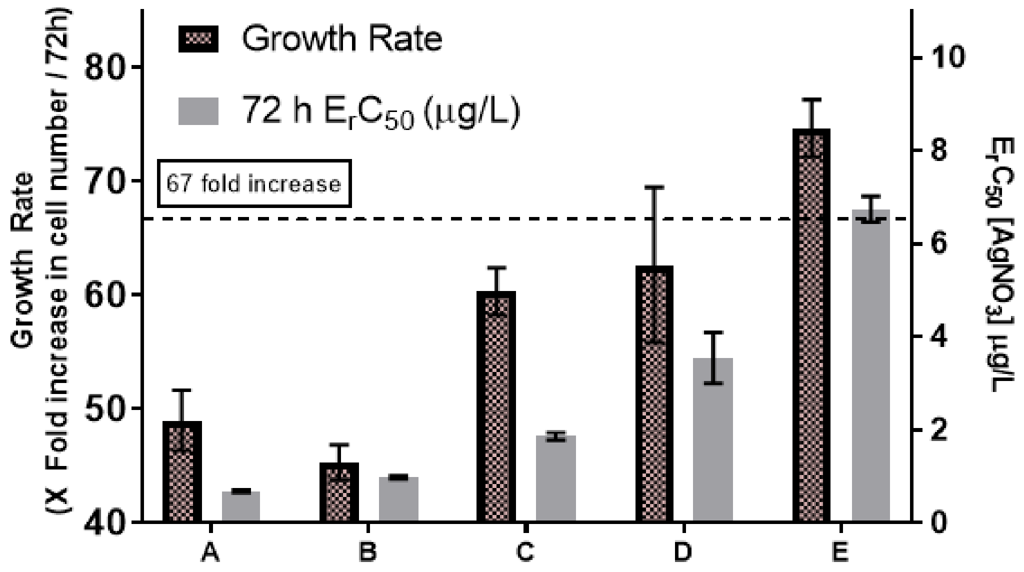
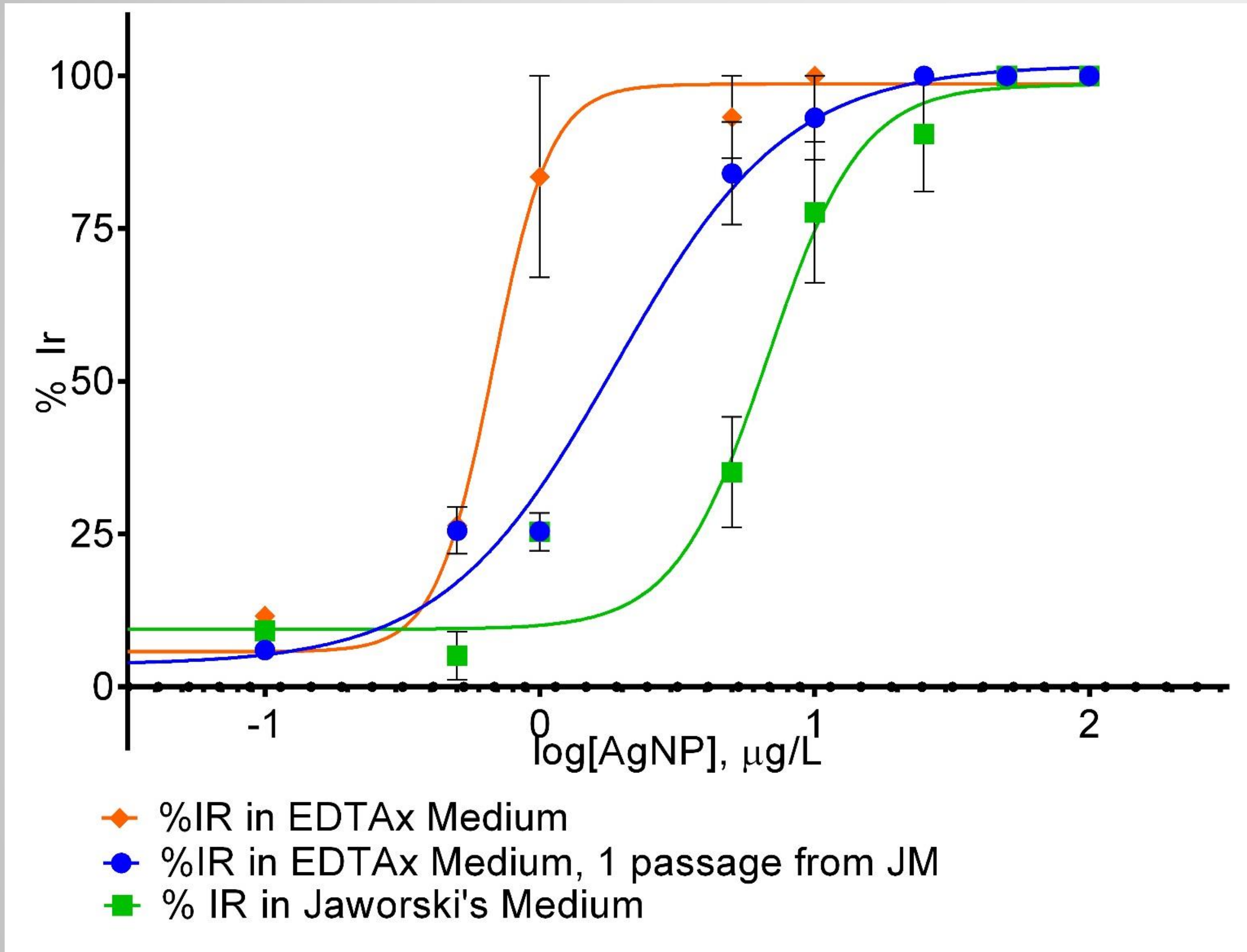


Figure Comparison of Test sensitivity to $AgNO_3$ and Algal Growth Rates under different media conditions. (n=3, SEM indicated).

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

43

Results – *Pseudokirchneriella subcapitata* (Algae)



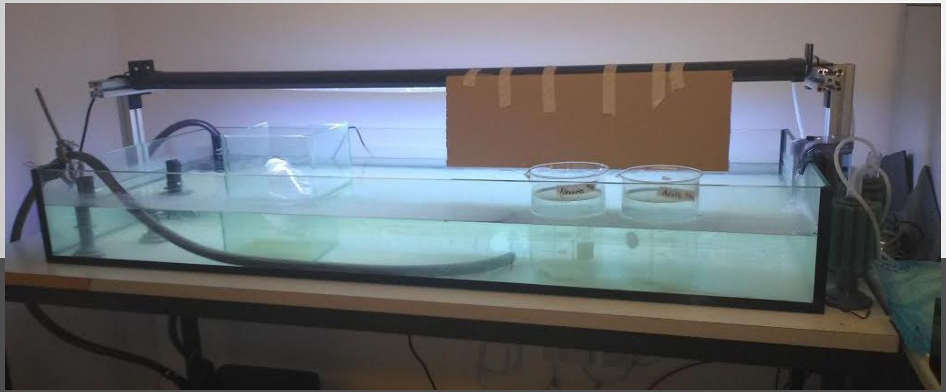
	Jaworski's Medium	EDTA Free Medium (Culture & Test)	EDTA Free Medium (Test only – Culture in JM)
E_rC_{50}	6.76 µg/L	0.68 µg/L	1.89 µg/L
95% CI	5.3 – 8.7 µg/L	0.58 – 0.79 µg/L	1.40 – 2.55µg/L
Mean Growth Rate	75X	45	59

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.

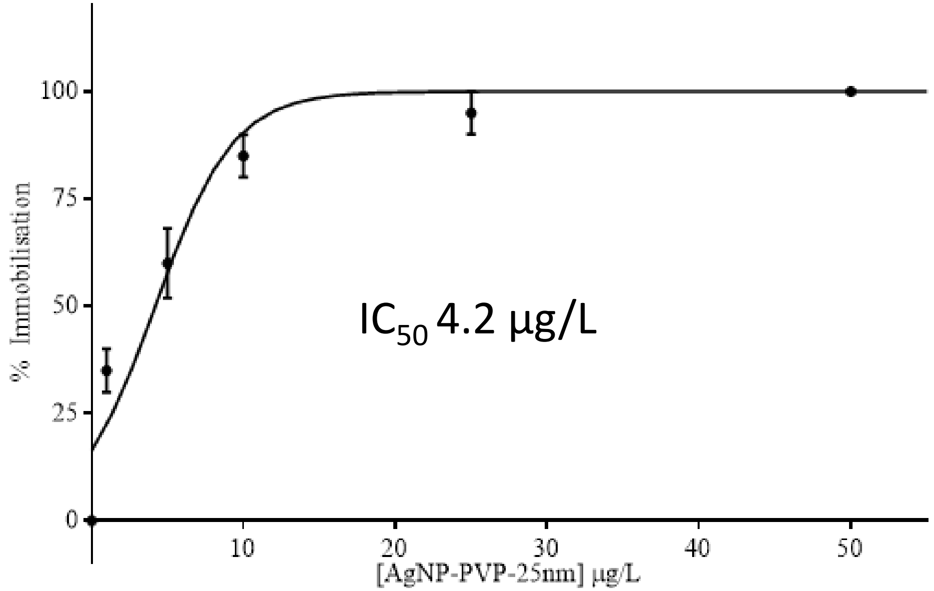


Tetrahymena thermophila

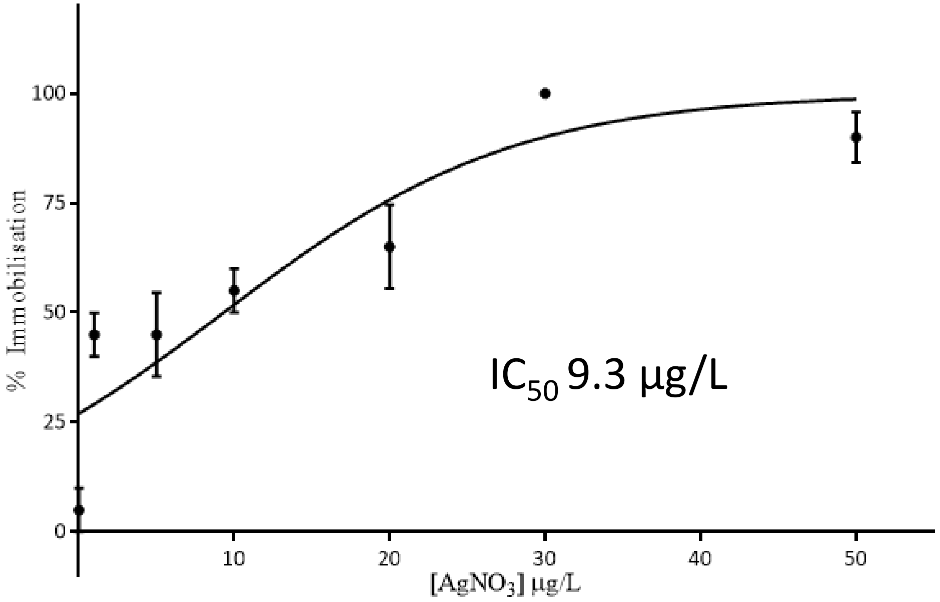
- Ciliate protozoan
  - Tested in both Artificial Freshwater (US EPA-Moderately hard) & Distilled water.
  - 24-32 hour Acute Substrate Utilisation

Primary Consumer

Daphnia pulex



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



Concentration response curve showing the acute toxic effect of $AgNO_3$ on *Daphnia pulex* over 24 hours. (SEM indicated, n=3)

Consumer Results

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

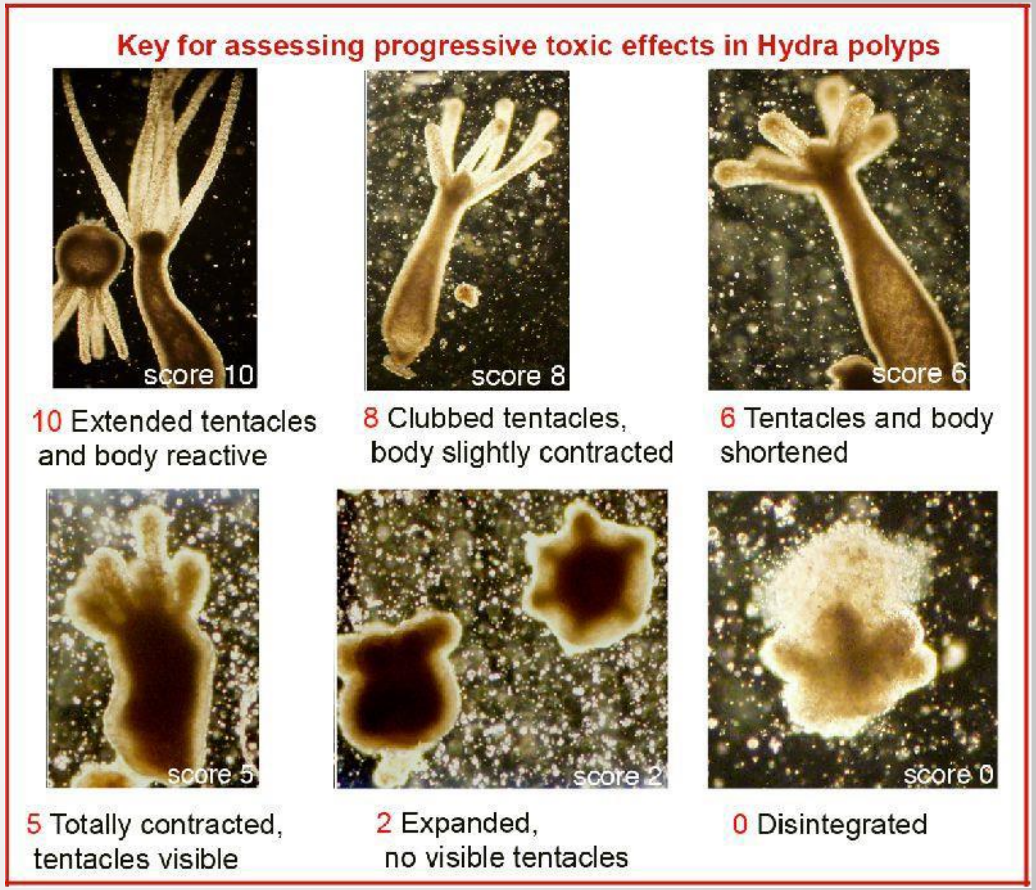
Tetrahymena thermophila

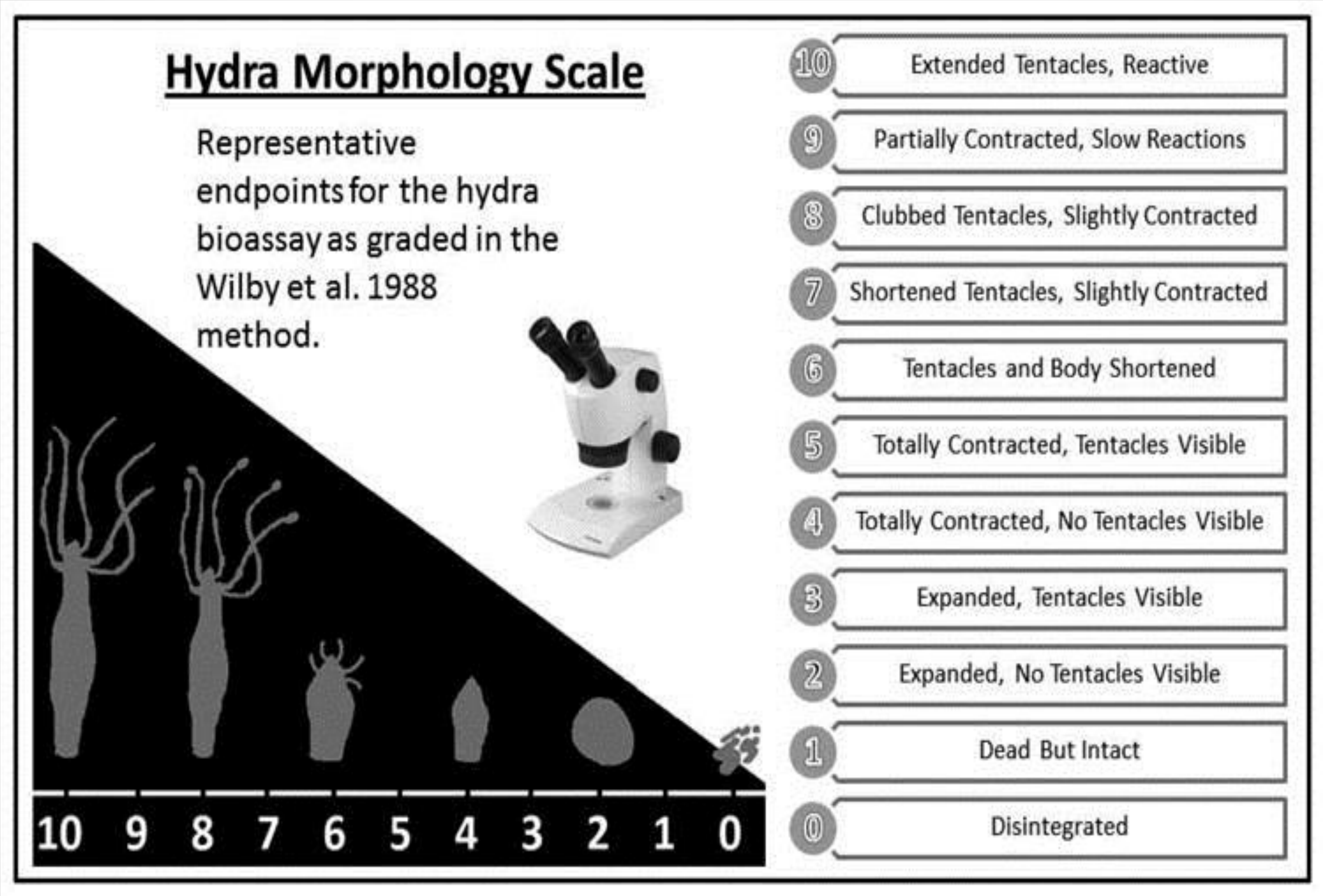
- Substrate Utilisation
- AgNP- 34hr –
  - IC_{50} 2.8mg/L in Artificial Freshwater
  - IC_{50} 1.9mg/L in ddH_2O
  - Not a significant difference

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

Secondary Consumer

- *Gammarus pulex:* Proving difficult to culture.
- *Hydra attenuata*
  - An "appropriate bioindicator species for use in environmental assessment" Quinn *et al.,* (2012).
  - Many diverse endpoints including teratogenicity, regeneration and both sexual and asexual reproduction.





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Results - *Hydra attenuata*


Control


12.5µg/L


25µg/L


50µg/L


75µg/L


100µg/L


<150µg/L

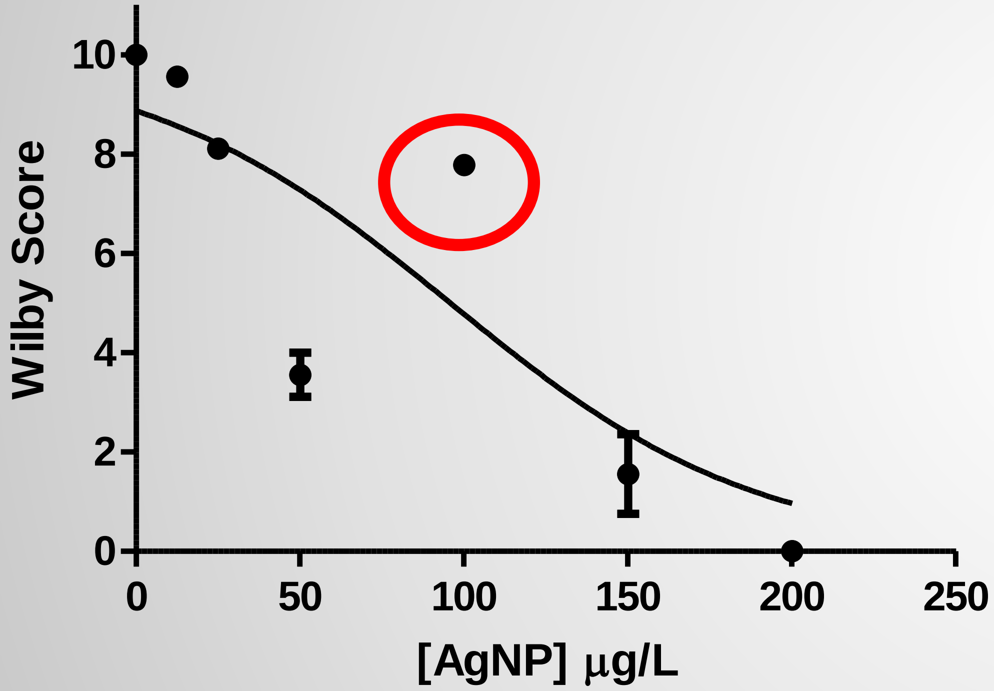
Control



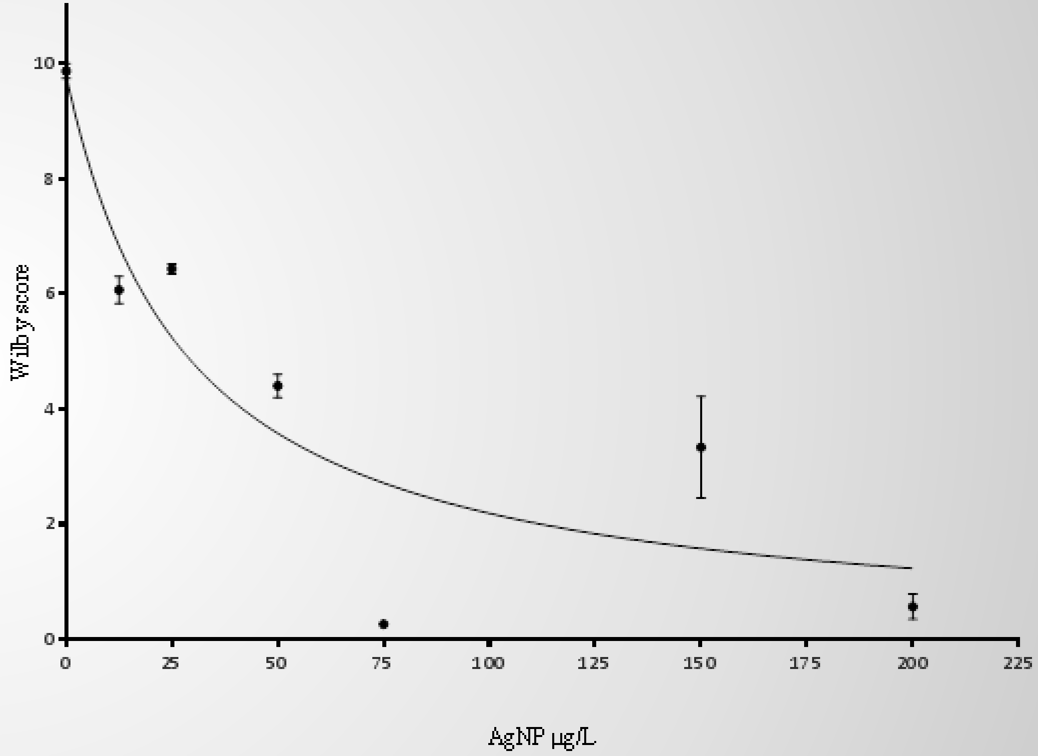
100µg/L

In preparation for publication in Science of the Total Environment

Results - *Hydra attenuata (outlier makes a difference)* *Morphology*



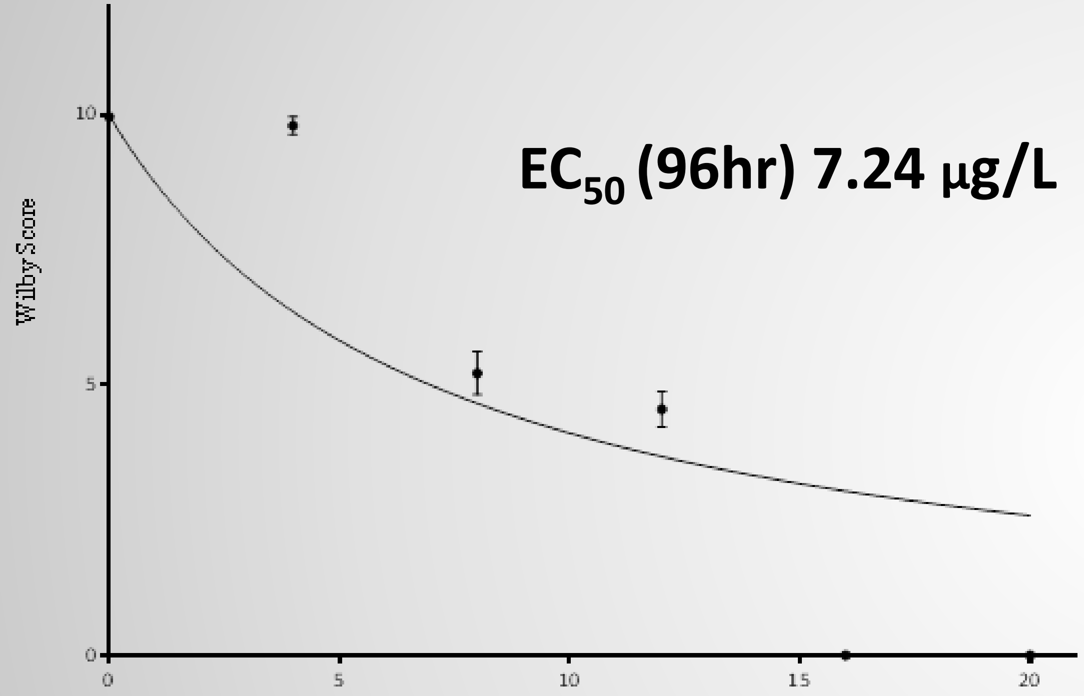
EC_{50} (96hr) 95.8 µg/L



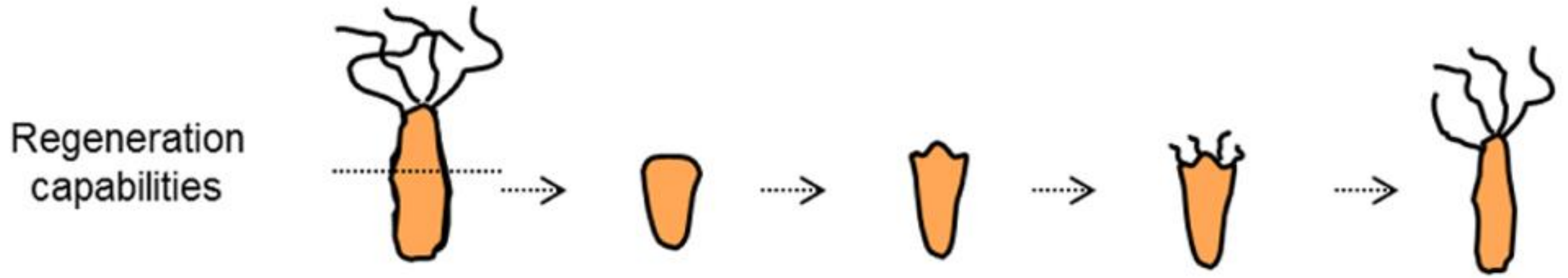
EC_{50} (96hr) 29 µg/L
(with 100µg/L omitted from analysis)

No significant difference between AgNP and $AgNO_3$

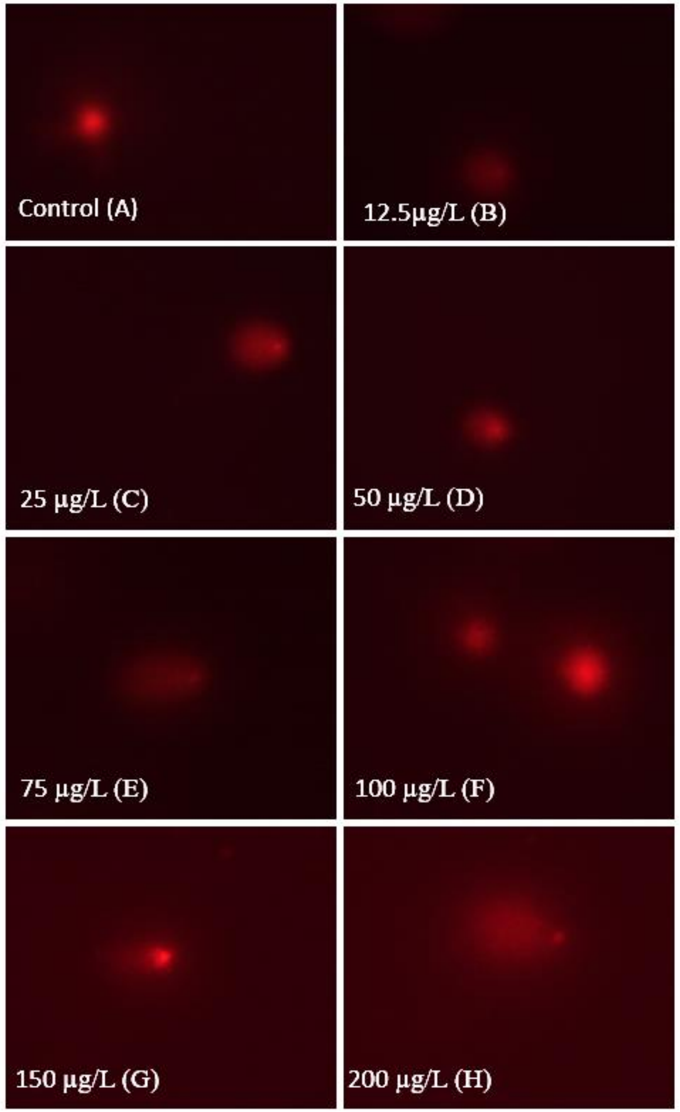
Results - *Hydra attenuata – regeneration*



- 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 $AgNO_3$



Hydra ssDNA damage Comet Assay



Comets formed in *Hydra attenuata* exposed *In-vivo* to AgNPs and performed under alkaline conditions, single cell gel electrophoresis. Cells stained with Sybr Gold 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 (tulip phase); (F) 100 µg/L (anomaly – No Comets observed; (G) 150 µg/L; (H) 200 µg/L (Disintegration or death of *Hydra*).

- 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

	[AgNP] µg/L
IC_{50}	92.4
NoEC	12.5
LoEC	25.0
MoEC	200.0

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.

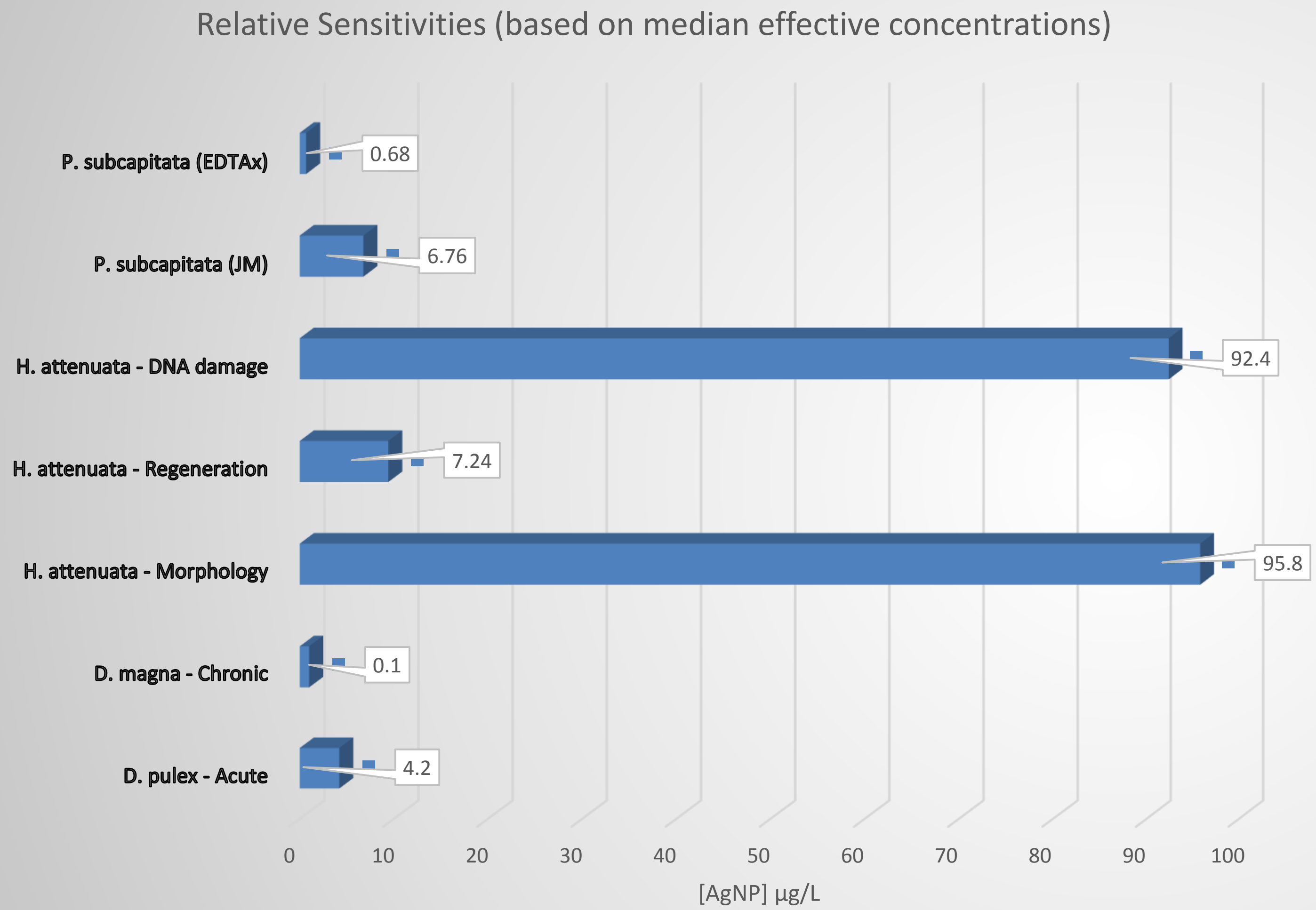
Conclusion

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*.

Test		**AgNP (25 nm PVP coated)**		**Ag^+ from $AgNO_3$**	
Species name	**Parameter**	**E_rC_{50} [µg/L]**	**95% CI**	**E_rC_{50} [µg/L]**	**95% CI**
Pseudokirchneriella subcapitata	JM(1)	6.76	5.28–8.66	6.74	5.72–7.94
	EDTA-X(2)	0.70	0.59-0.85	0.68	0.58-0.79
	Combination(3)	1.89	1.40-2.55	1.86	1.79-1.94
Daphnia magna	US EPA - Acute	7.85	5.8-10.7	1.2	0.97-1.55
Daphnia pulex	US EPA - Acute	4.2	3.4-5.0	9.3	5.8-13.0
	Fecundity	Reduced by 33% on day 8 and 80% on day 12 cultured in 0.1µg/L AgNP			
Hydra attenuata	Morphology	29	18-50	35	25-52
	Regeneration	6.98	4.9-9.7	7.24	5.2-10.0
	Comet Assay	Same as morphology			

(1) Jaworski'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



- *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

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