Short-term algal testing – a new approach for disclosing silver nanoparticle toxicity

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Short-term algal testing – a new approach for disclosing silver nanoparticle toxicity

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Introduction & objectives

Silver is the most common nanomaterial in commercially available products, used mainly for its antimicrobial properties.

In recent years, the environmental effects of silver nanoparticles (AgNPs) has been studied intensively, but still little is known about AgNP ecotoxicity and the underlying mechanisms.

A general challenge in aquatic toxicity testing of NPs is to control and describe the exposure (dose). Many processes affect NPs and causes the exposure dose to change during the exposure period.

The aim of this study is to:

1) Determine whether AgNP display ionic behaviour as measured by algal toxicity under various conditions
2) Investigate the effect of a shortened exposure period on AgNP and $AgNO_3$ toxicity

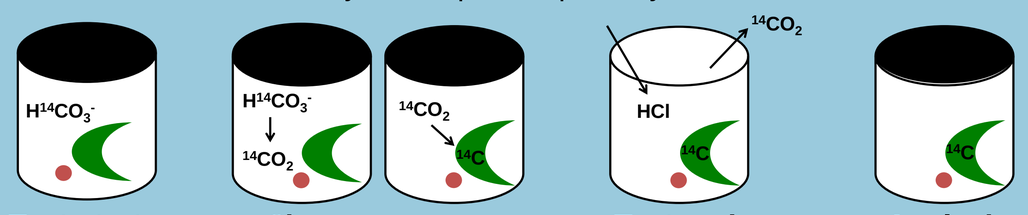
Materials & Methods

Tested materials:

AgNP-citrate	Citrate coated AgNPs of nominal size 30 nm
NM-300K	OECD reference AgNPs of nominal size 15 nm
$AgNO_3$	Dissolved silver reference

Algal tests with green algae *p. subcapitata:*

48h test	OECD 201 growth inhibition test
2h test	Newly developed 2h photosynthesis inhibition test:



Test start
Algae, NPs and $H^{14}CO_3^-$ is added to media

2h exposure
Via the carbonate system $^{14}CO_2$ is formed and via photosynthesis the alga takes up ^{14}C from $^{14}CO_2$

Test end
Added HCl stops photo-synthesis and excess $^{14}CO_2$ is evaporated

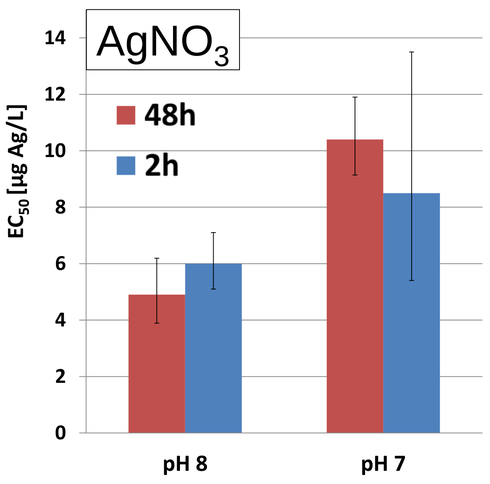
Analysis
The ^{14}C amount is a measure for biomass and exposed algae is related to the control

Results

For $AgNO_3$ the EC_{50} from 2h and 48h tests were not statistically significant different (p=0.05), meaning:

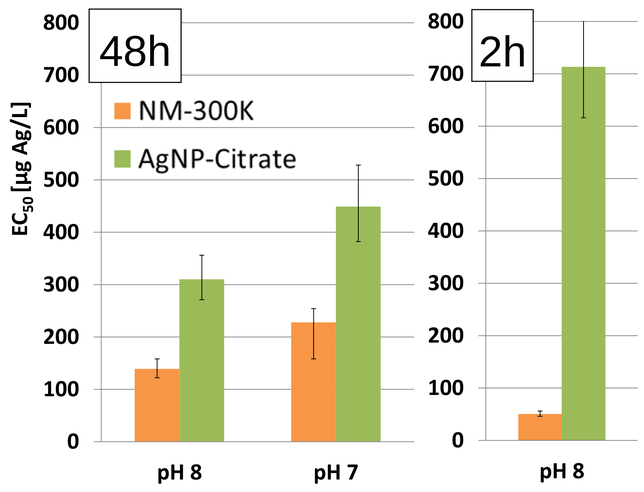
- The algal toxicity of dissolved silver occurs very rapidly
- The 2h test is applicable for testing toxicity of dissolved silver

The 2h test showed less precision at pH 7, due to interference with the ^{14}C-uptake from the carbon added as CO_2 in pH 7 tests.



EC_{50} values from 2h and 48h algal tests with $AgNO_3$. Error bars indicate the 95% confidence intervals.

Results



EC_{50} values from 2h and 48h algal tests with AgNPs. Error bars indicate the 95% confidence intervals.

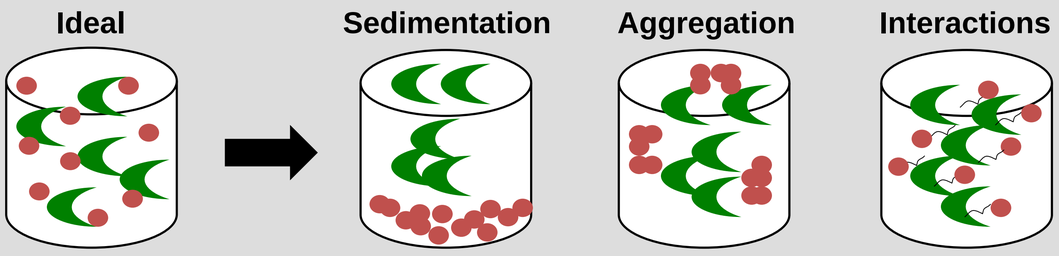
At pH 8, NM-300K was more toxic in the 2h test, while AgNP-citrate was less toxic - possibly due to the different test endpoints and different compositions of the AgNPs. NM-300K is smaller and has a greater ion release potential than AgNP-citrate.

The 2h test at pH 7, yielded poor dose-response relations-ships and no EC_{50} could be calculated - possibly due to competition in ^{14}C-uptake with carbon from added CO_2.

Test duration: The shorter – the better ?

The 2h algal test setup provides a measure for photosynthesis inhibition. Moreover, it allows for high throughput screening of nanoparticle toxicity.

The reasoning for doing a very short exposure period (2h) is to minimize the potential confounding factors often experienced in standard algal tests with NPs due to time-dependant processes such as:



The aim is better control of the test system, more stable exposure conditions and thus improved prerequisites for obtaining dose-response relations-ships.

Conclusions

- Overall, the changes in AgNP toxicity at various exposure conditions are in accordance with the expected outcome for ionic compounds
- AgNPs were less toxic than $AgNO_3$ based on total silver concentrations and the same order of toxicity was found in 2 and 48h tests: $AgNO_3$ > NM-300K > AgNP-citrate
- The 2h algal test was as sensitive as the 48h standard test to $AgNO_3$ toxicity at pH 8, demonstrating a fast toxic mechanism of dissolved silver in the algae *P. subcapitata*
- The 2h algal test setup provides a fast toxicity screening tool for AgNPs, and possibly other NPs – providing EC_{50} values greater or lower than from 48h testing, depending on the characteristics of the AgNPs