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# Aquatic toxicity testing of silver nanoparticles – a matter of timing

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In recent years, the ecotoxicity of silver nanoparticles (AgNPs) has been studied intensively due to their high toxicity and extensive use in consumer products. However, the field of aquatic nanotoxicology is generally challenged by poor reproducibility, lack of dose-response relationships, and difficulties in controlling and/or describing the characteristics of the tested NPs. These issues may be related to the widespread approach of using freshly prepared stock solutions for ecotoxicity testing, as the introduction of NPs into aqueous media initiates time-dependent processes that possibly interfere with the toxicity testing, e.g. dissolution, speciation, aggregation, sedimentation and interactions with media components.

The aim of this study was to investigate whether suspension of AgNPs in test media 24h prior to algal toxicity testing (a pre-suspension step) affects the toxicity and the reproducibility of the test. Ultimately, the aim is better control of the AgNPs in the algal test system and improved prerequisites for describing their toxicity to algae. The underlying hypothesis is that a large part in the variability of AgNPs toxicity to algae can be explained by the kinetics of dissolution and speciation of Ag ions in the test media. To reduce the amount of time in which changes to NPs may occur during testing, the exposure period was minimized. A recently proposed short-term (2h) algal test was applied, using <sup>14</sup>C-incorporation during photosynthesis as toxic endpoint [1]. For citrate coated spherical AgNPs with a nominal size of 30 nm, the resulting dose-response relationships from tests without (A) and with (B) the pre-suspension step are illustrated in figure 1.

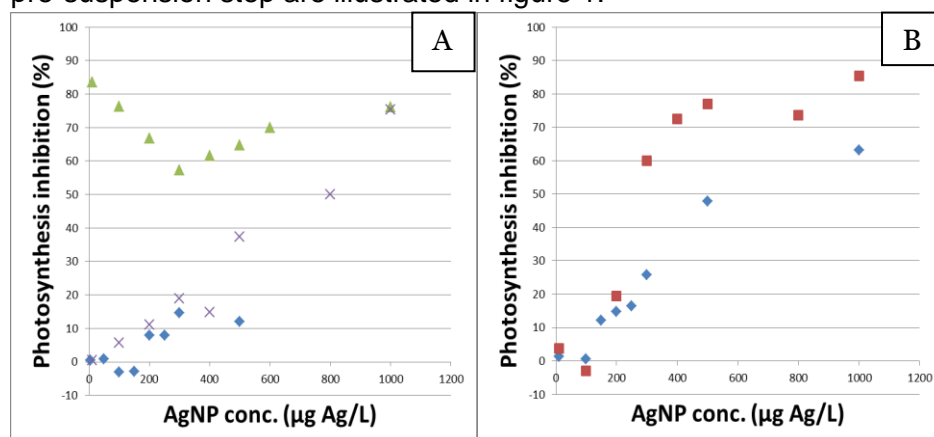


Figure 1. Inhibition of algal photosynthesis in 2h tests with citrate coated AgNPs prepared in ISO media A) Immediately before testing (three test runs) and B) 24h prior to testing (two test runs).

Without the pre-suspension step, poorly reproducible results were obtained and it was not possible to produce comparable EC<sub>50</sub> values from the three test runs. Introduction of the pre-suspension step resulted in a higher degree of reproducibility and in more comparable EC<sub>50</sub> values, indicating better control of the processes affecting AgNPs during the 2h testing. Moreover, the algal toxicity of AgNPs increased when extending the pre-suspension step period from 24 to 48h, suggesting that ionic dissolution of AgNPs into the media takes part in AgNP toxicity. Our results stress the importance of dealing with the time-dependent processes that NPs undergo in aquatic media when investigating their toxicity.

[1] Rosenkrantz R.T., Cupi D., Baun A., Kusk K.O. 2012. Phytoplankton <sup>14</sup>C-assimilation as a short term method to assess aquatic toxicity of difficult substances. (submitted).