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Pulse exposure of silver nanoparticles in acute and chronic toxicity tests with *D. magna*

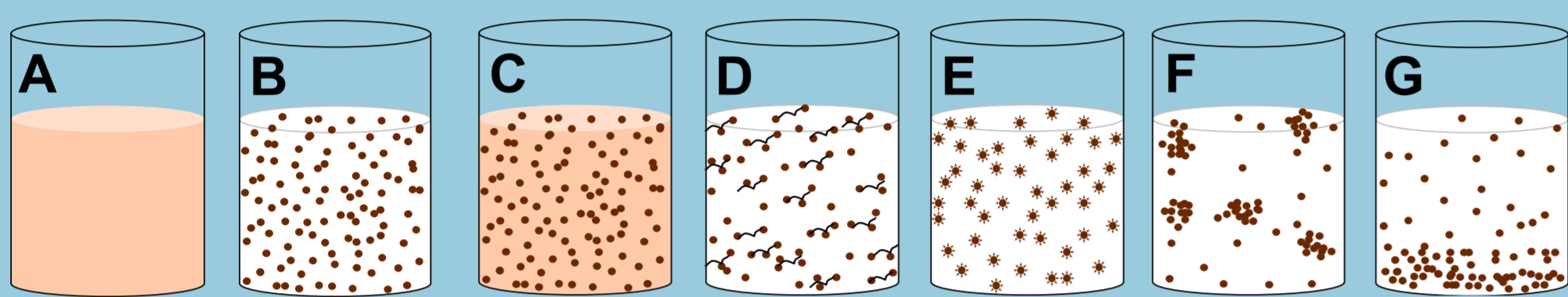


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Introduction & objectives

Nanoparticles in suspension (B) behave differently than soluble chemicals (A) and may undergo dissolution (C), interact with media components (D), be submitted to surface/coating alterations (E), aggregation (F) and sedimentation (G). These processes challenge our attempt to control or even describe the exposure concentration and characteristics of NPs during ecotoxicity testing (Sørensen et al., Integrated Environmental Assessment and Management *in press*).



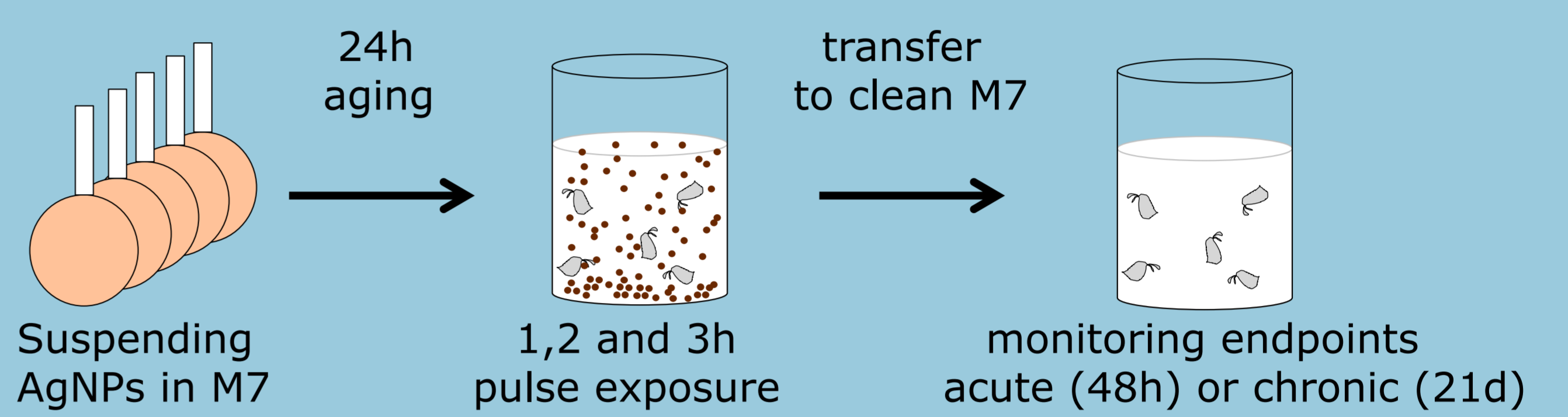
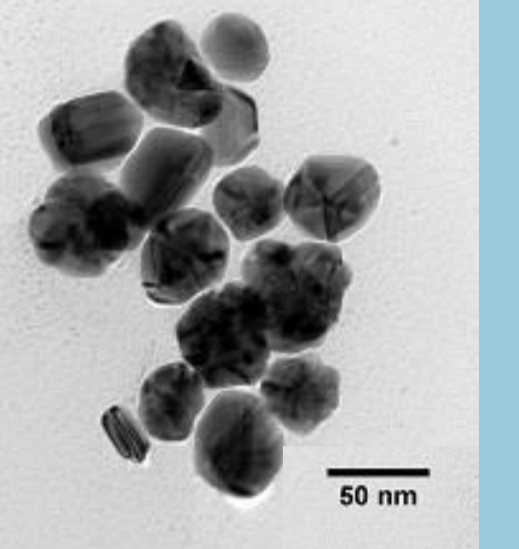
For silver nanoparticles (AgNPs), the use of aged test suspensions in a short-term (2h) algal test generated reproducible concentration-response data (Sørensen & Baun 2015, Nanotoxicology), indicating stable exposure concentrations. Here, we explore the same approach for another standard ecotoxicity test.

The aim is to investigate the applicability of a short-term (1-3h) pulse exposure to disclose acute and chronic effects of aged AgNP suspensions and dissolved silver (AgNO₃) in *Daphnia magna*.

Materials & Methods

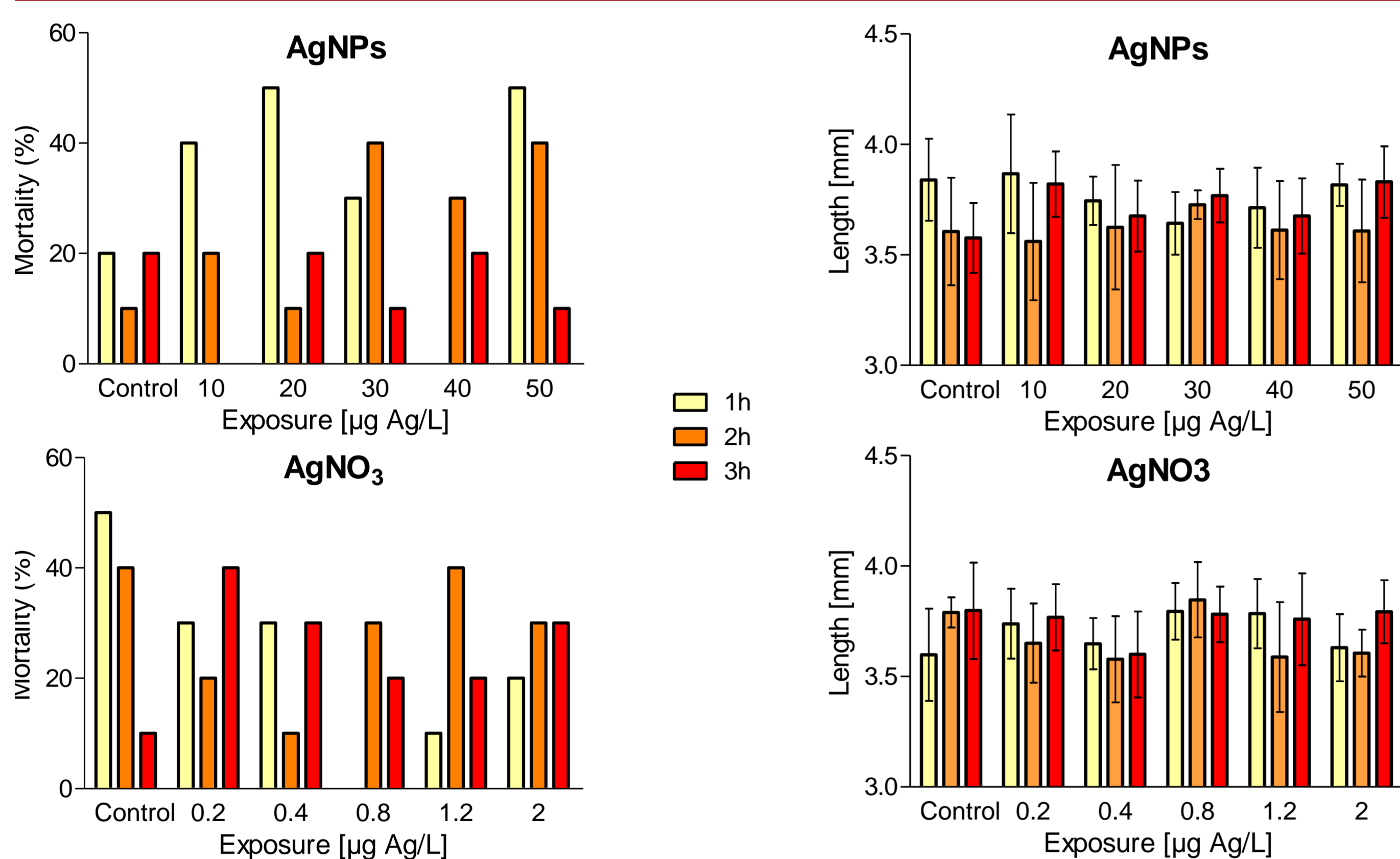
Tested NP Reference Citrate stabilized AgNPs nominal size 30 nm
Dissolved silver (AgNO₃)

Medium Organism Elenct M7 (OECD 211)
Daphnia magna neonates (<24h old)

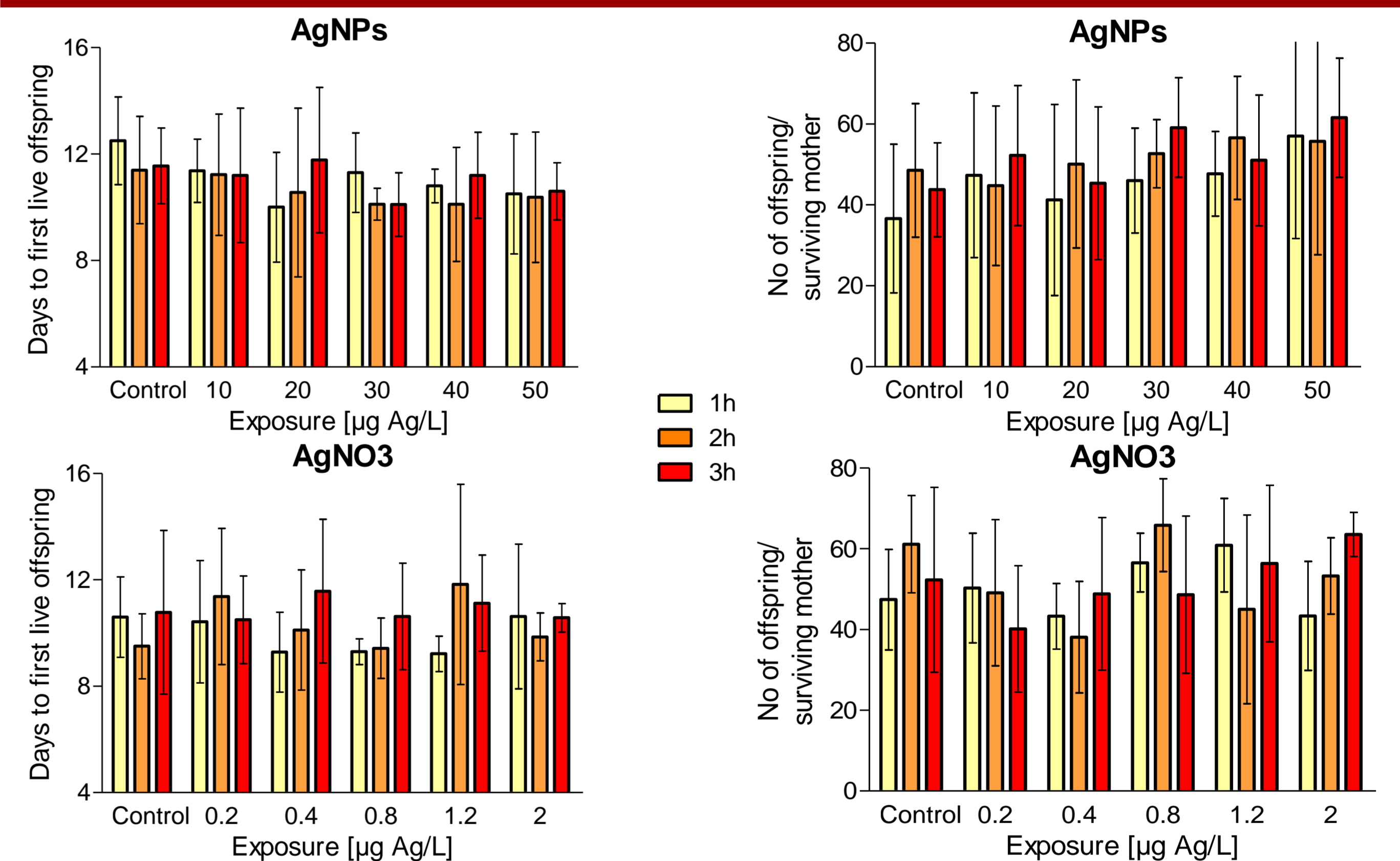


Acute endpoint (48h) Immobilization (according to OECD 202)
Chronic endpoints (21d) Mortality, growth and reproduction

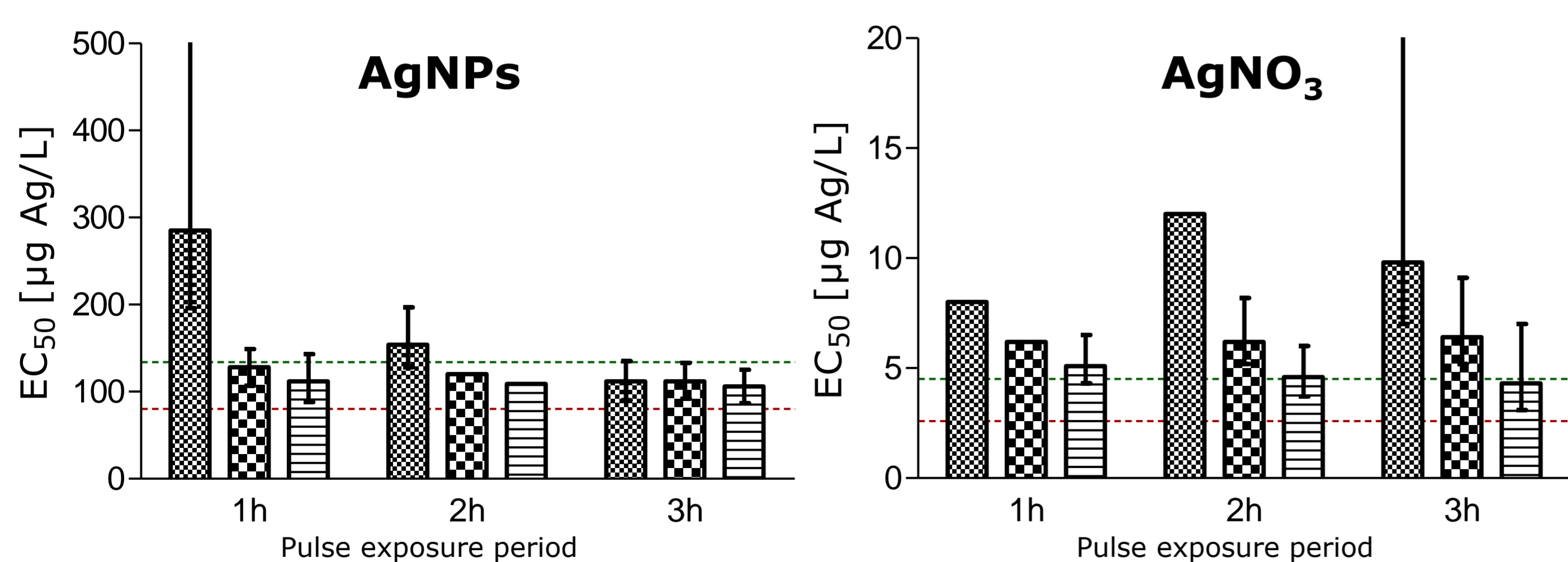
Chronic endpoints (mortality & growth)



Chronic endpoints (reproduction)



Acute endpoint (Immobilization)



EC_{50,24h} [µgAg/L] for continuous exposures (lines in graphs):

	AgNPs	AgNO ₃
Freshly made	134 [120;149]	4.5 [4.0;5.2]
Aged	< 80	2.6 [-]

Discussion & conclusion

ACUTE ENDPOINTS

- The aging prior to testing increases toxicity of AgNPs and AgNO₃, indicating that ions play a role
- The 24-48h immobility from 1-3h pulses of aged AgNPs are similar to that of 24h continuous exposure to freshly suspended AgNPs - i.e. this pulse setup is as sensitive as the continuous test but the short exposure makes monitoring and characterization of AgNPs during testing more feasible
- The toxicity generally increases slightly with pulse duration (1-3h) - this trend is less pronounced for AgNPs than AgNO₃ - i.e. 1h pulse can be applied in stead of 3h

CHRONIC ENDPOINTS

- Mortality decreases with pulse duration for AgNPs - not for AgNO₃. The double transfer of daphnids in short time may cause stress. High AgNO₃ control mortality (1-2h), so only 3h is considered
- No trends in growth between exposures
- Reproduction overall: No trend for pulse duration
- A very slight tendency for daphnids to reproduce sooner and have more offspring with AgNP-conc. Linear regression (pooled data 1-3h) confirms this (slope ≠ 0, P=0.04), but only the 50 µg/L (No of offspring) and 30 µg/L (Days to offspring) differs significantly from controls (P<0.05) by oneway ANOVA. For AgNO₃ (3h pulse) the same trend is observed for No of offspring
- The stimulation in reproduction may result from silver's antibacterial effect: Higher concentrations and/or other endpoints may be further tested

