The aquatic ecotoxicity of a marketed nanosilver product - a direct comparison with ionic silver.

Katrien Arijs[1,3], Dean Leverett[2], Koen Oorts[3], Jelle Mertens[1], Karsten Schlich[4], Maria Brüggeman[4]

INTRODUCTION & OBJECTIVES

- **Nanomaterials:** engineered for their specific phys-chem and biological characteristics » reactivity and/or behaviour can differ from the bulk form of the same material.
- Reaction rate of nanoparticles often relates to available surface area » chemical reactivity per mass dose may increase with decreasing particle size.
- In general, aquatic ecotoxicity of metal compounds is based on data for the dissolved metal ion.
- As part of the **REACH Substance Evaluation for silver**, further justification of the read-across from ionic silver to silver nanoforms was required » aquatic ecotoxicity and fate and behaviour of ionic silver and smallest silver nanoform with highest specific surface area registered under REACH were compared in standardised tests.

MATERIALS AND METHODS

- Silver nanoform (**Ag-NP**): aqueous suspension (Heraeus, 37% Ag), mean primary particle size = 8.4 nm, volume specific surface area = 714 m^2/cm^3. (Fig. 1)
- Silver nitrate (**$AgNO_3$**, Heraeus, 63.5% Ag, purity > 99.9%) was used as reference compound for ionic silver.
- Aquatic ecotoxicity tests were performed comparing effects of Ag-NP with $AgNO_3$:
  - Toxicity to the alga ***Pseudokirchneriella subcapitata*** (OECD TG No. 201).
  - Long-term toxicity to ***Daphnia magna*** (OECD TG No. 211).
- Test media were adapted to minimise complexation of Ag ions (amount of $Na_2EDTA.2(H_2O)$ minimised and chloride salts replaced by nitrate salts).
- **Total silver, 'conventional' dissolved silver** (0.45 µm membrane filtered) and **'truly' dissolved silver** (3 kDa centrifuge filtered) were measured (ICP-MS).
- **Dissolution rate** of Ag-NP was determined for specific test media used in ecotoxicity tests over 28 days (following OECD TG No. 29) with measurements of the same three silver fractions.

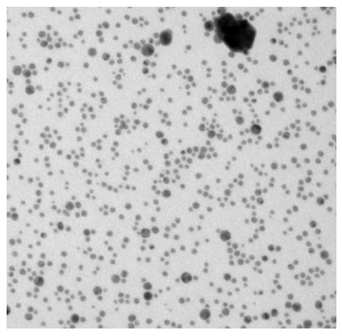

Figure 1: TEM image of the Ag-NP sample (68000x)

RESULTS

Algae

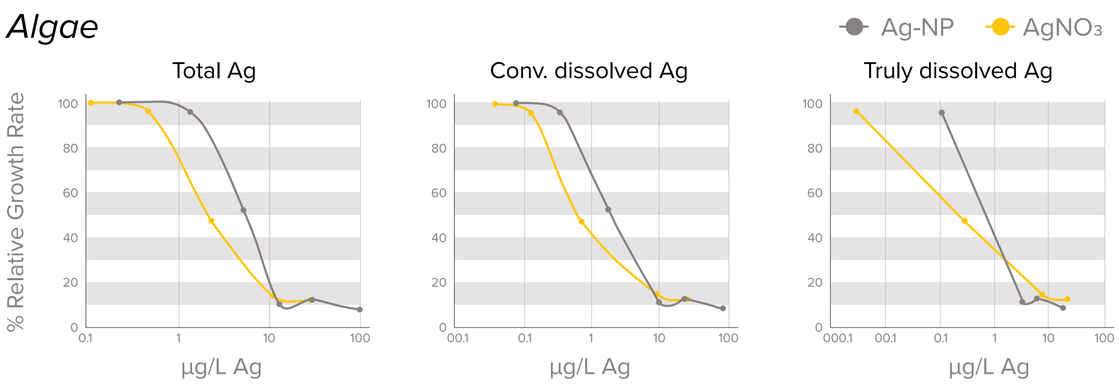

Figure 2: Growth rate response curves algae toxicity test based on geometric mean measured Ag concentration

Measured fraction	Test substance	EC10 (µg Ag/L)	EC50 (µg Ag/L)
Total Ag	$AgNO_3$	0.46 (0.31-0.62)	2.52 (2.13-2.98)
	Ag-NP	1.92 (1.2-2.54)	5.36 (4.57-6.17)
Conventional dissolved Ag	$AgNO_3$	0.1 (0.05-0.16)	0.96 (0.72-1.32)
	Ag-NP	0.37 (0.21-0.54)	2.13 (1.69-2.68)
Truly dissolved Ag	$AgNO_3$	0.005 (0.003-0.008)	0.285 (0.219-0.365)
	Ag-NP	0.17 (0.06-0.3)	0.89 (0.55-1.28)

Table 1: Results algae toxicity test (growth rate) based on geometric mean measured Ag concentration (with 95% confidence limits)

Daphnia

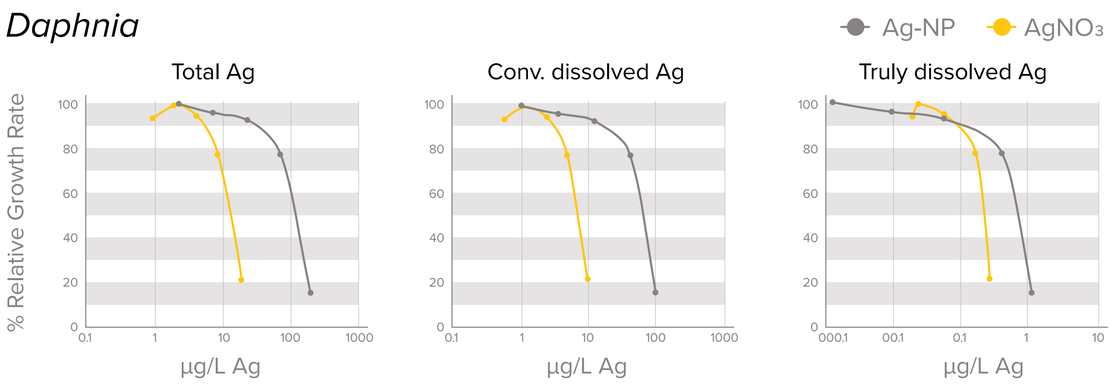

Figure 3: Reproduction response curves Daphnia reproduction test based on arithmetic mean measured Ag concentration

Measured fraction	Test substance	EC10 (µg Ag/L)	EC50 (µg Ag/L)
Total Ag	$AgNO_3$	5.371 (2.888 - 7.119)	12.01 (9.793 - 14.95)
	Ag-NP	50.68 (26.07 - 67.88)	106.51 (83.54 - 139.08)
Conventional dissolved Ag	$AgNO_3$	3.494 (1.981 - 4.485)	6.97 (5.808 - 8.437)
	Ag-NP	33.39 (18.40 - 43.06)	62.54 (50.39 - 79.44)
Truly dissolved Ag	$AgNO_3$	0.059 (0.022 - 0.088)	0.195 (0.151 - 0.266)
	Ag-NP	0.292 (0.143 - 0.396)	0.616 (0.475 - 0.821)

Table 2: Results Daphnia reproduction test based on arithmetic mean measured Ag concentration (with 95% confidence limits)

Dissolution Rate Testing

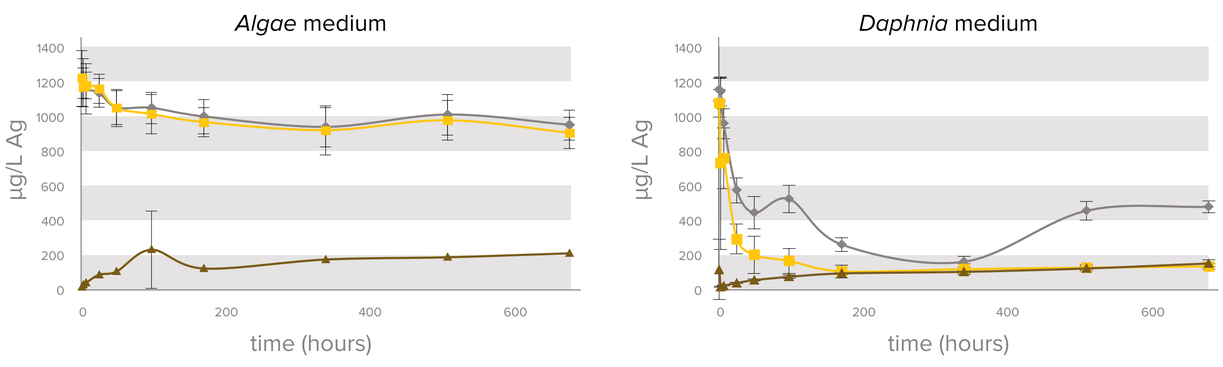

Figure 4: Ag concentrations during T/D tests in algae and Daphnia media (28 days)

CONCLUSIONS

- **$AgNO_3$ was more toxic than Ag-NP to both algae growth and *Daphnia* reproduction**, regardless of whether silver concentrations were expressed as total, conventional dissolved or truly dissolved silver.
- The measurement of **truly dissolved silver** (3 kDa centrifuge filtered) was intended to reflect the actual concentration of silver ions to which the organisms were exposed in each test. If toxicity is caused only by ionic Ag exposure, the ECx values for $AgNO_3$ and Ag-NP should be similar in tests with $AgNO_3$ and Ag-NP using the same test method, when expressed as truly dissolved silver. However, in the present study, the ECx values expressed as truly dissolved silver indicated greater toxicity in the tests with $AgNO_3$ than for Ag-NP. This likely reflects the degree of uncertainty in the measurement of dissolved silver ion concentrations in the test media, especially at the very low exposure concentrations applied in this study.
- The different **(dissolution) behaviour** of Ag-NP between test media partly explains the relative differences in toxicity thresholds between ionic and nano-forms for the different fractions in these media: lower dissolution of Ag-NP in *Daphnia* medium is reflected in higher thresholds when expressed as total or conventional dissolved silver.
- **The data shows that aquatic ecotoxicity data for ionic silver are conservative for aquatic ecotoxicity of nanosilver.**

[1] European Precious Metal Federation (EPMF), Brussels, Belgium - [2] wca environment, Faringdon, UK - [3] ARCHE Consulting, Gent, Belgium - [4] Fraunhofer IME, Schmallenberg, Germany - E-mail contact: katrien.arijs@arche-consulting.be

Precious Metals Consortium

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ARCHE CONSULTING

Fraunhofer IME