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NanoValid

Biological effects of nanoparticles of silver, gold, TiO_2 and nanoporous silica to selected invertebrate species and bacteria: FP7 project NanoValid

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BACKGROUND

- The number of industrial and consumer products which contain engineered nanomaterials (ENMs, materials with at least one dimension 1-100 nm) are increasing exponentially and there is a concern regarding their occupational and environmental safety
- FP7 project NanoValid (www.nanovalid.eu) aims to design well-characterized representative ENMs and develop reference bioassays for evaluation of the safety of ENMs. The role of our Institute therein is the development of ecotoxicological methods based on (i) organisms presumably resistant to the internalization of NPs such as bacteria, yeast, algae and (ii) particle-ingesting organisms such as crustaceans and protozoa.

MATERIALS & METHODS

CHEMICALS:

ENMs TiO_2, Au, Ag, SiO_2 were provided by the partners in NanoValid.

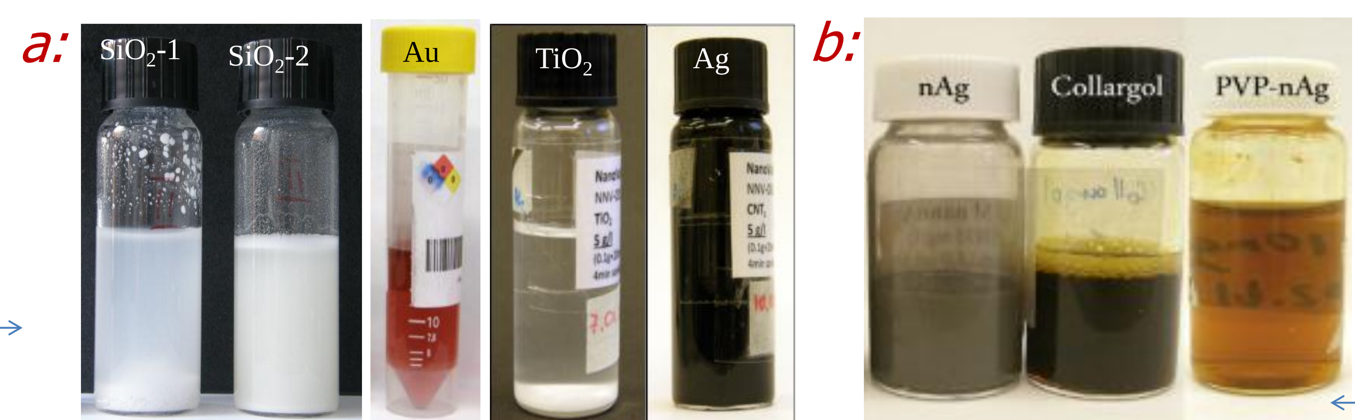

Fig 1. Stock-suspensions of ENMs studied: a: NanoValid ENMs; b: additionally studied nanosilver ENMs

Additional studied nanosilver preparations included uncoated Ag (nAg) from Sigma-Aldrich, protein (casein)-coated colloidal AgNPs (nAg-Col) from Laboratorios Argenol S. L. Polyvinylpyrrolidone-coated Ag ENMs (nAg-PVP) (a gift from Prof. Tenhu; Univ. Helsinki).

TEST ORGANISMS:

PARTICLE-INGESTING ORGANISMS | Presumably PARTICLE-"PROOF" ORGANISMS

EUKARYOTIC | PROKARYOTIC

CHARACTERIZATION OF ENMs:

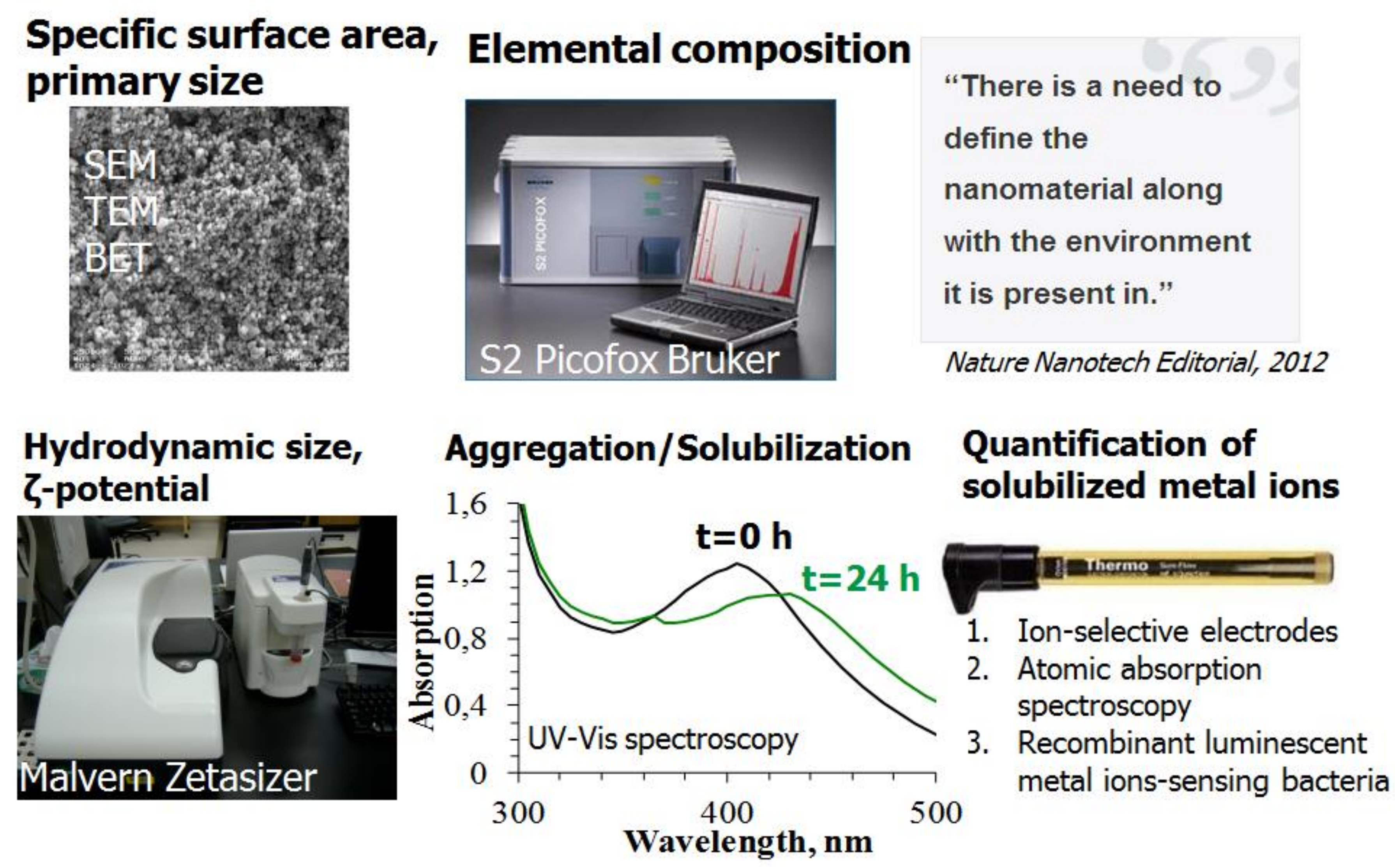

Fig 2. Physico-chemical methods and instruments used to characterize ENMs prior the test

RESULTS & CONCLUSIONS

Table 1. Toxicity of NanoValid ENMs (Fig 1a) to a battery of test organisms (L(E)C50 or MIC, mg/L)

Test species	Endpoint	SiO_2 -1	SiO_2-2	TiO_2	Au-citrate	Ag-PVP
CRUSTACEANS *Daphnia magna*, *Thamnocephalus platyurus*	48-h EC50, 24-h LC50 (immobilization)	>100 ☺	>100 ☺	>100 ☺	>10 ☺?	0.001 ☠
PROTOZOA *Tetrahymena thermophila*	24-h EC50 (viability by ATP)	>100 ☺	>100 ☺	>100 ☺	>30 ☺?	1.3 ☠
ALGAE *Pseudokirchneriella subcapitata*	97-h EC50 (growth inhibition)	73 ☹	>100 ☺	n.t	n.t* ?	0.08 ☠
YEAST *Saccharomyces cerevisiae*	24-h EC50 (growth inhibition)	>100 ☺	>100 ☺	>100 ☺	>10 ☺?	7.6 ☠
BACTERIA *Vibrio fischeri*	30-min EC50 (inhibition of bioluminescence)	>100 ☺	>100 ☺	>100 ☺	n.t* ?	2.9 ☠
Gram (-) bacteria *(P. aeruginosa, P. fluorescens, E. coli, P. putida)*	4-h minimal inhibitory concentration (MIC)	>100 ☺	>100 ☺	>100 ☺	>10 ☺?	4.5-5.0 ☠
Gram (+) bacteria *(B. subtilis, S. aureus)*		>100 ☺	>100 ☺	>100 ☺	>10 ☺?	6.6-8.1 ☠

**n.t=not tested*

Table 2. Classification of ENMs to different hazard categories (performed according to EU-Directive 93/67/EEC)

L(E)C50 or MIC*		EU classification
> 100 mg/L	☺	Not harmful/not classified
10-100 mg/L	☹	Harmful
1-10 mg/L	☠	Toxic

**MIC=minimal inhibitory concentration*

Conclusion 1 (on NanoValid ENMs, Fig 1a):

- In general, at 100 mg/L level NanoValid SiO_2 and TiO_2 **ENMs** (Fig 1a) proved not toxic to test organisms. **Au ENMs** were not toxic at 10 mg/L level for tested organisms (Table 1).
- NanoValid **SiO_2-1 ENMs** were harmful to algae *Pseudokirchneriella subcapitata* (72-h EC_{50}=80 mg/l) assumingly due to adsorption of growth media components to the porous surface of SiO_2.
- All studied Ag ENMs proved toxic to all the test species (EC_{50} 0.001-20 mg/L) being most toxic to crustacean *Daphnia magna*.

*Table 3. Minimal inhibitory concentration of different studied Ag ENMs (Fig 1b) and $AgNO_3$ (mg Ag/L) to various bacteria**

Bacterial strains (Gram staining)	nAg (Sigma)	nAg-Col	nAg-PVP	$AgNO_3$
Bacillus subtilis, Gram (+)	>100	40	20	5
Staphylococcus aureus, Gram (+)	>100	>100	>100	100
Escherichia coli , Gram (-)	>100	40	40	5
Pseudomonas fluorescens, ram(-)	>100	100	40	5
Pseudomonas putida, Gram (-)	>100	100	100	5
Pseudomonas aeruginosa, Gram (-)	>100	10	40	10

**incubated with toxicant for 4 h and plated onto Luria-Bertani medium for 24 h*

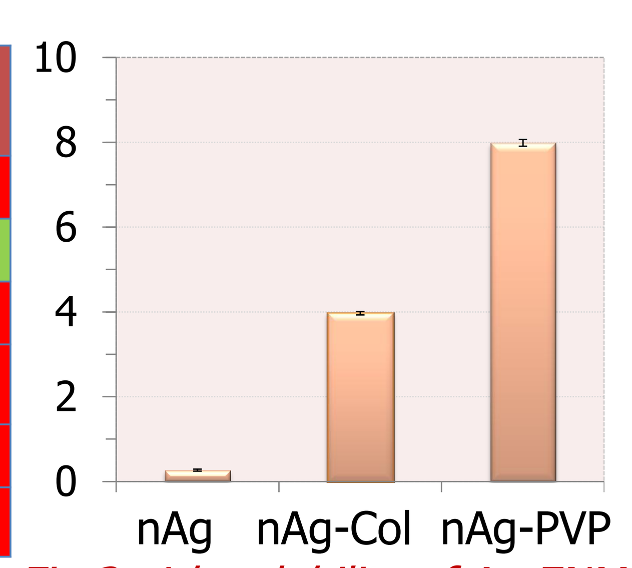

Fig 3. 4-h solubility of Ag ENMs incubated with E. coli, %

Conclusion 2 (on differently coated Ag ENMs, Fig 1b):

- Three Ag ENMs with different coatings (Fig 1b) were used to address the effect of coating on nanosilver toxicity
- Uncoated nAg was not toxic to bacteria. Toxicity of $AgNO_3$ (ionic control) was the highest, followed by nAg-PVP and nAg-Col (Table 3)
- Compared to uncoated nAg, remarkably more Ag ions was dissolved from PVP and collargol-coated nano-Ag contributing to their toxicity (Fig 3)
- To *P. aeruginosa* strain nAg-Col was as toxic as Ag ions showing bacterial strain-specific mechanism in addition to dissolution

For more information see our open-access papers:

Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review

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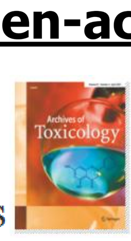

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RESEARCH ARTICLE

Particle-Cell Contact Enhances Antibacterial Activity of Silver Nanoparticles

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