Supplementary Data

Silver Nanoparticle Toxicity in the Embryonic Zebrafish is Governed by Particle Dispersion and Ionic Environment

Ki-Tae Kim[†], Lisa Truong[†], Leah Wehmas, Robert L. Tanguay*

Department of Environmental and Molecular Toxicology, the Sinnhuber Aquatic Research Laboratory and the Environmental Health Sciences Center at Oregon State University, and Safer Nanomaterials and Nanomanufacturing Initiative, Oregon Nanoscience and Microtechnologies Institute, Corvallis, OR

[†] Kim and Truong contributed equally to this work and are listed in alphabetical order.

Number of pages: 5 (this page is not included)

Number of figures: 4

Number of table: 1

NCL report (NCL-NIEH201111A)

The interest in engineered nanomaterials (ENMs) is growing at the National Institute of Environmental Health Sciences (NIEHS). To address the concerns of the ENMs safety, an interdisciplinary program, NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) Consortium was created. The NCNHIR Consortium consists of eight cooperative centers funded through Nanotechnology Environmental Health and Safety program for toxicity evaluation, the Nanotechnology Characterization Laboratory (NCL) for nanomaterial characterization and the National Institute of Biomedical Imaging and Bioengineering to create an informational database.

NCL characterized 20 and 110 nm AgNPs, with either PVP or citrate surface coatings, synthesized by nanoComposix (San Diego, CA). They stored materials 4 °C without freezing in a sterile environment. NCL confirmed the sterility threshold for endotoxin contamination, less than 2.5 EU/mL. They reported unstability of citrate-stabilized AgNPs in high ionic strength buffers. Citrate-stabilized AgNPs precipitate immediately (within minutes) while PVP-stabilized AgNPs precipitate gradually (in hours) in saline and PBS buffers. They reported that size of PVP-stabilized AgNPs is increased as a function of time in saline and PBS buffers. Also, they reported unstability of materials under gamma irradiation. The details for hydrodynamic diameter and zeta potential of four AgNPs are provided in the following pages.

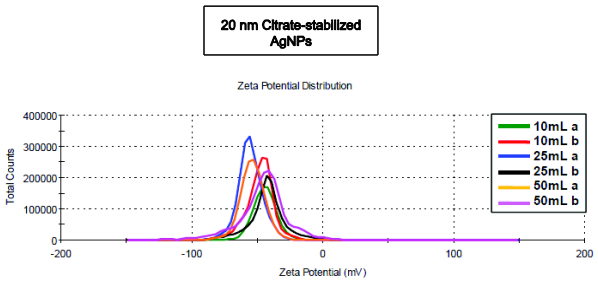
Table S1. LC_{50} values (the concentration causing 50% mortality) and EC_{50} values (the concentration causing 50% of adverse effects including mortality and any malformations) calculated using the Probit analysis over 6 exposure concentrations.

Silver Nanoparticle (AgNPs)	Test Media	LC_{50} (mg/L)	EC_{50} (mg/L)
20nm Citrate-stabilized AgNPs	UP	36.78	13.06
	$CaCl_2$	42.97	45.85
	EM	44.78	outside of testing range
110nm Citrate-stabilized AgNPs	UP	9.98	18.00
	$CaCl_2$	10.20	20.20
	EM	N/A	outside of testing range
20nm PVP-stabilized AgNPs	UP	11.92	10.10
	$CaCl_2$	4.14	2.14
	EM	42.49	outside of testing range
110nm PVP-stabilized AgNPs	UP	0.08	44.59
	$CaCl_2$	44.53	outside of testing range
	EM	N/A	N/A
10 kDa PVP UP		N/A	N/A
10 kDa PVP CaCl2		N/A	N/A
10 kDa PVP EM		N/A	N/A
40 kDa PVP UP		N/A	N/A
40 kDa PVP CaCl2		N/A	N/A
40 kDa PVP EM		N/A	N/A

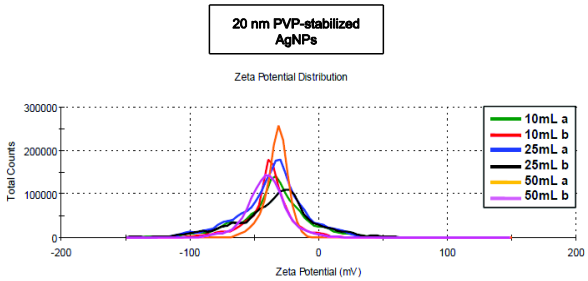
EM: embryo media; $CaCl_2$: 62.5 μM $CaCl_2$; UP: ultrapure water

N/A: not calculated due to low toxicity

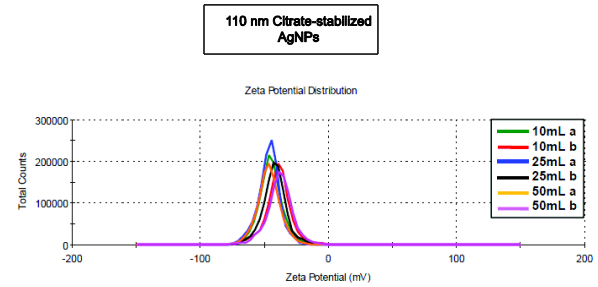
Figure S1. NCL characterization results (Hydrodynamic diameter). Taken from the NCL report (NCL-NIEHS201111A).



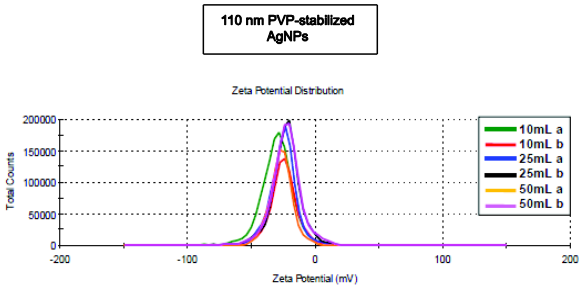
NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-1	20 nm, citrate stabilized silver	10 mL	a	-44.3 ± 1.3	6.6
			b	-48.3 ± 1.6	6.5
		25 mL	a	-55.5 ± 1.0	6.5
			b	-43.1 ± 1.7	6.5
		50 mL	a	-53.6 ± 2.1	6.6
			b	-46.2 ± 1.0	6.6



NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-2	20 nm, PVP stabilized silver	10 mL	a	-38.2 ± 1.6	6.0
			b	-38.7 ± 0.8	6.0
		25 mL	a	-36.8 ± 1.3	6.2
			b	-35.9 ± 2.3	6.2
		50 mL	a	-32.5 ± 1.9	6.2
			b	-40.6 ± 1.6	6.2



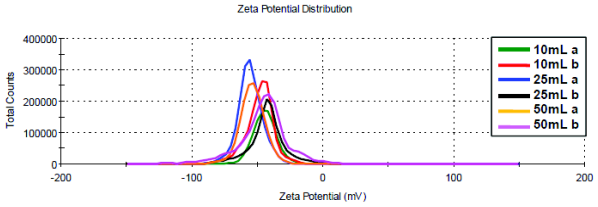
NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-3	110 nm, citrate stabilized silver	10 mL	a	-45.2 ± 0.4	6.4
			b	-39.7 ± 1.1	6.5
		25 mL	a	-46.4 ± 1.0	6.6
			b	-42.3 ± 0.8	6.6
		50 mL	a	-46.5 ± 1.2	6.6
			b	-38.0 ± 2.7	6.6



NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-4	110 nm, PVP stabilized silver	10 mL	a	-31.6 ± 2.2	5.6
			b	-25.0 ± 0.8	5.8
		25 mL	a	-25.5 ± 0.9	6.0
			b	-23.6 ± 0.9	6.0
		50 mL	a	-26.5 ± 0.8	6.0
			b	-23.3 ± 1.5	6.0

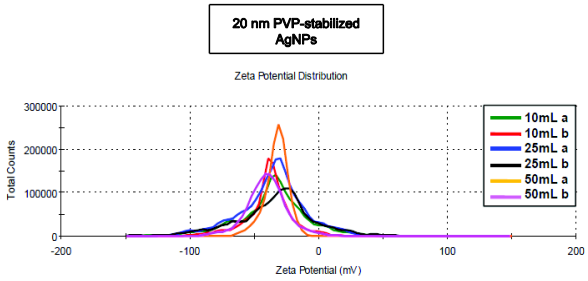
Figure S2. NCL characterization results (zeta-potential). Taken from the NCL report (NCL-NIEHS201111A).

20 nm Citrate-stabilized AgNPs



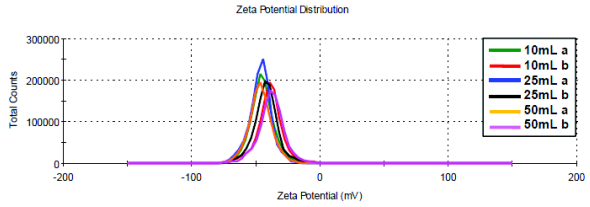
NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-1	20 nm, citrate stabilized silver	10 mL	a	-44.3 ± 1.3	6.6
			b	-48.3 ± 1.6	6.5
		25 mL	a	-55.5 ± 1.0	6.5
			b	-43.1 ± 1.7	6.5
		50 mL	a	-53.6 ± 2.1	6.6
			b	-46.2 ± 1.0	6.6

20 nm PVP-stabilized AgNPs



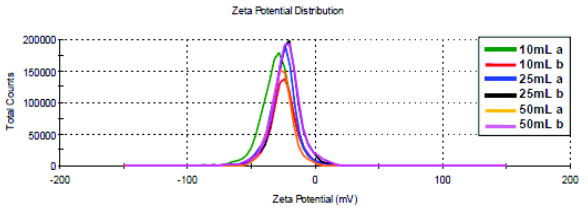
NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-2	20 nm, PVP stabilized silver	10 mL	a	-38.2 ± 1.6	6.0
			b	-38.7 ± 0.8	6.0
		25 mL	a	-36.8 ± 1.3	6.2
			b	-35.9 ± 2.3	6.2
		50 mL	a	-32.5 ± 1.9	6.2
			b	-40.6 ± 1.6	6.2

110 nm Citrate-stabilized AgNPs



NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-3	110 nm, citrate stabilized silver	10 mL	a	-45.2 ± 0.4	6.4
			b	-39.7 ± 1.1	6.5
		25 mL	a	-46.4 ± 1.0	6.6
			b	-42.3 ± 0.8	6.6
		50 mL	a	-46.5 ± 1.2	6.6
			b	-38.0 ± 2.7	6.6

110 nm PVP-stabilized AgNPs



NCL Particle Designation	Nanomaterial	Test Aliquot		Zeta Potential (mV)	pH
NIEHS-4	110 nm, PVP stabilized silver	10 mL	a	-31.6 ± 2.2	5.6
			b	-25.0 ± 0.6	5.8
		25 mL	a	-25.5 ± 0.9	6.0
			b	-23.6 ± 0.9	6.0
		50 mL	a	-26.5 ± 0.8	6.0
			b	-23.3 ± 1.5	6.0

Figure S3. Silver concentration of the supernatant of 4 AgNPs suspended in embryo media, 62.5 µM $CaCl_2$ and ultrapure water. The initial concentration was 5 µg/L.

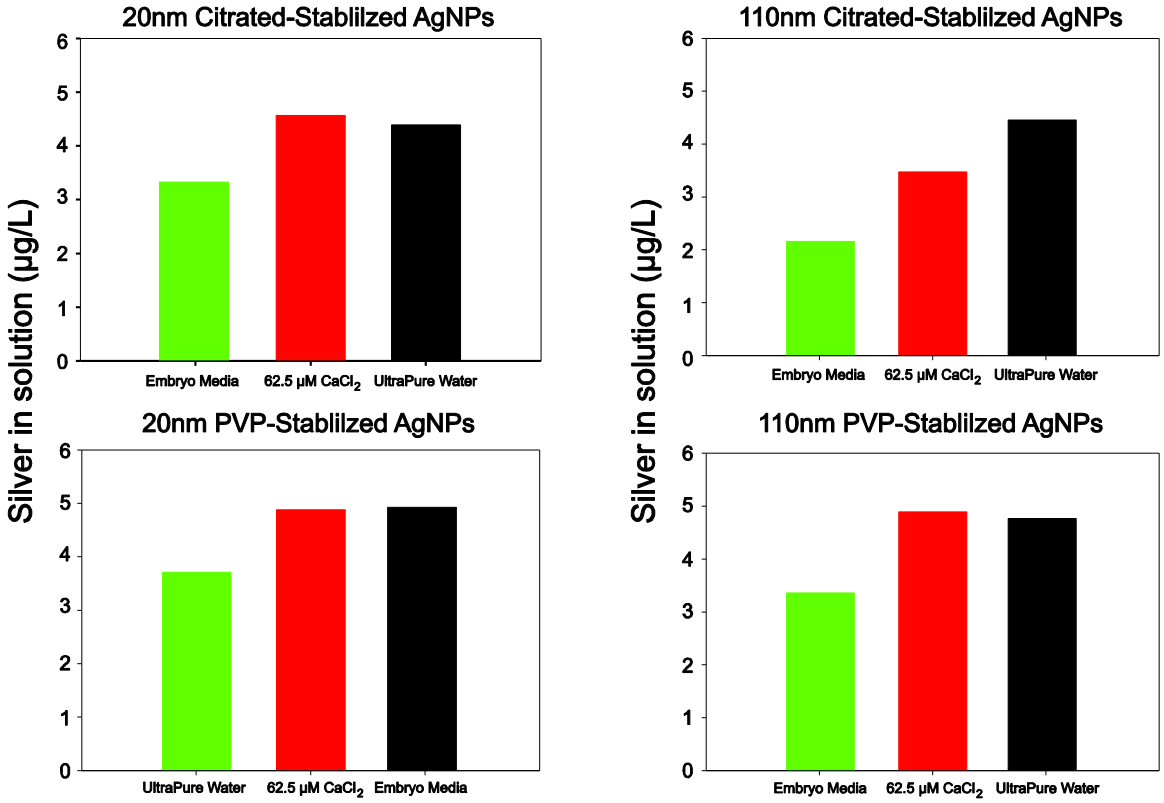


Figure S4. Relationship between silver body burden and toxicity in embryo media, 62.5 µM $CaCl_2$ and ultrapure water at 10 and 50 mg/L.