Supplementary information

Authors: Carolin L. Schultz, Anye Wamucho, Olga Tsyusko, Jason Unrine, Alison Crossley, Claus Svendsen, David J. Spurgeon

Title: Multigenerational exposure to silver ions and silver nanoparticles reveals heightened sensitivity and epigenetic memory in *Caenorhabditis elegans*

Summary: 10 pages including 7 tables and 3 figures

Details of nanoparticle synthesis

Pristine polyvinylpyrrolidone (PVP) coated Ag-ENPs (Ag-PVP) and a sulfidised form of these particles (Ag_2S) were synthesised and supplied by Jason Unrine, University of Kentucky, USA as described in Starnes et al. (2015).

Ag-PVP synthesis
Polyvinylpyrrolidone (PVP) coated Ag-ENPs were prepared by a modified polyol method, for details see Cheng et al (2011) DOI: 10.1021/jp109789j.

Ag_2S synthesis
Ag_2S-ENPs were prepared by combining the Ag-PVP ENPs with Na_2S at a molar ratio of 2:1 S to Ag, brief incubation in open air and sealed for 4 days. Particles were subsequently purified by washing three times in 18 MΩ deionized water and sulfidation verified using EDX and UVvis analysis. For details see Starnes et al. (2015) Supporting Information. DOI: 10.1016/j.envpol.2014.10.009

Supplementary Table 1: Exposure characterisation of Ag-PVP and Ag_2S ENPs over 96 h continuous exposure duration at 10 mg Ag/l in SSPW medium (pH 8.2), NTA: mean hydrodynamic diameter [nm] and zeta potential [mV]; averages ± SE

	0 h	**24 h**	**48 h**	**72 h**	**96 h**
Ag-PVP					
NTA diameter	79 ± 1	79 ± 4	92 ± 2	105 ± 18	73 ± 3
ζpotential	-2.8 ± 0.1	-5.1 ± 0.2	-5.1 ± 0.6	-3.7 ± 0.3	-5.2 ± 0.5
Ag_2S					
NTA diameter	243 ± 20	281 ± 24	297 ± 22	281 ± 37	213 ± 122
ζpotential	-2.3 ± 0.0	-3.4 ± 0.1	-4.1 ± 0.3	-4.3 ± 1.1	-5.3 ± 0.3

Supplementary Table 2: F statistic and p-values comparing logistic model fits of concentration response for the effects on reproduction in the nematode *C. elegans* of each sliver forms following continuous exposure over different numbers of multiple generations. Shaded areas and black writing indicate significant F-statistic for each response curve comparison, dark shading: p=0.01, light shading p=0.05.

		Parent	F2	F5	F8	F10	Recovery
$AgNO_3$							
F2	F-value	10.747					
	p-value	0.000					
F5	F-value	10.193	0.149				
	p-value	0.000	0.930				
F8	F-value	14.140	0.707	1.083			
	p-value	0.000	0.551	0.363			
F10	F-value	6.161	2.035	1.737	2.092		
	p-value	0.001	0.118	0.168	0.110		
Recovery	F-value	16.202	0.449	0.970	1.652	4.381	
	p-value	0.000	0.719	0.412	0.186	0.007	
Ag-PVP							
F2	F-value	14.287					
	p-value	0.000					
F5	F-value	10.553	2.019				
	p-value	0.000	0.120				
F8	F-value	23.618	2.112	2.457			
	p-value	0.000	0.107	0.071			
F10	F-value	13.586	7.065	1.472	6.471		
	p-value	0.000	0.000	0.231	0.001		
Recovery	F-value	18.609	2.991	0.102	3.571	3.375	
	p-value	0.000	0.037	0.958	0.019	0.024	
Ag_2S							
F2	F-value	0.735					
	p-value	0.535					
F5	F-value	3.838	5.542				
	p-value	0.014	0.002				
F8	F-value	2.762	2.428	4.233			
	p-value	0.049	0.073	0.009			
F10	F-value	5.574	7.797	1.308	3.546		
	p-value	0.002	0.000	0.281	0.020		
Recovery	F-value	3.677	6.188	0.305	4.830	1.275	
	p-value	0.017	0.001	0.822	0.004	0.292	

Supplementary Table 3: Effect concentration for each generation corresponding to the chosen continuous exposures concentrations ($AgNO_3$=0.1 mg Ag/l, Ag-PVP=0.9 mg Ag/l, Ag_2S=15 mg Ag/l).

	Parent	**F2**	**F5**	**F8**	**F10**	**Recovery**
$AgNO_3$	EC35	EC66	EC63	EC67	EC19	EC70
Ag-PVP	EC25	EC72	EC55	EC73	EC13	EC48
Ag_2S	EC56	EC80	EC55	EC73	EC65	EC50

Supplementary Table 4: Results of the statistical analysis of lifespan data using GLM, with factors: generation, concentration and the interaction term generation*concentration. Test of assumptions: Kolmogorov-Smirnov (Normality: p>0.05) and Levene's Test (Homoscedasticity: p>0.05). Comparisons of *Parent, F2-F10* were made to assess the effect of continuous exposure and of *Parent, F5, F10, Recovery* to assess the effect of the transfer into a recovery environment and subsequent re-exposure

		$AgNO_3$	Ag-PVP	Ag_2S
Parent, F2-F10				
Model fit	R^2	15.93%	17.03%	22.64%
	Normality	<0.01	<0.01	<0.01
	Homoscedasticity	0.057	0.087	0.060
Generation	df	4	4	4
	p-value	0.286	0.262	0.262
	F- value	1.26	1.32	1.32
Concentration	df	5	5	5
	p-value	0.006	0.003	0.000
	F- value	3.34	3.77	7.34
Generation*Concentration	df	20	20	20
	p-value	0.143	0.102	0.077
	F value	1.36	1.44	1.51
Parent, F5, F10, Recovery				
Model fit	R^2	10.06%	17.03%	21.94%
	Normality	<0.01	<0.01	<0.01
	Homoscedasticity	0.108	0.073	0.153
Generation	df	3	3	3
	p-value	0.816	0.369	0.219
	F- value	0.31	1.06	1.49
Concentration	df	5	5	5
	p-value	0.492	0.213	0.001
	F- value	0.88	1.44	4.61
Generation*Concentration	df	15	15	15
	p-value	0.299	0.106	0.021
	F value	1.17	1.50	1.94

Supplementary Table 5: Results of the statistical analysis of lifespan data using the non-parametric Scheirer-Ray-Hare test. Comparisons of *Parent, F2-F10* were made to assess the effect of continuous exposure and of *Parent, F5, F10, Recovery* to assess the effect of the transfer into a recovery environment and subsequent re-exposure

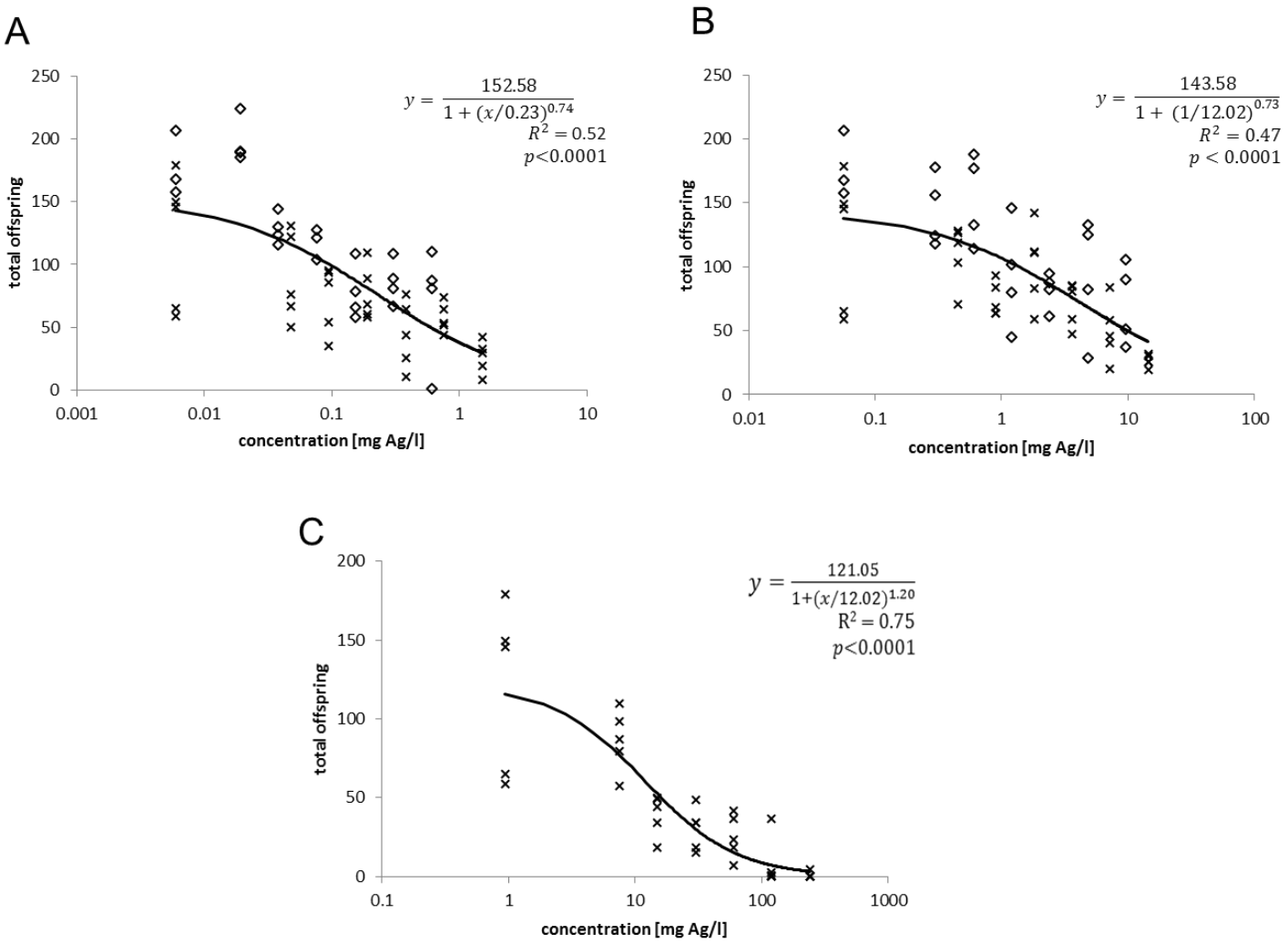
		$AgNO_3$	Ag-PVP	Ag_2S
Parent, F2-F10				
Generation	SS	31585	34880	32355
	SS/MS_{total}	4.51	4.91	4.96
	df	4	4	4
	p-value	0.341	0.296	0.292
Concentration	SS	123014	133811	212718
	SS/MS_{total}	17.57	18.86	32.59
	df	5	5	5
	p-value	0.004	0.002	<0.001
Generation*Concentration	SS	163387	202878	177775
	SS/MS_{total}	23.33	28.60	27.23
	df	20	20	20
	p-value	0.272	0.096	0.129
Parent, F5, F10, Recovery				
Generation	SS	3959	23634	17509
	SS/MS_{total}	0.90	5.19	4.23
	df	3	3	3
	p-value	0.826	0.158	0.237
Concentration	SS	19464	38087	81499
	SS/MS_{total}	4.42	8.36	19.70
	df	5	5	5
	p-value	0.490	0.137	0.001
Generation*Concentration	SS	78376	108773	113965
	SS/MS_{total}	17.80	23.89	27.55
	df	15	15	15
	p-value	0.272	0.067	0.025

Supplementary Table 6: Results of the statistical analysis of size data using GLM, with factors: generation, concentration and the interaction term generation*concentration. Test of assumptions: Kolmogorov-Smirnov (Normality: p>0.05) and Levene (Homoscedasticity: p>0.05). Comparisons of *Parent, F2-F10* were made to assess the effect of continuous exposure and of *Parent, F5, F10, Recovery* to assess the effect of the transfer into a recovery environment and subsequent re-exposure

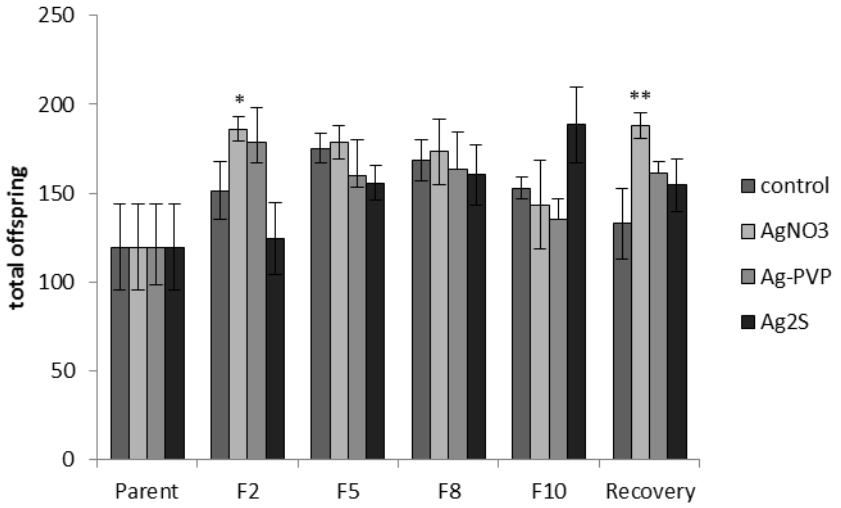
		$AgNO_3$	Ag-PVP	Ag_2S
Parent, F2-F10				
Model fit	R^2	57.04%	61.40%	67.98%
	Normality	<0.01	<0.01	<0.01
	Homoscedasticity	<0.01	<0.01	<0.01
Generation	df	4	4	4
	p-value	<0.001	<0.001	<0.001
	F- value	129.90	157.96	265.99
Concentration	df	5	5	5
	p-value	<0.001	<0.001	<0.001
	F- value	55.96	55.50	53.26
Generation*Concentration	df	20	20	20
	p-value	<0.001	<0.001	<0.001
	F value	9.39	12.00	3.70
Parent, F5, F10, Recovery				
Model fit	R^2	56.23%	59.02%	63.27%
	Normality	0.033	0.041	<0.01
	Homoscedasticity	<0.01	<0.01	<0.01
Generation	df	3	3	3
	p-value	<0.001	<0.001	<0.001
	F- value	80.57	77.59	182.63
Concentration	df	5	5	5
	p-value	<0.001	<0.001	<0.001
	F- value	88.55	104.62	69.93
Generation*Concentration	df	15	15	15
	p-value	<0.001	<0.001	<0.001
	F value	4.88	7.44	5.14

Supplementary Table 7: Results of the statistical analysis of size data using the non-parametric Scheirer-Ray-Hare test. Comparisons of *Parent, F2-F10* were made to assess the effect of continuous exposure and of *Parent, F5, F10, Recovery* to assess the effect of the transfer into a recovery environment and subsequent re-exposure

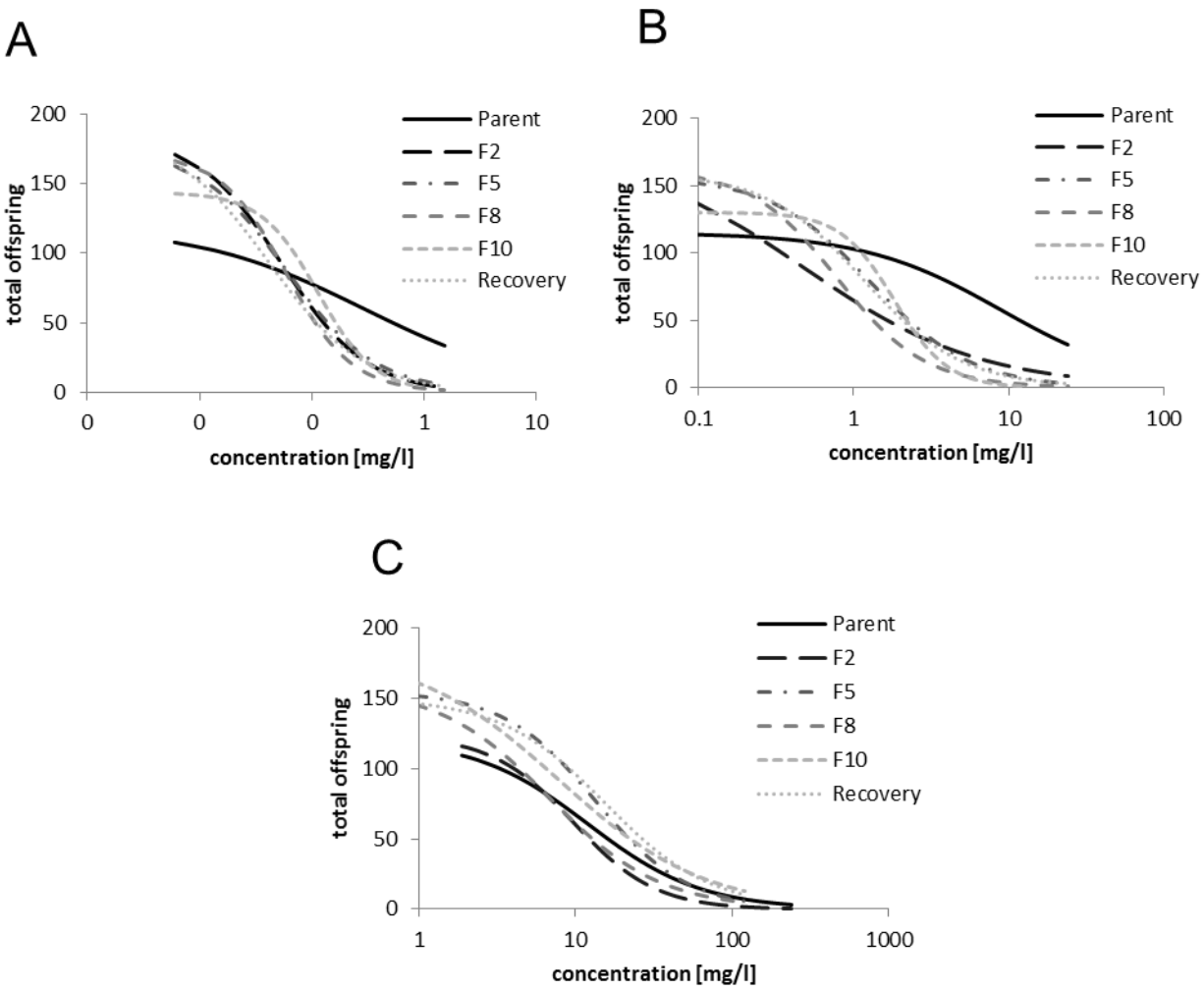
		$AgNO_3$	Ag-PVP	Ag_2S
Parent, F2-F10				
Generation	SS	12052732	15113990	15384851
	SS/MS_{total}	269.56	300.03	379.48
	df	4	4	4
	p-value	<0.001	<0.001	<0.001
Concentration	SS	4917395	5508812	3555316
	SS/MS_{total}	109.98	109.36	87.69
	df	5	5	5
	p-value	<0.001	<0.001	<0.001
Generation*Concentration	SS	3837551	4873809	1158018
	SS/MS_{total}	85.83	96.75	28.56
	df	20	20	20
	p-value	<0.001	<0.001	0.097
Parent, F5, F10, Recovery				
Generation	SS	4106488	4106354	5945045
	SS/MS_{total}	129.65	123.56	208.82
	df	3	3	3
	p-value	<0.001	<0.001	<0.001
Concentration	SS	6323310	7451365	3762621
	SS/MS_{total}	199.65	224.22	132.16
	df	5	5	5
	p-value	<0.001	<0.001	<0.001
Generation*Concentration	SS	1385475	1917126	1119340
	SS/MS_{total}	43.74	57.69	39.32
	df	15	15	15
	p-value	<0.001	<0.001	<0.001



Supplementary Figure 1: Reproductive toxicity of a) $AgNO_3$, b) Ag-PVP, c) Ag_2S to *C. elegans*. Data points represent the total number of offspring produced by individual nematodes in response to exposure to Ag in ◊ pre-trial and x parent generation as a function of Ag exposure concentration. Lines represents 3 parameter log logistic regression fitted to combined parent and pre-trial data for $AgNO_3$, Ag-PVP, or in parent generation for Ag_2S each with corresponding regression equation, R^2 and p-values to show goodness of fit. Since 0 mg Ag/l cannot be displayed on log scale controls are displayed as the lowest values in each figure, i.e. for $AgNO_3$: 0.006 mg Ag/l, Ag-PVP: 0.05 mg Ag/l, Ag_2S: 0.9 mg Ag/l.



Supplementary Figure 2: Number of offspring produced in control (MGExp-control) for each generation after continuous exposure, average ± SE. * indicate significant differences of controls from continuously Ag exposed populations compared to the continuously unexposed population, * p=0.05, ** p=0.01.



Supplementary Figure 3: The 3 parameter logistic regression curves of total number of offspring per adult after exposure to a) $AgNO_3$, b) Ag-PVP, c) Ag_2S at each of the different generations continuously exposed F2, F5, F8, F10 and the R generation.