

Exposure medium and particle ageing moderate the toxicological effects of nanomaterials to *Daphnia magna* over multiple generations: a case for standard test review?

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1. Methodology

1.1 Media and representative waters

Table SI.1 Media compositions			
Class V river water		HH combo media	
Compound	Stock mg L ⁻¹	Compound	Stock (g L ⁻¹)
Calcium Sulfate Dihydrate	155.02	Calcium Chloride, Dihydrate	110.28
Calcium carbonate	8.007	Magnesium sulphate heptahydrate	113.5
Magnesium carbonate	45.517	Potassium phosphate dibasic	1.742
	μL L ⁻¹	Sodium nitrate	17
Calcium Nitrate Tetrahydrate*	165	Sodium metasilicate nonahydrate	28.42
Sodium bicarbonate*	696.6	Boric acid	24
Calcium chloride*	363.5	Potassium chloride	5.96
Potassium bicarbonate *	85.9	Sodium Bicarbonate	63
	mL L ⁻¹		
Natural organic matter (NOM) (1g/L)	4.6		
pH	7.8	pH	7.6-7.8
Ionic strength without animate	6	Ionic strength without animate	11.07
Ionic strength with animate	15.76	Ionic strength with animate	20.83

*Stock solutions of 1 mol L⁻¹

Table SI.1A: Animate	
Compound	Stock (g/100mL)
Lithium chloride	31
Rubidium chloride	7
Strontium chloride hexahydrate	15
Sodium bromide	1.6
Potassium iodide	0.33

*add 1mL of each to 1L ultra pure water (UPW).

Add 1mL of each compound reported in Table SI.1A to prepare 1L stock solution of animate. From the 1L stock solution of animate, 1 mL is added to every 1 L of either the HH combo media or the Class V water.

Table SI.1B: Vitamin stock solution	
Compound	Stock
Biotin	10 mg in 96 mL
B ₁₂	10 mg in 89 mL
Thiamine HCl	10 mg in 50 mL

To make the vitamin stock, aliquot 1.5 mL of the biotin and the B12 vitamins into 50 mL with 10 mg of Thiamine HCl (Table SI.1B). A total of 0.5mL from the vitamin stock is added to every 1 L of either the HH combo media or the Class V water.

1.2 Nanomaterials and characterization

The NMs used in this study include PVP-coated and uncoated TiO₂ NMs (both supplied by Promethean Particles Ltd. www.prometheanparticles.co.uk), Ag₂S PVP coated Ag (AppNano Ltd, Spain) and described in, uncoated (bare) Ag (Promethean Particles Ltd) and PVP coated Ag (Amepox Ltd., Poland projects www.amepox-mc.com) via the EU H2020 project NanoFASE. Samples were analysed for size and size distribution at the exposure concentrations by dynamic light scattering (DLS), using a Malvern Nanosizer 5000 instrument, with results reported as the average mean size of 10 runs.

1.3 Range-finding study (*Daphnia* acute immobilization test, OECD 202)

When selecting test concentrations for NMs toxicity testing, there is always a trade-off between utilising environmentally realistic concentrations, and effective concentrations (ECs) at which some effect from the test material is observed. Our justification for using ECs in this study is because regulation and environmental risks are assessed by characterizing the effects in biological receptors. Furthermore, the concentrations ranges at which toxicity has been observed for various TiO₂ and Ag NMs have been reported elsewhere (1, 2). To confirm that our particles behave in a broadly similar manner, a range finding study was undertaken to determine the EC₅₀, using acute 48 hour immobilization tests. The results are shown in Figure SI.1. The pristine uncoated TiO₂ were overall less toxic compared to the PVP coated TiO₂ NMs, particularly at higher concentrations (Figure SI.1A) and as expected the Ag₂S NMs were much less toxic than the uncoated Ag NMs (Figure SI.1B). Using the range finding curves (Figure SI.1), EC₃₀ values were established for use in the pilot 28-day studies, using exposure concentrations of 45mg L⁻¹ (TiO₂ PVP), 30 mg L⁻¹ (TiO₂ uncoated), 20 µg L⁻¹ (PVP Ag), 20 µg L⁻¹ (uncoated Ag) and 100 µg L⁻¹ (Ag₂S NMs), respectively. The pilot studies were conducted to identify any issues with the study design and/or the NM concentrations used. The EC₃₀ concentration from the acute studies showed high mortality in the TiO₂ NM pilot chronic studies with almost 100% mortality at the EC₃₀ after 6 days of exposure for the pristine uncoated TiO₂, possibly due to the effects of feeding. For this reason the test concentrations for the multi-generational studies were reduced further to the EC₅ values of 5 mg L⁻¹ for the TiO₂ NMs, which matched the exposure concentrations reported in other *Daphnia* toxicity studies utilising TiO₂ NMs (3). No adjustments were required for the Ag NM studies. All the studies reported in the main manuscript utilise the EC₃₀ values for Ag NMs and the EC₅ values for TiO₂ NMs.

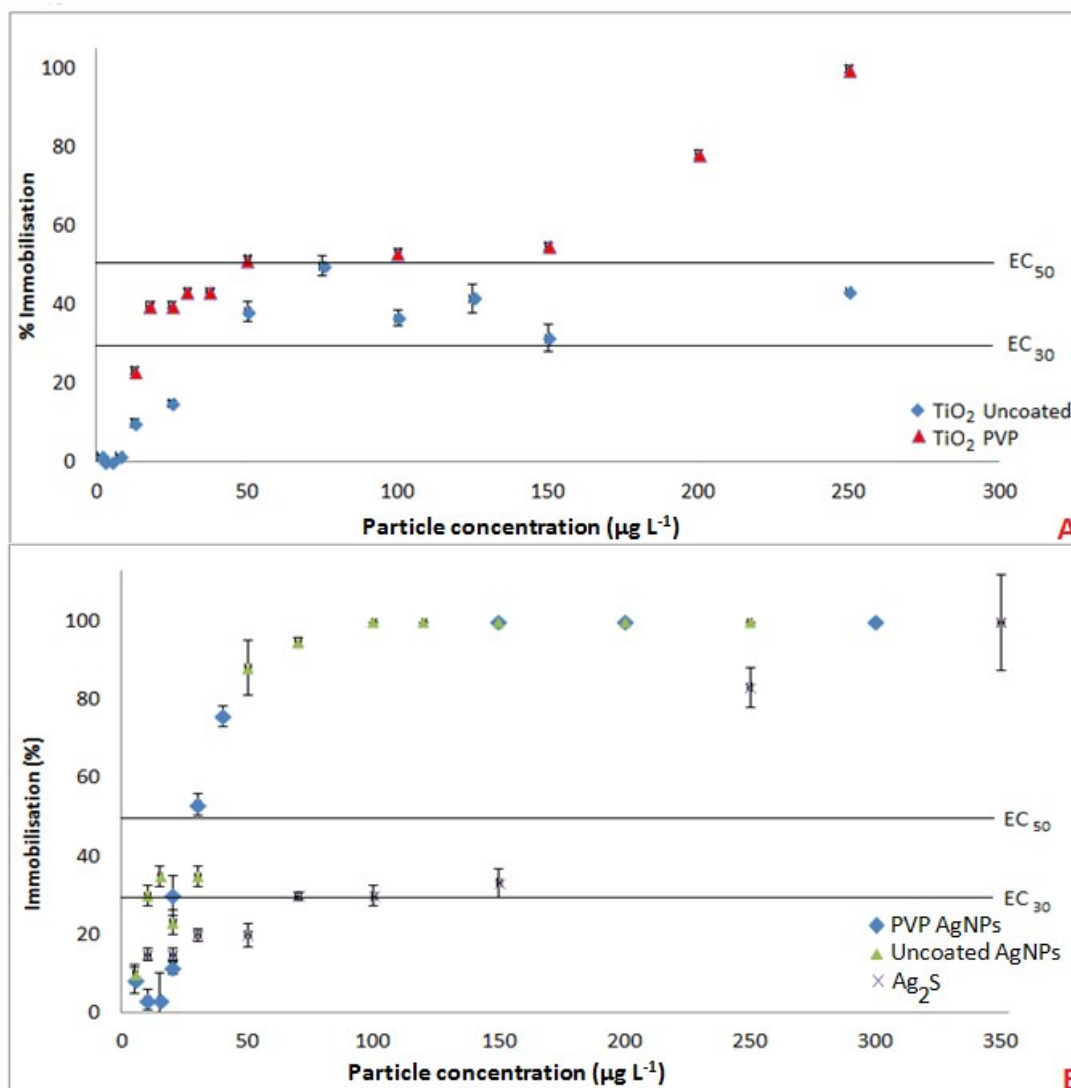


Figure SI.1A: EC_{50} range finding concentrations versus immobilization (%) for *Daphnia* exposed to the differently coated TiO_2 NMs over 48 hours and **B:** range finding concentrations versus immobilization (%) for *Daphnia* exposed to the differently coated AgNP over 48 hours.

1.4 Multigenerational study design

The description of the multigeneration study, is described in the main paper, and is shown schematically in Figure SI.2.

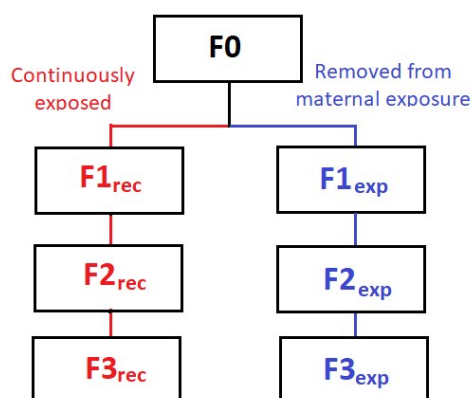


Figure SI.2: Multigenerational study design showing the exposed ($_{\text{exp}}$) and recovery ($_{\text{rec}}$) generations following the F0 parental exposure. Note, the F1_{rec} generations are born into exposure and then removed (within 24 hours post birth) to assess the recovery and effects of parental exposure on the following generations.

2. Results

2.1 Survival

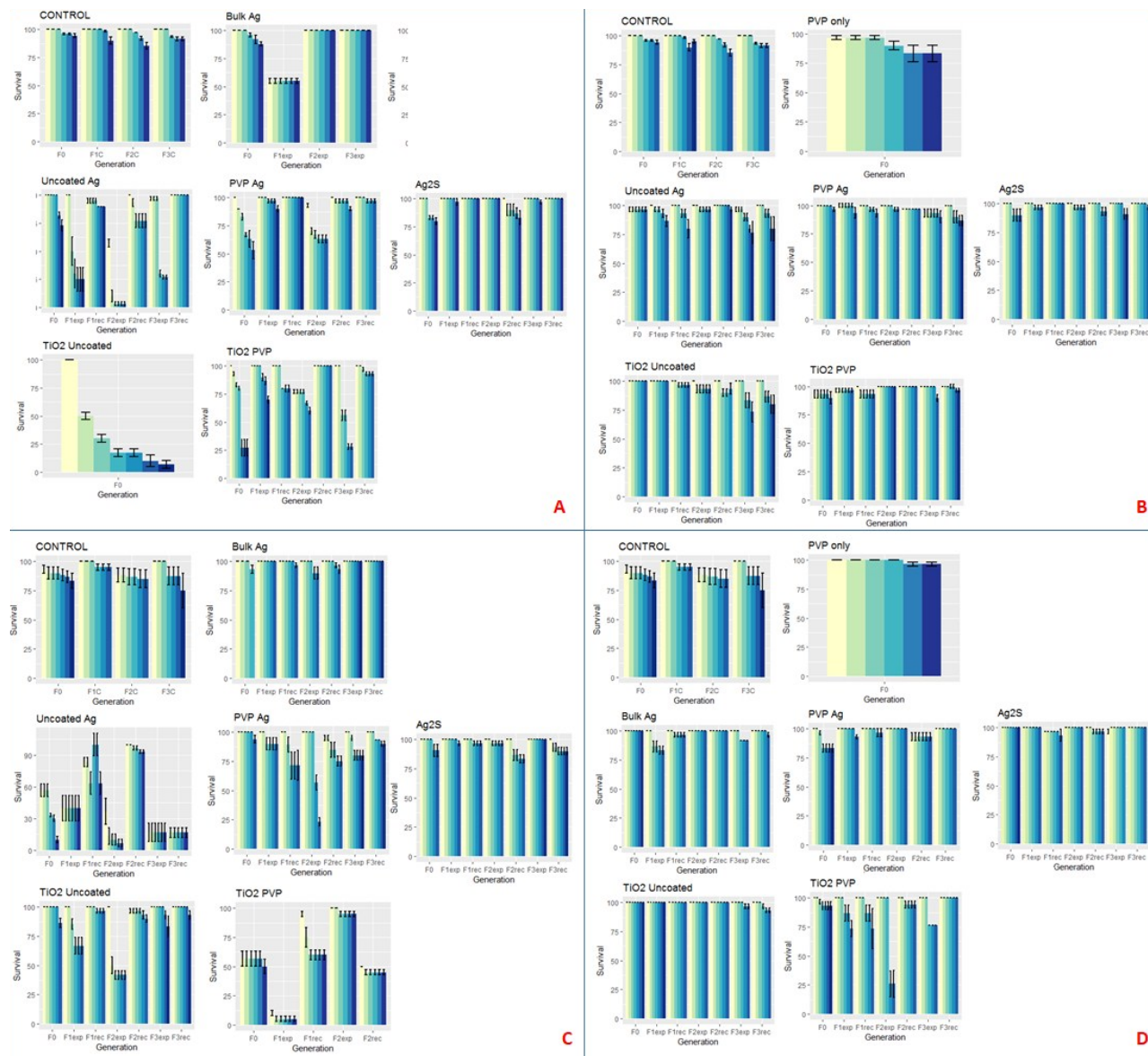


Figure SI.3: Multigenerational survival for daphnids exposed to A) Pristine Ag NPs in HH combo media, B) aged Ag NPs in HH combo media, C) pristine Ag NPs in Class V water, and D) aged Ag NPs in Class V water. The colour bar for Age is *D. magna* age in Days at the time of measurement. F0 = Parent exposure to the particular NP noted in at the top of the plot; F1-F3_{exp} are the continuously exposed organisms, while F1-F3_{rec} are the recovery generations grown in medium only (no further NP exposure).

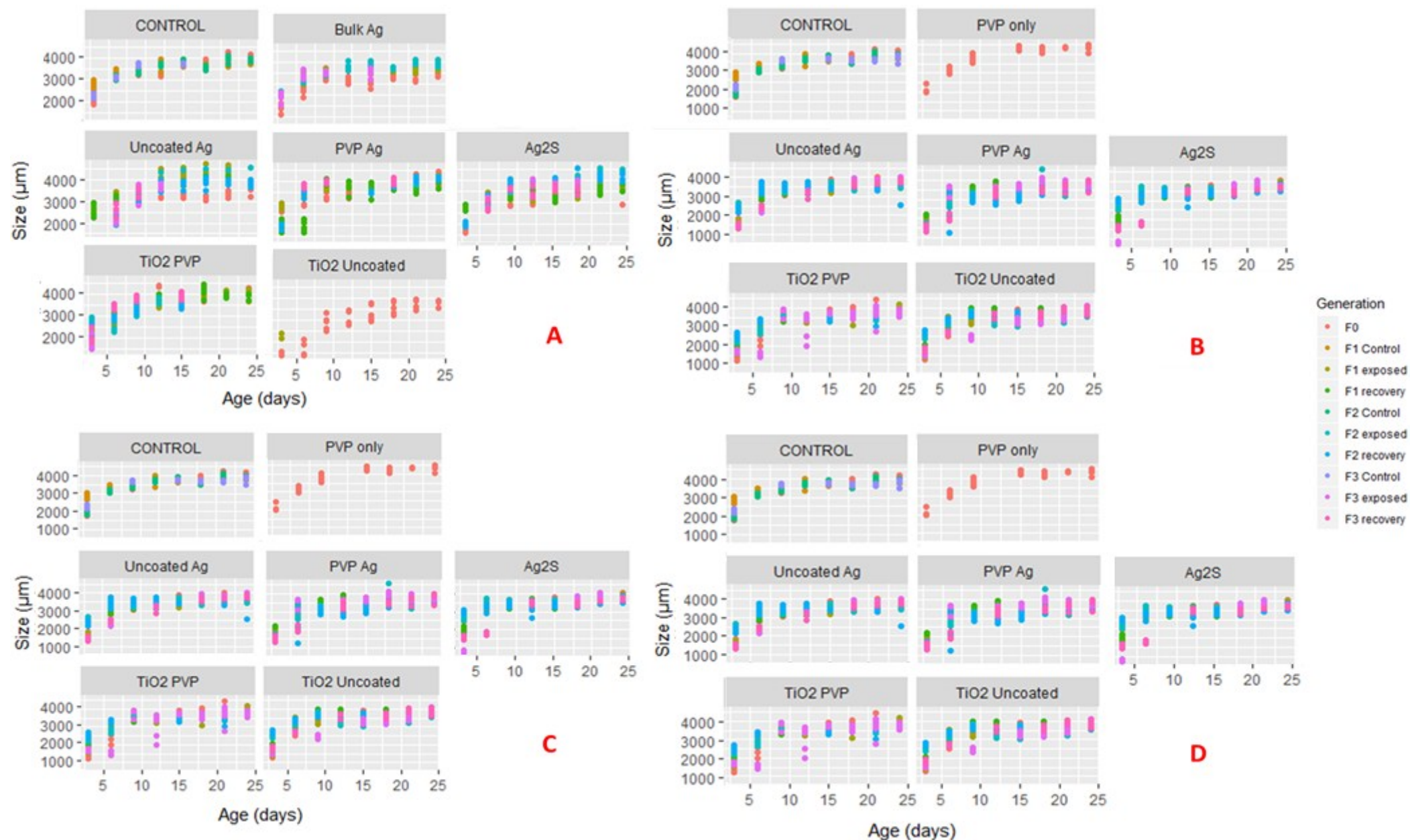


Figure SI.4: Size of daphnids over time in all generations following exposure to **A)** pristine NMs in HH combo medium, **B)** aged NMs in HH combo medium, **C)** pristine NMs in Class V river water and **D)** aged NMs in Class V river water. Exposure to the pristine NMs had the most negative effects on the daphnids body length in the HH combo. Here, size reductions in the presence of pristine and aged NMs under the same conditions were observed. Interestingly these effects were also trans-generational and reductions in sizes were still observed after 2 generations removed from exposure, suggesting that the NMs have an epigenetic effect on the development of the daphnids.

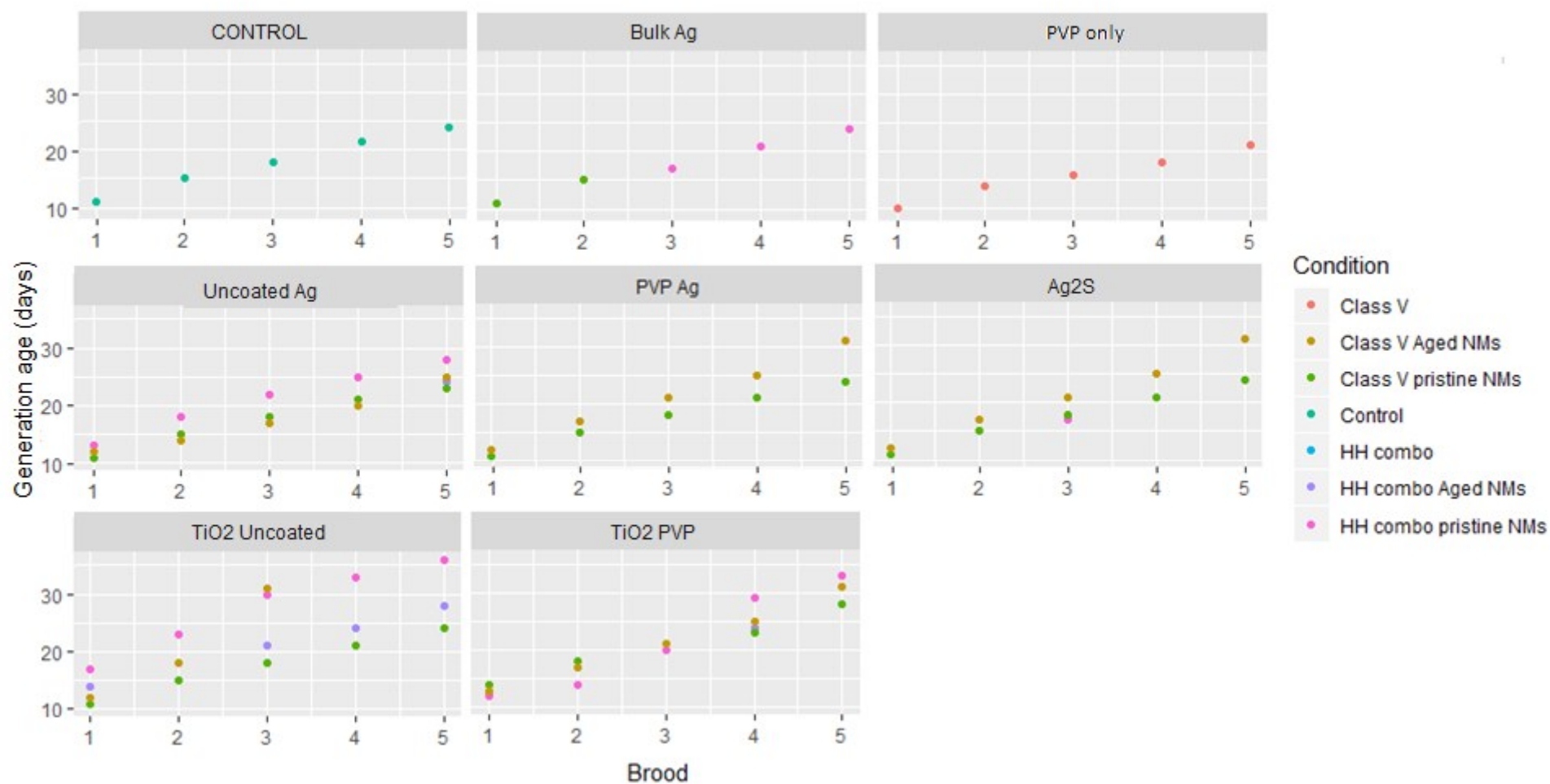


Figure SI.5: Brood timings for the F0 generation exposed to each NM (pristine versus aged) in each water condition (HH Combo versus Class V). Controls were not exposed to any NMs, and the PVP exposed organisms were exposed to the PVP polymer NM surface coating in the absence of NMs to rule out any contribution to the toxicity from the polymer coating.

Biodistribution

Table SI.2: Dissolved and NP solution concentrations Vs total concentrations internalized by Daphnids (HH combo)

Exposure condition and generation	Pristine Ag NM exposure						Aged Ag NM exposure					
	Most Freq.	Mean NP Size in solution (nm)	NM. Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution ($\mu\text{g L}^{-1}$)	Starting concentration ($\mu\text{g L}^{-1}$)	Measured Ag Daphnia uptake ($\mu\text{g L}^{-1}$)	Most Freq.	Mean NP Size in solution (nm)	NM. Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution ($\mu\text{g L}^{-1}$)	Starting concentration ($\mu\text{g L}^{-1}$)	Measured Ag Daphnia uptake ($\mu\text{g L}^{-1}$)
	Size (nm)						Size (nm)					
Uncoated Ag	32	43	5132	0.62	20	0.392	31	54	735	0.24	20	0.699
Ag F1	34	49	4500	0.64	20	0.455	41	63	715	0.70	20	0.768
Ag F2	29	38	8793	0.60	20	0.345	37	37	835	0.28	20	0.766
Ag F3	33	47	3596	0.68	20	0.708	41	57	358	0.69	20	0.655
PVP Ag	15	19	16457	0.20	20	0.673	11	23	216786	1.11	20	0.485
F1	16	23	7447	0.18	20	0.916	16	21	6497	0.06	20	0.322
F2	13	17	14918	0.19	20	0.652	17	43	32547	1.13	20	0.201
F3	15	18	34870	0.22	20	0.591	16	21	3576	0.06	20	0.289
Ag₂S	26	33	160144	0.36	100	0.716	25	32	42813	0.06	100	0.174
F1	27	33	125908	0.36	100	0.658	24	30	41903	0.06	100	0.199
F2	26	32	150524	0.35	100	0.668	24	30	45181	0.06	100	0.145
F3	23	29	206762	0.18	100	0.833	24	30	44526	0.06	100	0.241

Table SI.3: Dissolved Ag and Ag NP solution concentrations Vs total concentrations internalized by Daphnids (class V water exposures)

Exposure condition and generation	Pristine Ag NM exposure						Aged Ag NM exposure					
	Most Freq.	Mean NP Size in solution (nm)	NM. Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution ($\mu\text{g L}^{-1}$)	Starting concentration ($\mu\text{g L}^{-1}$)	Measured Ag Daphnia uptake ($\mu\text{g L}^{-1}$)	Most Freq.	Mean NP Size in solution (nm)	NM. Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution ($\mu\text{g L}^{-1}$)	Starting concentration ($\mu\text{g L}^{-1}$)	Measured Ag Daphnia uptake ($\mu\text{g L}^{-1}$)
	Size (nm)						Size (nm)					
Uncoated Ag	30	441	6247	0.47	20	0.554	35	40	59417	0.09	20	0.336
F1	32	42	6577	0.45	20	0.465	43	50	57854	0.11	20	0.273
F2	30	41	5901	0.39	20	0.498	45	54	60543	0.19	20	0.433
F3	33	41	7332	0.50	20	0.341	42	49	51873	0.23	20	0.549
PVP Ag	24	31	452141	0.05	20	0.687	21	30	105739	0.06	20	0.258
F1	25	31	47685	0.06	20	0.736	21	30	123921	0.07	20	0.289
F2	25	32	39400	0.06	20	0.554	24	31	94952	0.06	20	0.301
F3	23	30	41367	0.06	20	0.972	24	31	92091	0.06	20	0.245
Ag₂S	47	50	276947	1.27	100	1.342	54	61	298493	2.34	100	0.974
F1	44	49	284738	1.30	100	1.478	54	60	304752	2.28	100	0.804
F2	54	59	285679	2.32	100	1.248	54	61	295407	2.31	100	0.981
F3	46	49	258181	1.29	100	1.573	53	63	275393	2.35	100	0.987

Table SI.4: Dissolved and NM solution concentrations versus total concentrations internalized by exposed daphnids in HH combo media												
Exposure condition and generation	Pristine TiO ₂ NM exposure						Aged TiO ₂ NM exposure					
	Most Freq. Size (nm)	Mean NM Size in solution (nm)	NM Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution (µg L ⁻¹)	Starting concentration (µg L ⁻¹)	Measured Ti <i>Daphnia</i> uptake (µg L ⁻¹)*	Most Freq. Size (nm)	Mean NM Size in solution (nm)	NM Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution (µg L ⁻¹)	Starting concentration (mg L ⁻¹)	Measured Ti <i>Daphnia</i> uptake (µg L ⁻¹)*
F0 Control	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F1	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F2	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F3	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F0 TiO₂ PVP	151	152	5006.90	140.21	5000	1.496	143	151	6735.48	100.00	5000	1.161
F1 _{exp}	150	154	6228.82	113.45	5000	3.583	129	144	7093.12	81.69	5000	0.814
F2 _{exp}	153	154	6028.92	112.01	5000	3.245	141	147	8523.66	86.52	5000	1.981
F3 _{exp}	151	153	5877.54	130.22	5000	1.733	138	147	7450.75	89.40	5000	0.861
F0 TiO₂ uncoated	192	206	11384.75	288.47	5000	4.279	202	212	12119.89	302.30	5000	1.143
F1 _{exp} **	No data						116	121	17365.22	235.51	5000	1.078
F2 _{exp} **							209	217	12338.45	316.92	5000	1.754
F3 _{exp} **							116	122	18477.87	289.10	5000	1.304

Table SI.5: Dissolved and NM solution concentrations versus total concentrations internalized by exposed daphnids in Class V media												
Exposure condition and generation	Pristine TiO ₂ NM exposure						Aged TiO ₂ NM exposure					
	Most Freq. Size (nm)	Mean NM Size in solution (nm)	NM Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution (µg L ⁻¹)	Starting concentration (mg L ⁻¹)	Measured Ti <i>Daphnia</i> uptake (µg L ⁻¹)*	Most Freq. Size (nm)	Mean NM Size in solution (nm)	NM Conc. In solution (particles/mL)	Diss. Conc. Remaining in solution (µg L ⁻¹)	Starting concentration (mg L ⁻¹)	Measured Ti <i>Daphnia</i> uptake (µg L ⁻¹)*
F0 Control	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F1	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F2	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F3	0	0	0	0	0	0	0	0	0.00	0.00	0	0
F0 TiO₂ PVP	169	173	5901	128	5000	1.534	158	160	5769	88	5000	1.235
F1 _{exp}	172	175	5245	115	5000	2.05	161	162	5216	87	5000	1.773
F2 _{exp}	170	177	3874	101	5000	1.183	153	158	6035	90	5000	1.021
F3 _{exp}	No data						169	163	6840	85	5000	1.385
F0 TiO₂ uncoated												
F1 _{exp}	112	115	18955	22	5000	1.79	78	92	68487	3	5000	1.385
F2 _{exp}	110	116	18418	22	5000	1.031	76	91	71866	3	5000	1.014
F3 _{exp}	112	115	19372	23	5000	1.854	80	92	66937	3	5000	1.132

Appendix 1: Statistical information

Table AP.1. Two tailed t-test for unequal variance of growth compared to the control generations for the Ag NP exposures								
Generation and exposure type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Bulk Ag in HH combo								
F0 Bulk Ag	0.450	0.033	0.215	0.174	0.002	0.005	0.015	0.016
F1_{exp} Bulk Ag	0.002	0.886	0.407	0.895	0.156	0.486	0.460	0.129
F2_{exp} Bulk Ag	0.001	0.767	0.194	0.273	0.625	0.022	0.514	0.238
F3_{exp} Bulk Ag	0.495	0.035	0.010	0.010	0.052	0.025	0.011	0.033
Bulk Ag in Class V water								
F0 Bulk Ag	0.000	0.029	0.012	0.001	0.002	0.000	0.000	0.001
F1_{exp} Bulk Ag	0.000	0.006	0.002	0.003	0.039	0.000	0.005	0.000
F2_{exp} Bulk Ag	0.000	0.229	0.622	0.156	0.039	0.169	0.997	0.013
F3_{exp} Bulk Ag	0.011	0.001	0.000	0.009	0.060	0.950	0.029	0.129
F1_{rec} Bulk Ag	0.046	0.002	0.616	0.025	0.032	0.000	0.001	0.002
F2_{rec} Bulk Ag	0.208	0.200	0.151	0.002	0.012	0.451	0.000	0.005
F3_{rec} Bulk Ag	0.002	0.000	0.000	0.002	0.000	0.236	0.106	0.113

Table AP.2. Two tailed t-test for unequal variance of growth compared to the control generations for the Ag NP exposures								
Generation and exposure type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Pristine Ag NPs in HH combo								
F0 uncoated Ag	0.033	0.059	0.000	0.123	0.000	0.000	0.000	0.001
F1_{exp} Uncoated Ag	0.148	0.795	0.653	0.009	0.001	0.001	0.000	0.004
F2_{exp} Uncoated Ag	0.022	0.082	0.013	0.000	0.033	0.021	0.009	0.005
F3_{exp} Uncoated Ag	0.025	0.000	0.050	0.050	0.021	0.029	0.043	0.016
F1_{rec} Uncoated Ag	0.165	0.311	0.591	0.083	0.500	0.000	0.001	0.005
F2_{rec} Uncoated Ag	0.016	0.001	0.429	0.601	0.440	0.051	0.343	0.437
F3_{rec} Uncoated Ag	0.013	0.025	0.015	0.047	0.025	0.013	0.000	0.009
Aged Ag NPs in HH combo								
F0 uncoated Ag	0.142	0.044	0.502	0.043	0.592	0.774	0.061	0.179
F1_{exp} Uncoated Ag	0.003	0.022	0.011	0.079	0.000	0.411	0.805	0.110
F2_{exp} Uncoated Ag	0.002	0.022	0.831	0.013	0.002	0.984	0.008	0.015
F3_{exp} Uncoated Ag	0.000	0.000	0.000	0.123	0.188	0.087	0.178	0.175
F1_{rec} Uncoated Ag	0.000	0.024	0.025	0.016	0.025	0.489	0.903	0.569
F2_{rec} Uncoated Ag	0.004	0.028	0.172	0.147	0.000	0.935	0.005	0.220
F3_{rec} Uncoated Ag	0.000	0.000	0.000	0.002	0.002	0.827	0.879	0.029
Pristine Ag NPs in Class V water								
F0 uncoated Ag	0.000	0.031	0.190	0.012	0.109	0.227	0.701	0.000
F1_{exp} Uncoated Ag	0.016	0.458	0.030	0.053	0.796	0.029	0.084	0.045
F2_{exp} Uncoated Ag	0.000	0.000	0.069	0.010	0.904	0.131	0.109	0.150
F3_{exp} Uncoated Ag	NA	NA	NA	NA	NA	NA	NA	NA
F1_{rec} Uncoated Ag	0.275	0.043	0.535	0.514	0.321	0.001	0.005	0.032
F2_{rec} Uncoated Ag	0.677	0.757	0.157	0.123	0.009	0.556	0.014	0.024
F3_{rec} Uncoated Ag	0.000	0.163	0.000	0.000	0.000	0.016	0.009	0.057
Aged Ag NPs in Class V water								
F0 uncoated Ag	0.820	0.355	0.024	0.001	0.247	0.005	0.003	0.001
F1_{exp} Uncoated Ag	0.085	0.878	0.119	0.006	0.002	0.014	0.000	0.005
F2_{exp} Uncoated Ag	0.349	0.121	0.007	0.437	0.000	0.000	0.000	0.001
F3_{exp} Uncoated Ag	0.353	0.133	0.000	0.395	0.254	0.019	0.004	0.199
F1_{rec} Uncoated Ag	0.042	0.961	0.245	0.000	0.014	0.125	0.007	0.004
F2_{rec} Uncoated Ag	0.058	0.000	0.005	0.000	0.000	0.002	0.001	0.012
F3_{rec} Uncoated Ag	0.003	0.391	0.770	0.868	0.064	0.398	0.004	0.685

Table AP.3. Two tailed t-test for unequal variance of growth compared to the control generations for the Ag NP exposures								
Generation and exposure type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Pristine Ag NPs in HH combo								
F0 PVP Ag	0.177	0.878	0.618	0.723	0.864	0.038	0.719	0.897
F1 _{exp} PVP Ag	0.997	0.086	0.051	0.678	0.497	0.022	0.000	0.004
F2 _{exp} PVP Ag	0.299	0.023	0.260	0.012	0.304	0.516	0.362	
F3 _{exp} PVP Ag	NA	NA	NA	NA	NA	NA	NA	NA
F1 _{rec} PVP Ag	0.000	0.122	0.001	0.853	0.978	0.025	0.426	0.988
F2 _{rec} PVP Ag	0.806	0.004	0.010	0.054	0.000	0.009	0.050	0.001
F3 _{rec} PVP Ag	0.001	0.000	0.000	0.001	0.010	0.004	0.001	0.001
PVP only	0.009	0.064	0.050	0.002	0.000	0.001	0.003	0.014
Aged Ag NPs in HH combo								
F0 PVP Ag	0.017	0.029	0.239	0.125	0.006	0.143	0.001	0.057
F1 _{exp} PVP Ag	0.000	0.010	0.001	0.073	0.013	0.166	0.320	0.511
F2 _{exp} PVP Ag	0.002	0.000	0.019	0.011	0.001	0.336	0.000	0.000
F3 _{exp} PVP Ag	0.000	0.026	0.037	0.522	0.086	0.001	0.297	0.905
F1 _{rec} PVP Ag	0.000	0.912	0.260	0.607	0.516	0.383	0.299	0.117
F2 _{rec} PVP Ag	0.090	0.590	0.005	0.002	0.001	0.018	0.001	0.013
F3 _{rec} PVP Ag	0.002	0.000	0.000	0.040	0.005	0.022	0.304	#DIV/0!
Pristine Ag NPs in Class V water								
F0 PVP Ag	0.000	0.001	0.091	0.007	0.002	0.001	0.029	0.001
F1 _{exp} PVP Ag	0.000	0.752	0.264	0.026	0.014	0.011	0.018	0.048
F2 _{exp} PVP Ag	0.261	0.865	0.822	0.009	0.002	0.682	0.696	0.265
F3 _{exp} PVP Ag	0.000	0.000	0.000	0.002	0.692	0.378	0.188	0.112
F1 _{rec} PVP Ag	0.936	0.849	0.540	0.668	0.161	0.001	0.031	0.010
F2 _{rec} PVP Ag	0.035	0.269	0.151	0.091	0.809	0.349	0.299	0.067
F3 _{rec} PVP Ag	0.000	0.000	0.001	0.054	0.007	0.441	0.819	0.904
PVP only	0.001	0.388	0.092	0.015	0.001	0.011	0.001	0.012
Aged Ag NPs in Class V water								
F0 PVP Ag	0.372	0.404	0.028	0.000	0.003	0.006	0.001	0.001
F1 _{exp} PVP Ag	0.183	0.043	0.973	0.000	0.000	0.003	0.002	0.001
F2 _{exp} PVP Ag	0.002	0.575	0.001	0.000	0.054	0.000	0.049	0.059
F3 _{exp} PVP Ag	NA	NA	NA	NA	NA	NA	NA	NA
F1 _{rec} PVP Ag	0.307	0.343	0.192	0.000	0.000	0.219	0.001	0.029
F2 _{rec} PVP Ag	0.418	0.435	0.311	0.726	0.001	0.000	0.002	0.071
F3 _{rec} PVP Ag	0.001	0.219	0.031	0.951	0.803	0.075	0.002	0.536

Table AP.4. Two tailed t-test for unequal variance of growth compared to the control generations for the Ag NP exposures								
Generation and exposure type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Pristine Ag NPs in HH combo								
F0 Ag2S	0.022	0.511	0.119	0.088	0.006	0.945	0.008	0.116
F1 _{exp} Ag2S	0.046	0.527	0.039	0.015	0.127	0.233	0.876	0.486
F2 _{exp} Ag2S	0.771	0.783	0.052	0.019	0.007	0.000	0.001	0.000
F3 _{exp} Ag2S	0.001	0.594	0.169	0.013	0.217	0.001	0.012	0.005
F1 _{rec} Ag2S	0.147	0.234	0.018	0.016	0.000	0.645	0.683	0.847
F2 _{rec} Ag2S	0.617	0.707	0.021	0.009	0.038	0.012	0.015	0.063
F3 _{rec} Ag2S	0.003	0.142	0.067	0.798	0.117	0.431	0.590	0.120
Aged Ag NPs in HH combo								
F0 Ag2S	0.615	0.090	0.694	0.057	0.676	0.211	0.010	0.036
F1 _{exp} Ag2S	0.000	0.652	0.021	0.895	0.047	0.073	0.360	0.502
F2 _{exp} Ag2S	0.001	0.000	0.086	0.030	0.000	0.088	0.009	0.000
F3 _{exp} Ag2S	0.000	0.000	0.394	0.108	0.565	0.090	0.003	0.611
F1 _{rec} Ag2S	0.000	0.823	0.045	0.012	0.003	0.001	0.034	0.024
F2 _{rec} Ag2S	0.000	0.045	0.048	0.000	0.003	0.012	0.007	0.000
F3 _{rec} Ag2S	0.000	0.000	0.000	0.027	0.007	0.004	0.073	#DIV/0!
Pristine Ag NPs in Class V water								
F0 Ag2S	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.003
F1 _{exp} Ag2S	0.000	0.015	0.003	0.051	0.000	0.012	0.017	0.006
F2 _{exp} Ag2S	0.001	0.461	0.027	0.026	0.093	0.003	0.135	0.259
F3 _{exp} Ag2S	0.027	0.000	0.000	0.005	0.000	0.837	0.004	0.002
F1 _{rec} Ag2S	0.000	0.003	0.002	0.006	0.000	0.000	0.000	0.000
F2 _{rec} Ag2S	0.000	0.029	0.212	0.034	0.191	0.271	0.090	0.120
F3 _{rec} Ag2S	0.024	0.000	0.000	0.000	0.000	0.056	0.917	0.495
Aged Ag NPs in Class V water								
F0 Ag2S	0.962	0.000	0.000	0.065	0.002	0.025	0.188	0.156
F1 _{exp} Ag2S	0.021	0.017	0.493	0.000	0.000	0.768	0.198	0.245
F2 _{exp} Ag2S	0.112	0.800	0.794	0.953	0.000	0.000	0.004	0.076
F3 _{exp} Ag2S	0.003	0.261	0.169	0.590	0.694	0.048	0.002	0.640
F1 _{rec} Ag2S	0.206	0.206	0.272	0.000	0.000	0.263	0.007	0.041
F2 _{rec} Ag2S	0.066	0.304	0.001	0.000	0.000	0.000	0.000	0.000
F3 _{rec} Ag2S	0.005	0.246	0.449	0.000	0.050	0.062	0.149	0.527

Table AP.5. Two tailed t-test for unequal variance of growth compared to the control generations for the TiO ₂ exposures								
Generation and exposure type	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24
Pristine NMs in HH combo								
PVP only	0.009	0.064	0.050	0.002	0.000	0.001	0.003	0.014
F0 TiO ₂ Uncoated	0.001	0.004	0.029	0.292	0.067	0.085	0.016	0.129
F0 TiO ₂ PVP	0.005	0.000	0.385	0.018	0.135	0.167	0.823	0.532
F1 _{exp} TiO ₂ PVP	0.001	0.000	0.771	0.300	0.052	0.005	0.001	0.166
F2 _{exp} TiO ₂ PVP	0.000	0.734	0.039	0.178	0.183	0.008	0.111	0.143
F1 _{rec} TiO ₂ PVP	0.047	0.109	0.007	0.094	0.052	0.014	0.007	0.424
F2 _{rec} TiO ₂ PVP	0.000	0.036	0.001	0.215	0.003	0.002	0.004	0.008
Aged NMs in HH combo								
F0 TiO ₂ Uncoated	0.001	0.256	0.043	0.866	0.131	0.218	0.004	0.004
F1 _{exp} TiO ₂ Uncoated	0.000	0.368	0.266	0.097	0.793	0.603	0.718	0.281
F2 _{exp} TiO ₂ Uncoated	0.001	0.534	0.090	0.000	0.001	0.141	0.000	0.004
F3 _{exp} TiO ₂ Uncoated	0.000	0.000	0.000	0.001	0.001	0.118	0.006	0.001
F1 _{rec} TiO ₂ Uncoated	0.000	0.968	0.193	0.208	0.008	0.118	0.000	0.903
F2 _{rec} TiO ₂ Uncoated	0.000	0.001	0.063	0.048	0.004	0.234	0.001	0.003
F3 _{rec} TiO ₂ Uncoated	0.000	0.000	0.186	0.794	0.299	0.287	0.018	0.000
F0 TiO ₂ PVP	0.001	0.031	0.025	0.936	0.253	0.693	0.467	0.009
F1 _{exp} TiO ₂ PVP	0.000	0.021	0.072	0.059	0.087	0.585	0.044	0.817
F2 _{exp} TiO ₂ PVP	0.027	0.009	0.446	0.008	0.006	0.031	0.019	0.011
F3 _{exp} TiO ₂ PVP	0.000	0.001	0.470	0.393	0.926	0.888	0.000	0.000
F1 _{rec} TiO ₂ PVP	0.000	0.704	0.066	0.178	0.189	0.299	0.015	0.224
F2 _{rec} TiO ₂ PVP	0.000	0.009	0.119	0.005	0.002	0.228	0.004	0.015
F3 _{rec} TiO ₂ PVP	0.000	0.000	0.001	0.116	0.082	0.118	0.553	0.007
Pristine NMs in Class V river water								
PVP only	0.001	0.388	0.092	0.015	0.001	0.011	0.001	0.012
F0 TiO ₂ Uncoated	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.001
F1 _{exp} TiO ₂ Uncoated	0.000	0.063	0.001	0.000	0.024	0.018	0.019	0.141
F2 _{exp} TiO ₂ Uncoated	0.001	0.138	0.043	0.090	0.262	0.986	0.168	0.002
F3 _{exp} TiO ₂ Uncoated	0.903	0.086	0.000	0.001	0.000	0.208	0.078	0.752
F1 _{rec} TiO ₂ Uncoated	0.027	0.000	0.008	0.052	0.002	0.000	0.022	0.027
F2 _{rec} TiO ₂ Uncoated	0.026	0.914	0.381	0.298	0.012	0.451	0.849	0.004
F3 _{rec} TiO ₂ Uncoated	0.000	0.000	0.007	0.007	0.000	0.150	0.004	0.323
F0 TiO ₂ PVP	0.000	0.000	0.028	0.016	0.089	0.030	0.009	0.087
F1 _{exp} TiO ₂ PVP	0.000	0.436	0.002	0.076	0.292	0.044	0.002	0.002
F2 _{exp} TiO ₂ PVP	0.002	0.004	0.000	0.002	0.044	0.002	0.004	0.000
F3 _{exp} TiO ₂ PVP	0.000	0.000	0.000	0.002	0.692	0.378	0.188	0.112
F1 _{rec} TiO ₂ PVP	0.007	0.317	0.007	0.183	0.394	0.082	0.092	0.000
F2 _{rec} TiO ₂ PVP	0.000	0.059	0.869	0.011	0.002	0.003	0.001	0.004
F3 _{rec} TiO ₂ PVP	0.000	0.000	0.001	0.054	0.007	0.441	0.819	0.904
Aged NMs in Class V river water								
F0 TiO ₂ Uncoated	0.306	0.020	0.009	0.002	0.025	0.000	0.002	0.021
F1 _{exp} TiO ₂ Uncoated	0.398	0.737	0.044	0.000	0.003	0.005	0.001	0.001
F2 _{exp} TiO ₂ Uncoated	0.001	0.182	0.000	0.000	0.000	0.000	0.000	0.000
F3 _{exp} TiO ₂ Uncoated	0.000	0.001	0.028	0.129	0.016	0.893	0.004	0.011
F1 _{rec} TiO ₂ Uncoated	0.100	0.017	0.420	0.000	0.000	0.049	0.186	0.098
F2 _{rec} TiO ₂ Uncoated	0.086	0.444	0.458	0.487	0.518	0.660	0.609	0.559
F3 _{rec} TiO ₂ Uncoated	0.001	0.249	0.001	0.070	0.392	0.753	0.626	0.527
F0 TiO ₂ PVP	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
F1 _{exp} TiO ₂ PVP	0.185	0.090	0.590	0.021	0.010	0.130	0.003	0.372
F2 _{exp} TiO ₂ PVP	0.292	0.473	0.037	0.147	0.954	0.002	0.001	0.048
F3 _{exp} TiO ₂ PVP	0.226	0.217	0.356	0.003	0.008	0.028	0.001	0.053
F1 _{rec} TiO ₂ PVP	0.079	0.407	0.060	0.000	0.000	0.034	0.017	0.077
F2 _{rec} TiO ₂ PVP	0.001	0.535	0.012	0.002	0.002	0.000	0.007	0.038
F3 _{rec} TiO ₂ PVP	0.001	0.076	0.236	0.158	0.299	0.197	0.288	0.294

Table AP.6: Statistical evidence for growth rate Vs age for the TiO ₂ NMs in each condition						
Generation and exposure condition	Media type	Log ₁₀ Transformations comparing the slope for growth rate versus age for each condition				
		Adjusted R ²	F-statistic	degrees of freedom	p-value	Rate of change coefficient
PVP in HH combo	HH combo	0.03858	2.565	38	0.1175	9.96E-04
F0 Control	HH combo	0.9784	1990	43	2.20E-16	0.00821
F1 Control	HH combo	0.9894	3651	43	2.20E-16	0.008398
F2 Control	HH combo	0.9894	3651	38	2.20E-16	0.008648
F3 Control	HH combo	0.9964	9431	38	2.20E-16	0.009439
F0 TiO ₂ Uncoated	pristine in HH combo	0.781	140.4	38	2.49E-14	0.006811
F0 TiO ₂ PVP	pristine in HH combo	0.847	223.9	39	2.20E-16	0.006257
F1 _{exp} TiO ₂ PVP	pristine in HH combo	0.8811	290	38	2.20E-16	0.008775
F2 _{exp} TiO ₂ PVP	pristine in HH combo	0.4153	14.49	18	1.29E-03	0.00561
F3 _{exp} TiO ₂ PVP	pristine in HH combo	0.8782	246.1	33	2.20E-16	0.006515
F1 _{rec} TiO ₂ PVP	pristine in HH combo	0.8282	184.2	37	6.11E-16	0.009983
F2 _{rec} TiO ₂ PVP	pristine in HH combo	0.8385	99.67	18	9.16E-09	0.009219
F0 TiO ₂ Uncoated	Aged in HH combo	0.988	3225	38	2.20E-16	0.008951
F1 _{exp} TiO ₂ Uncoated	Aged in HH combo	0.7452	115	38	4.70E-13	0.006706
F2 _{exp} TiO ₂ Uncoated	Aged in HH combo	0.5846	55.89	38	5.64E-09	0.006706
F3 _{exp} TiO ₂ Uncoated	Aged in HH combo	0.9363	573.8	38	2.20E-16	0.006706
F1 _{rec} TiO ₂ Uncoated	Aged in HH combo	0.9873	3037	38	2.20E-16	0.009067
F2 _{rec} TiO ₂ Uncoated	Aged in HH combo	0.9895	2921	30	2.20E-16	0.008411
F3 _{rec} TiO ₂ Uncoated	Aged in HH combo	0.9896	3694	38	2.20E-16	0.007165
F0 TiO ₂ PVP	Aged in HH combo	0.994	8356.7	38	2.20E-16	0.009753
F1 _{exp} TiO ₂ PVP	Aged in HH combo	0.83	191.4	38	2.20E-16	0.008303
F2 _{exp} TiO ₂ PVP	Aged in HH combo	0.8431	210.6	38	2.20E-16	0.008303
F3 _{exp} TiO ₂ PVP	Aged in HH combo	0.8782	246.1	33	2.20E-16	0.008303
F1 _{rec} TiO ₂ PVP	Aged in HH combo	0.9935	5980	38	2.20E-16	0.01117
F2 _{rec} TiO ₂ PVP	Aged in HH combo	0.9927	5269	38	2.20E-16	0.008341
F3 _{rec} TiO ₂ PVP	Aged in HH combo	0.9907	4153	38	2.20E-16	0.009349
PVP in Class V	Class V	0.9834	2310	38	2.20E-16	0.007356
F0 Control	Class V	0.9357	568.7	38	2.20E-16	0.0102
F1 Control	Class V	0.9282	505.3	38	2.20E-16	0.012
F2 Control	Class V	0.4329	30.78	38	2.38E-06	0.011
F3 Control	Class V	0.8994	349.6	38	2.20E-16	0.01002
F0 TiO ₂ Uncoated	Pristine Class V	0.9601	940.1	38	2.20E-16	0.00912
F1 _{exp} TiO ₂ Uncoated	Pristine Class V	0.813	170.6	38	1.20E-15	0.008988
F2 _{exp} TiO ₂ Uncoated	Pristine Class V	0.6566	66.02	33	2.23E-09	0.008817
F3 _{exp} TiO ₂ Uncoated	Pristine Class V	0.7385	108.3	37	1.53E-12	0.007313
F1 _{rec} TiO ₂ Uncoated	Pristine Class V	0.841	207.1	38	2.20E-16	0.0114
F2 _{rec} TiO ₂ Uncoated	Pristine Class V	0.7437	114.1	38	5.26E-13	0.01159
F3 _{rec} TiO ₂ Uncoated	Pristine Class V	0.8764	277.6	38	2.20E-16	0.00827
F0 TiO ₂ PVP	Pristine Class V	0.9548	233.6	10	2.92E-08	0.005769
F1 _{exp} TiO ₂ PVP	Pristine Class V	0.9548	233.6	10	2.92E-09	0.005769
F2 _{exp} TiO ₂ PVP	Pristine Class V	0.9057	183.6	18	6.98E-11	0.009442
F1 _{rec} TiO ₂ PVP	Pristine Class V	0.7517	119.1	38	2.85E-13	0.007655
F2 _{rec} TiO ₂ PVP	Pristine Class V	0.6752	40.5	18	5.39E-06	0.01153
F0 TiO ₂ Uncoated	Aged Class V	0.869	259.7	38	2.20E-16	0.008222
F1 _{exp} TiO ₂ Uncoated	Aged Class V	0.9122	406.2	38	2.20E-16	0.008222
F2 _{exp} TiO ₂ Uncoated	Aged Class V	0.7133	98.02	38	4.50E-12	0.005074
F3 _{exp} TiO ₂ Uncoated	Aged Class V	0.9545	818.6	38	2.20E-16	0.007492
F1 _{rec} TiO ₂ Uncoated	Aged Class V	0.9001	352.4	38	2.20E-16	0.009067
F2 _{rec} TiO ₂ Uncoated	Aged Class V	0.9134	327.8	30	2.20E-16	0.008411
F3 _{rec} TiO ₂ Uncoated	Aged Class V	0.7108	96.86	38	5.31E-12	0.007165
F0 TiO ₂ PVP	Aged Class V	0.8994	349.7	38	2.20E-16	0.009753
F1 _{exp} TiO ₂ PVP	Aged Class V	0.9048	371.8	38	2.20E-16	0.009158
F2 _{exp} TiO ₂ PVP	Aged Class V	0.9048	371.8	38	2.20E-16	0.009158
F3 _{exp} TiO ₂ PVP	Aged Class V	0.7548	121.1	38	2.25E-13	0.009851
F1 _{rec} TiO ₂ PVP	Aged Class V	0.9719	1350	38	2.20E-16	0.01117
F2 _{rec} TiO ₂ PVP	Aged Class V	0.8855	302.6	38	2.20E-16	0.008341
F3 _{ec} TiO ₂ PVP	Aged Class V	0.8219	181	38	4.96E-17	0.009349

Table AP.7 : Statistical evidence for growth rate Vs age for the Ag NMs in each condition

Generation and exposure condition	Media type	Log ₁₀ Transformations for growth rate versus age for each condition				
		Adjusted R ²	F-statistic	degrees of freedom	p-value	Slope coefficient denoting the Rate of change
PVP in HH combo	HH combo	0.03858	2.565	38	0.1175	9.96E-04
F0 Control	HH combo	0.9784	1990	43	2.20E-16	0.00821
F1 Control	HH combo	0.9894	3651	43	2.20E-16	0.008398
F2 Control	HH combo	0.9894	3651	38	2.20E-16	0.008648
F3 Control	HH combo	0.9964	9431	38	2.20E-16	0.009439
F0 Uncoated Ag NPs	pristine in HH combo	0.984	2810	42	2e-16	0.01174
F1 _{exp} Uncoated Ag NPs	pristine in HH combo	0.9683	1038	33	2e-16	0.005695
F2 _{exp} Uncoated Ag NPs	pristine in HH combo	0.9861	637.8	8	6.473e-09	0.005515
F3 _{exp} Uncoated Ag NPs	pristine in HH combo	0.9941	2364	13	4.311e-16	0.003353
F1 _{rec} Uncoated Ag NPs	pristine in HH combo	0.9924	4441	33	2.2e-16	0.01079
F2 _{rec} Uncoated Ag NPs	pristine in HH combo	0.9913	3862	33	2.2e-16	0.007262
F3 _{rec} Uncoated Ag NPs	pristine in HH combo	0.994	1486	8	2.256e-10	0.003171
F0 PVP Ag NPs	pristine in HH combo	0.9732	1523	41	2.2e-16	0.008115
F1 _{exp} PVP Ag NPs	pristine in HH combo	0.9953	8298	38	2.2e-16	0.01491
F1 _{rec} PVP Ag NPs	pristine in HH combo	0.9934	5851	38	2.2e-16	0.005918
F2 _{rec} PVP Ag NPs	pristine in HH combo	0.9898	2814	28	2.2e-16	0.008774
F3 _{rec} PVP Ag NPs	pristine in HH combo	0.9996	3.2983+04	13	2.2e-16	0.0218
F0 Ag2S	pristine in HH combo	0.98	2013	40	2.2e-16	0.007927
F1 _{exp} Ag2S	pristine in HH combo	0.9957	9045	38	2.2e-16	0.01614
F2 _{exp} Ag2S	pristine in HH combo	0.9847	2506	38	2.2e-16	0.008069
F3 _{exp} Ag2S	pristine in HH combo	0.9977	8210	18	2.2e-16	0.0106
F1 _{rec} Ag2S	pristine in HH combo	0.9956	8787	38	2.2e-16	0.01575
F2 _{rec} Ag2S	pristine in HH combo	0.9894	3651	38	2.2e-16	0.00779
F3 _{rec} Ag2S	pristine in HH combo	0.9972	8592	23	2.2e-16	0.008378
F0 Bulk Ag	pristine in HH combo	0.9833	2121	35	2.2e-16	0.01086
F1 _{exp} Bulk Ag	pristine in HH combo	0.9976	1.601e+04	38	2.2e-16	0.01128
F2 _{exp} Bulk Ag	pristine in HH combo	0.9958	9067	37	2.2e-16	0.01246
F3 _{exp} Bulk Ag	pristine in HH combo	0.995	3746	18	2.2e-16	0.006155
F0 Uncoated Ag NPs	Aged in HH combo	0.9863	2441	33	2.2e-16	0.009071
F1 _{exp} Uncoated Ag NPs	Aged in HH combo	0.9887	3403	38	2.2e-16	0.01172
F2 _{exp} Uncoated Ag NPs	Aged in HH combo	0.9963	1.054e+04	38	2.2e-16	0.01134
F3 _{exp} Uncoated Ag NPs	Aged in HH combo	0.989	2247	24	2.2e-16	0.007969
F1 _{rec} Uncoated Ag NPs	Aged in HH combo	0.9935	5956	38	2.2e-16	0.01253
F2 _{rec} Uncoated Ag NPs	Aged in HH combo	0.9955	8611	38	2.2e-16	0.008062
F3 _{rec} Uncoated Ag NPs	Aged in HH combo	0.9836	1685	27	2.2e-16	0.008597
F0 PVP Ag NPs	Aged in HH combo	0.989	3053	33	2.2e-16	0.009197
F1 _{exp} PVP Ag NPs	Aged in HH combo	0.8595	239.6	38	2.2e-16	2.812e-04
F2 _{exp} PVP Ag NPs	Aged in HH combo	0.9787	1700	36	2.2e-16	0.007545
F3 _{exp} PVP Ag NPs	Aged in HH combo	0.9975	1.12e+04	27	2.2e-16	0.01491
F1 _{rec} PVP Ag NPs	Aged in HH combo	0.9926	5198	38	2.2e-16	0.008984
F2 _{rec} PVP Ag NPs	Aged in HH combo	0.985	2490	37	2.2e-16	0.007499
F3 _{rec} PVP Ag NPs	Aged in HH combo	0.0986	2146	28	2.2e-16	0.007951
F0 Ag2S	Aged in HH combo	0.9861	2407	33	2.2e-16	0.01017
F1 _{exp} Ag2S	Aged in HH combo	0.9944	6923	38	2.2e-16	0.009746
F2 _{exp} Ag2S	Aged in HH combo	0.9987	2.9535e+04	38	2.2e-16	0.01253
F3 _{exp} Ag2S	Aged in HH combo	0.9976	7534	17	2.2e-16	0.005883
F1 _{rec} Ag2S	Aged in HH combo	0.9944	6932	38	2.2e-16	0.0104
F2 _{rec} Ag2S	Aged in HH combo	0.9966	1.13e+04	38	2.2e-16	0.01901
F3 _{rec} Ag2S	Aged in HH combo	0.9971	9831	28	2.2e-16	0.00775
PVP in Class V	Class V	0.981	NA	38	2.20E-16	0.009
F0 Control	Class V	0.9357	568.7	38	2.20E-16	0.0102
F1 Control	Class V	0.9282	505.3	38	2.20E-16	0.012
F2 Control	Class V	0.4329	30.78	38	2.38E-06	0.011
F3 Control	Class V	0.8994	349.6	38	2.20E-16	0.01002
F0 Uncoated	Pristine Class V	0.9853	1812	26	2.20E-16	0.007086
F1 _{exp} Uncoated	Pristine Class V	0.9932	5714	38	2.20E-16	0.007198
F2 _{exp} Uncoated	Pristine Class V	0.9795	1098	22	2.20E-16	0.005848
F1 _{rec} Uncoated Ag NPs	Pristine Class V	0.996	9653	38	2.38E-06	0.01309
F2 _{rec} Uncoated Ag NPs	Pristine Class V	0.9964	1.079e+04	38	2.20E-16	0.01465

F3 _{rec} Uncoated Ag NPs	Pristine Class V	0.9897	2786	28	2.20E-16	0.00564
F0 PVP Ag NPs	Pristine Class V	0.9917	4087	33	2.20E-16	0.008575
F1 _{exp} PVP Ag NPs	Pristine Class V	0.993	4802	33	2.20E-16	0.007782
F2 _{exp} PVP Ag NPs	Pristine Class V	0.8556	226.2	37	2.20E-16	0.002028
F3 _{exp} PVP Ag NPs	Pristine Class V	0.9846	1791	27	2.20E-16	0.005884
F1 _{rec} PVP Ag NPs	Pristine Class V	0.9878	3156	38	2.38E-06	0.01065
F2 _{rec} PVP Ag NPs	Pristine Class V	0.9986	2.611e+04	36	2.20E-16	0.01349
F3 _{rec} PVP Ag NPs	Pristine Class V	0.9886	2083	23	2.20E-16	0.007476
F0 Ag2S	Pristine Class V	0.9899	3334	33	2.20E-16	0.01022
F1 _{exp} Ag2S	Pristine Class V	0.9888	3441	38	2.20E-16	0.01071
F2 _{exp} Ag2S	Pristine Class V	0.9924	5116	38	2.20E-16	0.009148
F3 _{exp} Ag2S	Pristine Class V	0.9816	1548	28	2.20E-16	0.00739
F1 _{rec} Ag2S	Pristine Class V	0.994	6419	38	2.20E-16	0.01074
F2 _{rec} Ag2S	Pristine Class V	0.9929	5430	38	2.20E-16	0.008821
F3 _{rec} Ag2S	Pristine Class V	0.9907	3102	28	2.20E-16	0.005741
F0 Bulk Ag	Pristine Class V	0.9903	3454	33	2.20E-16	0.009967
F1 _{exp} Bulk Ag	Pristine Class V	0.9909	4130	38	2.20E-16	0.01026
F2 _{exp} Bulk Ag	Pristine Class V	0.9923	5025	38	2.20E-16	0.008034
F3 _{exp} Bulk Ag	Pristine Class V	0.9897	2775	28	2.20E-16	0.007313
F1 _{rec} Bulk Ag	Pristine Class V	0.9953	8317	38	2.20E-16	0.0112
F2 _{rec} Bulk Ag	Pristine Class V	0.9933	5742	38	2.20E-16	0.01195
F3 _{rec} Bulk Ag	Pristine Class V	0.7466	77.62	25	3.875e-09	-3.675e-05
F0 Uncoated Ag NPs	Aged Class V	0.9873	2638	33	2.20E-16	0.007534
F1 _{exp} Uncoated Ag NPs	Aged Class V	0.9857	2143	30	2.20E-16	0.007455
F2 _{exp} Uncoated Ag NPs	Aged Class V	0.9938	5929	36	2.20E-16	0.01099
F3 _{exp} Uncoated Ag NPs	Aged Class V	0.9956	7660	33	2.20E-16	0.01175
F1 _{rec} Uncoated Ag NPs	Aged Class V	0.9856	2330	33	2.20E-16	0.009097
F2 _{rec} Uncoated Ag NPs	Aged Class V	0.9917	2742	22	2.20E-16	0.005469
F3 _{rec} Uncoated Ag NPs	Aged Class V	0.9914	3899	33	2.20E-16	0.01013
F0 PVP	Aged Class V	0.9887	2966	33	2.20E-16	0.007856
F1 _{exp} PVP	Aged Class V	0.9901	3416	33	2.20E-16	0.01058
F2 _{exp} PVP	Aged Class V	0.9875	2996	37	2.20E-16	0.007254
F1 _{rec} PVP	Aged Class V	0.9889	3021	33	2.20E-16	0.01045
F2 _{rec} PVP	Aged Class V	0.9728	1394	38	2.20E-16	0.007078
F3 _{ec} PVP	Aged Class V	0.993	4805	33	2.20E-16	0.009646
F0 Ag2S	Aged Class V	0.9859	2378	33	2.20E-16	0.007936
F1 _{exp} Ag2S	Aged Class V	0.9922	4219	32	2.20E-16	0.01185
F2 _{exp} Ag2S	Aged Class V	0.995	7704	38	2.20E-16	0.01167
F3 _{exp} Ag2S	Aged Class V	0.9937	5366	33	2.20E-16	0.01014
F1 _{rec} Ag2S	Aged Class V	0.9792	1598	33	2.20E-16	0.0082
F2 _{rec} Ag2S	Aged Class V	0.9882	3255	38	2.20E-16	0.008102
F3 _{rec} Ag2S	Aged Class V	0.9867	2531	33	2.20E-16	0.008494

References

1. Kim KT, Klaine SJ, Cho J, Kim S-H, Kim SD. Oxidative stress responses of *Daphnia magna* exposed to TiO₂ nanoparticles according to size fraction. *Science of the Total Environment*. 2010;408(10):2268-72.
2. Zhu XC, Yung Chen, Yongsheng. Toxicity and bioaccumulation of TiO₂ nanoparticle aggregates in *Daphnia magna*. *Chemosphere*. 2010;78(3):209-15.
3. Kim K-T, Klaine SJ, Kim SD. Acute and chronic response of *daphnia magna* exposed to TiO₂ nanoparticles in agitation system. *Bulletin of environmental contamination and toxicology*. 2014;93(4):456-60.