



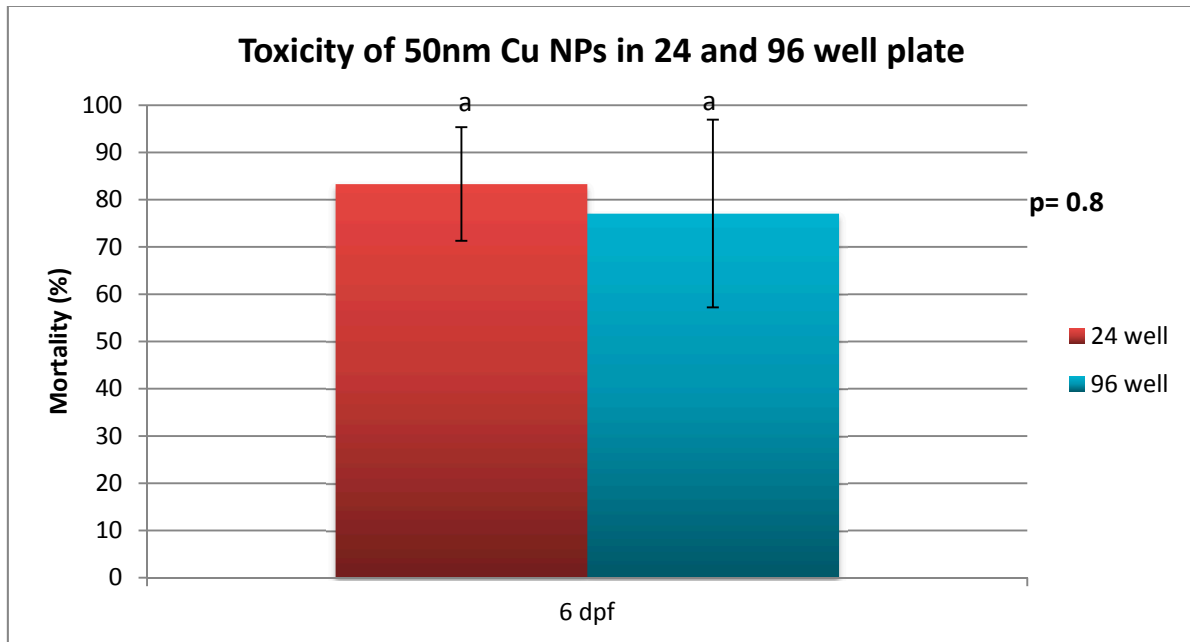
# Supplementary Materials: A Novel Experimental and Modelling Strategy for Nanoparticle Toxicity Testing Enabling the use of Small Quantities

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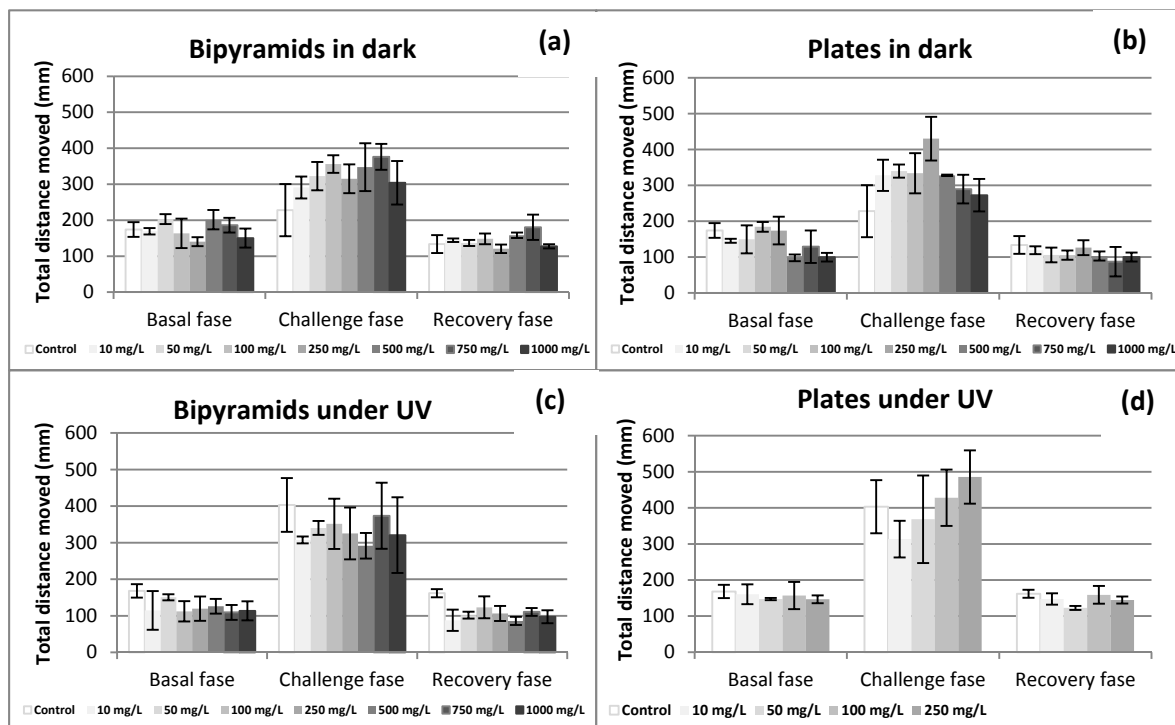
9 pages, 3 figures, 5 tables

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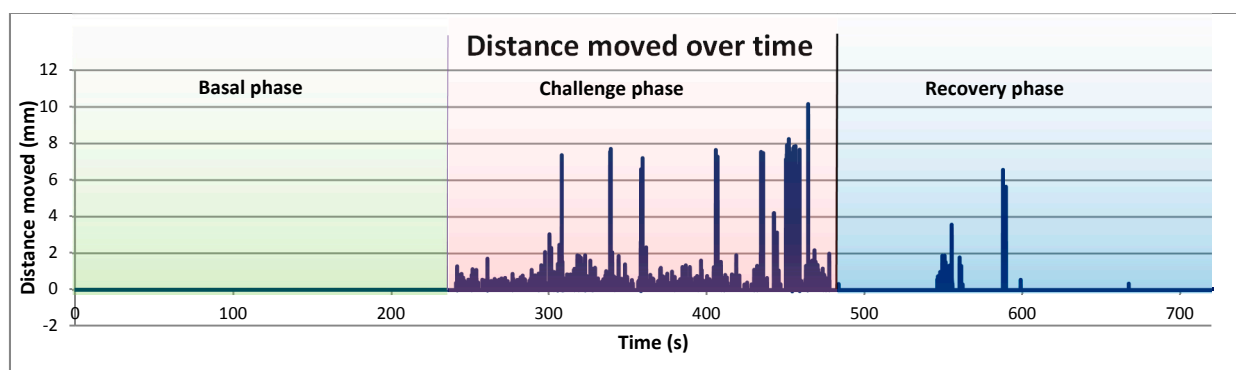
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**Figure S1.** Comparison of two experimental designs: 24-well plate (novel method) and 96-well plate (as in [15]). The mortality rate of zebrafish embryos at 6 dpf exposed to 2 mg/L, 50 nm Cu. Given is the average  $\pm$  SEM per exposure group and the *p*-value.



**Figure S2.** Behavioral performance in the light–dark challenge test. For each actual concentration of TiO<sub>2</sub> nanobipyramids (a and c) and nanoplates (b and d), the total distance moved was given in each phase. Set a and b were kept in the dark during the exposure period, set c and d were illuminated with UV during the exposure period. Data presented as mean  $\pm$  standard error of the mean (SEM; n = 9).



**Figure S3.** Example of light-dark challenge test recording of unexposed zebrafish embryos. In the figure, basal, challenge, and recovery phase are indicated.

**Table S1.** Formulas for calculating the surface area and volume of NPs for different shapes.

Shape	Surface Area	Volume
Spheres	$4\pi \left(\frac{d}{2}\right)^2$	$\frac{4}{3}\pi \left(\frac{d}{2}\right)^3$
Plates	$2lw$	$lw$
(Elongated) rods	$2\pi l \left(\frac{d}{2}\right) + 2\pi \left(\frac{d}{2}\right)^2$	$\pi \left(\frac{d}{2}\right)^2 l$
Bipyramids	$8 \times \text{side of pyramid} = 8 * \left(\frac{1}{2} lw\right)$	$2 \times \text{pyramid} = 2 * \left(\frac{1}{2} lw\right)$

**Table S2.** Size, surface to volume ratio, and Zeta-potentials for each NP tested. Based on TEM images, the average diameter, length (L) and width (W) of the NPs are calculated, and subsequently used to calculate the surface/volume ratio.

Particles	Size Variation Using TEM (nm)	Surface/Volume Ratio	Size Distribution Using Dynamic Light Scattering (50% Percentile in nm)		Zeta-Potential Using Dynamic Light Scattering (mV)	
			1 h	24 h	1 h	24 h
Ag nanospheres	36.2 ± 19.7	0.23 ± 0.15	4339	996.1	-9.79	-9.91
Ag nanoplates	96.9 ± 43.9 (rods) L 595.8 ± 471.0 W 73.9 ± 4.6)	0.85 ± 0.35	326.3	312.5	0.60	-1.20
Ag elongated nanorods	L 2712.0 ± 1726.6 W 148.9 ± 66.5	0.033 ± 0.013	7349	5352	-30.00	-26.60
TiO <sub>2</sub> nanoplates	28.3 ± 10.6	1 ± 0	2019	3945	-7.97	-11.90
TiO <sub>2</sub> nanobipyramids	L 68.2 ± 29.0 W 27.7 ± 13.1	0.20 ± 0.075	1934	2250	-3.51	-7.25
TiO <sub>2</sub> nanospheres	29.3 ± 37.4	0.24 ± 0.21	-	>1300	-	-20

**Table S3.** Overview of LC<sub>50</sub> and EC<sub>50</sub> values calculated for AgNPs and TiO<sub>2</sub>NPs, as well as the size and surface/volume ratio of the NPs. For TiO<sub>2</sub>, effect levels are calculated for ‘with UV’ treatment and ‘without UV’ treatment. Toxicity values are calculated on the basis of the actual average concentrations at T 0 and at T 24, as can be found in Table 1. Note that the values indicated with an asterisk are calculated values due to the lack of actual LC<sub>50</sub> and EC<sub>50</sub> data.

Particles	Size Variation Using TEM	Surface/Volume Ratio	LC <sub>50</sub> (95% Confidence Interval) (mg/L)		EC <sub>50</sub> (95% Confidence Interval) (mg/L)	
			NP <sub>total</sub> 0 h	NP <sub>total</sub> 24 h	NP <sub>total</sub> 0 h	NP <sub>total</sub> 24 h
AgNO <sub>3</sub>	-	-	0.09 (0.08–0.1)	0.09 (0.08–0.1)	na	na
Ag nanospheres	36.2 ± 19.7	0.23 ± 0.15	11.7 (9.9–13.6)	5.4 (4.5–6.2)	15.9 (14.0–17.8)	7.3 (6.4–8.2)
Ag nanoplates	96.9 ± 43.9 (rods) L 595.8 ± 471.0 W 73.9 ± 4.6	0.85 ± 0.35	4.9 (4.8–5.0)	1.4 (1.3–1.5)	6.3 (1.4–11.3)	1.5 (1.2–1.9)
Ag elongated nanorods	L 2712.0 ± 1726.6 W 148.9 ± 66.5	0.033 ± 0.013	9.2 (5.7–12.7)	0.019 (0.018–0.021)	10.9 (4.0–17.8)	0.020 (0.017–0.023)
TiO <sub>2</sub> nanoplates with UV	28.3 ± 10.6	1 ± 0	35.8* (–14.1–85.9)	1.5* (–0.6–3.6)	16.4 (9.5–23.4)	0.10 (0.10–1.11)
without UV			394.5* (394.5–394.5)	16.4* (16.4–16.4)	na	na
TiO <sub>2</sub> nanobipyramids with UV	L 68.2 ± 29.0	0.20 ± 0.075	na	na	na	na
without UV	W 27.7 ± 13.1		na	na	64.2* (–170.2–298.5)	0.25* (0.24–0.27)
TiO <sub>2</sub> nanospheres with UV	29.3 ± 37.4	0.24 ± 0.21	na	na	na	na
without UV			na	na	na	na

**Table S4.** Predicted particle number values and LC<sub>50</sub> values for TiO<sub>2</sub> nanospheres and nanobipyramids, calculated for four dose metrics.

TiO <sub>2</sub> NP		Total surface area	Surface to volume ratio	Minimal diameter	Effective diameter
LC <sub>50</sub> (Log <sub>10</sub> Particle number #/L)					
Nanosphere	Dark	15.1 (15.0–15.4)	13.4 (12.7–14.1)	15.9 (15.90–15.98)	14.4 (13.9–14.8)
	UV	14.2 (14.0–14.4)	12.7 (12.0–13.4)	15.0 (14.94–15.03)	13.4 (13.0–13.8)
Nanobipyramid	Dark	14.7 (14.5–15.0)	14.2 (13.6–14.7)	16.1 (16.14–16.16)	14.6 (14.3–15.0)
	UV	13.7 (13.5–14.1)	13.4 (12.9–14.0)	15.2 (15.19–15.20)	13.7 (13.3–14.0)
LC <sub>50</sub> (mg/L)					
Nanosphere	Dark	271.4 (190.3–493.3)	5.6 (1.1–28.7)	1826.1 (1660.1–2008.7)	47.3 (18.3–122.7)
	UV	30.0 (21.0–54.5)	1.1 (0.2–5.4)	201.6 (183.3–221.7)	5.2 (2.0–13.5)
Nanobipyramid	Dark	54.1 (32.5–127.7)	16.5 (4.7–58.8)	1605.8 (1580.4–1631.7)	46.8 (20.8–105.6)
	UV	6.0 (3.6–14.0)	3.1 (0.9–11.0)	177.3 (174.5–180.1)	5.2 (2.3–11.7)

**Table S5.** Overview of highest measured concentration, actual 50% effect concentrations (mortality and malformation), and predicted LC<sub>50</sub> values, calculated for four dose metrics. Note that the values indicated with an asterisk are calculated values due to the lack of actual LC<sub>50</sub> and EC<sub>50</sub> data.

Particles	Highest NP <sub>total</sub> Concentration 0 h (mg/L)	Actual Median Concentration (95% Confidence Interval) (mg/L)		Predicted LC <sub>50</sub> (95% Confidence Interval) (mg/L)				Behavioral Effects (EC <sub>50</sub> )
		LC <sub>50</sub>	EC <sub>50</sub>	Total Surface Area	Surface to Volume Ratio	Minimal Diameter	Effective Diameter	
Ag nanospheres	17.20	11.7 (9.9–13.6)	15.9 (14.0–17.8)	-	-	-	-	-
Ag nanoplates	14.00	4.9 (4.8–5.0)	6.3 (1.4–11.3)	-	-	-	-	-
Ag elongated nanorods	328.00	9.2 (5.7–12.7)	10.9 (4.0–17.8)	-	-	-	-	-
TiO <sub>2</sub> nanoplates UV	22.7	35.8* (-14.1–85.9)	16.4 (9.5–23.4)	-	-	-	-	na
Without UV		394.5* (394.5– 394.5)	na	-	-	-	-	na
TiO <sub>2</sub> nanobipyramids UV	20.7	na	na	6.0 (3.6–14.0)	3.1 (0.9–11.0)	177.3 (174.5–180.1)	5.2 (2.3–11.7)	na
Without UV		na	64.2* (-170.2–298.5)	54.1 (32.5–127.7)	16.5 (4.7–58.8)	1605.8 (1580.4– 1631.7)	46.8 (20.8–105.6)	na
TiO <sub>2</sub> nanospheres UV	50.9	na	na	30.0 (21.0–54.5)	1.1 (0.2–5.4)	201.6 (183.3–221.7)	5.2 (2.0–13.5)	-
Without UV		na	na	271.4 (190.3–493.3)	5.6 (1.1–28.7)	1826.1 (1660.1–2008.7)	47.3 (18.3–122.7)	-

