Through the magnifying glass: The effects of size and shape on the uptake, biodistribution and (eco)toxicity of nanoparticles

Pomeren, M. van

Citation

Pomeren, M. van. (2019, April 17). *Through the magnifying glass: The effects of size and shape on the uptake, biodistribution and (eco)toxicity of nanoparticles*. Retrieved from https://hdl.handle.net/1887/71375

Author: Pomeren, M. van
Title: Through the magnifying glass: The effects of size and shape on the uptake, biodistribution and (eco)toxicity of nanoparticles
Issue Date: 2019-04-17

Chapter 5

The impacts of interactions between TiO_2 nanoparticles and differently dissolving nanoparticles on mixture toxicity

M. van Pomeren[1], W.J.G.M. Peijnenburg[1,2], M.G. Vijver[1].

[1] Institute of Environmental Sciences (CML), Leiden University, 2300 RA, Leiden, The Netherlands,
[2] National Institute of Public Health and the Environment, Center for the Safety of Substances and Products, 3720 BA, Bilthoven, The Netherlands.

In preparation

Abstract

With the increasing numbers of products on the marked that contain mixtures of nanoparticles, the necessity to understand the toxicity of these mixtures becomes progressively important. With TiO_2 nanoparticles being used in various nanoparticle mixtures, it is important to gain more insight into the role of TiO_2 nanoparticles in mixture toxicity.

In this study, we aim to understand the joint effects of soluble NPs with non-reactive NPs based on the fate of the nanoparticles. By focusing on the co-agglomeration behavior and the adsorption of ions to the TiO_2 nanoparticles, we aim to distinguish the process with the highest contribution to the mixture toxicity.

In our experimental study, co-aggregation as well as adsorption of free ions to TiO_2 nanoparticles occurred to a limited extent. Moreover, the adsorption capacity of TiO_2 nanoparticles was found to be limited, indicating that the concentration, as typically used in toxicity tests, of TiO_2 nanoparticles in the mixtures was not sufficient to induce visible effects on mixture toxicity other than additivity. Thus, it is still unclear what the joint impact is that TiO_2 NPs have on soluble and non-reactive nanoparticles, as there is still a need to confirm or reject multiple hypotheses concerning the importance of co-agglomeration and adsorption of ions to TiO_2 nanoparticles.

1. Introduction

With the increase of nanotechnology, the use of nanomaterials in products has increased as well. Whereas at first only nanomaterials consisting of one type of nanoparticles (NPs) were used, current products commonly contain mixtures of NPs. For example, titanium dioxide nanoparticles (TiO_2 NPs; exhibiting photocatalytic properties under ultraviolet light) are often combined with silver NPs (Ag NPs) in order to enhance the antimicrobial effect of the Ag NPs[1]. Another example are sunscreens: both TiO_2 NPs and zinc oxide (ZnO) NPs are effective ultraviolet light (UV) blockers that do penetrate the skin[2], yet these NPs together show enhanced UV blocking capacity[3]. With the use or disposal of products containing mixtures of NPs, the mixtures will eventually be released into the environment. With TiO_2 NPs being used in a large variety of mixtures, it is important to gain more insight into the effects of TiO_2 NPs within the toxicity of mixtures of NPs in the environment.

Co-exposure of biota to two or more compounds may lead to either additive, antagonistic, or synergistic effects[4,5]. For TiO_2 NPs, not only additivity in mixtures has been found, but they can also have antagonistic effects and reduce the toxicity of compounds. Being used as a oxidation agent in Waste Water Treatment Plants, it breaks down organic compounds under UV radiation[6]. Furthermore, TiO_2 NPs appear the have an antagonistic effect on the toxicity of other nanoparticles[7]. In co-exposure, TiO_2 NPs reduced the toxicity of ZnO and CuO to algal cells[7]. Since TiO_2 is known to agglomerate quickly and subsequently settle efficiently[8], this sedimentation process may cause other nanoparticles to be trapped in large aggregates typically formed after emission of TiO_2 NPs, after which the particles are no longer bioavailable to pelagic organisms. On the other hand, adsorption of ions to TiO_2 NPs may also occur, reducing the amount of free ions and therewith decreasing the effective ionic concentration.

Whether nanoparticles in the environment induce toxicity depends on many factors, such as chemical factors that influence the fate of particles, but also the presence of other NPs. The toxicity of nanoparticles is highly dependent on the fate of the particles, which in turn is influenced by numerous factors and properties, including the size, composition, surface charge, concentration and coating of the particle (Vijver et al, in press ES nano). In addition, NPs have a tendency to agglomerate at higher concentrations, which subsequently alters the bioavailable dose[9]. Nanotoxicology is complicated even further by the fact that effects are not necessarily linearly related to dose[10]. Besides, the presence of multiple NPs in the environment may lead to mixture interactions.

As we have seen in previous examples[7], TiO_2 NPs are capable of influencing the fate of other NPs when co-exposed. In this study, we aimed to understand the influence of TiO_2 NPs on the toxicity of other NPs, considering their influence on the fate of the other NPs. Building upon the knowledge obtained in previous work[11], where TiO_2 reduced the toxicity of ZnO to zebrafish embryos, we ask the question whether the toxicity reducing capacity of TiO_2 NPs can be ascribed by enhancement of the aggregation/agglomeration and subsequent sedimentation of the co-exposed NPs or whether this capacity can be ascribed to reduction of the ion concentrations in suspension due to ion sorption to TiO_2 NPs. To unravel these hypotheses, we exposed zebrafish embryos to mixtures of TiO_2 NPs and a chemically inert NP (represented by polystyrene particles) as well as to TiO_2 NPs and a slowly dissolving NP (represented by silver particles).

Secondly, we aim to focus on the dissolution behavior of the non-stable ZnO particles. The question we asked is whether the toxicity-reducing capacity of TiO_2 NPs is stronger when the dissolution of the non-stable particle is higher. It can be hypothesized that if the most dominant way of reducing toxicity is via the reduction of the concentration of free ions in suspension, the overall reduction in toxicity is higher when there are more free ions to be removed from the exposure medium. For this question, we manipulated the dissolution and aggregation/agglomeration behavior of zinc nanoparticles by adding a coating (hydrophobic and hydrophilic) to the particles.

2. Materials and Methods

2.1 Preparation of particle suspensions

Elongated Ag rods with a nominal size of 50 nm × 0.6-12 μm suspended in H_2O were purchased from Fraunhofer ISC (Würzburg, Germany). Spherical TiO_2 NPs with a nominal size of 20 nm and ZnO nanosticks with a nominal size of 43 nm were obtained as powder from Io-Li-Tec nanomaterials (Heilbronn, Germany). Hydrophobic ZnO NPs were obtained in powder from the JRC repository (JRC NM01101a). Fluorescent polystyrene nanoparticles (PS NPs), internally dyed with FirefliTM Fluorescent Green (468/508nm), of size 25 nm suspended in H_2O were purchased from ThermoFisher Scientific (Catalog number R25; Waltham, USA).

Exposure suspensions were prepared either by adding the purchased stock suspensions to egg water (60 μg/ml Instant Ocean Sea Salt, Sera GmbH, Heinsberg, Germany) or by weighing dry powdered TiO_2 or ZnO particles into egg water. Thereafter, stock suspensions were diluted to obtain the desired concentrations. Zn^{2+} solutions were obtained by adding zinc nitrate (Sigma Aldrich, Zwijndrecht, The Netherlands) to egg water. The hydrophilic ZnO NPs were obtained by preparing a stock suspension in a 10 mg/L PVP (polyvinylpyrrolidone) solution. Immediately before exposure, the solutions were freshly prepared and sonicated for 10 min using an ultrasonic water bath (45kWh or 60W; USC200T, VWR, Amsterdam, The Netherlands).

2.2 Physicochemical characterization

Transmission electron microscopy (TEM; JEOL 1010, JEOL Ltd., Tokyo, Japan) was used directly after preparation (T0) to characterize the size and morphology of the particles in egg water. Additionally, a TEM image was obtained after 24 hours of incubation in egg water (T24). Using dynamic light scattering (DLS) assessments (Zetasizer Nano-ZS instrument; Malvern Instruments Ltd, Malvern, UK), the size distribution and zeta-potential of the particle suspensions in egg water at 0 h, 1h and 24 h were assessed.

Using atomic absorption spectroscopy (AAS; Perkin Elmer 1100B,Waltham, MA, USA), the actual total concentration of dissolved Ag ions as well as the total Ag concentration (Ag NP total) in egg water were analyzed. The same analysis was performed for the differently coated ZnO NPs. For this purpose, freshly prepared dispersions were measured at time point t= 0 h and after equilibration at t= 1 h and t= 24 h. The concentration of dissolved ions was obtained by sampling the supernatant after centrifugation at 5000× g for 30 min to remove the nanoparticle fraction and ultimately determine the soluble fraction in the suspension. After preparation, samples were stored in 1M HCl (CAS 7647-01-0, Sigma Aldrich, Zwijndrecht, The Netherlands) in order to digest the sample for measurement.

2.3 Experimental setup

2.3.1 Zebrafish husbandry

Zebrafish were handled as described by animal welfare regulations and maintained according to standard protocols (http://ZFIN.org). Adult zebrafish were maintained at 25 ± 0.5 °C in a 14 h light : 10 h dark cycle. Fertilized zebrafish eggs were obtained from an AB/TL wild-type zebrafish.

2.3.2 Set up

The tested mixtures were representing combinations of nanoparticles that show a high dissolution rate compared to those that are relatively non-reactive (Table 1).

Table 1. *Overview of the different mixture setups.*

NP	**Nominal dose** (mg/L or x time dilution)	**Type of stability**	**Dose of TiO_2 NPs** (mg/L)
PS NP	0; 25; 32,5; 40; 47,5; 55	Non-reactive: No dissolution or aggregation/agglomeration	0; 0.25; 1; 2.5; 4; 10; 40
Ag NP	0; 30x; 100x; 300x; 1000x; 3000x	Non stable: High dissolution of Ag	0; 2.5; 4; 10; 25; 40
ZnO NP uncoated	0; 2; 4; 8; 16; 32; 64	Non stable: High dissolution of Zn and aggregation/agglomeration	0; 1.5; 3; 6; 12; 24
ZnO NP hydrophilic coated	0; 2; 4; 8; 16; 32; 64	Non stable: Dissolution of Zn, limited aggregation/agglomeration	0; 1.5; 3; 6; 12; 24
ZnO NP hydrophobic coated	0; 2; 4; 8; 16; 32; 64	Hardly reactive: Low dissolution, high aggregation/agglomeration potency	0; 1.5; 3; 6; 12; 24

2.3.3 Toxicity assessment of zebrafish embryo life stages to different mixtures

2.3.2.1 Toxicity tests with free-swimming embryos

Free swimming embryo larvae (3dpf) were selected for this experiment, since this life stage has shown to be the most sensitive[12]. Per group, ten zebrafish embryos were exposed in 24-well plates and an exposure regime of 48 hours was maintained (see approach as described in Van Pomeren et al., (2017)[8]). Particle suspensions and egg water were renewed every 24 hours. At the final day of the experiment, the embryos were screened for survival and malformations. Temperature was maintained at 28 ± 0.8 °C during the experiments.

2.3.2.2 Toxicity test on the hatching success of embryos

As zinc is known to influence the hatching of zebrafish embryos [13], we aimed to include this feature as a more sensitive sub-lethal endpoint. For that reason, we adapted the exposure period from 4 hpf till 72 hpf. The remainder of the experimental setup was comparable to the previous mentioned setup, with the addition of hatching success as an extra sub-lethal endpoint.

For all non-stable NPs, the relative contribution to toxicity of particles and ions was calculated as explained in Van Pomeren et al. (2017)[8].

2.4 Statistical analysis

Significance ($p<0,05$) for effects on survival, hatching success and malformations among the different treatments was tested using a one way ANOVA using the SPSS 23 software package. Results are given as mean ± standard deviation (SD).

3. Results

3.1 Physico-chemical characterization of polystyrene, silver and titanium dioxide particles

TEM images showing the aggregation/agglomeration state of silver + TiO_2 NPs and polystyrene + TiO_2 NPs are provided in Figure 1. Over time, silver + TiO_2 NPs form intertwined clusters, with TiO_2 NPs accumulating at the tips of the elongated Ag nanorods (Figure 1, b). Remarkably, polystyrene does not form (large) aggregates/agglomerates with TiO_2 NPs, also not over time (Figure 1, c and d). This same trend is nicely visible when considering the hydrodynamic sizes of the samples (Table S1 and S2). The silver + TiO_2 NP mixture rapidly forms aggregates/agglomerates that increase in size over time (Table S1). Most of these aggregates/agglomerates settle out of the suspension, as can be deduced from the decreasing total concentrations as measured by AAS (Table S5). Yet the addition of TiO_2 NPs does only marginal affect the decrease of the total concentration of silver in suspension. In contrast, the polystyrene

+ TiO_2 NP mixture shows two individual peaks as measured by DLS: one peak representing the single polystyrene particles, and one peak representing the agglomerating TiO_2 NPs (Table S2).

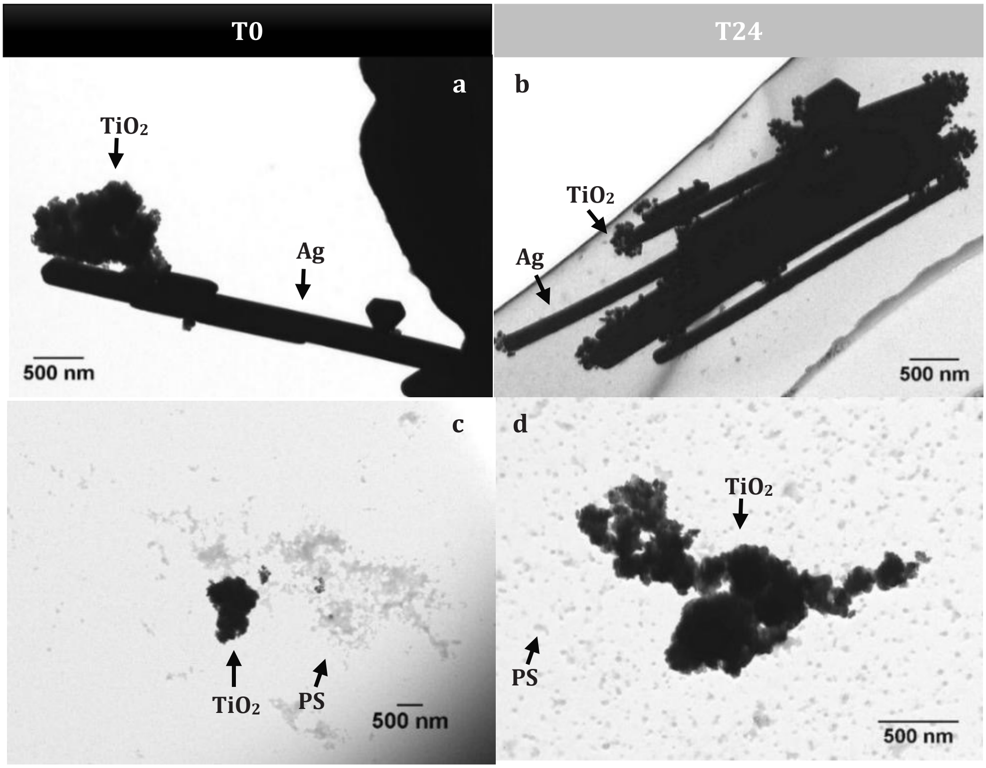


Figure 1. *TEM images of Ag NPs (a, b) and PS NPs (c,d) in mixture with TiO_2 NPs, at both T0 (a,c) and T24 (b,d).*

Considering the zeta potential of the mixtures (Table S3 and S4), we see indeed that the silver mixture is prone to agglomeration (zeta potential lies in between -30 and 30 mV; Table S3). In contrast, the polystyrene mixture is relatively stable, since the zeta potentials range from -25 till -35 mV (Table S4), even though the TiO_2 NPs still form aggregates/agglomerates (Table S2).

3.2 Physico-chemical characterization of differently coated zinc and titanium dioxide particles

Similar to the PS and Ag NP mixtures, Figure 2 shows TEM images of the aggregation/agglomeration state of the TiO_2 NP mixtures with differently coated ZnO NPs. All differently coated ZnO NPs form aggregates/agglomerates with TiO_2 NPs, the size of which increases over time (Figure 2). Morphologically, the aggregates/agglomerates of uncoated ZnO NPs (Figure 2, a and b) and the hydrophobic coated ZnO NPs (Figure 2, e and f) are relatively comparable. However, the hydrophilic coated particles (Figure 2, c and d) form aggregates/agglomerates with a much more open structure: there is more space around the particles. Evaluating the aggregate/agglomerate sizes obtained via the DLS measurements (Tables S6, S7 and S8) as well as the sedimentation as measured as decrease in total concentration (Table S9), it is to be concluded that only little differences are observable between the different coatings. Additionally, very little effect of the addition of TiO_2 NPs has been observed on the total concentration in suspension as well as on the amount of free ions (Table S9). When testing the sorption capability of TiO_2 NPs for Zn ions (Table S10), only minor differences were observed in the amount of free ions when TiO_2 NPs were added. This indicates that the sorption capacity is relatively low and explains why almost similar ion concentrations were observed in the different combinations of TiO_2 NPs and (coated) ZnO NPs.

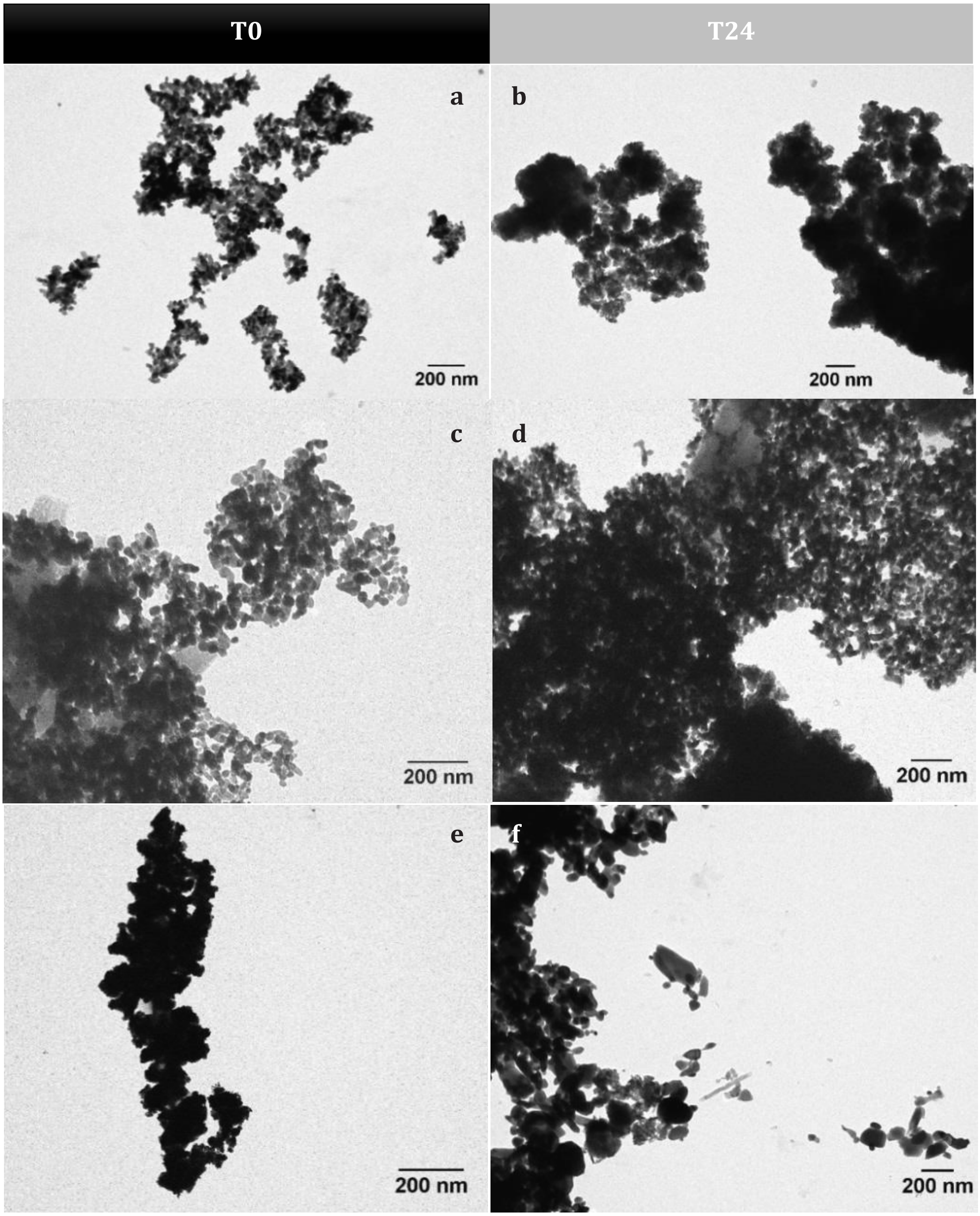


Figure 2. *TEM images of ZnO nanoparticles with different coatings in mixture with* TiO_2 *NPs, at both T0 (a,c,e) and T24 (b,d,f). The coatings were: uncoated (a,b), hydrophylic coated (c,d) and hydrophobic coated (e,f).*

3.3 Mixture toxicity of titanium dioxide with stable and non-stable particles

In Figures 3 and 4, the survival of zebrafish embryos is shown after 48h of exposure to suspensions of a mixture of TiO_2 NPs and polystyrene (Figure 3) or Ag (Figure 4) NPs. For both mixtures, the organisms were exposed from 3 dpf till 5 dpf. While both particles show a clear effect on survival of the organism, the addition of non-toxic[8] TiO_2 NPs was found not to significantly affect the toxicity of any of the particles. When further assessing the LC_{50} values for each treatment (Table 1 and 2), no statistically significant differences or patterns could be observed. Other endpoints such as malformations (Table S11) did not show any pattern linked to the addition of increasing concentrations of TiO_2 NPs either.

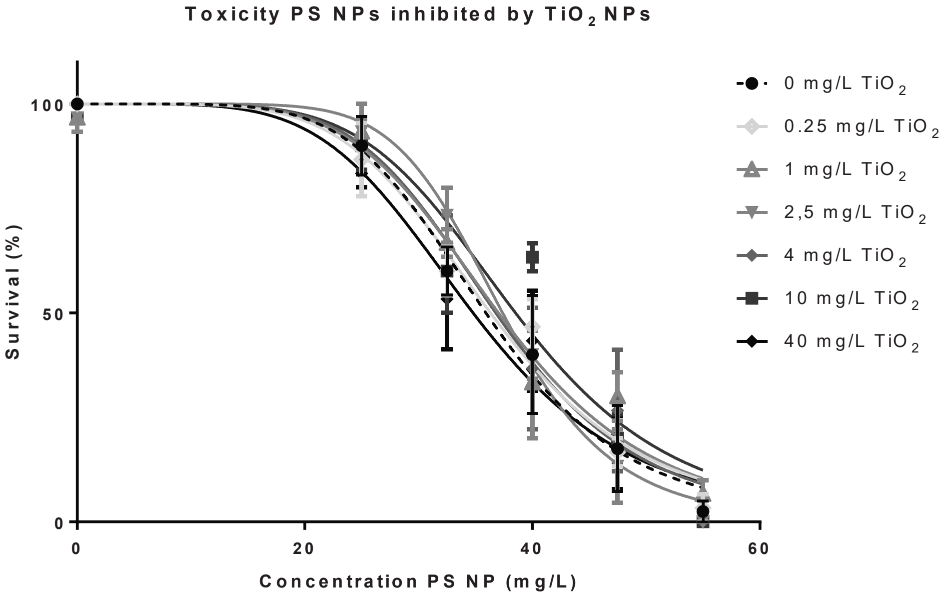


Figure 3. *Survival of zebrafish embryos after exposure to PS NPs in combination with different concentrations TiO_2 NPs after exposure from 3 dpf till 5 dpf.*

Table 1. *LC_{50} values of polystyrene NPs with different concentrations TiO_2 NPs added. SE = Standard Error*

Particle	TiO_2 (mg/L)	LC_{50} value (mg/L)	+ SE
Polystyrene	0	35,9	1,3
	0,25	36,3	1,0
	1	37,0	1,2
	2,5	37,4	0,8
	4	36,8	1,4
	10	38,5	1,5
	40	34,8	1,4

For the Ag NPs, we calculated the relative contribution of the ions (NP_{ion}) and the particles ($NP_{particle}$) to the overall toxicity observed. As can be seen in Table 2, the relative contribution did not show large differences as well, indicating that the addition of TiO_2 to the exposure medium containing either PS or Ag NPs induced only additive toxicity, although minor effects that were not observable in this test cannot be excluded.

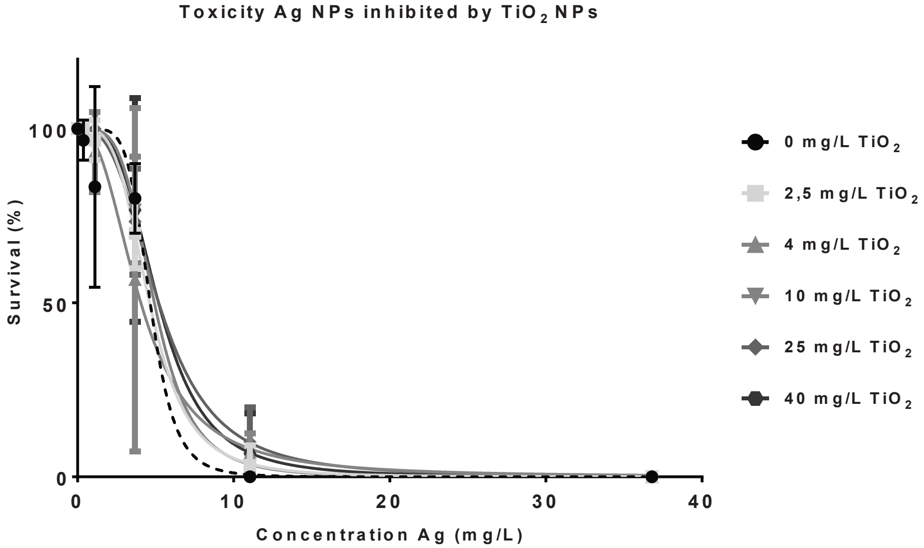


Figure 4. *Survival of zebrafish embryos after exposure to Ag NPs in combination with different concentrations TiO_2 NPs after exposure from 3 dpf till 5 dpf.*

***Table 2.** LC_{50} values of Ag NPs with different concentrations TiO_2 NPs added.*

				Relative contribution (%)	
Particle	**TiO_2 (mg/L)**	**LC_{50} value (mg/L)**	**+ SE**	**NP^{ion}**	**Np^{particle}**
Silver	0	4,7	2,1	79,6	20,4
	2,5	4,6	0,2	79,6	20,4
	4	4,1	0,7	79,6	20,4
	10	4,9	0,4	79,6	20,4
	25	5,2	0,3	79,6	20,4
	40	5,2	0,7	79,6	20,4

3.4 Mixture toxicity of titanium dioxide with differently coated particles

Figure 5 shows the survival and hatching success of zebrafish embryos after 3 days of exposure to differently coated ZnO NPs. As is apparent from the graph, no full dose-response models could be drawn since the survival rate at this time point has not reached 0 % for any of the coating types and shows large variation (Figure 5). Hatching success provides a more robust endpoint.

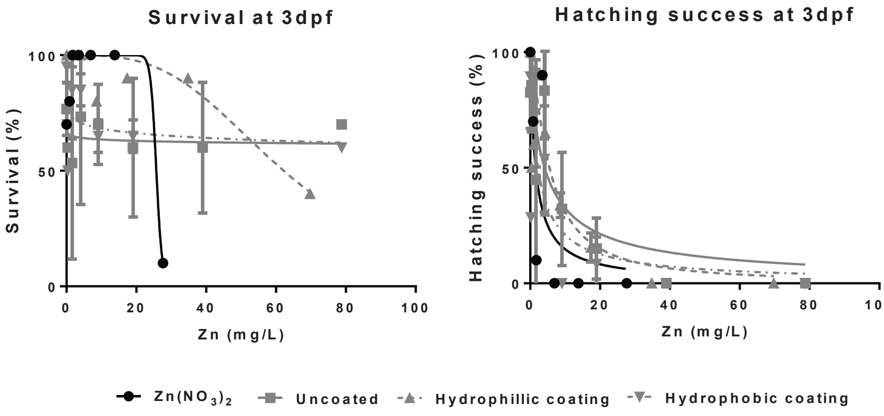


***Figure 5.** Survival and Hatching success of 3 day old zebrafish embryos after exposure to differently coated ZnO NPs.*

When comparing the EC_{50} of the different coatings, no clear difference could be observed (Table 3). Also apparent, is that the relative contribution of the ions is 100% for each of the coating types.

Table 3. *EC_{50} values of the hatching success of 3 dpf zebrafish embryos exposed to zinc ions and differently coated ZnO NPs.*

			Relative contribution (%)	
Coating	**EC_{50} value (mg/L)**	**+ SE**	**NP^{ion}**	**$Np^{particle}$**
Zn^{2+}	1,6	1,3	100	-
Uncoated	4,1	1,8	100,1	-0,1
Hydrophilic	7,6	0,4	100,2	-0,2
Hydrophobic	2,1	0,7	100,1	-0,1

For each different coating type, we assessed the effect of additional TiO_2 NPs in the medium. Looking at the most robust endpoint, hatching success, no clear effect could be observed (Figure 6). When we assessed the effect on the survival of the embryos, the variation in the data was found to be too large to see any pattern (Figure S1). Additionally, the relative contribution of the ions on the hatching success remained approximately 100% for each treatment (Table S12).

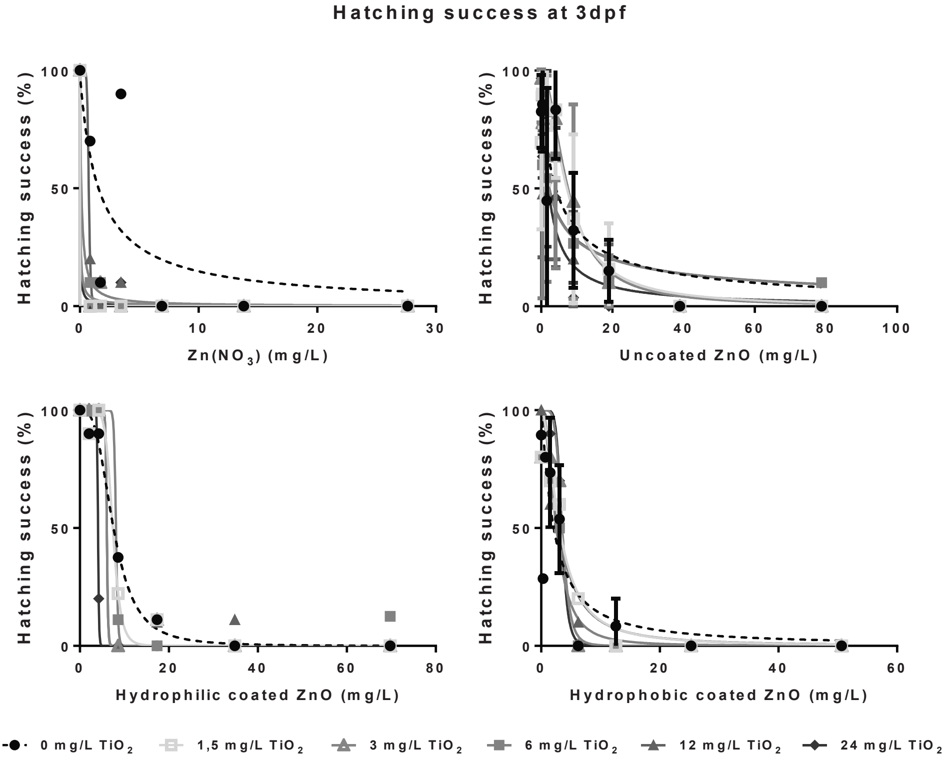


Figure 6. *Hatching success of zebrafish embryos at 3 dpf after exposure to a zinc-ion control ($Zn(NO_3)_2$; a) or differently coated ZnO NPs (b,c,d) in combination with different concentrations of TiO_2 NPs.*

4. Discussion

In this study, we aimed to understand the influence of TiO_2 NPs on the toxicity of other NPs, considering the influence of TiO_2 NPs on the fate of the other NPs. In order to tackle this aim, we formulated two research questions. Firstly, we asked the question whether the reducing capacity of TiO_2 NPs can be ascribed by enhancement of the aggregation/agglomeration and subsequent sedimentation of the co-exposed NPs or ascribed to reduction of the ion concentrations in suspension due to ion sorption to TiO_2 NPs. Secondly, the question we asked is whether the toxicity-reducing capacity of TiO_2 NPs is related to the dissolution behavior of non-stable particles.

Answering the first research question, we observed no interactive effects between PS NPs and TiO_2 NPs when assessing the fate of the particles (Table S3). The PS NPs were stable in the suspension, and no interactions with the added TiO_2 NPs were detected. The absence of interactions between the particles was also reflected in the toxicity experiments, as the toxicity of PS NPs was not affected by addition of TiO_2 NPs (Figure 3). A similar pattern was observed for the mixtures of silver NPs and TiO_2 NPs (Figure 4). Although there were indications of co-agglomeration based on TEM pictures and DLS measurements (Figure 1, Table S1), no clear effects of addition of TiO_2 NPs were observed on the total Ag concentration nor on the ion concentration in suspension (Table S5). Thus there were either no interactions (PS NPs mixtures) or the interactive effects were too small to be detected in our experimental design (as is the case for the Ag NPs mixtures).

For the second research question addressed in this study, we focused on the dissolution behavior of NPs and the subsequent effect on toxicity of mixtures of TiO_2 and ZnO NPs. By adding a hydrophobic or hydrophilic coating to the particles, we aimed to influence the dissolution behavior of the particles. Although the dissolution rate differed slightly after 1 hour of incubation, the total concentration and the percentage of ions in the suspension were similar at T24 (Table S9) in all mixtures. Due to the high dissolution rate of all differently coated NPs, the ionic form of Zn dominated the toxicity (Table 3). Most probably, this also explains why there was only a minor difference observable between the toxicity of the differently coated ZnO NPs. In addition it is to be noted that contradictory data concerning the effect of adding a coating to NPs are reported in literature. Coating NPs with PVP (which was in our study used as hydrophilic coating) was shown to reduce the toxicity of both Ag and ZnO NPs[14,15], yet Species Sensitivity Distribution modeling showed that a PVP coating increased the toxicity of Ag NPs[16]. For the hydrophobic coating used in our study the same pattern was observed: decreased[17] as well as increased[18] toxicity has been reported, and also no impacts on the toxicity compared to bare ZnO particles were reported[19]. These contradictory findings suggest that other chemical and environmental factors may play a much larger role in the effectiveness of coatings than previously thought.

As we have built our research questions upon the knowledge obtained from previous work[11], we wondered why we were not able to repeat the previous results. Whereas we observed high dissolution rates of the ZnO NPs, study of Hua et al (2014)[11] reported much lower rates of dissolution. This resulted in a relative contribution to toxicity of up to 38% for the ions, whereas we observed that toxicity was completely due to the presence of Zn^{2+}-ions formed in suspension. Upon comparing the experimental design, we noticed that the embryo media used differed significantly. Whereas the previous study reported a concentration of Instant Ocean of 210 mg/L, the egg water in our study contained 60 mg/L Instant Ocean. This difference in salt concentration may probably influence the equilibrium between ZnO NPs and Zn ions, shifting the equilibrium to the particulate form upon increasing salt concentration. Since in *in vitro* experiments, the reduction of ZnO NP toxicity by TiO_2 NPs was ascribed to the reduction of the concentration of Zn ions[20], this shift towards the particle form may be crucial. As we have observed that TiO_2 NPs can adsorb only limited amounts of ions, the added amount of TiO_2 NPs in our experiments is probably not sufficient to observe reducing effects on the toxicity of the co-exposed NP. In contrast, the high salt concentration may saturate the TiO_2 NPs, which makes them unable to adsorb Zn^{2+}-ions. This suggests that the observed reduction of toxicity in the study of Hua et al. (2014)[11] can be ascribed to the co-agglomeration of ZnO NPs with TiO_2 NPs. Since the observed toxicity in our experiment was fully due to the presence of Zn^{2+}-ions formed in suspension, it is not possible to confirm this hypothesis.

In our study, we observed that only limited interactive effects occurred in our experimental setups, whereas we expected to observe stronger interactions. For future research, it is worthwhile to explore which environmental factors have an influence on mixture interactions. Knowing which factors are driving mixture interactions may not only give an indication at which natural locations certain interactions may occur, but may also contribute to properly understanding and modeling mixture toxicity.

In conclusion, all of our tested mixture combinations resulted in additive responses. Based on previous studies, antagonistic effects were expected, but antagonistic interactions were not visible in zebrafish embryo responses nor was the fate of the NPs in the aquatic exposure medium modified upon addition of TiO_2 NPs. Co-agglomeration with stable PS NPs was not observed, whereas the Ag and the differently coated ZnO NPs showed some co-agglomeration. This suggests that adsorption of free ions to TiO_2 NPs has the highest contribution to the mixture impacts. Yet, given the high dissolution rates observed in our experiments, the adsorption capacity of the added TiO_2 NPs was not sufficient to diminish ion exposure. Conversely, reduction in toxicity ascribed to co-agglomeration could not be confirmed, since the observed toxicity was due to the ionic form of Zn. This means that there is still no conclusion to be drawn regarding the impact of TiO_2 NPs on the toxicity of slowly dissolving nanoparticles.

Acknowledgements

The authors would like to thank Julia Mars for her contribution during the experiments. The staff of the ZF facility of the Cell Observatory is thanked for providing the experimental work environment. Marinda van Pomeren and Martina G. Vijver were funded by NWO-VIDI 864.13.010 granted to Martina G. Vijver.

Supplementary Materials

Tables

S1 Hydrodynamic size distribution (nM) + standard deviation of Ag and TiO_2 NPs in eggwater

		0x diluted		100x			300x			1000x		Ag
		0 h	1 h	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2	Peak 1			0,0 ± 0,0	0,0 ± 0,0	128,2 ± 51,8	91,6 ± 104,1	251,6 ± 97,6	157,3 ± 30,7	0,0 ± 0,0	10,3 ± 17,8	239,5 ± 70,6
	Peak 2					4,2 ± 7,4	0,0 ± 0,0	21,4 ± 37,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
2,5 mg/L TiO_2	Peak 1	289,0 ± 38,1	303,5 ± 91,6	648,7 ± 46,2	450,0 ± 36,4	110,8 ± 59,3	427,1 ± 34,2	329,8 ± 39,4	262,3 ± 23,3	350,8 ± 95,1	304,1 ± 137,2	286,3 ± 96,3
	Peak 2					0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	151,4 ± 262,2	0,0 ± 0,0	0,0 ± 0,0	17,22 ± 29,8
4 mg/L TiO_2	Peak 1	470,0 ± 21,6	450,1 ± 35,8	635,1 ± 33,8	386,8 ± 16,1	132,7 ± 7,9	295,9 ± 13,6	336,6 ± 117,0	164,4 ± 20,3	276,2 ± 26,2	134,5 ± 27,7	207,0 ± 31,8
	Peak 2					0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
10 mg/L TiO_2	Peak 1	467,7 ± 42,4	530,3 ± 16,1	639,8 ± 56,3	409,4 ± 38,4	194,0 ± 17,4	310,0 ± 96,3	344,6 ± 14,0	197,3 ± 5,9	136,8 ± 134,4	334,3 ± 189,1	134,9 ± 17,1
	Peak 2					0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0

S2 Size distribution (nM) + standard deviation of PS and TiO_2 NPs in eggwater

		10 mg/L			25 mg/L			40 mg/L		PS
		0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2	Peak 1	25,9 ± 0,6	29,7 ± 0,8	28,8 ± 0,5	27,0 ± 0,2	28,5 ± 1,0	28,3 ± 0,4	28,0 ± 0,3	29,3 ± 0,4	29,7 ± 0,5
	Peak 2	0,0 ± 0,0	3210,7 ± 2780,9	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
	Peak 3	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
2,5 mg/L TiO_2	Peak 1	338,2 ± 142,7	94,2 ± 125,7	27,1 ± 1,2	19,3 ± 2,3	22,6 ± 0,4	26,7 ± 0,7	23,5 ± 0,4	23,6 ± 1,6	27,9 ± 0,7
	Peak 2	20,5 ± 0,5	122,0 ± 114,7	464,8 ± 164,5	130,9 ± 46,1	206,8 ± 66,9	299,4 ± 72,3	368,7 ± 67,4	232,7 ± 157,8	1625,0 ± 2814,8
	Peak 3	26,5 ± 45,9	0,0 ± 0,0	5217,0 ± 151,5	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0
4 mg/L TiO_2	Peak 1	252,4 ± 109,8	411,5 ± 140,3	25,2 ± 1,2	72,4 ± 91,3	349,8 ± 122,4	27,1 ± 0,4	24,5 ± 0,7	26,9 ± 0,1	30,1 ± 1,2
	Peak 2	20,0 ± 4,6	24,8 ± 5,4	178,4 ± 83,8	115,0 ± 101,4	23,1 ± 1,1	369,5 ± 103,9	377,9 ± 32,4	467,4 ± 39,6	2094,0 ± 1172,6
	Peak 3	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	1795,7 ± 3110,2	0,0 ± 0,0	3609,3 ± 3125,8	1551,0 ± 2686,4
10 mg/L TiO_2	Peak 1	269,0 ± 32,4	403,1 ± 32,2	25,2 ± 1,4	269,5 ± 52,5	533,7 ± 50,4	28,5 ± 0,4	506,5 ± 38,5	618,0 ± 32,7	28,3 ± 0,6
	Peak 2	13,5 ± 1,5	18,4 ± 1,7	168,9 ± 34,3	19,7 ± 1,7	27,2 ± 1,0	224,8 ± 45,8	24,6 ± 0,5	26,8 ± 1,1	297,1 ± 48,8
	Peak 3	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	0,0 ± 0,0	1799,0 ± 3116,0	0,0 ± 0,0	1844,0 ± 3193,9	1808,3 ± 3132,1

S3 Zeta potential (mV) + standard error of Ag and TiO_2 NPs in eggwater

	0x		100x			300x			1000x		Ag
	0 h	1 h	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2					-7,6 ± 2,9	-31,6 ± 2,0	-22,4 ± 0,7	-15,5 ± 1,1	-17,0 ± 3,0	-12,4 ± 9,8	-9,4 ± 0,4
2,5 mg/L TiO_1	16,8 ± 0,3	8,2 ± 0,9	-27,0 ± 1,0	-25,4± 1,3	-20,1 ± 0,5	-18,7 ± 0,8	-18,5 ± 0,6	-19,4 ± 0,8	-19,4 ± 1,1	-18,9 ± 0,8	-20,4 ± 1,2
4 mg/L TiO_2	19,8 ± 0,4	7,8 ± 0,4	-26,6 ± 1,5	-25 ± 0,4	-20,5 ± 1,8	-17,7 ± 1,2	-18,5± 0,1	-20,6 ± 0,4	-17,7 ± 1,5	-19,8 ± 0,9	-22,0 ± 0,8
10 mg/L TiO_2	20,2 ± 1,0	12,5 ± 0,8	-26,4 ± 1,1	-26,4 ± 0,5	-22,1 ± 0,7	-17,8 ± 0,8	-20,1 ±0,8	-20,5 ± 0,6	-18,4 ± 0,7	-19,8 ± 0,7	-22,9 ± 0,7

S4 Zeta potential (mV) + standard error of PS and TiO_2 NPs in eggwater

	10 mg/L			25 mg/L			40 mg/L		PS
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2	-21,1 ± 2,8	-22,3 ± 2,3	-24,3 ± 1,7	-26,3 ± 2,0	-27,7 ± 0,4	-24,4 ± 4,7	-29,2 ± 1,0	-31,1 ± 1,0	-30,2 ± 1,4
2,5 mg/L TiO_1	-25,9 ± 0,4	-25,5 ± 2,7	-25,8 ± 2,8	-30,5 ± 0,2	-29,8 ± 0,1	-30,1 ± 1,0	-32,1 ± 0,6	-32,9 ± 0,2	-30,6 ± 0,3
4 mg/L TiO_2	-25,3 ± 1,8	-23,6 ± 1,2	-26,2 ± 0,7	-33,3 ± 1,1	-31,4 ± 0,5	-29,6 ± 1,2	-34,4 ± 1,4	-33,3 ± 0,2	-32,3 ± 0,8
10 mg/L TiO_2	-30,4 ± 1,3	-27,9 ± 0,7	-27,1 ± 1,0	-33,3 ± 0,6	-31,7 ± 0,2	-31,6 ± 0,3	-34,3 ± 0,4	-35,1 ± 0,4	-33,1 ± 0,3

S5 Actual concentrations of silver in mixtures of Ag NPs with TiO_2 NPs in eggwater

		0h				1h				24h			
		TiO_2 (mg/L)											
	Dilution	0	2,5	4	10	0	2,5	4	10	0	2,5	4	10
ions	100x	0,07	0,04	0,05	0,06	0,04	0,04	0,06	0,06	0,02	0,03	0,02	0,02
	300x	0,02	0,03	0,00	0,15	0,02	0,03	0,03	0,03	0,04	0,04	0,03	0,03
	1000x	0,23	0,16	0,22	0,19	0,02	0,01	0,02	0,03	0,05	0,05	0,05	0,05
Total	100x	52,48	55,52	54,08	61,12	2,50	2,36	2,53	2,50	0,11	0,07	0,04	0,06
	300x	8,35	7,65	10,85	9,85	2,01	1,93	2,07	2,05	0,15	0,11	0,05	0,05
	1000x	2,40	2,34	2,38	2,74	1,39	1,77	1,42	1,48	0,18	0,10	0,12	0,12
% ions	100x	0,13	0,08	0,10	0,09	1,44	1,78	2,22	2,32	20,75	40,00	50,00	34,38
	300x	0,28	0,37	0,01	1,54	1,00	1,45	1,35	1,66	25,00	9,36	61,54	64,00
	1000x	9,43	4,37	9,14	6,78	1,15	0,79	1,26	1,75	27,17	48,08	42,37	40,98

S6 Hydrodynamic size distribution (nm) and zeta potential (mV) of uncoated ZnO and TiO_2 NPs in eggwater

Size distribution (nm) + standard error of uncoated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				716 ± 261	906 ± 529	467 ± 33	1463 ± 221	1006 ± 43	842 ± 196
1,5 mg/L TiO_2	2723 ± 498	1800 ± 335	1161 ± 418	1530 ± 237	1617 ± 282	632 ± 17	871 ± 60	2016 ± 361	1018 ± 341
24 mg/L TiO_2	690 ± 52	683 ± 22	263 ± 8	697 ± 17	675 ± 21	278 ± 15	2643 ± 1210	1828 ± 388	1126 ± 446

Zeta potential (mV) + standard error of uncoated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				-21 ± 8	-25 ± 3	-16 ± 1	-6 ± 2	-21 ± 1	-21 ± 0
1,5 mg/L TiO_2	-4 ± 4	-24 ± 1	-25 ± 1	-19 ± 0	-22 ± 3	-23 ± 1	6 ± 3	-22 ± 1	-21 ± 1
24 mg/L TiO_2	-15 ± 1	-19 ± 1	-25 ± 1	-14 ± 0	-19 ± 1	-23 ± 0	0 ± 1	-23 ± 1	-22 ± 0

S7 Hydrodynamic size distribution (nm) and zeta potential (mV) of hydrophobic coated ZnO and TiO_2 NPs in eggwater

Size distribution (nm) + standard error of hydrophobic coated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				2158 ± 153	1187 ± 598	911 ± 336	746 ± 54	951 ± 118	914 ± 193
1,5 mg/L TiO_2	2723 ± 498	1800 ± 335	1161 ± 418	996 ± 232	1313 ± 187	937 ± 197	792 ± 82	747 ± 58	589 ± 80
24 mg/L TiO_2	690 ± 52	683 ± 22	263 ± 8	797 ± 49	804 ± 83	248 ± 9	1059 ± 25	1089 ± 5	499 ± 61

Zeta potential (mV) + standard error of hydrophobic coated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				-7 ± 5	-11 ± 3	-7 ± 3	-13 ± 2	-14 ± 1	-19 ± 5
1,5 mg/L TiO_2	-4 ± 4	-24 ± 1	-25 ± 1	-10 ± 7	-26 ± 2	-9 ± 3	5 ± 2	-18 ± 0	-22 ± 4
24 mg/L TiO_2	-15 ± 1	-19 ± 1	-25 ± 1	-17 ± 3	-22 ± 1	-24 ± 1	3 ± 2	-12 ± 0	-21 ± 1

S8 Hydrodynamic size distribution (nm) and zeta potential (mV) of hydrophilic coated ZnO NPs and TiO_2 NPs in eggwater

Size distribution (nm) + standard error of hydrophilic coated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				1020 ± 380	1567 ± 1319	2544 ± 1947	2650 ± 1724	995 ± 229	1220 ± 153
1,5 mg/L TiO_2	2723 ± 498	1800 ± 335	1161 ± 418	2226 ± 278	1534 ± 45	1985 ± 213	1660 ± 159	1264 ± 281	1176 ± 215
24 mg/L TiO_2	690 ± 52	683 ± 22	263 ± 8	750 ± 113	816 ± 81	316 ± 13	1463 ± 164	1096 ± 165	379 ± 34

Zeta potential (mV) + standard error of hydrophilic coated ZnO and TiO_2 NPs in eggwater

	0 mg/L ZnO			2 mg/L ZnO			32 mg/L ZnO		
	0 h	1 h	24 h	0 h	1 h	24 h	0 h	1 h	24 h
0 mg/L TiO_2				-9 ± 2	-12 ± 3	-9 ± 3	7 ± 0	-16 ± 2	-17 ± 1
1,5 mg/L TiO_2	-4 ± 4	-24 ± 1	-25 ± 1	-17 ± 3	-26 ± 3	-4 ± 2	-2 ± 1	-18 ± 2	-20 ± 0
24 mg/L TiO_2	-15 ± 1	-19 ± 1	-25 ± 1	-24 ± 3	-28 ± 3	-27 ± 1	-15 ± 1	-20 ± 1	-11 ± 5

S9 Actual concentrations of uncoated, hydrophilic coated and hydrophobic coated ZnO in mixtures with TiO_2 in eggwater

S9. Actual concentrations of uncoated ZnO NPs

		0 h				1 h				24 h			
		TiO_2 (mg/L)											
	ZnO (mg/L)	0	1,5	6	24	0	1,5	6	24	0	1,5	6	24
Ions	2	0,51	0,50	0,58	0,38	0,48	0,48	0,45	0,39	0,59	0,57	0,54	0,43
	8	1,32	1,37	1,22	1,04	1,30	1,39	0,97	0,74	2,02	2,03	1,90	1,29
	32	2,60	2,88	2,72	2,51	2,67	2,87	2,63	2,44	3,65	3,39	3,30	2,90
Total	2	0,73	0,76	0,85	0,80	0,46	0,47	0,46	0,42	0,60	0,58	0,54	0,43
	8	3,00	3,71	3,63	3,82	1,46	1,41	1,28	1,19	2,05	2,09	1,93	1,32
	32	21,87	17,33	20,55	16,80	3,94	3,86	4,08	3,41	3,98	3,79	4,32	3,00
% Ions	2	69,88	65,31	68,21	46,85	103,72	100,49	97,69	92,00	98,44	99,19	100,70	100,33
	8	43,93	36,98	33,59	27,11	89,28	98,18	75,45	61,76	98,18	97,21	98,91	97,70
	32	11,91	16,64	13,22	14,92	67,81	74,32	64,46	71,51	91,85	89,35	76,54	96,65

S9. Actual concentrations of hydrophilic coated ZnO NPs

		0 h				1 h				24 h			
		TiO$_2$ (mg/L)											
	ZnO (mg/L)	0	1,5	6	24	0	1,5	6	24	0	1,5	6	24
Ions	2	0,48	0,53	0,52	0,49	0,39	0,50	0,52	0,50	0,65	0,90	0,88	0,84
	8	1,25	1,29	1,15	0,92	1,39	1,43	1,41	1,18	2,17	2,32	0,61	1,67
	32	1,65	1,76	1,67	1,53	2,24	2,06	1,48	1,95	3,51	3,58	2,09	2,60
Total	2	1,13	1,08	1,02	1,02	0,39	0,43	0,43	0,41	0,37	0,74	0,69	0,61
	8	4,19	4,16	4,12	4,06	1,23	1,37	1,32	1,32	1,87	1,94	1,79	1,42
	32	17,25	17,53	17,63	17,07	4,38	4,57	2,81	3,66	2,96	3,06	2,14	2,15
% Ions	2	42,54	48,70	51,10	48,48	99,88	114,84	119,35	120,00	177,32	121,13	127,68	137,34
	8	29,77	30,98	27,89	22,76	113,14	104,62	107,26	89,20	116,25	120,00	0,00	117,05
	32	9,58	10,06	9,45	8,94	51,20	45,00	52,78	53,12	118,58	117,25	97,70	121,30

S9. Actual concentrations of hydrophobic coated ZnO NPs

		0 h				1 h				24 h			
		TiO₂ (mg/L)											
	ZnO (mg/L)	0	1,5	6	24	0	1,5	6	24	0	1,5	6	24
Ions	2	1,05	1,04	0,96	0,76	1,09	1,08	1,02	0,86	1,15	1,10	1,00	0,77
	8	1,79	1,83	1,71	1,68	1,57	1,76	1,92	1,90	2,93	2,72	2,67	2,37
	32	1,92	1,90	2,06	1,95	2,38	2,46	2,77	2,84	3,70	3,78	3,99	3,56
Total	2	1,16	1,17	1,16	1,16	1,15	1,10	1,09	1,04	1,18	1,13	1,03	0,78
	8	4,41	4,33	4,35	4,34	4,09	4,09	3,96	3,64	2,94	2,81	2,60	2,46
	32	18,06	18,07	17,95	18,03	16,17	15,09	15,08	13,62	3,77	3,92	4,06	3,65
% Ions	2	90,14	88,52	82,69	65,30	94,65	98,22	92,84	82,97	97,38	97,27	97,55	99,28
	8	40,63	42,39	39,23	38,71	38,36	43,15	48,41	52,31	99,52	96,52	102,69	96,02
	32	10,62	10,53	11,47	10,79	14,72	16,33	18,38	20,86	98,14	96,43	98,28	97,32

S10 Actual concentrations of Zn ions with and without TiO_2 after 1-hour incubation in eggwater

Nominal concentration Zn ions (mg/L)	TiO_2 0 mg/L	TiO_2 6 mg/L	Difference
3	2,548	2,518	0,03
1,8	1,552	1,540	0,012
0,9	0,776	0,758	0,018
0,3	0,262	0,258	0,004
0,1	0,100	0,096	0,004

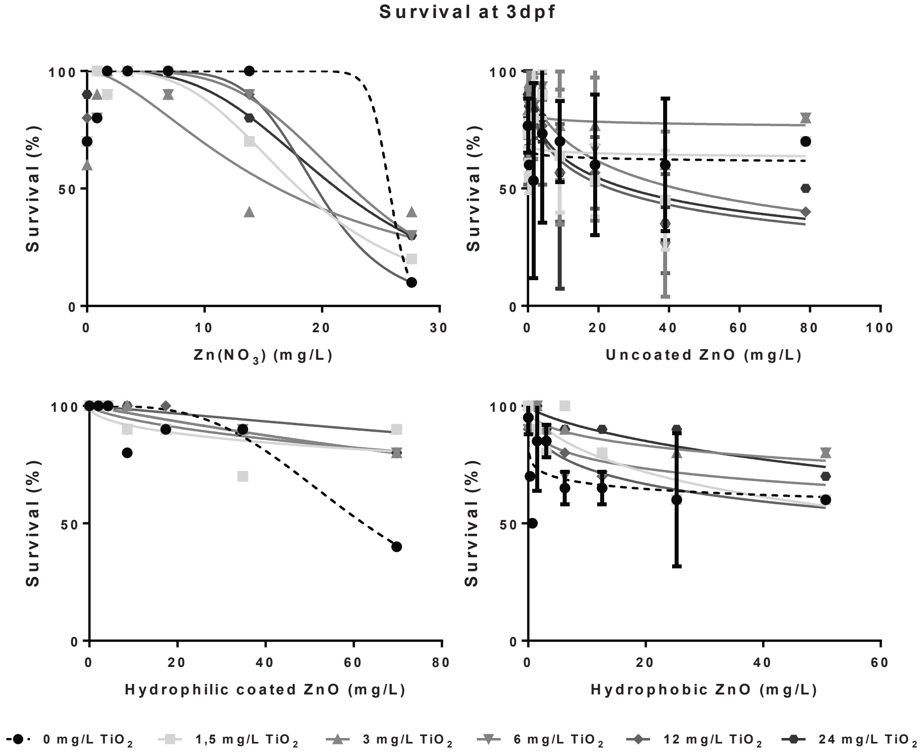
S11 EC_{50} values of Ag NPs with different concentrations of TiO_2 NPs added

Endpoint	**TiO_2 (mg/L)**	**EC_{50} value (mg/L)**	**+ SE**
Malformations	0	2,2	0,7
	2,5	4,6	1,3
	4	7,1	8,7
	10	3,0	0,6
	25	3,4	0,5
	40	2,7	0,4
Non-inflated swimbladder	0	0,7	0,2
	2,5	0,7	0,2
	4	0,8	0,6
	10	0,8	0,1
	25	1,0	0,1
	40	1,4	0,2

S12 EC_{50} values of differently coated ZnO NPs with different concentrations of TiO_2 NPs added and the relative contribution of ions and particles

Coating	TiO_2 (mg/L)	EC_{50} value (mg/L)	+ SD	Relative contribution (%)	
				NP^{ion}	$Np^{particle}$
Uncoated	0	4,1	1,8	100,1	-0,1
	1,5	7,2	1,9	99,8	0,2
	3	7,6	1,4	99,8	0,2
	6	2,2	1,6	100,5	-0,5
	12	2,3	1,4	100,3	-0,3
	24	2,0	0,6	100,4	-0,4
Hydrophilic	0	7,6	0,4	100,2	-0,2
	1,5	7,4	1,3	100,2	-0,2
	3	6,1	10839,0	100,2	-0,2
	6	~ 8,12	~ 10315442	#VALUE!	#VALUE!
	12	6,1	12332,0	100,2	-0,2
	24	4,07900	0,0	100,4	-0,4
Hydrophobic	0	2,1	0,7	100,1	-0,1
	1,5	3,1	0,6	100,0	0,0
	3	3,6	0,5	100,0	0,0
	6	2,56	0,42	100,1	-0,1
	12	2,97	0,66	100,0	0,0
	24	3,5	0,5	100,0	0,0

Figure



S1 *Survival of zebrafish embryos at 3 dpf after exposure to a zinc-ion control ($Zn(NO_3)_2$; a) or differently coated ZnO NPs (b,c,d) in combination with different concentrations of TiO_2 NPs.*

References

(1) Wilke, C. M.; Wunderlich, B.; Gaillard, J. F.; Gray, K. A. Synergistic bacterial stress results from exposure to nano-Ag and nano-TiO2 mixtures under light in environmental media. *Environ. Sci. Technol.* **2018**, *52* (5), 3185–3194.

(2) Leite-Silva, V. R.; Liu, D. C.; Sanchez, W. Y.; Studier, H.; Mohammed, Y. H.; Holmes, A.; Becker, W.; Grice, J. E.; Benson, H. A.; Roberts, M. S. Effect of flexing and massage on in vivo human skin penetration and toxicity of zinc oxide nanoparticles. *Nanomedicine* **2016**, *11* (10), 1193–1205.

(3) Sajid, M.; Ilyas, M.; Basheer, C.; Tariq, M.; Daud, M.; Baig, N.; Shehzad, F. Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects. *Environ. Sci. Pollut. Res.* **2015**, *22* (6), 4122–4143.

(4) Altenburger, R.; Backhaus, T.; Boedeker, W.; Faust, M.; Scholze, M. Simplifying complexity: Mixture toxicity assessment in the last 20 years. *Environ. Toxicol. Chem.* **2013**, *32* (8), 1685–1687.

(5) Klaine, S. J.; Alvarez, P. J. J.; Batley, G. E.; Fernandes, T. F.; Handy, R. D.; Lyon, D. Y.; Mahendra, S.; McLaughlin, M. J.; Lead, J. R. Nanomaterials in the environment: behaviour, fate, bioavailabilty, and effects. *Environ. Toxicol. Chem.* **2008**, *27* (9), 1825.

(6) Chong, M. N.; Jin, B.; Chow, C. W. K.; Saint, C. Recent developments in photocatalytic water treatment technology: A review. *Water Res.* **2010**, *44* (10), 2997–3027.

(7) Ko, K.-S.; Koh, D.-C.; Kong, I. Toxicity Evaluation of Individual and Mixtures of Nanoparticles Based on Algal Chlorophyll Content and Cell Count. *Materials (Basel).* **2018**, *11* (1), 121.

(8) van Pomeren, M.; Peijnenburg, W.; Brun, N.; Vijver, M. A novel experimental and modelling strategy for nanoparticle toxicity testing enabling the use of small quantities. *Int. J. Environ. Res. Public Health* **2017**, *14* (11), 1348.

(9) Suttiponparnit, K.; Jiang, J.; Sahu, M.; Suvachittanont, S.; Charinpanitkul, T.; Biswas, P. Role of Surface Area, Primary Particle Size, and Crystal Phase on Titanium Dioxide Nanoparticle Dispersion Properties. *Nanoscale Res. Lett.* **2011**, *6* (1), 1–8.

(10) Auffan, M.; Rose, J.; Proux, O.; Borschneck, D.; Masion, A.; Chaurand, P.; Hazemann, J.-L.; Chaneac, C.; Jolivet, J.-P.; Wiesner, M. R.; et al. Enhanced Adsorption of Arsenic onto Maghemites Nanoparticles: As(III) as a Probe of the Surface Structure and Heterogeneity. *Langmuir* **2008**, *24* (7), 3215–3222.

(11) Hua, J.; Peijnenburg, W. J. G. M.; Vijver, M. G. TiO2nanoparticles reduce the effects of ZnO nanoparticles and Zn ions on zebrafish embryos (Danio rerio). *NanoImpact* **2016**, *2*, 45–53.

(12) van Pomeren, M.; Brun, N. R.; Peijnenburg, W. J. G. M.; Vijver, M. G. Exploring uptake and biodistribution of polystyrene (nano) particles in zebrafish embryos at different developmental stages. *Aquat. Toxicol.* **2017**, *190* (June), 40–45.

(13) Hua, J.; Vijver, M. G.; Richardson, M. K.; Ahmad, F.; Peijnenburg, W. J. G. M. Particle-specific toxic effects of differently shaped zinc oxide nanoparticles to zebrafish embryos (Danio rerio). *Environ. Toxicol. Chem.* **2014**, *33* (12), 2859–2868.

(14) Poynton, H. C.; Lazorchak, J. M.; Impellitteri, C. A.; Blalock, B. J.; Rogers, K.; Allen, H. J.; Loguinov, A.; Heckman, J. L.; Govindasmawy, S. Toxicogenomic responses of nanotoxicity in Daphnia magna exposed to silver nitrate and coated silver nanoparticles. *Environ. Sci. Technol.* **2012**, *46* (11), 6288–6296.

(15) Bonfanti, P.; Moschini, E.; Saibene, M.; Bacchetta, R.; Rettighieri, L.; Calabri, L.; Colombo, A.; Mantecca, P. Do nanoparticle physico-chemical properties and developmental exposure window influence nano ZnO embryotoxicity in Xenopus laevis? *Int. J. Environ. Res. Public Health* **2015**, *12* (8), 8828–8848.

(16) Chen, G.; Peijnenburg, W. J. G. M.; Xiao, Y.; Vijver, M. G. Developing species sensitivity distributions for metallic nanomaterials considering the characteristics of nanomaterials, experimental conditions, and different types of endpoints. *Food Chem. Toxicol.* **2018**, *112*, 563–570.

(17) Bermejo-Nogales, A.; Fernández-Cruz, M. L.; Navas, J. M. Fish cell lines as a tool for the ecotoxicity assessment and ranking of engineered nanomaterials. *Regul. Toxicol. Pharmacol.* **2017**, *90*, 297–307.

(18) Farcal, L.; Andón, F. T.; Di Cristo, L.; Rotoli, B. M.; Bussolati, O.; Bergamaschi, E.; Mech, A.; Hartmann, N. B.; Rasmussen, K.; Riego-Sintes, J.; et al. Comprehensive in vitro toxicity testing of a panel of representative oxide nanomaterials: First steps towards an intelligent testing strategy. *PLoS One* **2015**, *10* (5), 1–34.

(19) Li, X.; Fang, X.; Ding, Y.; Li, J.; Cao, Y. Toxicity of ZnO nanoparticles (NPs) with or without hydrophobic surface coating to THP-1 macrophages: interactions with BSA or oleate-BSA. *Toxicol. Mech. Methods* **2018**, *28* (7), 520–528.

(20) Kathawala, M. H.; Ng, K. W.; Loo, S. C. J. TiO2 nanoparticles alleviate toxicity by reducing free Zn2+ ion in human primary epidermal keratinocytes exposed to ZnO nanoparticles. *J. Nanoparticle Res.* **2015**, *17* (6), 263.

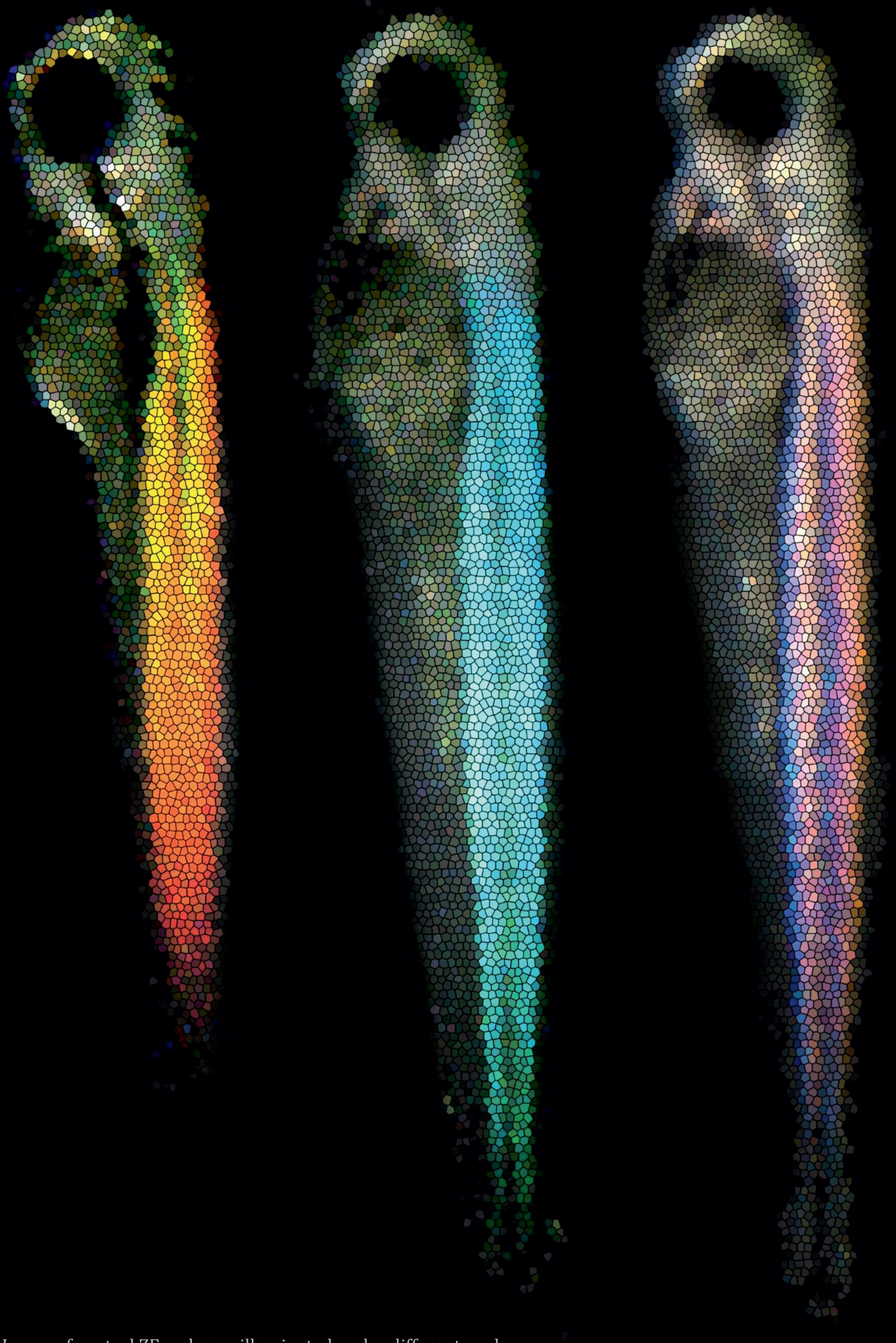


Image of control ZF embryos illuminated under different angles