Electronic Supplementary Information for:

Effect and mechanism of zinc oxide nanoparticle transformations on toxicity to zebrafish embryo

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Table S1. The neoformation level of sulfidation and phosphation. The values are calculated using intensities of XRD peaks. The neoformation level in sulfidation is obtained by dividing XRD peak intensity at 29° by that at 36° (P_{29}/P_{36}), and it is obtained by dividing XRD peak intensity at 31° by that at 36° (P_{31}/P_{36}) in phosphation. The neoformation levels increased with increasing the molar ratios. The neoformation was not observed in Zn-FeOOH; thus the neoformation level of Zn-FeOOH cannot be calculated.

Neoformation level									
Sulfidation					Phosphation				
Ratio	0	0.484	0.968	1.936	Ratio	0	0.484	0.968	1.936
P_{29}	35	215	420	524	P_{31}	101	123	110	152
P_{36}	1001	286	77	34	P_{36}	1001	432	227	263
P_{29}/P_{36}	0.03	0.75	5.45	15.41	P_{31}/P_{36}	0.10	0.28	0.48	0.58

Table S2. Percentage of malformation induced by three kinds of transformed zinc oxide nanoparticle. The values were calculated by the number of malformed larvae per the number of alive larvae.

	Molar ratios					
	0.484	0.968	1.936	4.84	9.68	19.36
Sulfidation (S/Zn)	60% (3/5)	19% (12/62)	21% (10/48)			
Phosphation (PO_4/Zn)	39% (22/56)	24% (15/62)	22% (14/65)			
Zn-FeOOH (Fe/Zn)	50% (3/6)	89% (8/9)	56% (10/18)	29% (7/24)	46% (18/39)	32% (18/56)

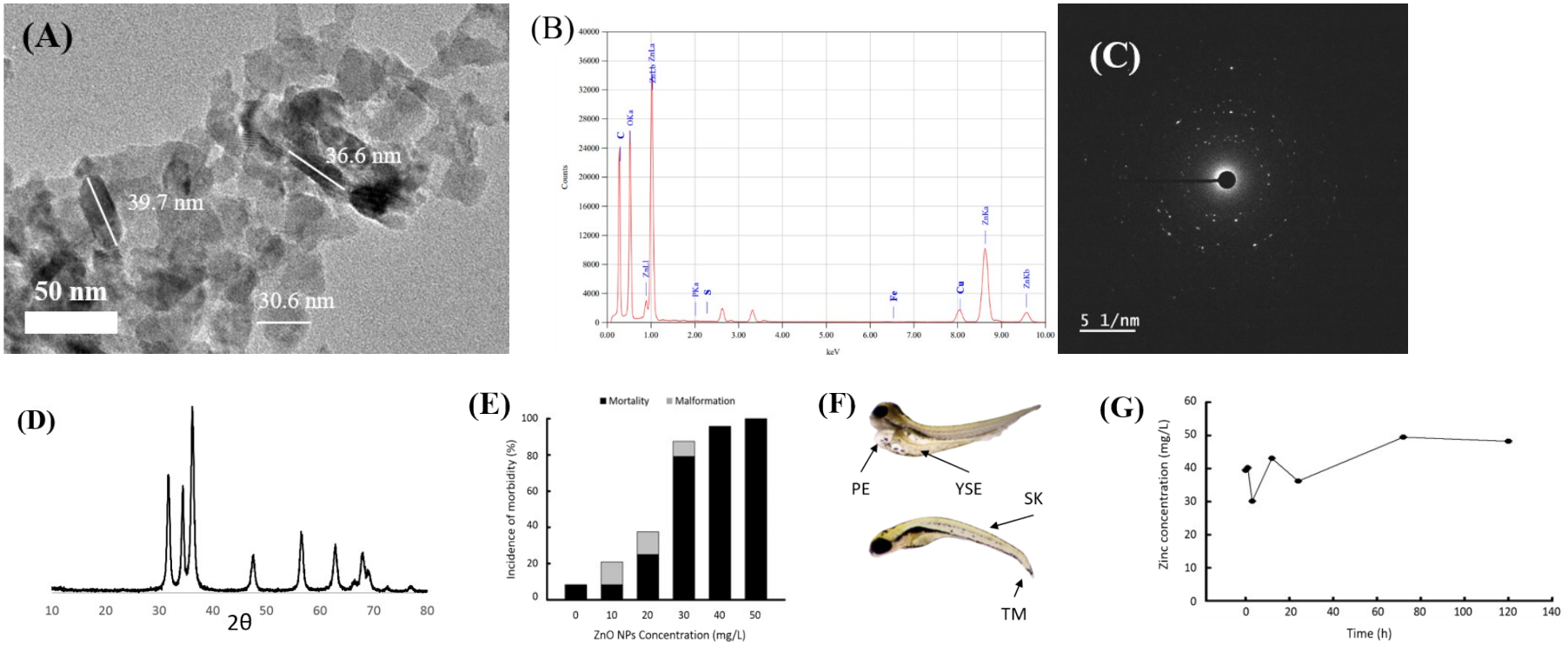
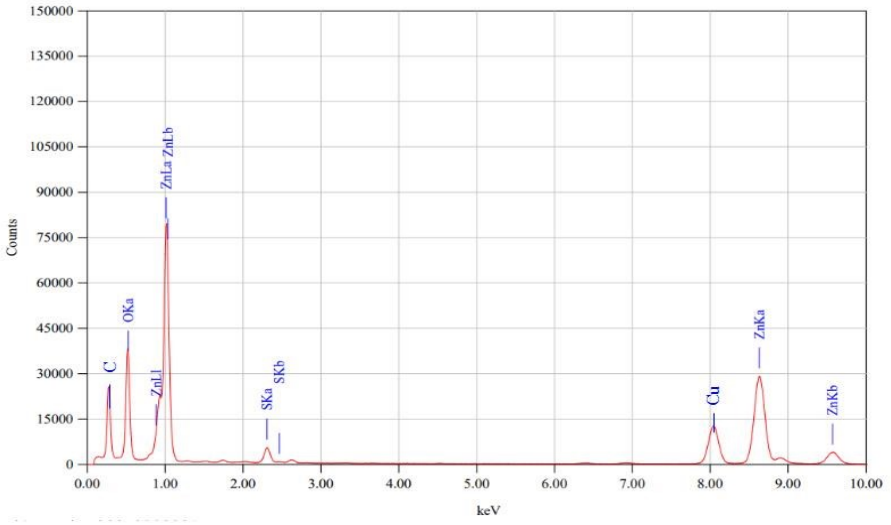
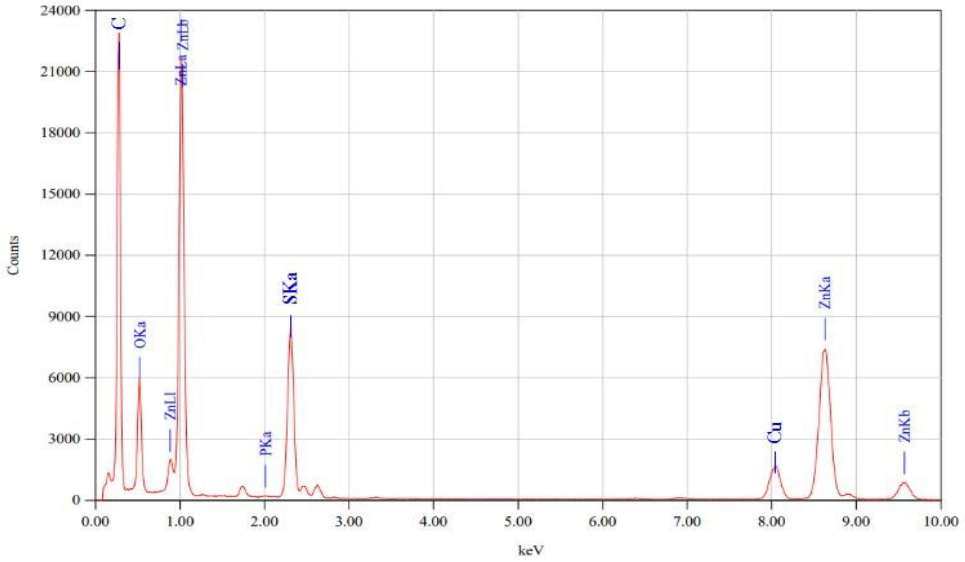
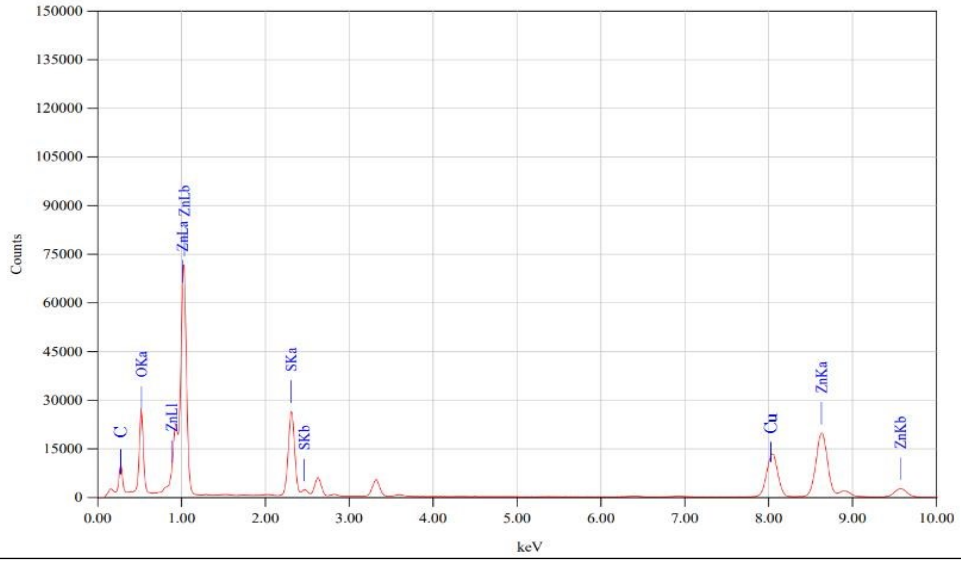
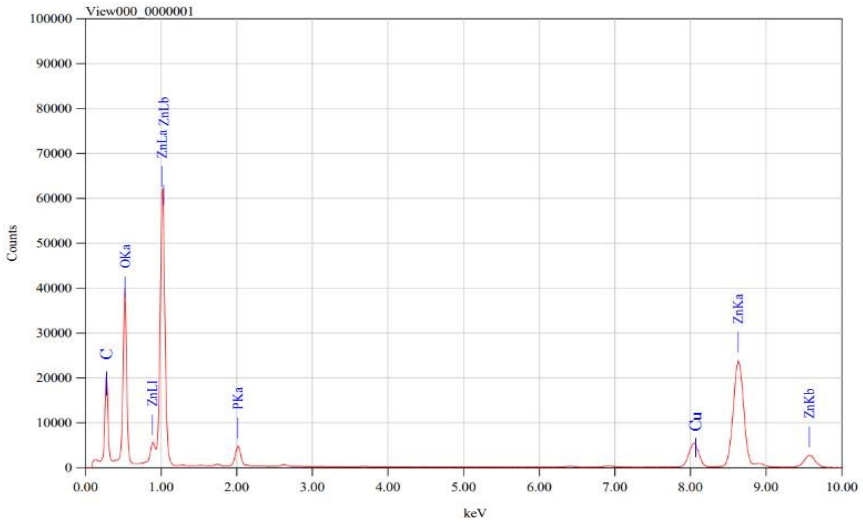
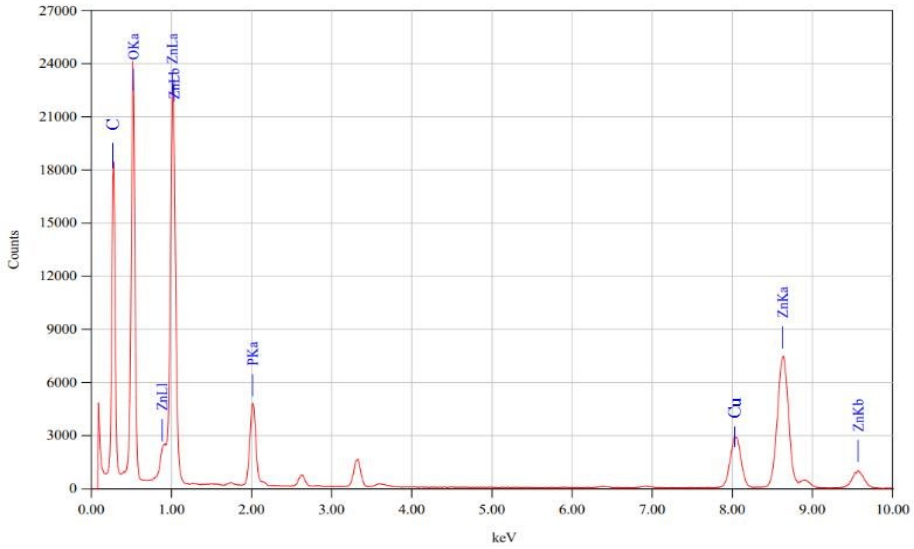
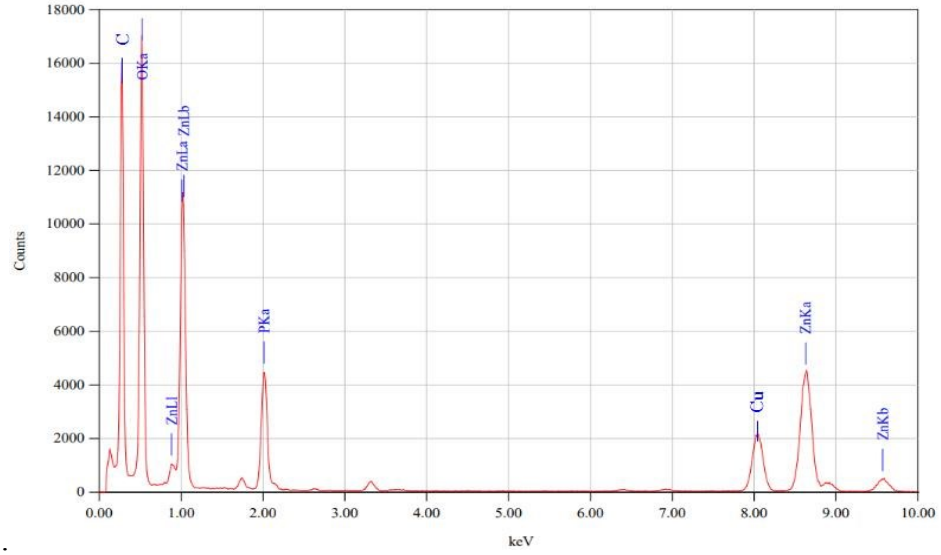


Figure S1. Properties of pristine zinc oxide nanoparticle: (A) TEM image; (B) EDX result; (C) SAED pattern image (D) XRD peaks; (E) embryonic toxicity at different concentrations; (F) images for malformed embryos pericardial edema (PE), yolk sac edema (YSE), spinal kyphosis (SK), and tail malformation (TM); (G) zinc concentration in bulk solution over time.

Molar ratios	Sulfidation EDX results
(A) 0.484	
(B) 0.968	
(C) 1.936	

Molar ratios	Phosphation EDX results
(D) 0.484	
(E) 0.968	
(F) 1.936	

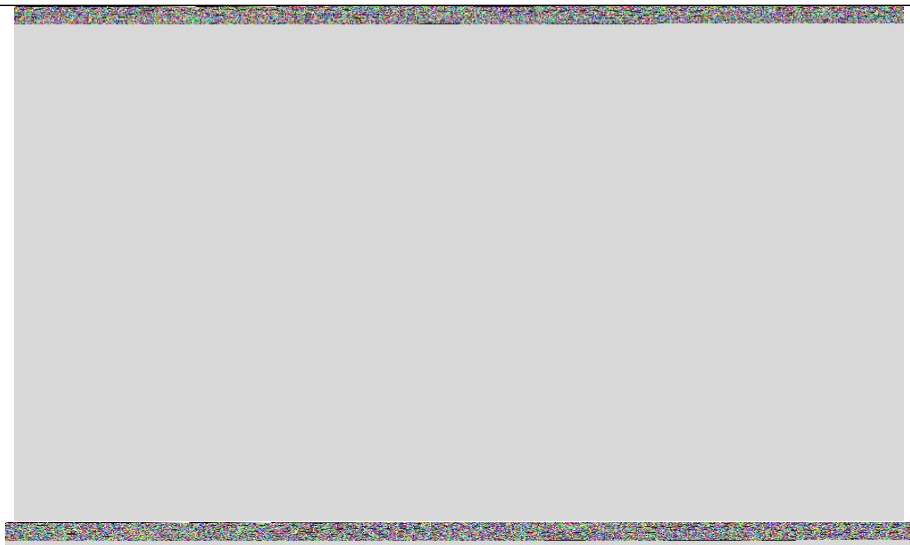
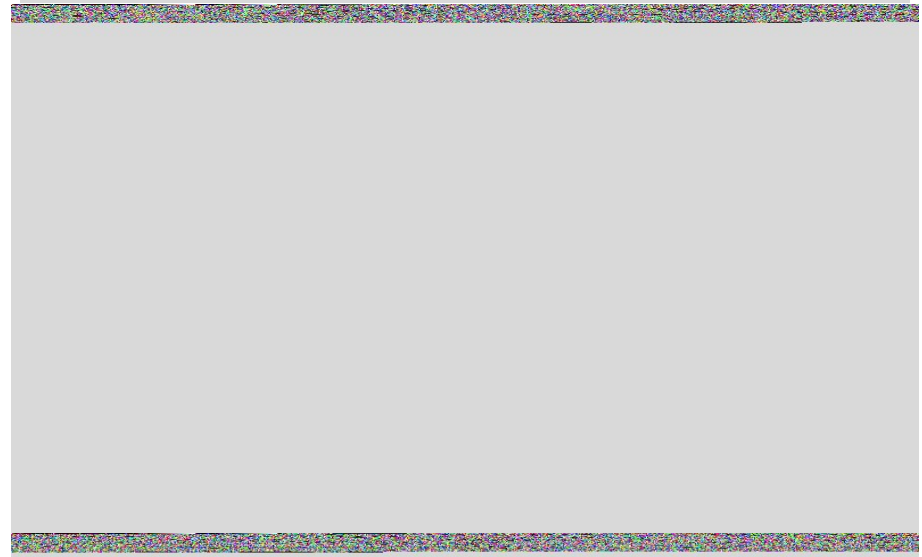
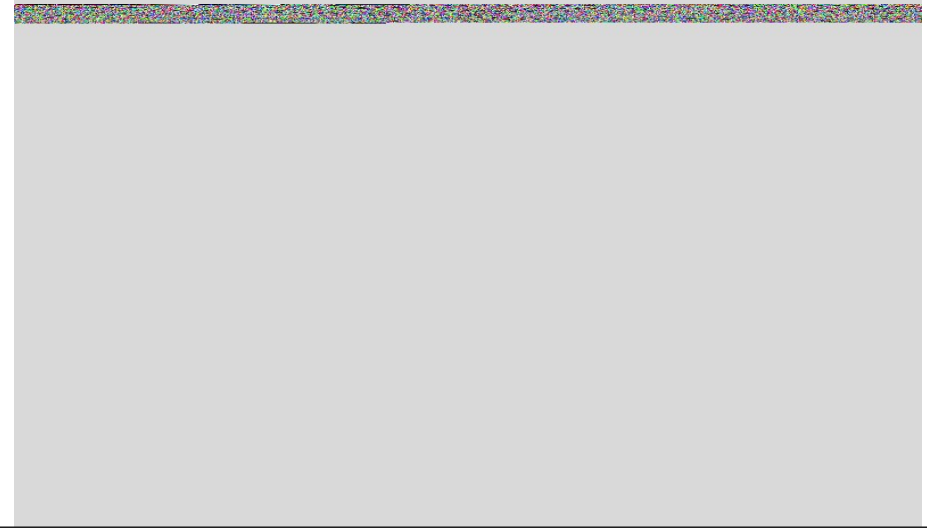
Molar ratios	Zn-FeOOH EDX results
(G) 0.484	
(H) 0.968	
(I) 1.936	

Figure S2. Elemental composition of each transformed ZnO NPs is analyzed by EDX for (A-C) sulfidation; (D-F) phosphation; and (G-I) Zn-FeOOH.

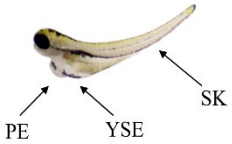
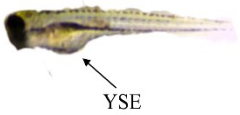
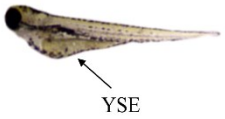
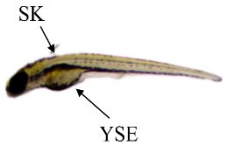
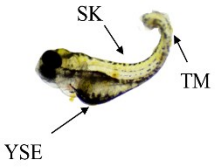
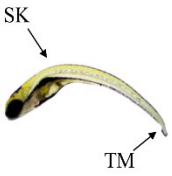
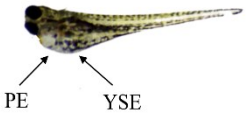
		Molar ratios		
		0.484	0.968	1.936
(A)	Sulfidation (S/Zn)			
(B)	Phosphation (PO_4/Zn)			
(C)	Zn-FeOOH (Fe/Zn)			

Figure S3. Malformations of zebrafish larvae exposed to transformed zinc oxide nanoparticles (ZnO NPs) at different molar ratios of S/Zn, PO_4/Zn, and Fe/Zn for (A) sulfidation; (B) phosphation; and (C) Zn-FeOOH. Pericardial edema (PE), Yolk sac edema (YSE), spinal kyphosis (SK), and tail malformation (TM).

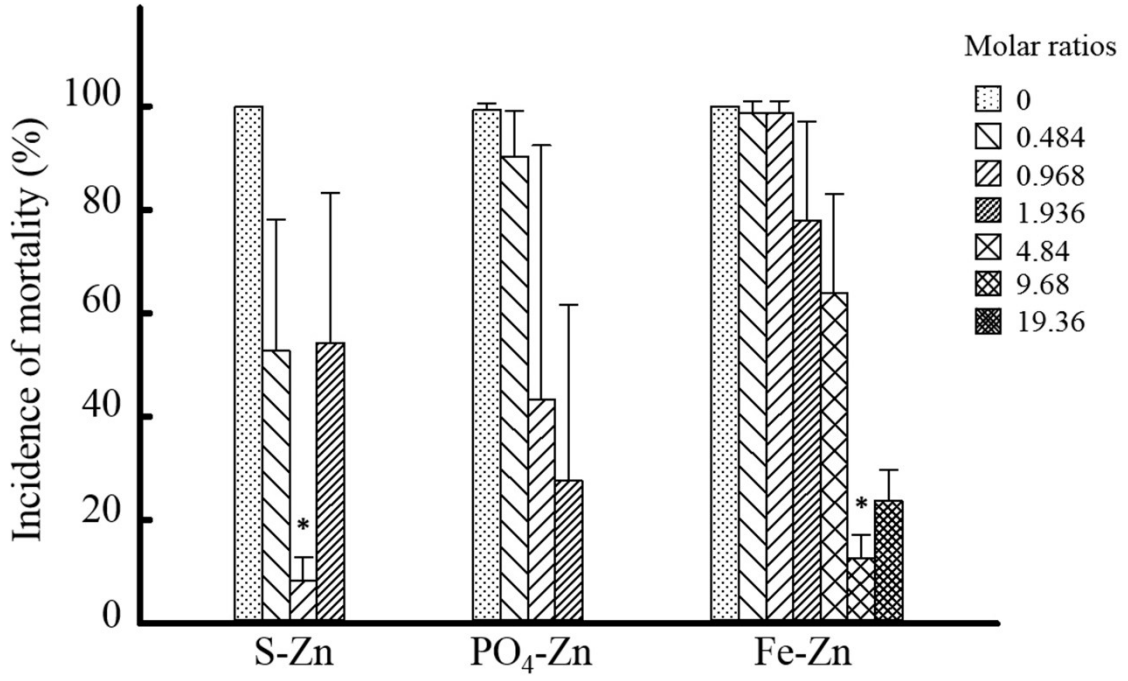


Figure S4. Embryonic toxicity of solutions in which each sulfur, phosphate, and hydrous ferric oxide were added to zinc chloride ($ZnCl_2$) at different molar ratios of S/Zn, PO_4/Zn, and Fe/Zn. The concentration of $ZnCl_2$ was fixed at 84 mg/L, which corresponds to the same zinc molar concentration in 50 mg/L zinc oxide nanoparticles. For toxicity of Zn-FeOOH, Kruskal-Wallis followed by Turkey`s post-hoc was used for multiple comparisons because the equal variance test was failed. Twenty-four embryos were exposed to each solution. (*$p < 0.05$)

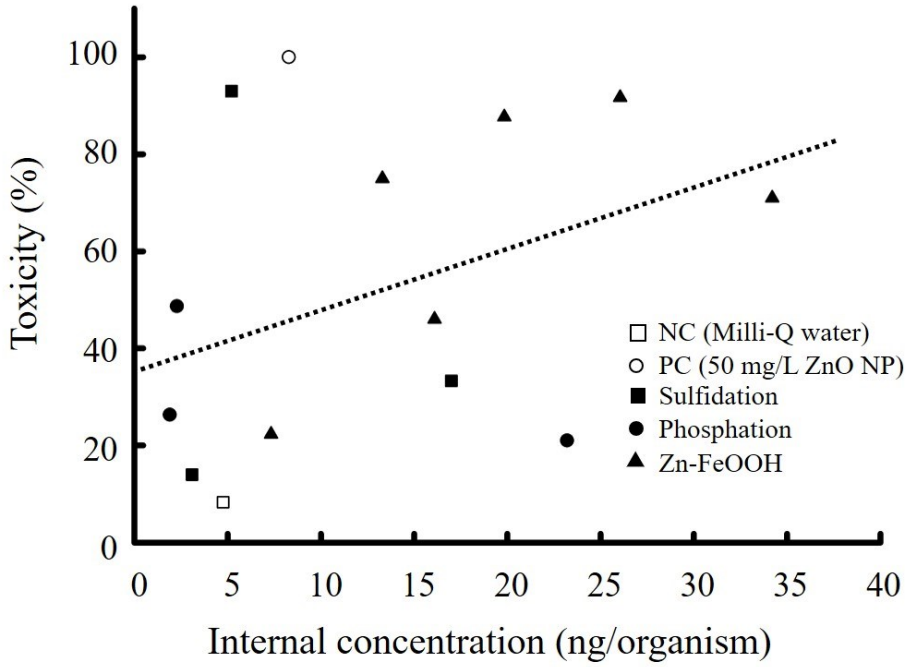


Figure S5. Correlation between internal concentration of zinc and toxicity of transformed ZnO NPs. The correlation between internal concentration of zinc in larvae and observed toxicity varied by transformation. Toxicity is not well matched in sulfidation and phosphation, while toxicity is correlated with internal concentration of zinc in Zn-FeOOH.

[Method]

We measured the internal concentration of zinc ions in larvae by using ICP-MS. We collected 60 larvae (20 larvae per each treatment) exposed to pristine and three types of transformed ZnO NPs from 4 to 72 hpf. Because of high mortality in pristine and sulfide ZnO NP at 72 and 96 hpf, respectively, we determined the time point of 72 hpf for internal zinc analysis. After exposure, the pooled larvae were rinsed three times with Milli-Q water and digested with nitric acid on 100°C heat block. The digested was diluted by Milli-Q water to 10 mL and the concentration of zinc ions was analyzed. The internal concentration of zinc was correlated with toxicity observed at 120 hpf.