Electronic Supplementary Information (ESI) for *Environmental Science: Nano*

Nickel oxide nanoparticles induce developmental neurotoxicity in zebrafish by triggering apoptosis and ferroptosis.

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Figure S2. The median lethal concentration (LC50) of NiO-NPs to zebrafish larvae.

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Table S1. Sequences of primers used in this study.

	gene name	Sequences of the primers (5' – 3')
Neurodevelopment-related	*elavl3*	F: AGACAAGATCACAGGCCAGAGCTT
		R: TGGTCTGCAGTTTGAGACCGTTGA
	gap43	F: GCAGCAGGAAGTGGAGAAGCCA
		R: GGATTCCTCAGCAGCGTCTGGT
	syn2α	F: GTGACCATGCCAGCATTTC
		R: TGGTTCTCCACTTTCACCTT
	mbpα	F: AATCAGCAGGTTCTTCGGAGGAGA
		R: AAGAAATGCACGACAGGGTTGACG
Apoptosis-related	*bax*	F: GGCTATTTCAACCAGGGTTCC
		R: TGCGAATCACCAATGCTGT
	bcl2	F: AACCCAAATTCTGCGCAACG
		R: ATCTACCTGGGACGCCATCT
	caspase3b	F: ATCTACGGCACGGACAACAG
		R: AACCAGGAGCCATTAGCGAC
	caspase8	F: TGTCTCAGCCTATAGAAAGATGC
		R: AAATGAAGTAGATCTTCCCAGCT
	caspase9	F: TCTGCCAGTCCAAAATCTAAATC
		R: GCATCCATCTTGTAGCACTGTAT
Ferroptosis-related	*ptgs2a*	F: TGAGGAGATGACAGGAGACA
		R: CACAAGAAGGCCAGGATACA
	ptgs2b	F: GCTGACATCACGTGCCCATC
		R: TACCTGCGACTCCCATTGGC
	nrf2	F: GACAAAATCGGCGACAAAAT
		R: TTAGGCCATGTCCACACGTA
	dmt1	F: CTGCGCTCTACATCTGGGCT
		R: CTGCACGTCCTGAAACACGG
	tf	F: AAGGAAAGGACTGGGCAAAGGA

		R: TCACAGCATGAACTGGCACTTG
	tfr	F: ATGGATCAAGCCAGGACAACC
		R: CTAAAGAGGTGAGCTGAAG
	fth	F: CAGGACGTGAAGAAACCAGAGAAGG
		R: ATGTGAGGGTCGTTGTGTTGAGATG
	gap4a	F: TGCGACACCACAACTGACTGAAC
		R: ACCAATGATCCCACTGCTCGATAAAG
	slc7all	F: CGCCATTGCCATTATCATT
		R: TTCTCCACCTCCTCAGTC
	ho-1	F: ATGCCCTTGTTTCCAGTCAGC
		R: GGACTTGGAGCACTTCTTCGG

Table S2. Detailed statistical information in this study.

Figure	Test method		Significance		N
Figure. 2B Survival rate					
6 dpf	Ctrl	One-way ANOVA	F=2.919; P=0.04 *		N=3
	5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.10	ns	
	10 mg/L vs. Ctrl		P=0.29	ns	
	20 mg/L vs. Ctrl		P=0.50	ns	
	50 mg/L vs. Ctrl		P=0.02	*	
	100 mg/L vs. Ctrl		P=0.02	*	
□					
Figure. 2C Hatching rate					
60 hpf	Ctrl	One-way ANOVA	F=0.8533; P=0.53 ns		N=4
	5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.7236	ns	
	10 mg/L vs. Ctrl		P=0.6070	ns	
	20 mg/L vs. Ctrl		P=0.0693	ns	
	50 mg/L vs. Ctrl		P=0.0387	*	
	100 mg/L vs. Ctrl		P=0.0270	*	
□					
Figure. 2D Body length					
Ctrl		Kruskal-Wallis	H=11.99; P=0.03 *		N=19
5 mg/L vs. Ctrl		Dunn's multiple comparisons	P=0.13	ns	N=18
10 mg/L vs. Ctrl			P=0.01	*	N=22
20 mg/L vs. Ctrl			P=0.04	*	N=23
50 mg/L vs. Ctrl			P=0.01	*	N=28
100 mg/L vs. Ctrl			P=0.01	*	N=20
□					
Figure. 2E Heart rate					

Ctrl	One-way ANOVA	F=34.62; P<0.001 ***		N=11
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.05	*	N=11
10 mg/L vs. Ctrl		P<0.001	***	N=11
20 mg/L vs. Ctrl		P<0.001	***	N=11
50 mg/L vs. Ctrl		P<0.001	***	N=11
100 mg/L vs. Ctrl		P<0.001	***	N=11
□				
Figure. 2F spontaneous movement (26 hpf)				
Ctrl	Kruskal-Wallis	H=2.824; P=0.73 ns		N=19
5 mg/L vs. Ctrl	Dunn's multiple comparisons	P>0.99	ns	N=17
10 mg/L vs. Ctrl		P=0.92	ns	N=19
20 mg/L vs. Ctrl		P>0.99	ns	N=16
50 mg/L vs. Ctrl		P=0.01	*	N=18
100 mg/L vs. Ctrl		P=0.02	*	N=18
□				
Figure. 3C Distance moved by larvae under locomotion test				
Ctrl	One-way ANOVA	F=6.042; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.06	ns	N=16
10 mg/L vs. Ctrl		P=0.02	*	N=16
20 mg/L vs. Ctrl		P=0.02	*	N=16
50 mg/L vs. Ctrl		P=0.02	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3D Velocity of larvae under locomotion test				
Ctrl	One-way ANOVA	F=6.042; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.06	ns	N=16
10 mg/L vs. Ctrl		P=0.02	*	N=16
20 mg/L vs. Ctrl		P=0.02	*	N=16
50 mg/L vs. Ctrl		P=0.02	*	N=16

100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3E Cumulative mobility of larvae under locomotion test				
Ctrl	Kruskal-Wallis	H=25.99; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunn's multiple comparisons	P=0.06	ns	N=16
10 mg/L vs. Ctrl		P=0.02	*	N=16
20 mg/L vs. Ctrl		P=0.04	*	N=16
50 mg/L vs. Ctrl		P=0.03	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3F Maximum acceleration of larvae under locomotion test				
Ctrl	One-way ANOVA	F=1.606; P=0.17 ns		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.5	ns	N=16
10 mg/L vs. Ctrl		P=0.42	ns	N=16
20 mg/L vs. Ctrl		P=0.42	ns	N=16
50 mg/L vs. Ctrl		P=0.42	ns	N=16
100 mg/L vs. Ctrl		P=0.04	*	N=16
□				
Figure. 3I Distance moved of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=3.659; P=0.005 **		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.02	*	N=16
10 mg/L vs. Ctrl		P=0.01	**	N=16
20 mg/L vs. Ctrl		P=0.03	*	N=16
50 mg/L vs. Ctrl		P=0.04	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3J Velocity of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=3.659; P=0.005 **		N=16
5 mg/L vs. Ctrl	Dunnett's multiple	P=0.02	*	N=16

10 mg/L vs. Ctrl	comparisons	P=0.01	**	N=16
20 mg/L vs. Ctrl		P=0.03	*	N=16
50 mg/L vs. Ctrl		P=0.04	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3K Cumulative mobility of larvae during whole tapping stimulation test				
Ctrl	Kruskal-Wallis	H=16.59; P=0.005 **		N=16
5 mg/L vs. Ctrl	Dunn's multiple comparisons	P=0.03	*	N=16
10 mg/L vs. Ctrl		P=0.02	**	N=16
20 mg/L vs. Ctrl		P=0.04	*	N=16
50 mg/L vs. Ctrl		P=0.20	ns	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3L Maximum acceleration of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=74.33; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P>0.99	ns	N=16
10 mg/L vs. Ctrl		P<0.001	***	N=16
20 mg/L vs. Ctrl		P<0.001	***	N=16
50 mg/L vs. Ctrl		P<0.001	***	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3M Distance moved of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=9.191; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.97	ns	N=16
10 mg/L vs. Ctrl		P=0.75	ns	N=16
20 mg/L vs. Ctrl		P=0.02	*	N=16
50 mg/L vs. Ctrl		P=0.02	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				

Figure. 3N Velocity of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=9.191; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.97	ns	N=16
10 mg/L vs. Ctrl		P=0.75	ns	N=16
20 mg/L vs. Ctrl		P=0.02	*	N=16
50 mg/L vs. Ctrl		P=0.02	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3O Cumulative mobility of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=9.191; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.03	*	N=16
10 mg/L vs. Ctrl		P=0.07	ns	N=16
20 mg/L vs. Ctrl		P<0.001	***	N=16
50 mg/L vs. Ctrl		P=0.005	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 3P Maximum acceleration of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=5.493; P<0.001 ***		N=16
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.69	ns	N=16
10 mg/L vs. Ctrl		P=0.21	ns	N=16
20 mg/L vs. Ctrl		P=0.02	*	N=16
50 mg/L vs. Ctrl		P=0.02	*	N=16
100 mg/L vs. Ctrl		P<0.001	***	N=16
□				
Figure. 4C Relative density of periventricular neurons (%)				
Ctrl	One-way ANOVA	F=6.801; P<0.001 ***		N=6
5 mg/L vs. Ctrl	Dunnett's multiple comparisons	P=0.02	*	N=6
10 mg/L vs. Ctrl		P<0.001	***	N=6
20 mg/L vs. Ctrl		P<0.001	***	N=6

50 mg/L vs. Ctrl			P<0.001	***	N=6
100 mg/L vs. Ctrl			P=0.009	**	N=6
□					
Figure. 4D-G Relative expression levels of neurodevelopment-related genes					
elavl3	Ctrl	One-way ANOVA	F=92.96; P<0.001 ***		N=4
	10 mg/L vs. Ctrl	Dunnett's multiple comparisons	P<0.001	***	
	100 mg/L vs. Ctrl		P<0.001	***	
gap43	Ctrl		F=33.16; P<0.001 ***		N=4
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P=0.001	**	
syn2α	Ctrl		F=27.33; P<0.001 ***		N=4
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P=0.01	*	
mbpa	Ctrl		F=7.530; P=0.01 *		N=4
	10 mg/L vs. Ctrl		P=0.02	*	
	100 mg/L vs. Ctrl		P=0.02	*	
□					
Figure. 5B Relative density of apoptotic cells					
Ctrl		One-way ANOVA	F=25.86; P<0.001 ***		N=10
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P<0.001	***	N=8
100 mg/L vs. Ctrl			P<0.001	***	N=8
□					
Figure. 5C Relative expression levels of apoptosis-related genes					
bax	Ctrl	One-way ANOVA	F=141.1; P<0.001 ***		N=3
	10 mg/L vs. Ctrl	Dunnett's multiple comparisons	P<0.001	***	
	100 mg/L vs. Ctrl		P<0.001	***	
bcl	Ctrl		F=46.57; P<0.001 ***		N=3
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P<0.001	***	

caspase3b	Ctrl		F=167.9; P<0.001 ***		N=3
	10 mg/L vs. Ctrl		P=0.009	**	
	100 mg/L vs. Ctrl		P<0.001	***	
caspase8	Ctrl		F=10.70; P=0.01 *		N=3
	10 mg/L vs. Ctrl		P=0.01	*	
	100 mg/L vs. Ctrl		P=0.02	*	
caspase9	Ctrl		F=40.10; P<0.001 ***		N=3
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P=0.002	**	
□					
Figure. 5D Relative content of iron in zebrafish larvae (%)					
Ctrl		One-way ANOVA	F=78.17; P<0.001 ***		N=3
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P=0.001	**	
100 mg/L vs. Ctrl			P<0.001	***	
Figure. 5D Relative content of MDA in zebrafish larvae (%)					
Ctrl		One-way ANOVA	F=9.221; P=0.01 *		N=3
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P=0.01	*	
100 mg/L vs. Ctrl			P=0.05	*	
□					
Figure. 5E Relative content of total glutathione in zebrafish larvae (%)					
Ctrl		One-way ANOVA	F=6.784; P=0.03 *		N=3
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P=0.83	ns	
100 mg/L vs. Ctrl			P=0.03	*	
Figure. 5E Relative content of GSH in zebrafish larvae (%)					
Ctrl		One-way ANOVA	F=6.286; P=0.03 *		N=3
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P=0.84	ns	
100 mg/L vs. Ctrl			P=0.03	*	
Figure. 5E Relative ratio of GSH/GSSG in zebrafish larvae (%)					
Ctrl		One-way ANOVA	F=8.265; P=0.02 *		N=3

10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P=0.20	ns	
100 mg/L vs. Ctrl			P=0.01	*	
□					
Figure. 5F Relative expression levels of ferroptosis-related genes					
ptgs2a	Ctrl	One-way ANOVA	F=10.95; P=0.004 **		N=4
	10 mg/L vs. Ctrl		P=0.79	ns	
	100 mg/L vs. Ctrl		P=0.004	**	
ptgs2b	Ctrl		F=12.26; P<0.004 **		N=4
	10 mg/L vs. Ctrl		P=0.02	*	
	100 mg/L vs. Ctrl		P=0.002	**	
nrf2	Ctrl		F=14.58; P=0.002 **		N=4
	10 mg/L vs. Ctrl		P=0.001	**	
	100 mg/L vs. Ctrl		P=0.05	*	
dmt1	Ctrl		F=12.87; P=0.01 *		N=4
	10 mg/L vs. Ctrl		P=0.03	*	
	100 mg/L vs. Ctrl		P=0.008	**	
tf	Ctrl		F=74.78; P<0.001 ***		N=4
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P<0.001	***	
tfr	Ctrl		F=4.434; P=4.434 ns		N=4
	10 mg/L vs. Ctrl		P=0.66	ns	
	100 mg/L vs. Ctrl		P=0.05	*	
fth	Ctrl		F=13.83; P=0.006 **		N=4
	10 mg/L vs. Ctrl		P=0.005	**	
	100 mg/L vs. Ctrl		P=0.009	**	
gpx4	Ctrl		F=596.3; P<0.001 ***		N=4
	10 mg/L vs. Ctrl		P<0.001	***	
	100 mg/L vs. Ctrl		P<0.001	***	
scl7a11	Ctrl		F=8.986; P=0.01 *		N=4

	10 mg/L vs. Ctrl		P=0.02	*	
	100 mg/L vs. Ctrl		P=0.01	*	
ho-1	Ctrl		F=23.10; P<0.001 ***		N=4
	10 mg/L vs. Ctrl		P=0.41	ns	
	100 mg/L vs. Ctrl		P=0.003	**	
Figure. 5H Relative number of mitochondria in the brain (%)					
Ctrl		One-way ANOVA	F=26.44; P<0.001 ***		N=6
10 mg/L vs. Ctrl		Dunnett's multiple comparisons	P<0.001	***	N=6
100 mg/L vs. Ctrl			P<0.001	***	N=7
Figure. 5I Relative area of mitochondria in the brain (%)					
Ctrl		Kruskal-Wallis	F=8.265; P<0.001 ***		N=77
10 mg/L vs. Ctrl		Dunn's multiple comparisons	P<0.001	***	N=66
100 mg/L vs. Ctrl			P<0.001	***	N=85
Figure. 5J Relative diameter of mitochondria in the brain (%)					
Ctrl		Kruskal-Wallis	H=124.4; P<0.001 ***		N=77
10 mg/L vs. Ctrl		Dunn's multiple comparisons	P<0.001	***	N=66
100 mg/L vs. Ctrl			P<0.001	***	N=85
Figure. 6B Relative fluorescence of DFC					
Ctrl		Unpaired t-test			N=6
Ctrl vs. NAC			P=0.2419	ns	N=6
Ctrl vs. NiO-NPs (10mg/L)			P=0.0023	**	N=6
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + DFO			P<0.0001	***	N=6
Ctrl vs. NiO-NPs (100mg/L)			P=0.0271	*	N=6
NiO-NPs (100mg/L) vs.			P=0.0458	*	N=6

NiO-NPs (100mg/L) + DFO				
□				
Figure. 6D Relative density of apoptotic cells				
Ctrl	Unpaired t-test			N=13
Ctrl vs. NAC		P=0.3038	ns	N=6
Ctrl vs. NiO-NPs (10mg/L)		P<0.0001	***	N=8
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + DFO		P<0.0001	***	N=6
Ctrl vs. NiO-NPs (100mg/L)		P<0.0001	***	N=8
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + DFO		P<0.0001	***	N=7
□				
Figure. 6E Relative expression levels of apoptosis-related genes				
bax	Ctrl	One-way ANOVA	F=141.1; P<0.001 ***	N=3
	NiO-NPs (10 mg/L) vs. Ctrl	Dunnett's multiple comparisons	P<0.001 ***	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +NAC		P<0.001 ***	
bcl	Ctrl		F=46.57; P<0.001 ***	N=3
	NiO-NPs (10 mg/L) vs. Ctrl		P<0.001 ***	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +NAC		P<0.001 ***	
caspase3b	Ctrl		F=167.9; P<0.001 ***	N=3
	NiO-NPs (10 mg/L) vs.Ctrl		P=0.009 **	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +NAC		P<0.001 ***	
caspase8	Ctrl		F=10.70; P=0.01 *	N=3
	NiO-NPs (10 mg/L) vs. Ctrl		P=0.01 *	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +NAC		P=0.02 *	

caspase9	Ctrl		F=40.10; P<0.001 ***		N=3
	NiO-NPs (10 mg/L) vs. Ctrl		P<0.001	***	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +NAC		P=0.002	**	
□					
Figure. 6G Relative density of periventricular neurons (%)					
Ctrl		One-way ANOVA	F=20.90; P<0.001 ***		N=15
Ctrl vs. NAC		Dunnett's multiple comparisons	P=0.9220	ns	N=9
Ctrl vs. NiO-NPs (10mg/L)			P<0.0001	***	N=12
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC			P<0.0001	***	N=10
Ctrl vs. NiO-NPs (100mg/L)			P<0.0001	***	N=10
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC			P=0.0159	*	N=8
□					
Figure. 6J Distance moved by larvae under locomotion test					
Ctrl		Kruskal-Wallis	H=54.18; P<0.001 ***		N=32
Ctrl vs. NAC		Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)			P=0.05	*	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC			P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)			P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC			P=0.05	*	N=32
□					
Figure. 6K Velocity of larvae under locomotion test					
Ctrl		Kruskal-Wallis	H=54.18; P<0.001 ***		N=32
Ctrl vs. NAC		Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)			P=0.05	*	N=32

NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.05	*	N=32
□				
Figure. 6L Cumulative mobility of larvae under locomotion test				
Ctrl	Kruskal-Wallis	H=56.15; P<0.001 ***		N=32
Ctrl vs. NAC	Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.05	*	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.05	*	N=32
□				
Figure. 6M Maximum acceleration of larvae under locomotion test				
Ctrl	Kruskal-Wallis	F=22.05; P<0.001 ***		N=32
Ctrl vs. NAC	Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.89	ns	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P=0.001	**	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.002	**	N=32
□				
Figure. 6P Distance moved of larvae during whole tapping stimulation test				
Ctrl	Kruskal-Wallis	F=45.69; P<0.001 ***		N=32
Ctrl vs. NAC	Dunn's multiple	P>0.99	ns	N=32

Ctrl vs. NiO-NPs (10mg/L)	comparisons	P=0.05	*	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.03	*	N=32
□				
Figure. 6Q Velocity of larvae during whole tapping stimulation test				
Ctrl	Kruskal-Wallis	F=45.69; P<0.001 ***		N=32
Ctrl vs. NAC	Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.05	*	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.03	*	N=32
□				
Figure. 6R Cumulative mobility of larvae during whole tapping stimulation test				
Ctrl	Kruskal-Wallis	H=51.91; P<0.001 ***		N=32
Ctrl vs. NAC	Dunn's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.03	*	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.02	*	N=32
□				
Figure. 6S Maximum acceleration of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=2.521; P=0.03 *		N=32

Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.98	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.29	ns	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.98	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P=0.21	ns	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.87	ns	N=32
□				
Figure. 6T Distance moved of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=16.44; P<0.001 ***		N=32
Ctrl vs. NAC	Dunnett's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.98	ns	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.91	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.75	ns	N=32
□				
Figure. 6U Velocity of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=16.44; P<0.001 ***		N=32
Ctrl vs. NAC	Dunnett's multiple comparisons	P>0.99	ns	N=32
Ctrl vs. NiO-NPs (10mg/L)		P=0.98	ns	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.91	ns	N=32
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.75	ns	N=32
□				
Figure. 6V Cumulative mobility of larvae within 1s immediately after the vibrations				

Ctrl		One-way ANOVA	F=74.97; P<0.001 ***	N=16
Ctrl vs. NAC		Dunnett's multiple comparisons	P=0.42 ns	N=16
Ctrl vs. NiO-NPs (10mg/L)			P=0.40 ns	N=16
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC			P=0.87 ns	N=16
Ctrl vs. NiO-NPs (100mg/L)			P<0.001 ***	N=16
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC			P=0.11 ns	N=16
□				
Figure. 6W Maximum acceleration of larvae within 1s immediately after the vibrations				
Ctrl		One-way ANOVA	F=56.56; P=0.03 *	N=32
Ctrl vs. NAC		Dunnett's multiple comparisons	P=0.28 ns	N=32
Ctrl vs. NiO-NPs (10mg/L)			P=0.77 ns	N=32
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC			P=0.92 ns	N=32
Ctrl vs. NiO-NPs (100mg/L)			P<0.001 ***	N=32
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC			P=0.56 ns	N=32
□				
Figure. 7A Relative expression levels of apoptosis-related genes				
gpx4	Ctrl	Unpaired t-test		N=3
	NiO-NPs (10 mg/L) vs. Ctrl		P=0.0033 **	
	NiO-NPs (10 mg/L) vs. NiO-NPs (10 mg/L) +DFO		P=0.0027 **	
slc7a11	Ctrl			N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0042 **	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P<0.001 ***	
nrf2	Ctrl			N=3

	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0403	*	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0492	*	
ho-1	Ctrl				N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P<0.001	***	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0077	**	
dmt1	Ctrl				N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0044	**	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0235	*	
fth	Ctrl				N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0011	**	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0088	**	
tfr	Ctrl				N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0138	*	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0200	*	
tf	Ctrl				N=3
	NiO-NPs (100 mg/L) vs. Ctrl		P=0.0368	*	
	NiO-NPs (100 mg/L) vs. NiO-NPs (100 mg/L) +DFO		P=0.0497	*	
□					
Figure. 7 Relative density of periventricular neurons (%)					
Ctrl		One-way ANOVA	F=9.622; P=0.03 *		N=31
Ctrl vs. NAC		Dunnett's multiple comparisons	P=0.85	ns	N=18
Ctrl vs. NiO-NPs (10mg/L)			P=0.02	*	N=28
NiO-NPs (10mg/L) vs.			P>0.99	ns	N=18

NiO-NPs (10mg/L) + NAC				
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=18
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.0284	*	N=20
□				
Figure. 7F Distance moved by larvae under locomotion test				
Ctrl	One-way ANOVA	F=17.11; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.83	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.006	**	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.83	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.03	*	N=24
□				
Figure. 7G Velocity of larvae under locomotion test				
Ctrl	One-way ANOVA	F=17.11; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.83	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.006	**	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.83	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.03	*	N=24
□				
Figure. 7H Cumulative mobility of larvae under locomotion test				
Ctrl	One-way ANOVA	F=16.79; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple	P=0.73	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.02	*	N=24

NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC	comparisons	P=0.23	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.04	*	N=24
□				
Figure. 7I Maximum acceleration of larvae under locomotion test				
Ctrl	One-way ANOVA	F=2.799; P=0.02 *		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.85	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.62	ns	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.99	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P=0.02	*	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.05	*	N=24
□				
Figure. 7L Distance moved of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=10.66; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P>0.99	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.01	*	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.62	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.05	*	N=24
□				
Figure. 7M Velocity of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	L=10.66; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple	P>0.99	ns	N=24

Ctrl vs. NiO-NPs (10mg/L)	comparisons	P=0.01	*	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P>0.62	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.05	*	N=24
□				
Figure. 7N Cumulative mobility of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=14.20; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P>0.99	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.04	*	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.30	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.03	*	N=24
□				
Figure. 7O Maximum acceleration of larvae during whole tapping stimulation test				
Ctrl	One-way ANOVA	F=4.597; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P>0.99	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.25	ns	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.96	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P=0.005	**	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P=0.02	*	N=24
□				
Figure. 7P Distance moved of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=24.48; P<0.001 ***		N=24

Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.06	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.05	ns	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.71	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P<0.001	***	N=24
□				
Figure. 7Q Velocity of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=24.48; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.06	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.05	ns	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.71	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P<0.001	***	N=24
□				
Figure. 7R Cumulative mobility of larvae within 1s immediately after the vibrations				
Ctrl	One-way ANOVA	F=49.48; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.06	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.19	ns	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=0.08	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P<0.001	***	N=24
□				
Figure. 7S Maximum acceleration of larvae within 1s immediately after the vibrations				

Ctrl	One-way ANOVA	F=11.36; P<0.001 ***		N=24
Ctrl vs. NAC	Dunnett's multiple comparisons	P=0.06	ns	N=24
Ctrl vs. NiO-NPs (10mg/L)		P=0.02	*	N=24
NiO-NPs (10mg/L) vs. NiO-NPs (10mg/L) + NAC		P=>0.99	ns	N=24
Ctrl vs. NiO-NPs (100mg/L)		P<0.001	***	N=24
NiO-NPs (100mg/L) vs. NiO-NPs (100mg/L) + NAC		P<0.001	***	N=24

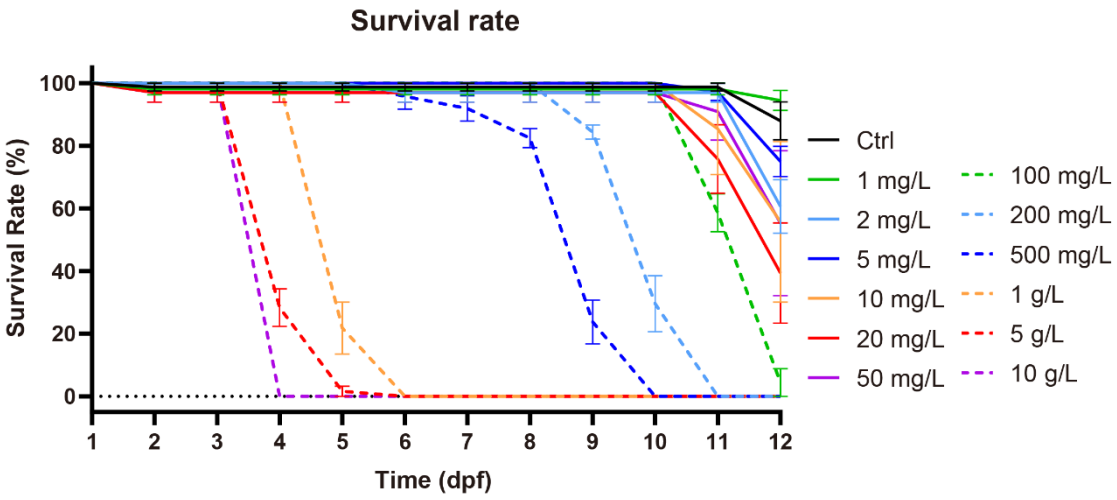

Figure S1. Survival rate of zebrafish embryos/larvae exposed to NiO-NPs from 1 to 12 dpf.

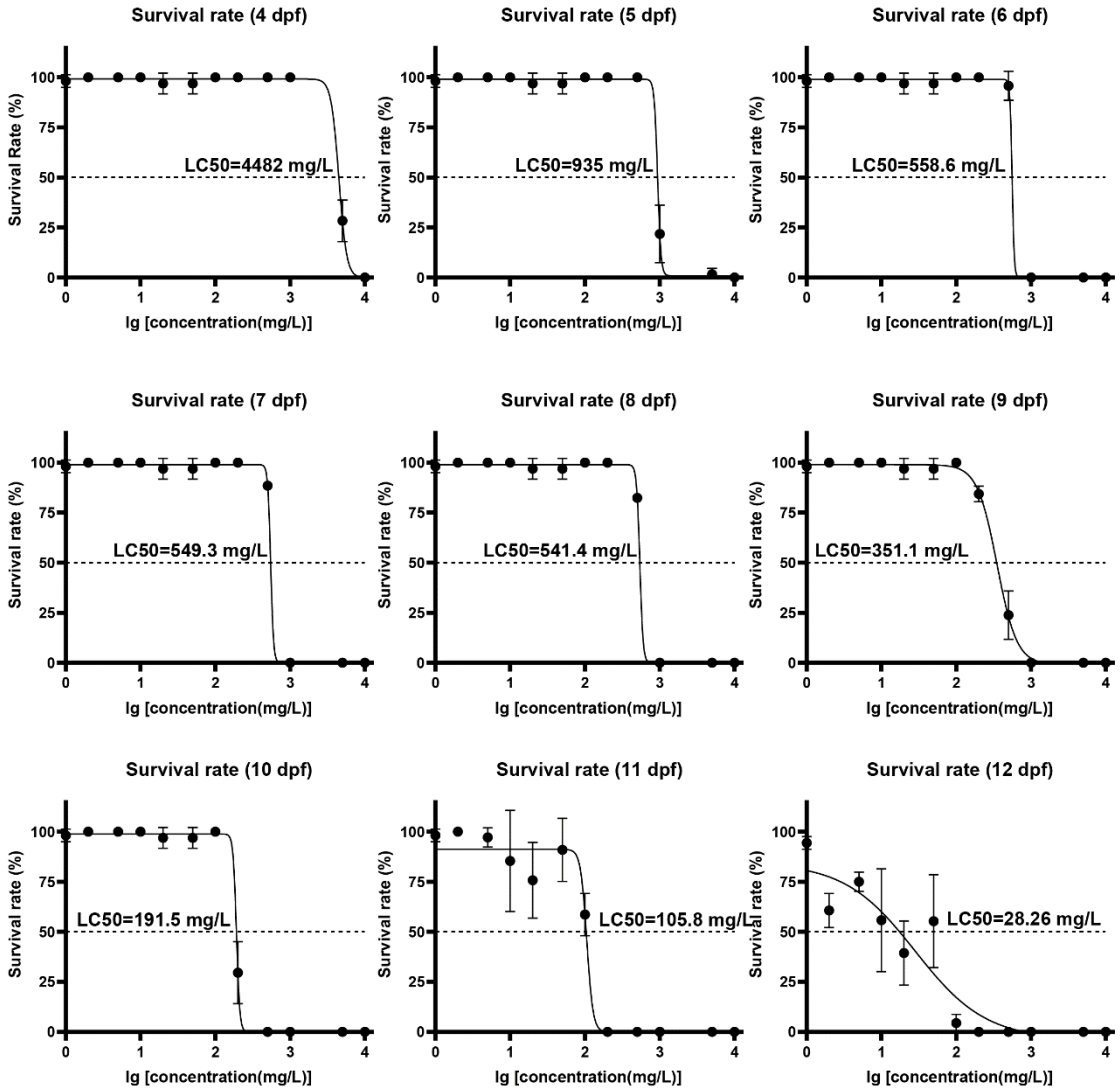

Figure S2. The median lethal concentration (LC50) of NiO-NPs to zebrafish larvae.