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Supplementary information

Toxicity screening and ranking of diverse engineered nanomaterials using established hierarchical testing approaches with a complementary in vivo zebrafish model

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Results

Table S1. Water-soluble ion concentration of nanoparticle dispersions in Holtfreter's medium at 50 µg/mL

Nanoparticles	**Concentration of ions in nanoparticle suspension (µg/mL)**	**% Dissolution in case of nanoparticles**
CuO	29.3	73.4
CeO_2	ND	-
TiO_2	7.2	14.3
ZnO	27.0	67.2
$ZnFe_2O_4$	7.1	51.6

Methods

Table S2. Summary of major uses of tested nanoparticles in this study.

Nanoparticles	**Applications**
CuO	Burning rate catalyst in rocket propellants, catalyst, superconducting materials, ceramics resistors, gas sensors, semiconductors, antibacterial applications, etc.
CeO_2	Oxidation resistant coatings, oxygen sensors, oxygen pumps, heat resistant alloy coatings, coating for infrared filters, biomedicines, drug delivery, catalysts etc.
TiO_2-A	Electrodes, solar cells, sunscreens, whitening agent such as in paints, paper, laser toners, antibacterial coatings, cancer treatment, light emitting diodes, solar cells etc.
TiO_2-R	Antiseptic and antibacterial compositions, UV resistant materials, cosmetic products, paper industry printing ink, laser toners, glass coating etc.
Qdots	Semiconductor devices, imaging, miniature lasers, TV or computer displays, etc.
SiO_2	*Amorphous:* Protein adsorption and separation, drug and gene delivery, fungal resistant materials, molecular imaging, etc. *Crystalline:* Filler in numerous industrial coatings and mortar applications.
$ZnFe_2O_4$	Gas sensors, magnetic materials, catalysis, foundry, etc.
ZrO_2	Ceramic pigments, artificial jewelry, abrasive materials, fire retarding material, optical storage, light shutters, etc.
ZnO	Manufacture of rubber and cigarettes, calamine lotion, ointments to treat skin diseases, concrete additive, food additive, sunscreen, whitening agent in paints, etc.
Au	Antibiotic, antifungal, nanowires, catalyst applications, delivery of therapeutic agents, photodynamic therapy, sensors, etc.

Table S3. Primers used for carrying out qPCR reactions for oxidative stress and hatching rate.

Gene	**Sequence**
18s rRNA	Forward – 5' TCGCTAGTTGGCATCGTTTATG 3' Reverse – 5' CGGAGGTTCGAAGACGATCA 3'
GAPDH	Forward – 5' GTGGAGTCTACTGGTGTCTTC 3' Reverse – 5' GTGCAGGAGGCATTGCTTACA 3'
SOD1	Forward – 5’ AGACCTGGGTAATGTGACCG 3’ Reverse – 5’ CGGGCTAAGTGCTTTCAGAG 3’
HMOX1	Forward – 5’ GGAAGAGCTGGACAGAAACG 3’ Reverse – 5’GACAGATCTCCGAGGTAGCG 3’
GPX1	Forward – 5’ GAGGCACAACAGTCAGGGAT 3’ Reverse – 5’ TCTCCCATAAGGGACACAGG 3’
HN1	Forward – 5’CTGAACTTCTCTACACACTGAGG 3’ Reverse – 5’CCTTATCACC ATCACCTCACTTC 3’

Table S4. LC50 lethal concentration of toxic nanomaterials in zebra fish embryos.

Nanoparticle	**LC 50 (µg/ml)**	**IC50 (Percentage tail DNA) (µg/ml)**
CuO	41.90	4.86
CeO_2	24.26	15.08
TiO_2-A	795.57	163.09
Qdots	512.8	-
SiO_2	41.16	13.66
ZnO	216.41	5.63

Figures

Figure S1. SEM images of the nanomaterials in both dry state and after dispersions in the Holtfreter's medium at 50 µg/mL concentration.

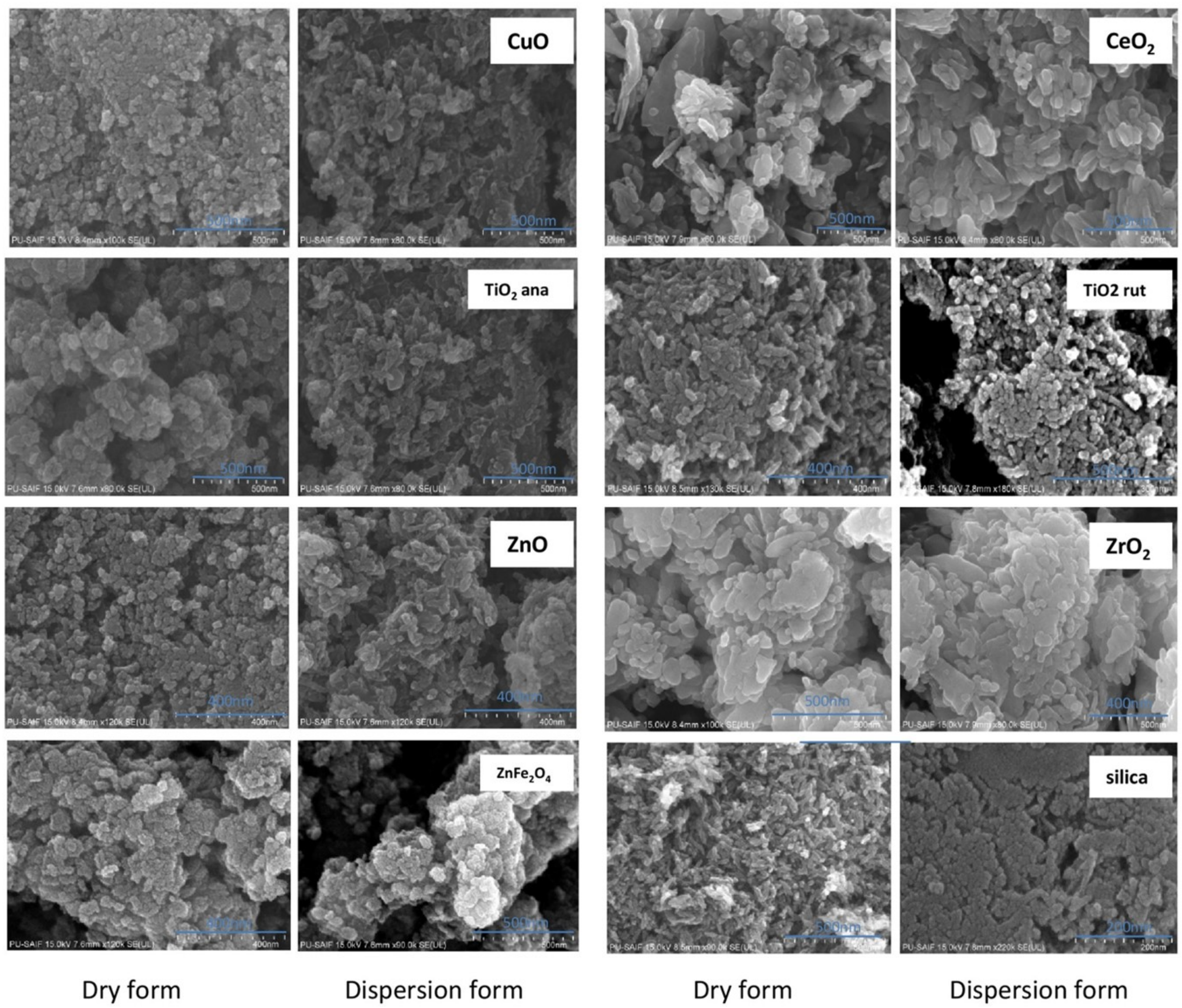


Figure S2. Fraction of administered dose delivered over a time period of 24 hours for all ENMs in RPMI media.

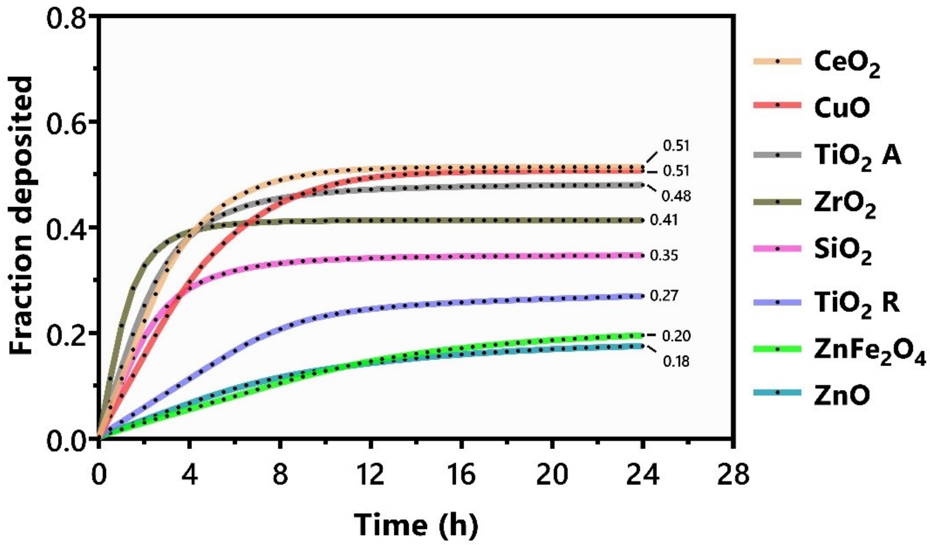


Figure S3. Fraction of administered dose delivered over a time period of 24 hours for all ENMs in Holtfreter's media.

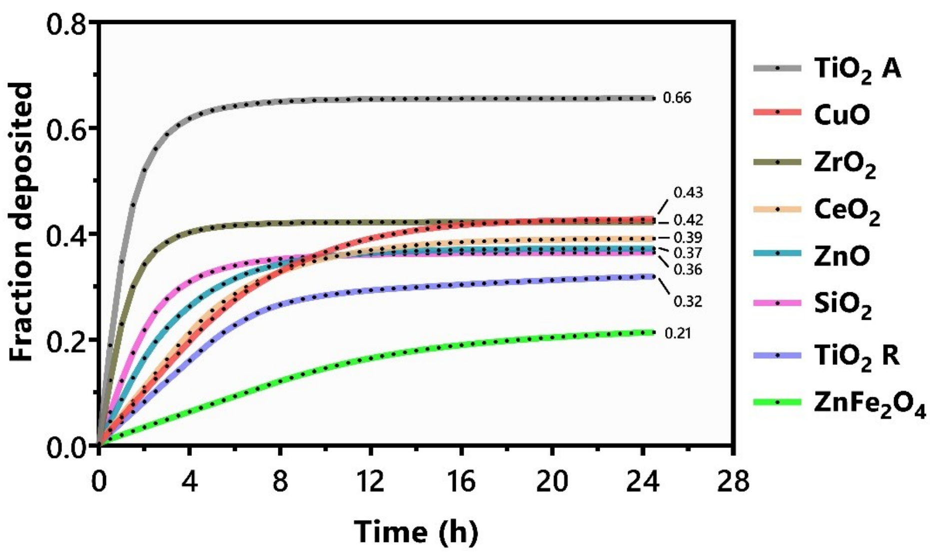
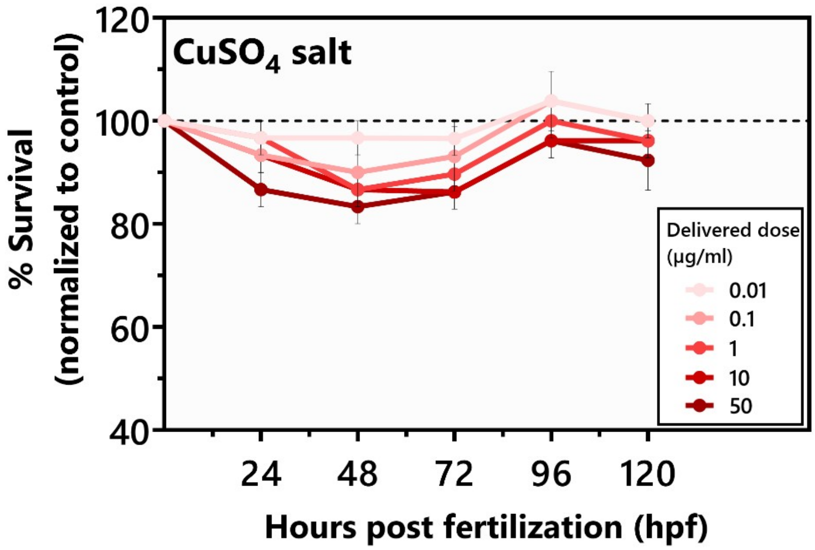


Figure S4. Survival rate of zebrafish embryos after treatment with salts of respective ENMs (**A**) CuO and (**B**) ZnO at 24, 48, 72, 96 and 120 hrs post fertilization.



Research ethics approval

Certificate

This is certify that the project title **Chronic nanomaterial toxicity screening using zebra fish model** has been approved by the PU/45/99/CPCSEA/IAEC/2019/ 387 by the IAEC, Panjab University, Chandigarh (160014).

Number of Animals approved: 20 male/female Dario Rerio/ Zebra fish per year for 24 months

(Chairman IAEC) Member Secretary IAEC CPCSEA Nominee

Panjab University

Chandigarh

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)