Low toxicity and accumulation of zinc oxide nanoparticles in mice after 270-day consecutive dietary supplement

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1. Bioaccessiblity of Zn in animal food by the RIVM method

The RIVM method was used to assess the Zn bioaccessibility of animal food containing ZnO NPs, ZnO MPs, and Zinc ion, respectively. The animal food (0.1 g) was used in this in vitro digestion. The constituents and concentrations of all synthetic juices are shown in Table S1. All digestive juices were heated to 37 °C before digestion. The digestions were carried out in a Tabletop Orbital Shaker (SPH-100B, Shanghai Shiping Laboratory Equipment Co., LTD, China) at 100 rpm. The pH values of the digestive juices were adjusted with $NaHCO_3$ (1M) or HCl (37% w/w). Digestion started by introducing 4 mL gastric juice into 0.1 g animal food. The mixture was adjusted to pH 1.3 ± 0.2 and shaked for 2 h. After that, 4 mL duodenal juice, 2 mL bile juice were added. The mixture was adjusted to pH 6.5 ± 0.2, and shaked for another 2 h. Then, the mixture was centrifuged at 3000 g for 10 min (MIKRO 200R, Hettich, Germany). Finally, 0.5 mL of the supernatant was used to determine Zn concentration by an atomic absorption spectroscopy (900T, PerkinElmer, USA) after digestion with 1 mL HNO_3 and 1 mL H_2O_2, and diluted to 2% HNO_3 solution. The Zn bioaccessibility in animal food was calculated following the completion of intestinal phase extraction according to the following equation:

$$\text{Zn bioaccessibility (\%)} = \text{Zn in supernatant} / \text{total Zn} \times 100$$

where Zn in supernatant is Zn (μg) extracted from 0.1 g of sample, and total Zn is Zn (μg) in 0.1 g animal food.

Table S1. Composition of the synthetic juices for the in vitro digestion model (Amounts based on 1000 mL of juice) with minor modification (R. Peters, E. Kramer, A. G. Oomen, Z. E. Herrera Rivera, G. Oegema, P. C. Tromp, R. Fokkink, A. Rietveld, H. J. P. Marvin, S. Weigel, A. A. C. M. Peijnenburg, H. Bouwmeester, *ACS Nano* **2012**, 6, 2441).

	Gastric juice *PH 1.3±0.1*	*Duodenal juice* *pH 8.1±0.1*	*Bile juice* *pH 8.2±0.1*
Inorganic constituents	2752 mg NaCl	7012 mg NaCl	5259 mg NaCl
	306 mg $NaH_2PO_4 \cdot H_2O$	3388 mg $NaHCO_3$	5785 mg $NaHCO_3$
	824 mg KCl	80 mg KH_2PO_4	376 mg KCl
	302 mg $CaCl_2$	564 mg KCl	150 μL HCl(37%)
	6.5 mL glucose	50 mg $MgCl_2 \cdot 6H_2O$	250 mg urea
	650 mg glucose	180 μL HCl(37%)	167.5 mg $CaCl_2$
organic constituents	20 mg glucuronic acid		
	85 mg urea	100 mg urea	1.8 g BSA
	330 mg glucosaminhydrochloride	151 mg $CaCl_2$	30 g bile
	1 g BSA	1 g BSA	milli-Q water
	2.5g pepsin	4.5 g pancreatin	
	3 g mucin	1.5 g lipase	
	milli-Q water	milli-Q water	
			sodium carbonate solution
			84.7 g $NaHCO_3$
			milli-Q water

Table S2. Zn bioaccessibility of food replenished with Zn ion, ZnO-NP and ZnO-MP by the RIVM method.

Sample	Gastric		Intestine	
	Dissolved content (μg/0.1g)	Bioaccessibility (%)	Dissolved content (μg/0.1g)	Bioaccessibility (%)
Zinc ion	119.9±5.3	74.9	15.9±2.0	9.9
ZnO-NP	104.0±1.1	65.0	15.1±1.8	9.4
ZnO-MP	110.5±2.0	69.1	15.3±1.2	9.5

2. XRD patterns of ZnO particles

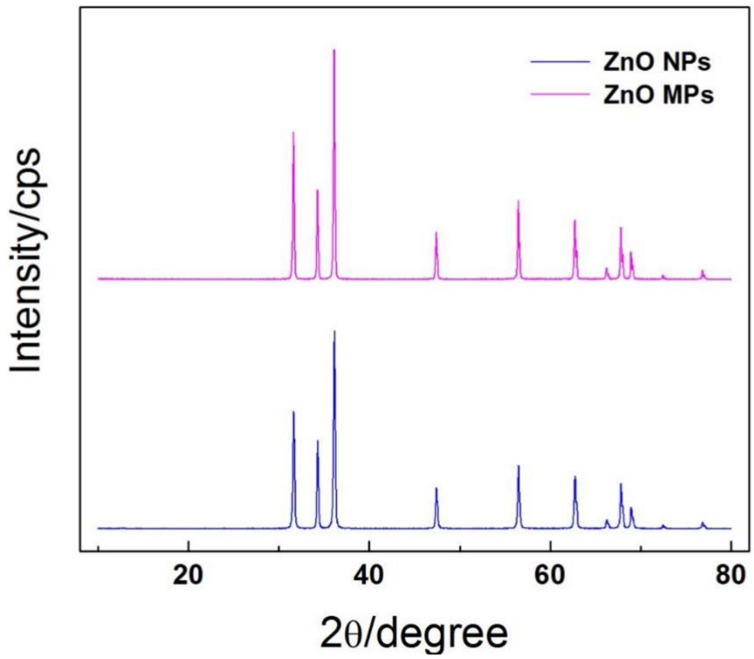


Fig. S1 XRD spectra of ZnO NPs and ZnO MPs.

3. TEM images of ZnO particles separated from the animal food samples

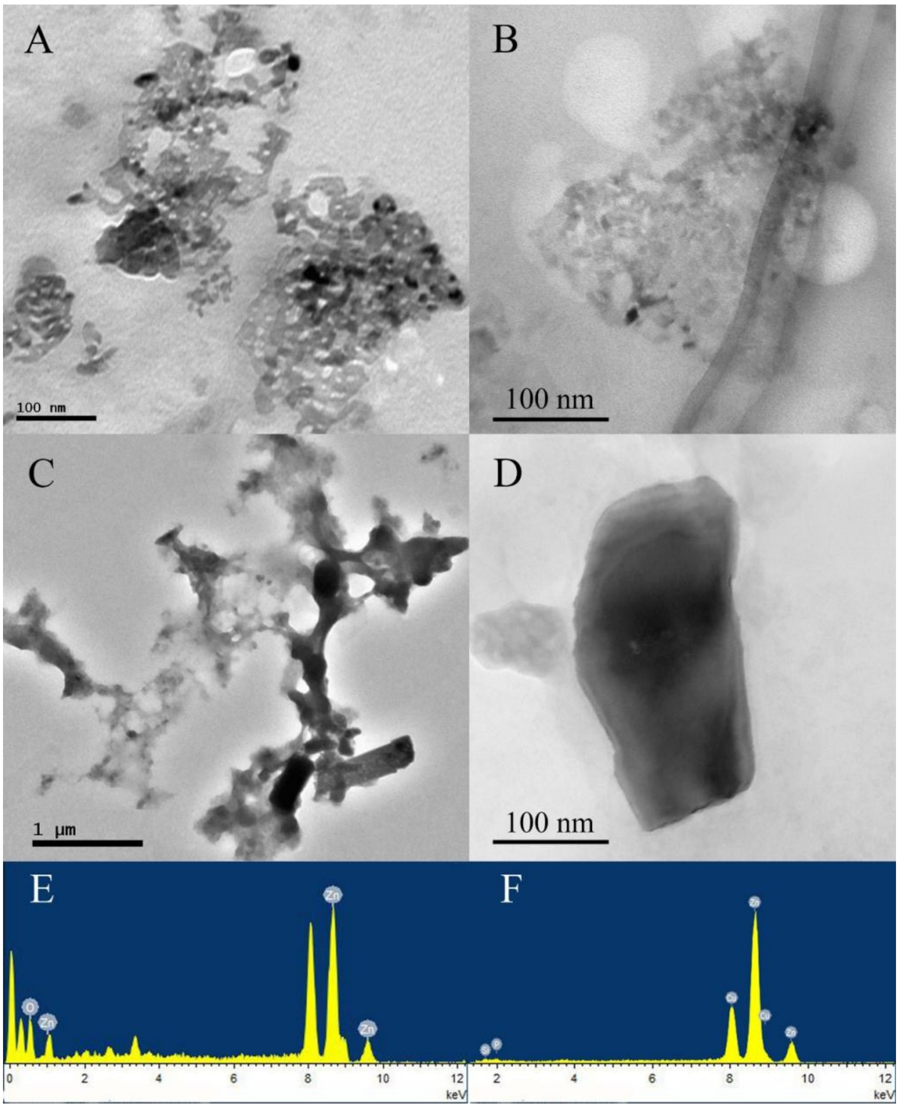


Fig. S2 The morphology and size of ZnO-NPs and ZnO-MPs separated from the animal food. (A&B) TEM images of ZnO-NPs; (C&D) TEM images of ZnO-MPs; (E) EDS data confirming the chemicals from image B; (F) EDS data confirming the chemicals from image D.

4. Ultra-thin pathological photos of stomach of female mice

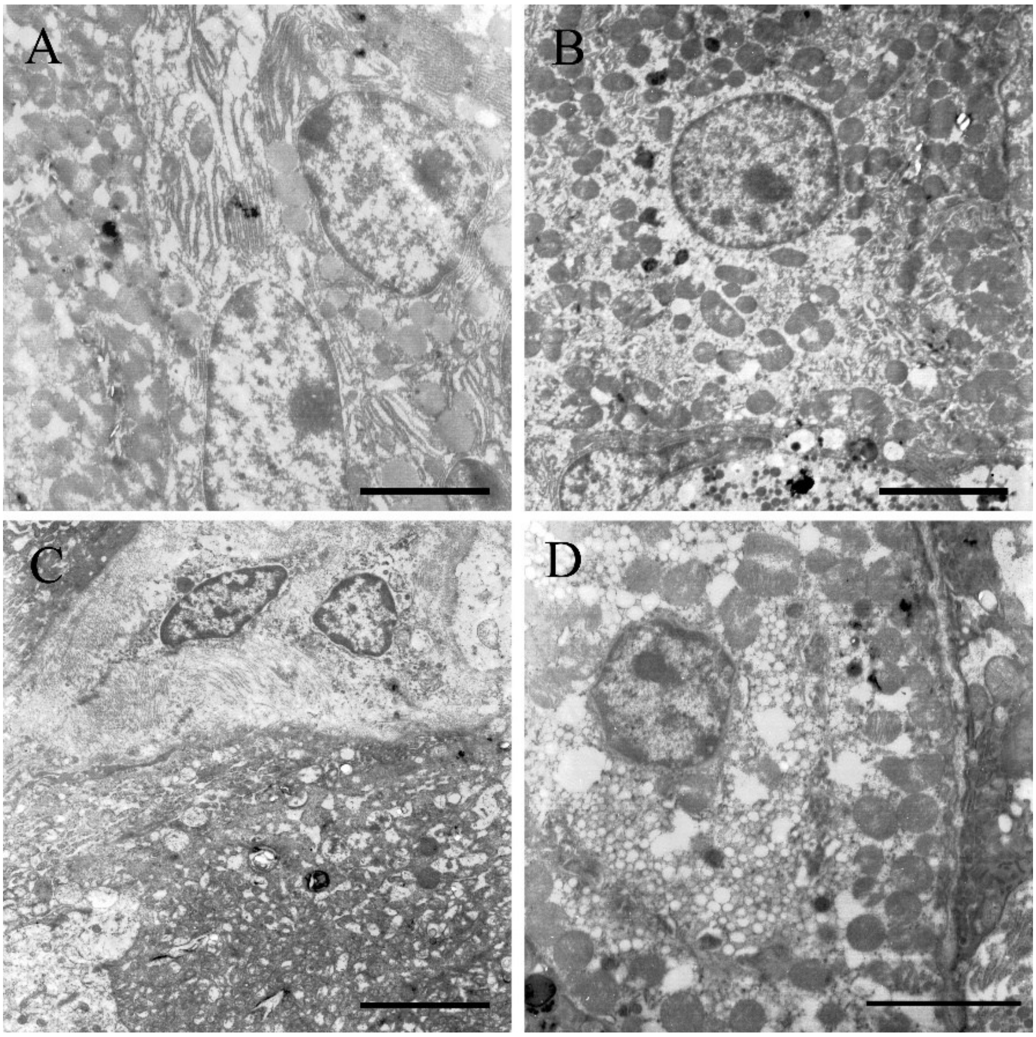
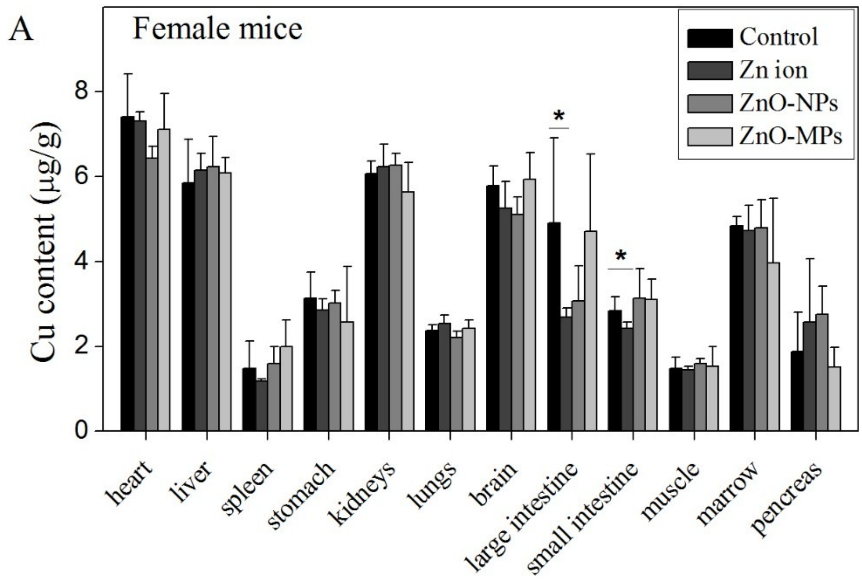


Fig. S3 Representative ultra-thin pathological photos of stomach of female mice after 270 days consecutive zinc dietary supplement. (A) control group, (B) Zn ion group, (C) ZnO-NPs group, (D) ZnO-MPs group. The scale bar is 2.5 μm.

5. Copper distribution in mice after exposed to zinc oxide particles and zinc ion



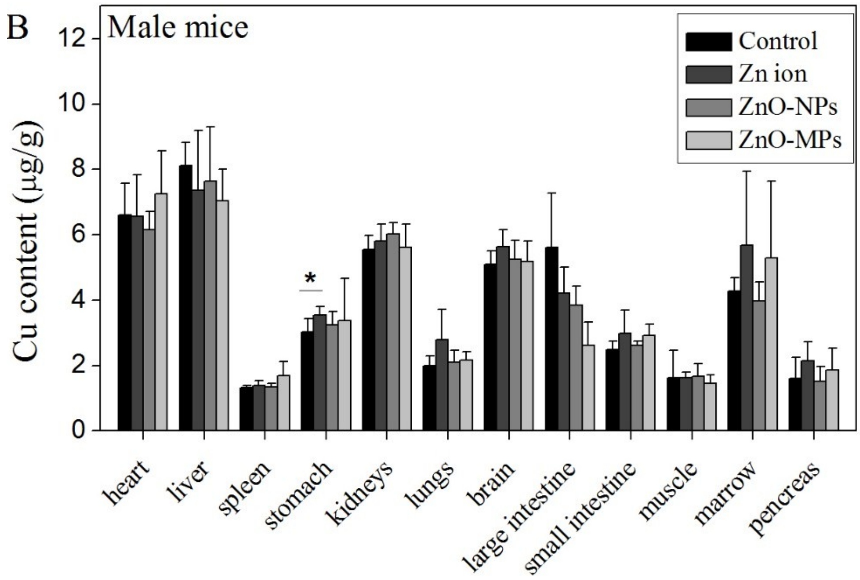


Fig. S4 Copper contents in tissues in the control, Zn ion, ZnO-NPs, and ZnO-MPs mice after 270 days consecutive exposure to zinc oxide particles or zinc ion (n=5). *p<0.05, compared to the control. (A) female mice; (B) male mice.

The consumption of excessive zinc ion increased the copper content in stomach in male mouse, but decreased the copper content in intestine in female mouse. However, the exposure of ZnO-NPs and ZnO MPs didn’t influence the copper distribution in mice.