Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Material

S1. The determination of mental content of nTSBs using inductively coupled plasma mass spectrometry (ICP-MS) assay

ICP-MS (Elan6100; PerkinElmer, Shelton, CT, USA) was used to determine the concentrations of metals in nTSB. Briefly, the nTSB (n=6) were liquefied with 0.5 ml nitric acid and subjected to microwave digestion (Anton Paar GmbH, Graz, Austria). The microwave digestion temperature was programmed to increase from 30 to 75 °C at a rate of 7.5 °C/min with a 1 min hold at 75 °C and up to 130 °C at a rate of 11 °C/min with a hold of 30 min at 130 °C. The digested solution was diluted with deionized water. This sample solution was then directly analyzed by ICP-MS.

S2 Collection of nTSBs

It has been demonstrated that nanoparticles often form agglomerates (<500 nm) in air during production. In the present work, the airborne nTSBs (<500 nm) from our manufacturing location of nTSBs were collected on Zefluor after-filter (Pall Life Sciences, 1.0 μm pore) using a micro-orifice uniform deposited impactor (MOUDI) (MSP Corporation, MN, USA). The sampling flow rate for the MOUDI was 30 L/min. For each sample, the collected air volume approximated 43.2 m^3/day. Before and after each sampling, the filters were equilibrated in a dust-free desiccator (RH=60%; temperature=25°C) for 24h. The daily mean nTSBs (<500 nm) concentration was 171.75 ± 21.45 μg/m^3 (147.48–188.16 μg/m^3) during the sampling period.

S3 Calculation of the daily nTSBs alveolar deposition dose

The human daily nTSBs alveolar deposition dose was calculated using the following equation:

$$Human\ alveolar\ deposition\ dose =$$

$$nTSBs\ aerosol\ concentration \times V_E \times exposure\ duration \times alveolar\ deposition\ efficiency =$$

$$nTSBs\ aerosol\ concentration \times (10\frac{\mathrm{L}}{\min}) \times (10^{-3}\frac{m^3}{L}) \times (8\frac{hr}{d}) \times (60\frac{\min}{h}) \times 50\%$$

where VE is the respiratory volume/min. The alveolar deposition efficiency of nTSBs was calculated according to International Commission on Radiological Protection Deposition Model. For nanoparticles, the alveolar deposition efficiency was assuming as 50%. The human daily nTSBs alveolar deposition dose was estimated as 412.21±51.48 (353.95-509.85) μg/day. In addition, 412.21 μg nTSBs per day deposition with a conservative estimate of human lung surface area (70 m^2) would amount to 0.59 ng/cm^2 per day. Considering a 5-day workweek, 52 weeks a year, and the surface area (0.32 cm^2) of a well in a 96-well plate, it would take 20 working years to reach the tested dose of 10 μg/mL nTSBs, assuming 100% deposition of the administered dose.

Table S1. Element Composition of TSBs

Element	(N) %	(C) %	(H) %	(O) %	H/C	O/C	(N+O)/C
nTSBs	1.27±0.02	70.5±0.02	2.55±0.01	10.22±0.083	0.04	0.14	0.16

Table S2. Metal Composition of TSBs

Metal Concentration (µg/g)	nTSBs
Mg	4571
Al	1819
K	43408
Cr	ND
Co	ND
Ni	ND
Cu	<52.36
Zn	<52.36
As	ND
Cd	ND
Pb	ND

Table S3.Primer sequences for the qRT-PCR assays

Primer	Sense	Antisense
GAPDH	GAGTCAACGGATTTGGTCGT	TTCATTTTGGAGGGATCTCG
HO-1	AACAAAGTGCAAGATTCTGCCC	AGCTGAGTGTAAGGACCCATCG

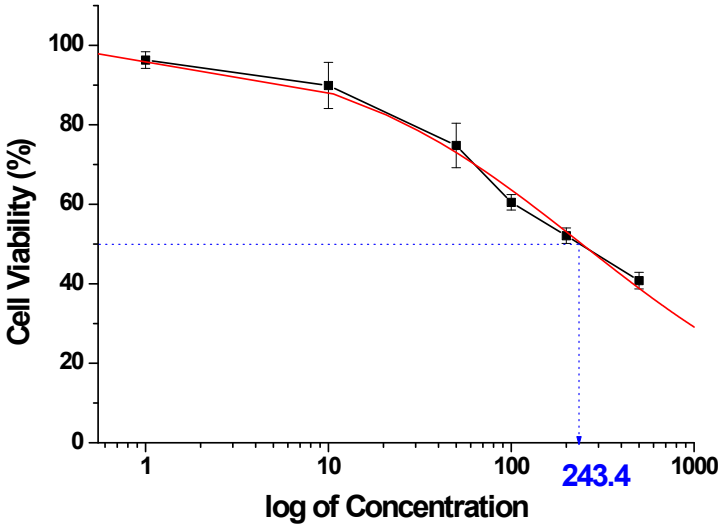

Fig. S1. The effective concentrations (EC_{50}) value of nTSB.

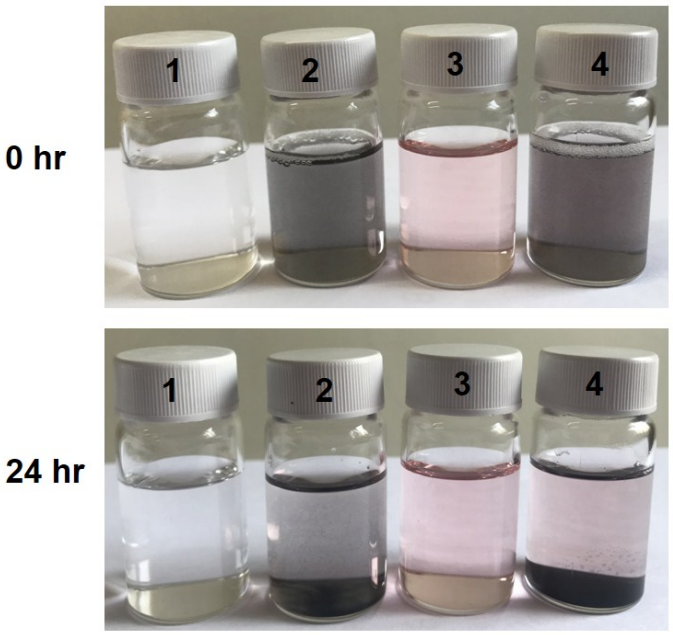

Figure S2. Photographs of nTSBs dispersions in ddH_2O and LHC-9 medium, respectively. 1. ddH_2O; 2. nTSBs dispersed in ddH_2O; 3. LHC-9 medium; 4. nTSBs dispersed in LHC-9 medium; Images marked '0 hr' show dispersions immediately after addition, while images marked '24 hr' is from dispersions after standing still for 1day, respectively.

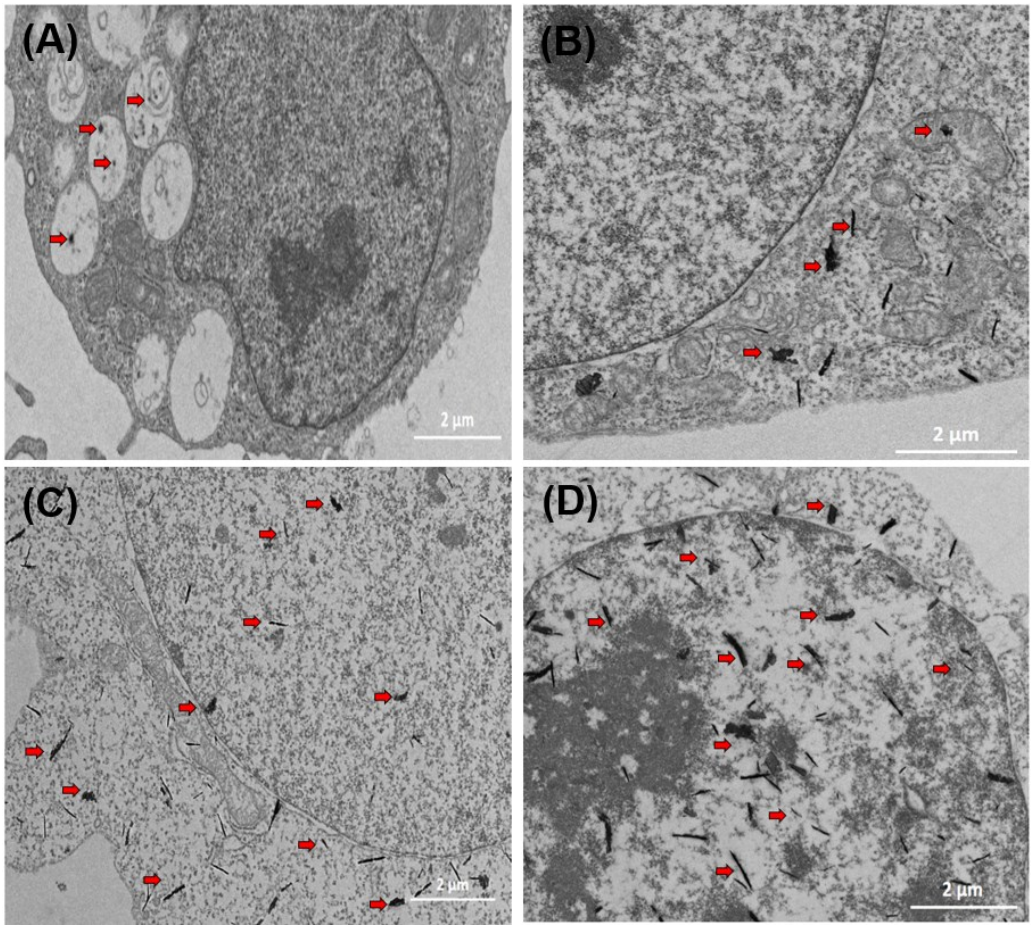

Figure S3. Uptake of nTSBs by BEAS-2B cells. Transmission electron microscopic (TEM) images of ultrathin sections of BEAS-2B cells treated with (A, B) 10 µg/mL nTSBs and (C, D) 100 µg/mL nTSBs. Red arrows denote nTSBs, scale bar: 2 µm.