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

Ceramic fibers do not exhibit larger toxicity in pulmonary epithelial cells than nanoparticles of same chemical composition

Supplementary Information

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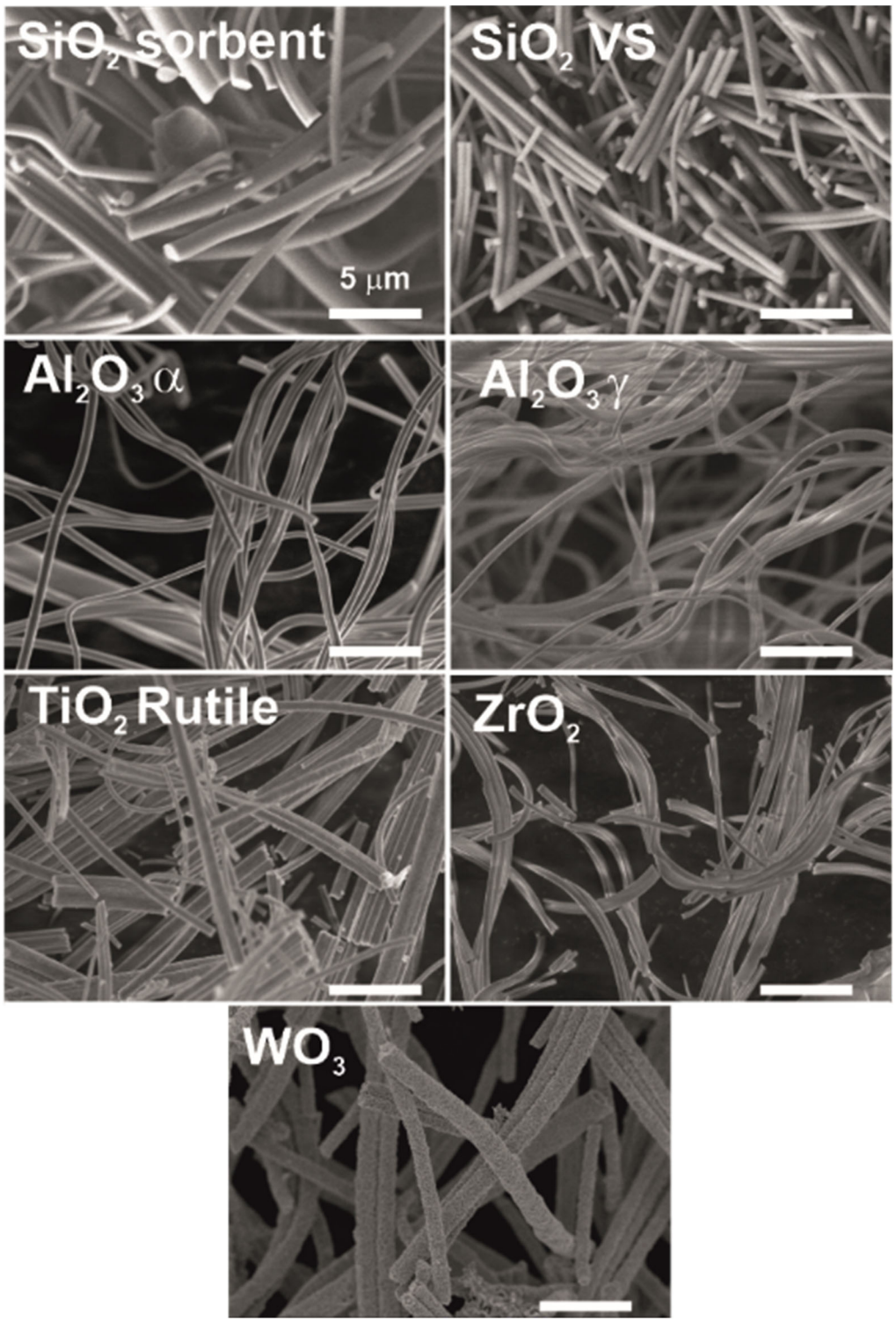

Figure S1. SEM images of as-prepared inorganic fibers (scale bar for all images represent 5 μm). Samples are identical to samples shown in Figure 1, but the magnification of these images is substantially higher.

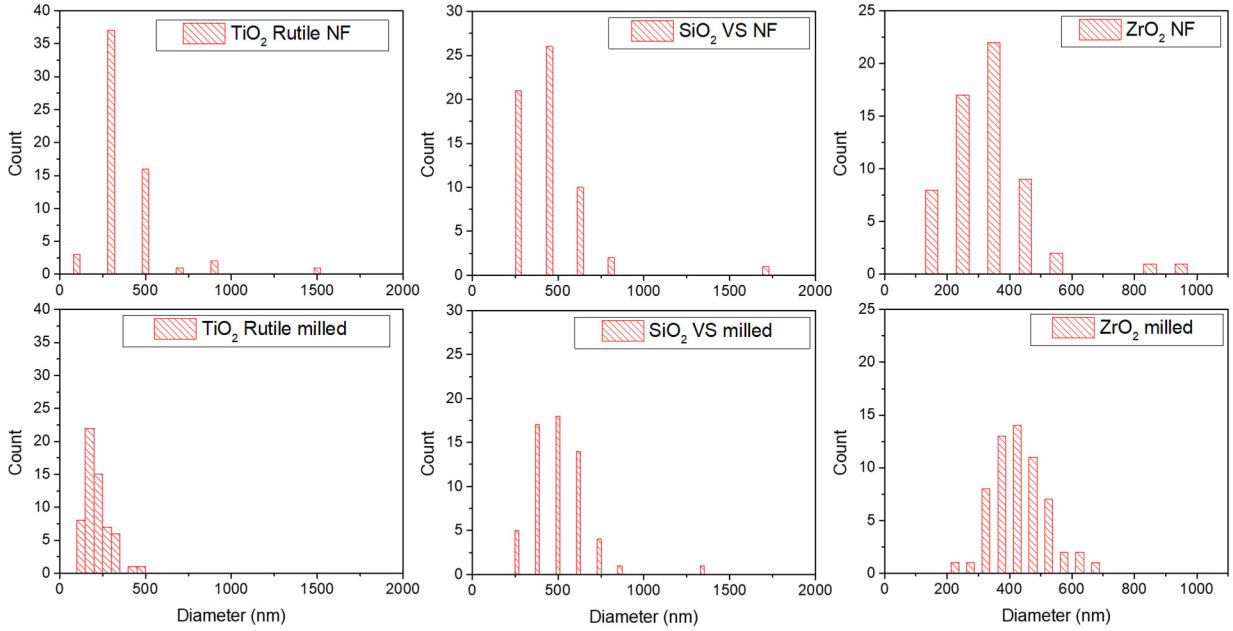

Figure S2. Statistical analyses of fiber diameters before (upper line) and after milling (lower line) for selected fibers. The number of measurements for each samples was n = 60.

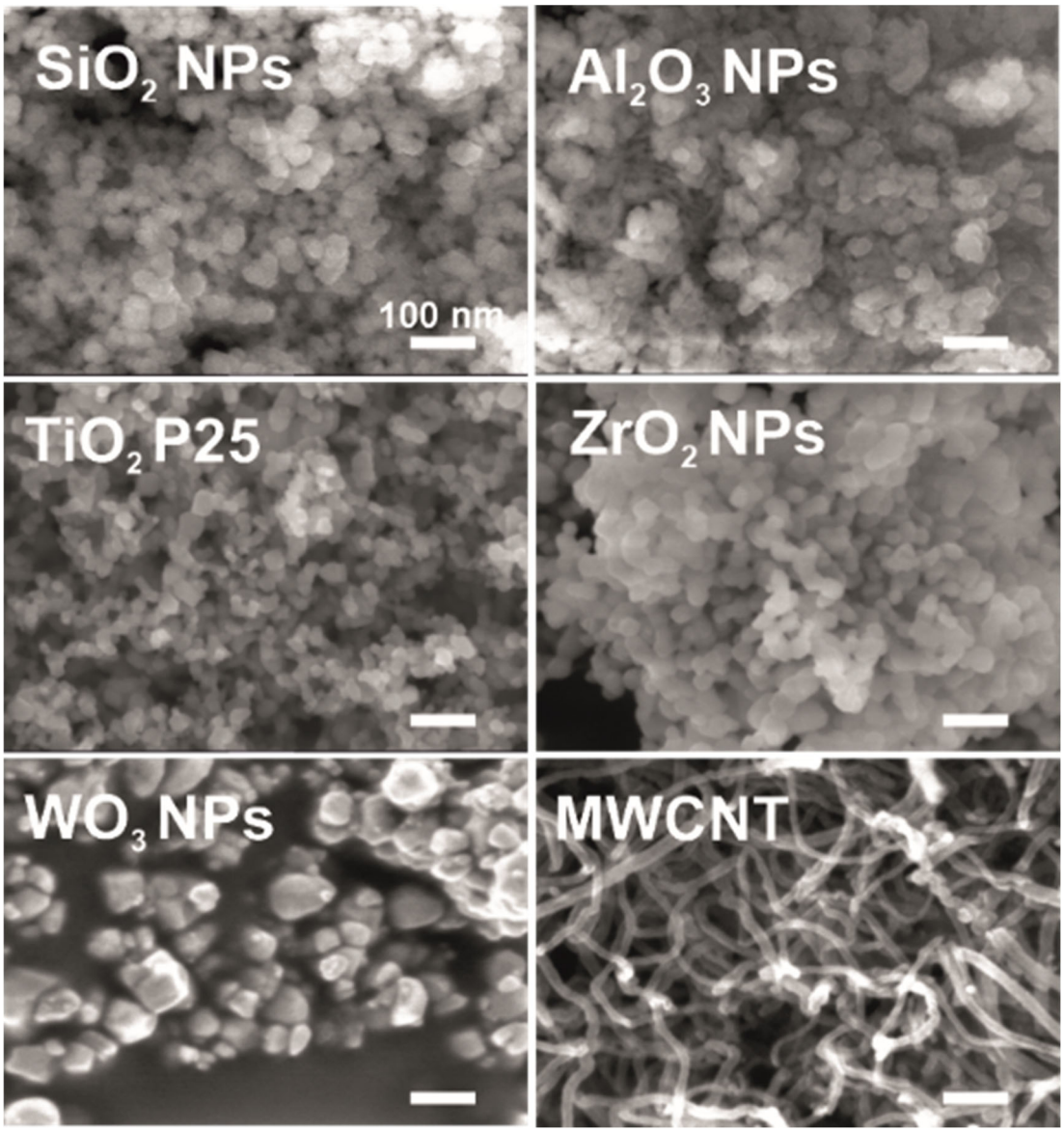

Figure S3. SEM images of all reference nanomaterials used in this work (scale bar for all images represent 100 nm).

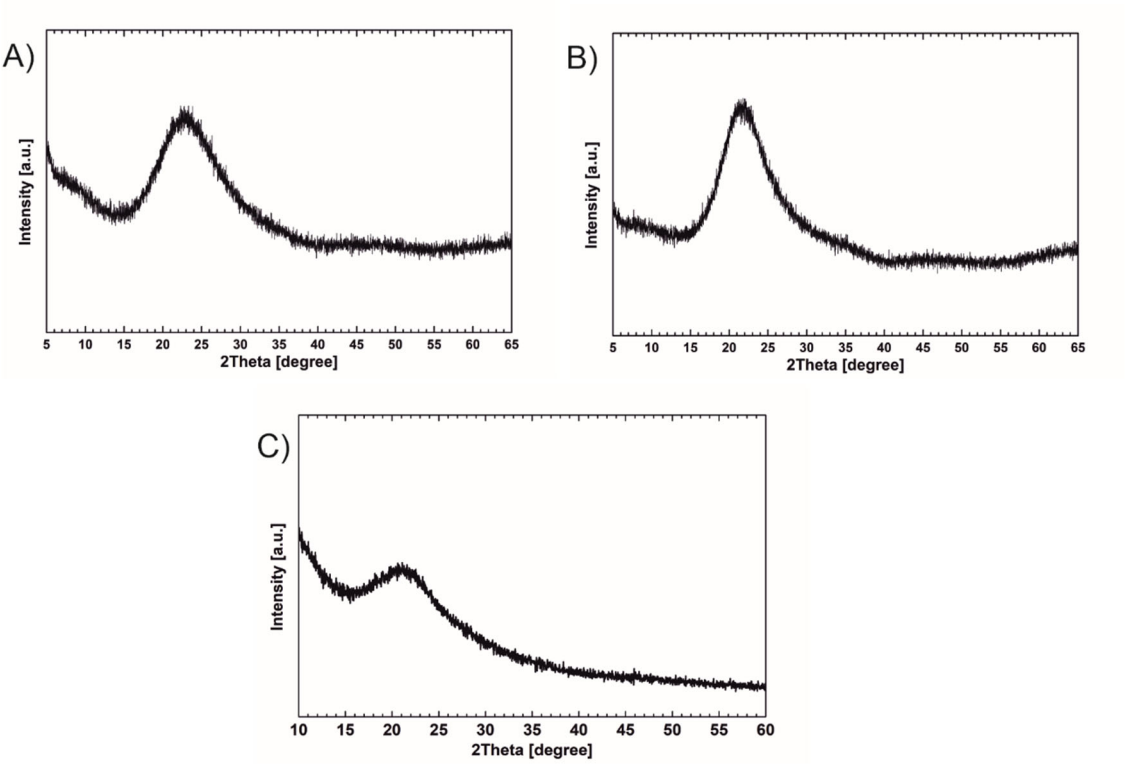

Figure S4. XRD patterns of amorphous samples of a) SiO_2 sorbent, b) SiO_2 VS, c) SiO_2 nanoparticles.

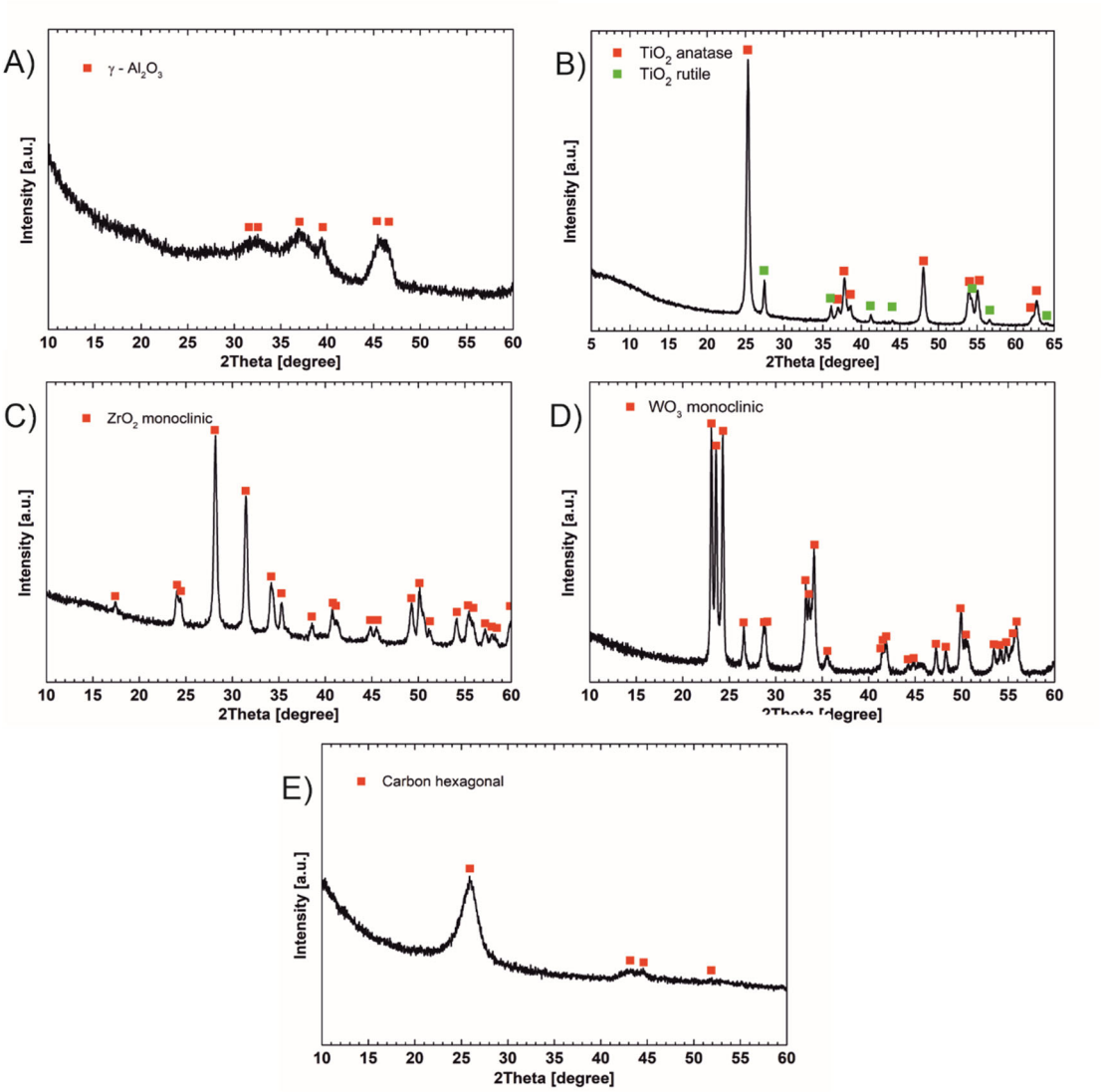

Figure S5. X-ray diffractograms of reference crystalline nanomaterials: a) Al_2O_3 γ nanoparticles, b) TiO_2 P25 nanoparticles, c) ZrO_2 nanoparticles, d) WO_3 nanoparticles, e) multi-wall carbon nanotubes (MWCNT).

Table S1. Dehydrogenase activity in pulmonary cells A549 after incubation with materials. A549 cells were treated with fibers and nanoparticles for 24 and 48 h at concentrations 0-100 µg.mL^{-1}. Multiwalled carbon nanotubes *(MWCNT)* were used as a positive control and untreated cells as a negative control. Dehydrogenase activity of A549 cells was assayed using the WST-1 test. The results are expressed as mean ± SD (control = 100%; n = 12); *, *P < 0.05*; **, *P < 0.01*; ***, *P < 0.001* vs. untreated cells (*P = 0.05*).

Sample		Concentration [µg.mL^{-1}]	Incubation period	
			24 h	48 h
Control		0	100 ± 2%	100 ± 4%
Fibers	**Al_2O_3 α**	1	101 ± 4%	96 ± 7%
		10	99 ± 4%	93 ± 6%
		100	83 ± 7% *(P < 0.001)*	82 ± 7% *(P < 0.001)*
	Al_2O_3 γ	1	98 ± 3%	98 ± 5%
		10	106 ± 4%	106 ± 4%
		100	98 ± 5%	93 ± 5%
	SiO_2 Vs	1	104 ± 3%	99 ± 4%
		10	97 ± 10%	90 ± 5% *(P = 0.006)*
		100	96 ± 2%	87 ± 4% *(P < 0.001)*
	SiO_2 Sorbent	1	101 ± 3%	102 ± 5%
		10	105 ± 4%	107 ± 4%
		100	102 ± 2%	93 ± 5%
	ZrO_2	1	97 ± 7%	98 ± 6%
		10	99 ± 4%	94 ± 6%
		100	98 ± 3%	97 ± 6%
	TiO_2 Rutile	1	102 ± 5%	101 ± 5%
		10	99 ± 5%	100 ± 6%
		100	91 ± 5% *(P = 0.004)*	87 ± 5% *(P < 0.001)*
	WO_3	1	100 ± 8%	105 ± 5%
		10	92 ± 11% *(P = 0.013)*	98 ± 9%
		100	91 ± 5% *(P = 0.005)*	105 ± 8%
Nanoparticles	**Al_2O_3**	1	100 ± 7%	95 ± 3%
		10	97 ± 3%	92 ± 5%
		100	90 ± 5% *(P < 0.001)*	86 ± 5% *(P < 0.001)*
	SiO_2	1	104 ± 6%	98 ± 5%
		10	102 ± 5%	94 ± 4%
		100	96 ± 5%	86 ± 2% *(P < 0.001)*
	ZrO_2	1	100 ± 5%	98 ± 7%
		10	100 ± 6%	105 ± 4%
		100	99 ± 5%	97 ± 8%
	TiO_2 P25	1	100 ± 4%	100 ± 3%
		10	98 ± 5%	106 ± 3%
		100	92 ± 5% *(P = 0.014)*	86 ± 5% *(P < 0.001)*
	WO_3	1	94 ± 7%	96 ± 5%
		10	94 ± 7%	97 ± 6%
		100	91 ± 5% *(P = 0.002)*	101 ± 7%
MWCNT		1	101 ± 6%	98 ± 4%
		10	95 ± 5%	90 ± 3% *(P < 0.003)*
		100	73 ± 5% *(P < 0.001)*	63 ± 8% *(P < 0.001)*

Table S2. Glutathione levels in pulmonary cells A549 after incubation with materials. A549 cells were treated with fibers and nanoparticles for 24 and 48 h at concentrations 0-100 µg.mL^{-1}. Multiwalled carbon nanotubes *(MWCNT)* were used as a positive control and untreated cells as a negative control. Glutathione levels in cells were measured using the monochlorobimane assay. The results are expressed as mean ± SD (control = 100%; n = 12); *, *P < 0.05*; **, *P < 0.01*; ***, *P < 0.001* vs. untreated cells (*P = 0.05*).

Sample		Concentration [µg.mL^{-1}]	Incubation period	
			24 h	48 h
Control		0	100 ± 4%	100 ± 4%
Fibers	**Al_2O_3 α**	1	103 ± 4%	100 ± 5%
		10	101 ± 5%	98 ± 4%
		100	86 ± 6% *(P < 0.001)*	82 ± 5% *(P < 0.001)*
	Al_2O_3 γ	1	102 ± 4%	98± 6%
		10	103 ± 5%	100 ± 4%
		100	83 ± 5% *(P < 0.001)*	82 ± 4% *(P < 0.001)*
	SiO_2 Vs	1	102 ± 8%	101 ± 5%
		10	101 ± 3%	105 ± 5%
		100	97 ± 4%	92 ± 5% *(P = 0.001)*
	SiO_2 Sorbent	1	105 ± 6%	106 ± 4%
		10	98 ± 3%	99 ± 4%
		100	101 ± 5%	99 ± 3%
	ZrO_2	1	97 ± 7%	101 ± 5%
		10	99 ± 4%	100 ± 3%
		100	94 ± 3%	95 ± 4%
	TiO_2 Rutile	1	93 ± 11%	98 ± 3%
		10	99 ± 8%	100 ± 3%
		100	80 ± 7% *(P < 0.001)*	77 ± 6% *(P < 0.001)*
	WO_3	1	105 ± 5%	102 ± 3%
		10	103 ± 3%	101 ± 4%
		100	93 ± 8%	90 ± 2% *(P < 0.001)*
Nanoparticles	**Al_2O_3**	1	107 ± 4%	106 ± 3%
		10	106 ± 5%	102 ± 5%
		100	99 ± 7%	95 ± 6%
	SiO_2	1	105 ± 5%	107 ± 3%
		10	105 ± 9%	107 ± 6%
		100	99 ± 3%	99 ± 5%
	ZrO_2	1	105 ± 10%	105 ± 2%
		10	95 ± 2%	96 ± 2%
		100	95 ± 5%	95 ± 4%
	TiO_2 P25	1	104 ± 6%	101 ± 5%
		10	91 ± 4% *(P = 0.017)*	92 ± 4% *(P = 0.004)*
		100	87 ± 4% *(P < 0.001)*	83 ± 3% *(P < 0.001)*
	WO_3	1	107 ± 4%	106 ± 4%
		10	101 ± 6%	104 ± 6%
		100	100 ± 4%	103 ± 5%
MWCNT		1	106 ± 5%	105 ± 5%
		10	104 ± 5%	103 ± 6%
		100	78 ± 12% *(P < 0.001)*	74 ± 11% *(P < 0.001)*

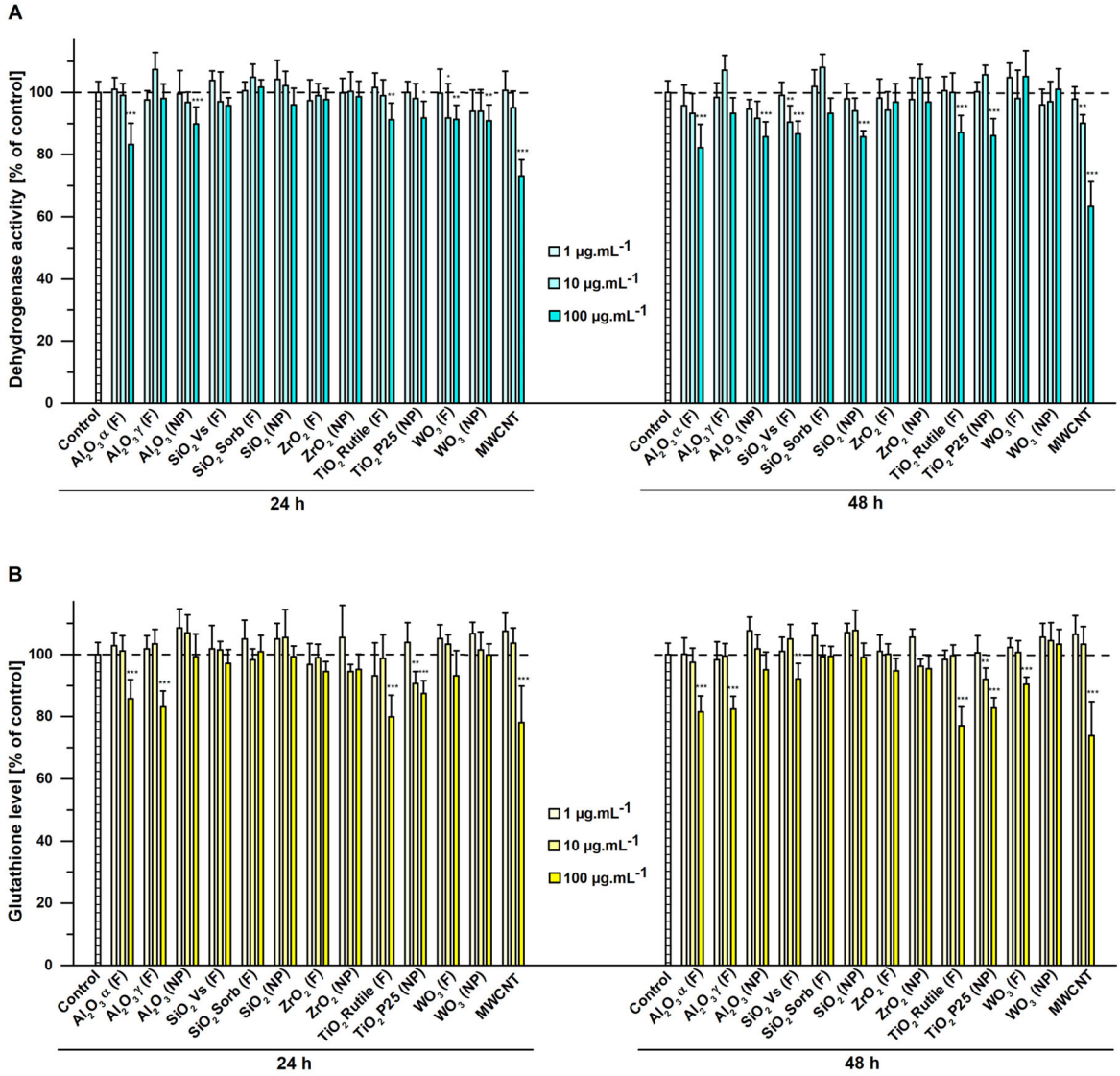

Figure S6. Effect of fibers (F) and nanoparticles (NP) on dehydrogenase activity (A) and glutathione levels (B) in A549 cells after 24 and 48 h of treatment. Data are expressed as means ± SD (n = 12). *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ vs. untreated control cells.