Co-exposure with CeO2NPs results in an antagonistic effect on the cytotoxicity and genotoxicity of TiO2NP on A549 cells

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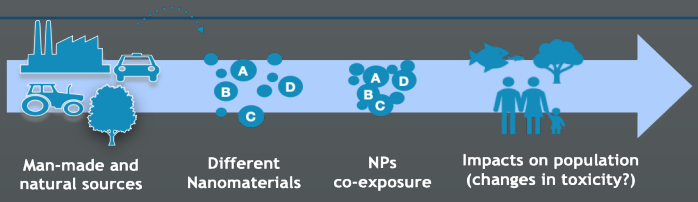
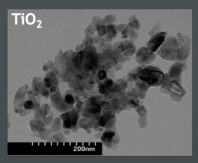
NanoLegaTox

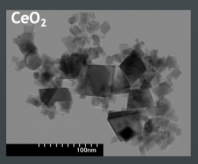


OBJECTIVE Assess the CYTOTOXIC and GENOTOXIC POTENTIAL resulting from titanium dioxide nanoparticles (TiO_2NP) and cerium oxide nanoparticles (CeO_2NP) co-exposure in human lung epithelial cell line A549.



HRTEM analysis
size: 26.6 ± 10.3 nm
crystalline particles
heterogeneous morphology

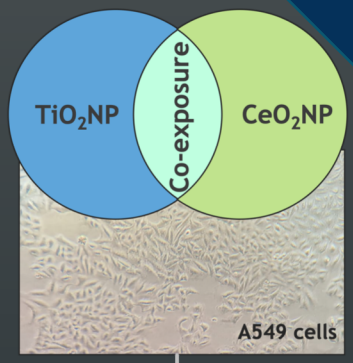
DLS analysis
ζ-potential (water): 41.1 ± 1.6 mV
z-average (culture media): 61.7 ± 1.2 nm (1 mg/L)
115.0 ± 0.8 nm (75 mg/L)



HRTEM analysis
size: 14.0 ± 7.7 nm
crystalline particles
cubic morphology

DLS analysis
ζ-potential (water): 14.9 ± 0.7 mV
z-average (culture media): 18.5 ± 0.7 nm (0.1 µg/L)
32.8 ± 1.2 nm (10 µg/L)

TiO2 and CeO2 NPs mixture increased z-average
exp.: TiO2NP 75 mg/L + CeO2NP 10 µg/L 250.1 ± 1.9 nm



24h exposure at 37 °C, 5% CO_2.

Fig. 1. A) Viability; B) cell cycle; C) DNA damage and D) % **H2AX** 24 h post-exposure of A549 cells to single and binary mixtures of TiO_2-CeO_2 NP.

*Values expressed as mean ± standard deviation (n≥3, each experiment in triplicate). Statistical significance of samples compared to C- is indicated by * (One-way ANOVA; p≤0.05).*

	Fig. 1A	Fig. 1B	Fig. 1C	Fig. 1D
	VIABILITY WST-1 assay	**CELL CYCLE** PI + RNase flow cytometry	**DNA DAMAGE** comet assay standard and FPG-modified	**γH2AX** DOUBLE STRAND BREAKS (DSB) Anti-hu/mo phospho-histone flow cytometry
individual	Cytotoxicity increased with concentration.	Overall, NPs did not affect the cell cycle, but TiO_2NP 75 mg/L induced an arrest at G0/G1 phase, with decreased S and G2/M phases.	No significant changes in DNA damage and oxidative damage in relation to negative control.	Both NPs significantly increased % γH2AX. TiO_2NP > CeO_2NP
co-exposure	CeO_2NP decreased TiO_2NP 75 mg/L cytotoxic potential.	CeO_2NP co-exposure partially reduced the negative effect TiO_2NP 75 mg/L had on the cell cycle progression.	Increased DNA damage. However, CeO_2NP 0.1 µg/L decreased oxidative damage compared to single exposure and to negative control.	When in mixture, the presence of CeO_2NPs resulted in the significant diminishing of DSBs induced by TiO_2NPs.

CONCLUSION Overall, an antagonistic effect of CeO2NP on TiO2NP was observed concerning the latter's toxicity. Regarding genotoxicity, A549 co-exposure to both NPs increased mechanisms of primary DNA damage, but base oxidation processes and cytotoxic lesions, such as DSB, were, somehow, prevented. These findings prove that joint toxicity of nanomaterials cannot be disregarded and must be further explored.

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