BRAIN-be

Belgian Research Action through Interdisciplinary Networks

2012-2017

To²DeNano

Towards a toxicologically relevant definition of nanomaterials

Peter Hoet (KU Leuven) – Lode Godderis (KU Leuven) – Sivakumar Murugadoss (KU Leuven) – Jan Mast (Sciensano) – Frederic Brasinne (Sciensano) – Dominique Lison (UCL) – Sybille van den Brule (UCL) –Jasmine Petry (FPS Economy) – Noham Sebaihi (FPS Economy)

Axis 4: Federal public strategies

Annex :Workpackage 3 and 4

A

B

C

D

E

F
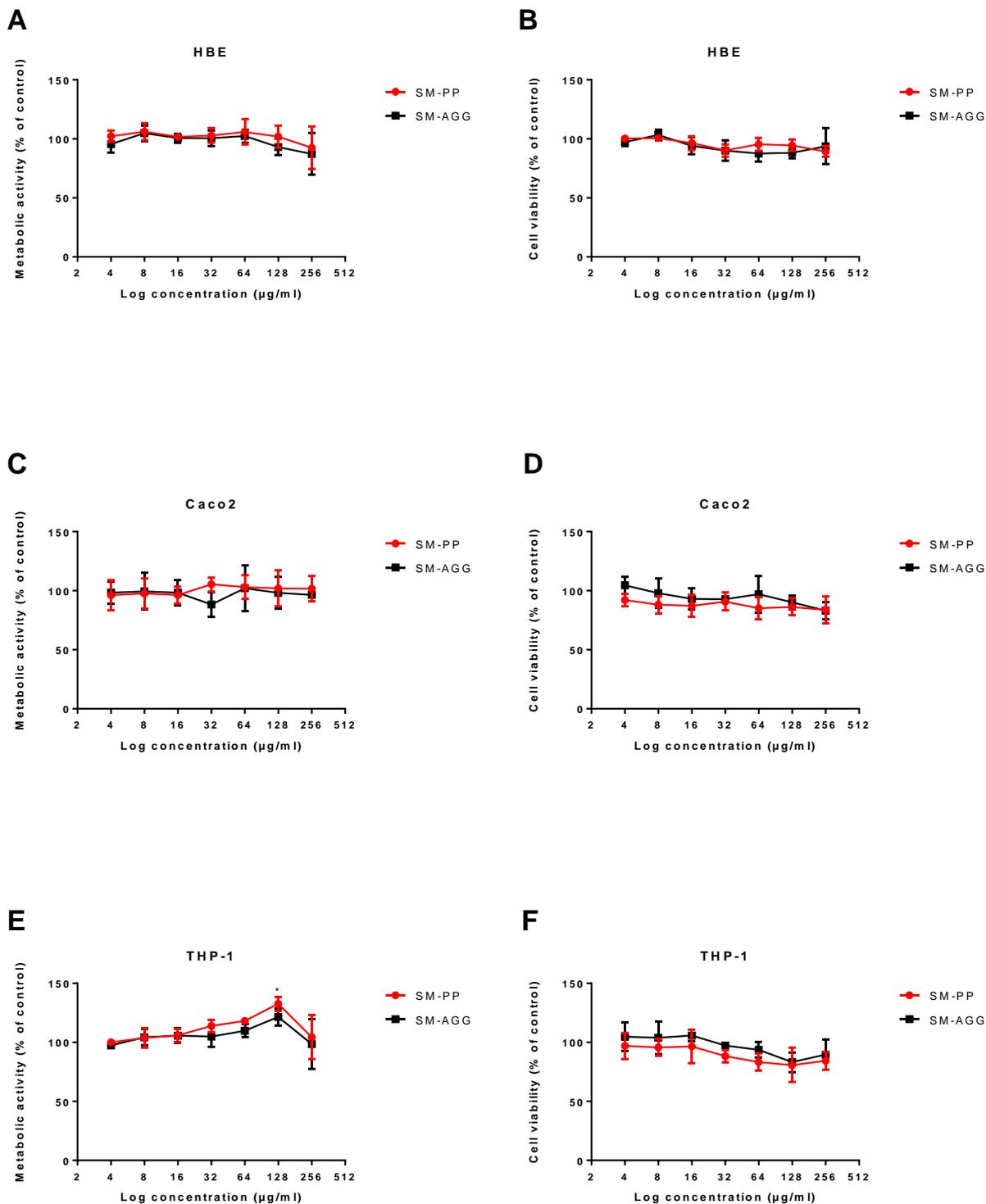
Figure 1: Effect of SM-TiO$_2$ NMs on cytotoxicity. WST-1 and LDH assay was used to measure the effect on metabolic activity (A-HBE;C-Caco2; E-THP1) and cell viability (B-HBE;D-Caco2; F-THP1) in cell cultures following 24 h exposure to SM-PP (red line) and SM-AGG (black line) dispersions. Data are expressed as means ± SD from three independent experiments performed in triplicates. Two way ANOVA followed by post-hoc tests were performed to determine statistical significances. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) represents significant difference compared to control; $p < 0.05$ (Δ), p

< 0.01 (ΔΔ) and p < 0.001 (ΔΔΔ) represents significant difference between PP and AGG at the same mass dose.
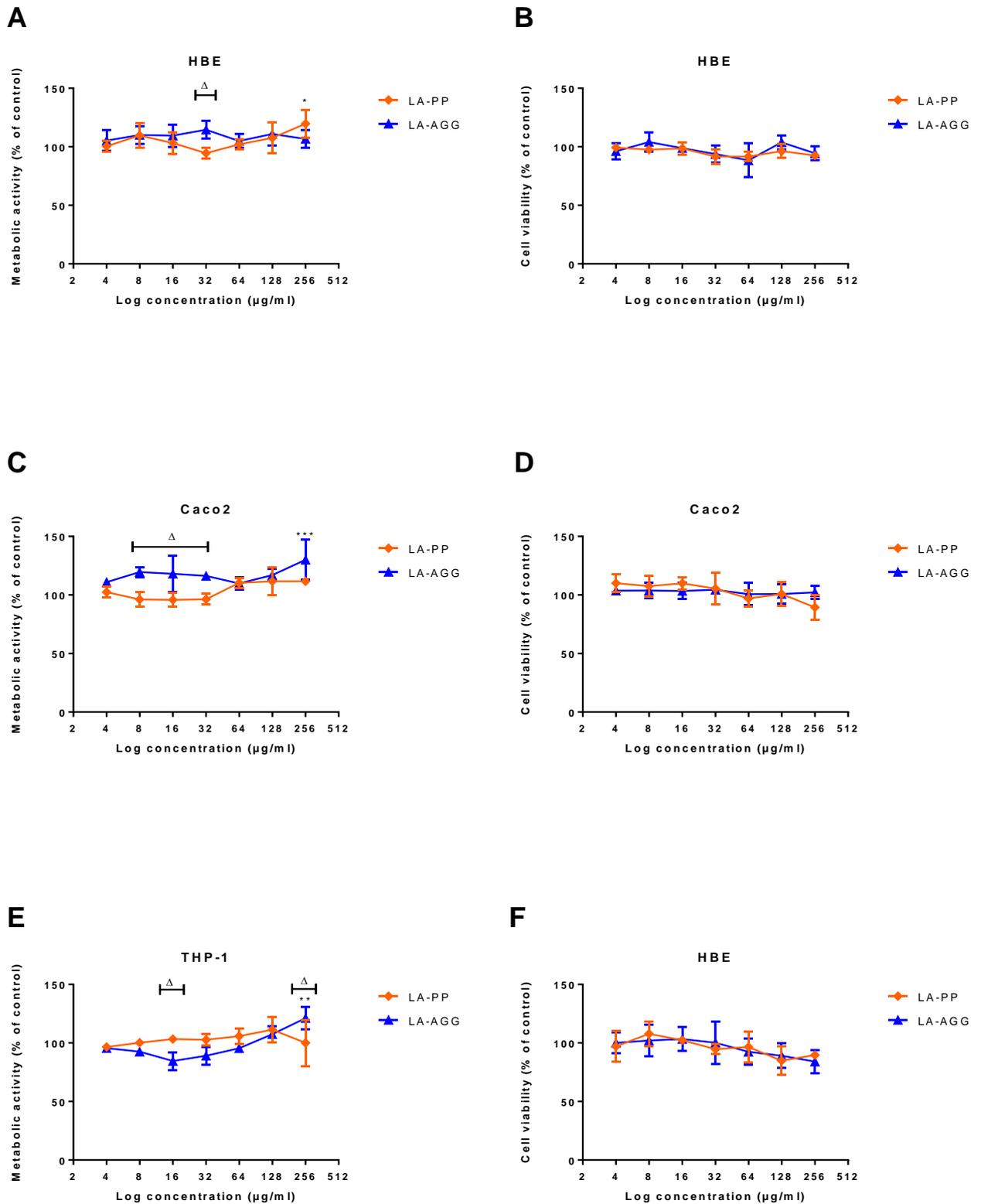
Figure 2: Effect of LA-TiO$_2$ NMs on cytotoxicity. WST-1 and LDH assay was used to measure the effect on metabolic activity (A-HBE;C-Caco2; E-THP1) and cell viability (B-HBE;D-Caco2; F-THP1) in cell cultures following 24 h exposure to LA-PP (orange line) and LA-AGG (blue line) dispersions. Data are expressed as means ± SD from three independent experiments performed in triplicates. Two way

ANOVA followed by post-hoc tests were performed to determine statistical significances. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) represents significant difference compared to control; $p < 0.05$ (Δ) represents significant difference between PP and AGG at the same mass dose.
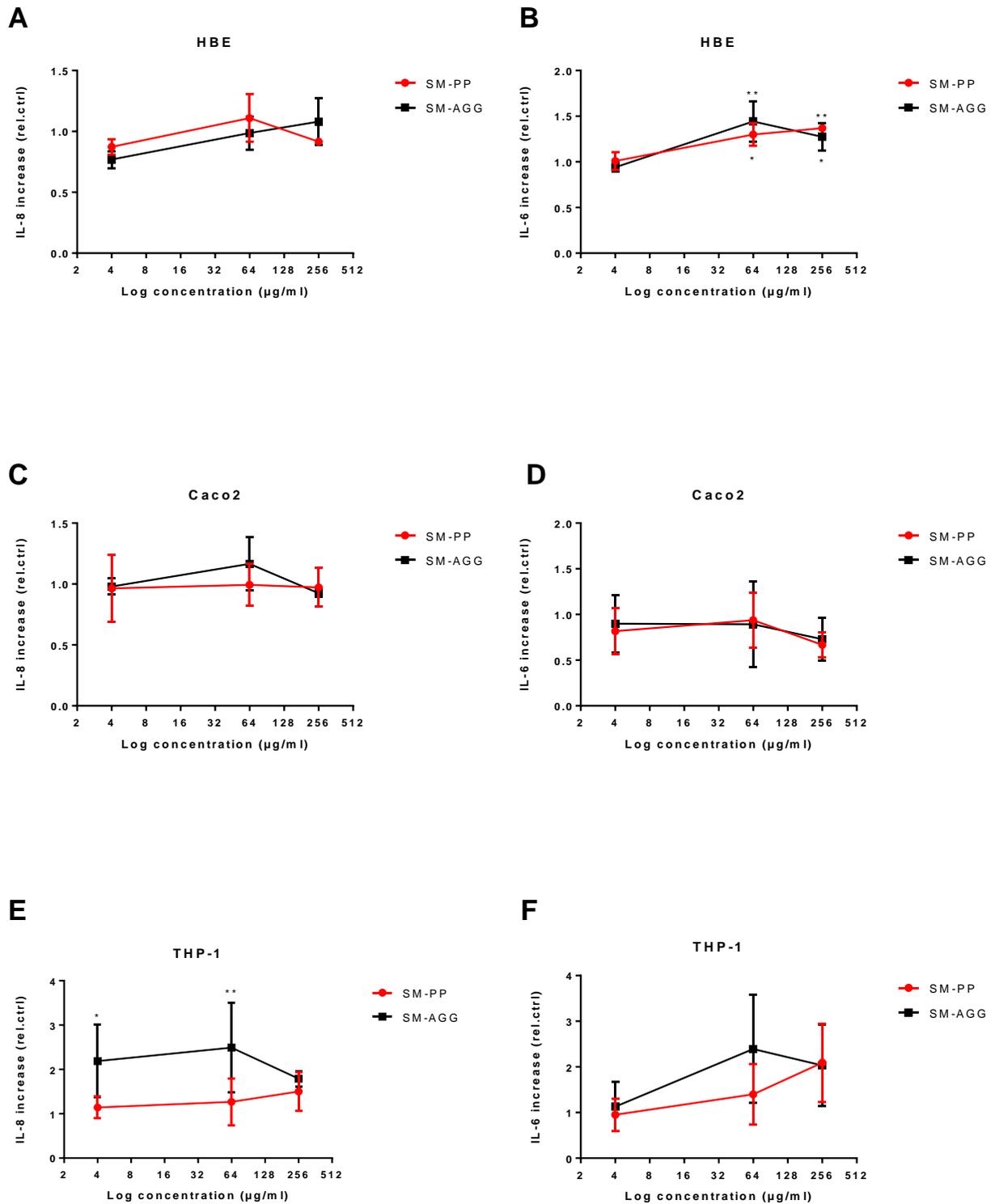
Figure 3: Effect of SM TiO$_2$ NMs on cytokine release. IL-8 (A-HBE; C-Caco2; E-THP1) and IL-6 (B-HBE; D-Caco2; F-THP1) levels were measured in the supernatant of the cell cultures following 24 h exposure to SM-PP (red line) and SM-AGG (black line) suspensions. Data are expressed as means ± SD from three independent experiments performed in duplicates. Two way ANOVA followed by post-

hoc tests were performed to determine statistical significances. $p < 0.05$ (*) and $p < 0.01$ (**) represents significant difference compared to control.
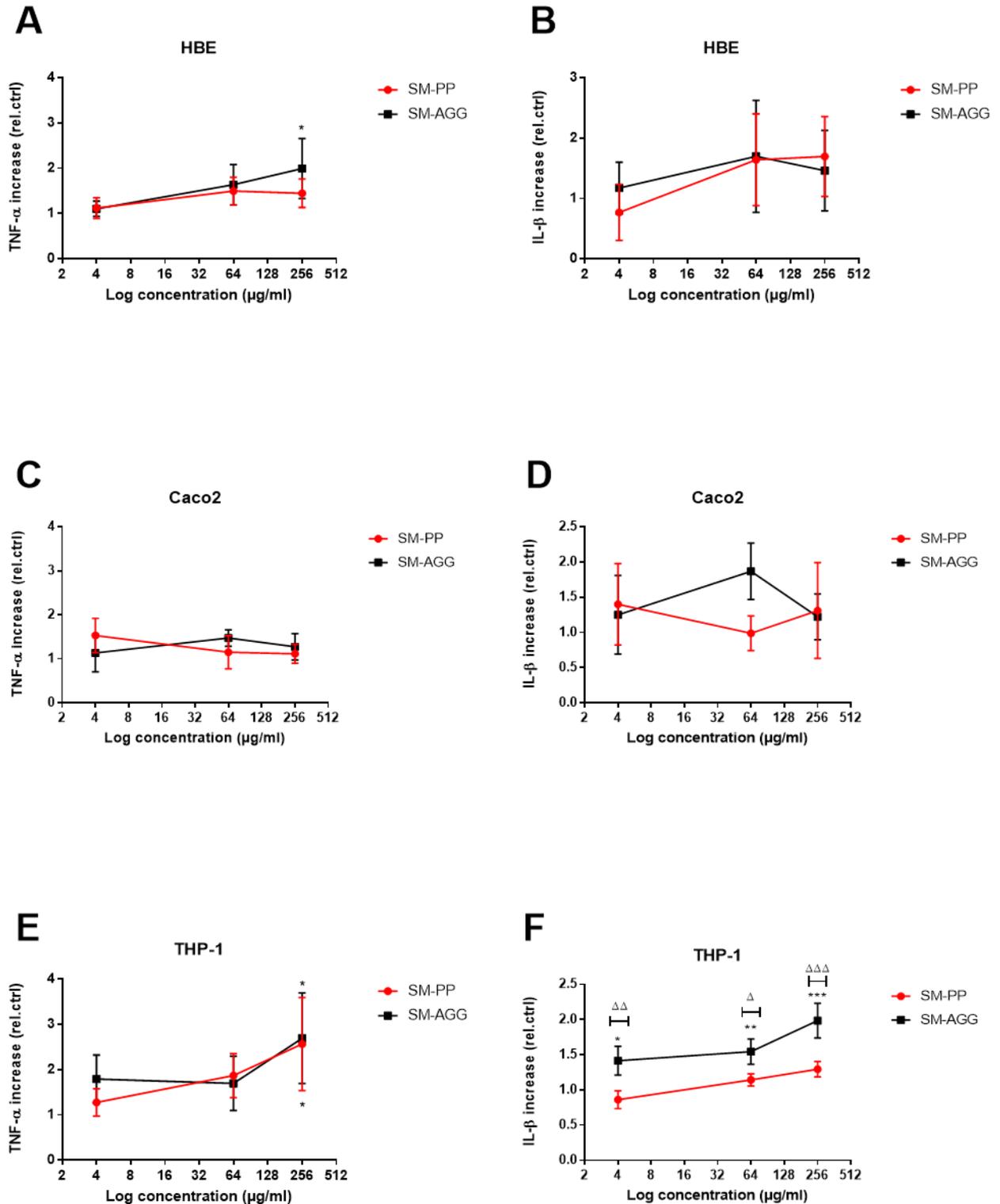
Figure 4: Effect of SM TiO$_2$ NMs on cytokine release. TNF-α (A-HBE; C-Caco2; E-THP1) and IL-1β (B-HBE; D-Caco2; F-THP1) levels were measured in the supernatant of the cell cultures following 24 h exposure to SM-PP (red line) and SM-AGG (black line) suspensions. Data are expressed as means ± SD from three independent experiments performed in duplicates. Two way ANOVA followed by

post-hoc tests were performed to determine statistical significances. $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) represents significant difference compared to control; $p < 0.05$ (Δ), $p < 0.01$ ($\Delta\Delta$) and $p < 0.001$ ($\Delta\Delta\Delta$) represents significant difference between PP and AGG at the same mass dose.

Figure 5: Effect of LA TiO$_2$ NMs on cytokine release. IL-8 (A-HBE; C-Caco2; E-THP1) and IL-6 (B-HBE; D-Caco2; F-THP1) levels were measured in the supernatant of the cell cultures following 24 h exposure to LA-PP (orange line) and LA-AGG (blue line) suspensions. Data are expressed as means ± SD from three independent experiments performed in duplicates. Two way ANOVA followed by

post-hoc tests were performed to determine statistical significances. p < 0.05 (*) represents significant difference compared to control.

A

HBE

B

HBE

C

Caco2

D

Caco2

E

THP-1

F
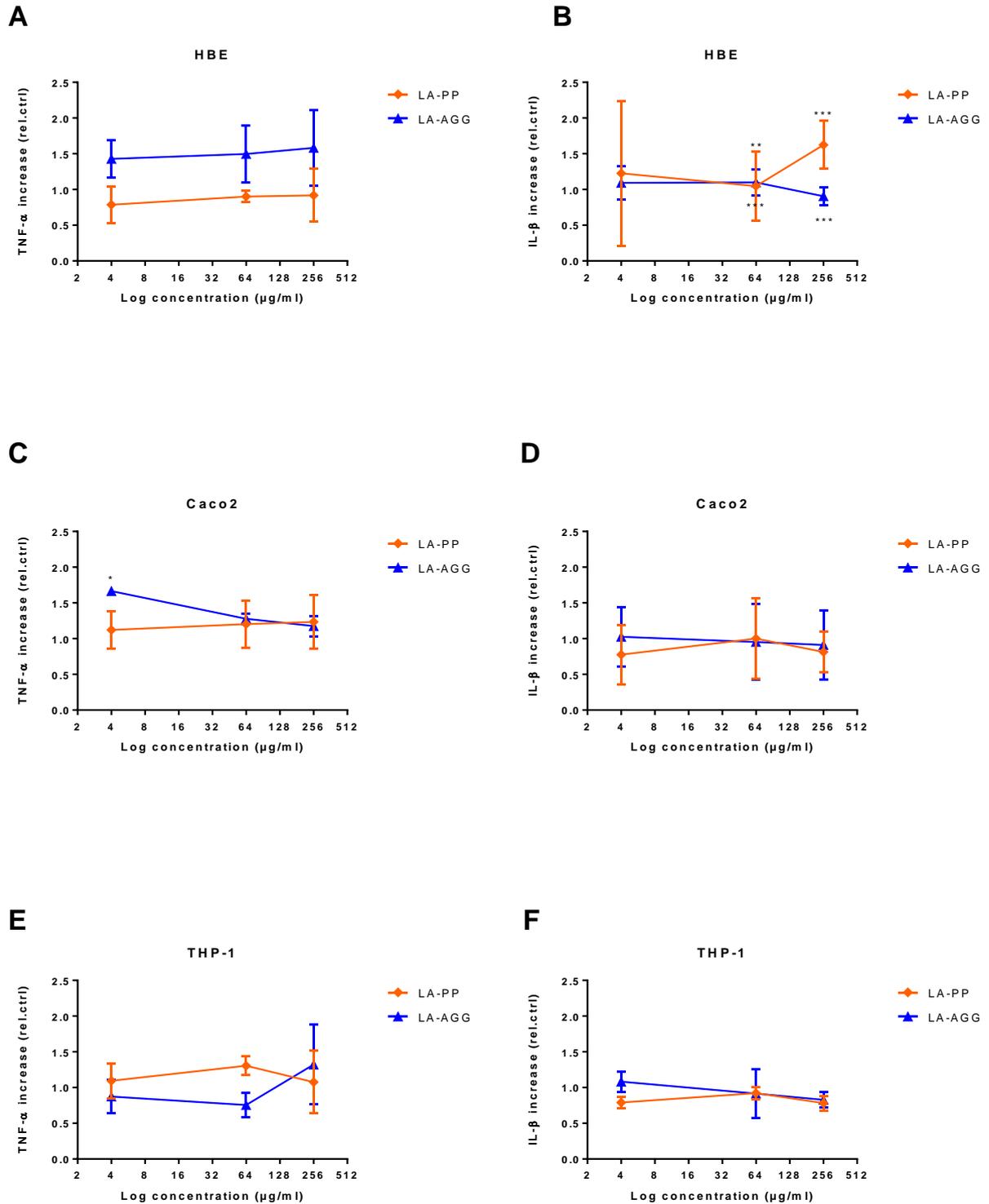
THP-1

Figure 6: Effect of LA TiO$_2$ NMs on cytokine release. TNF-α (A-HBE; C-Caco2; E-THP1) and IL-1β (B-HBE; D-Caco2; F-THP1) levels were measured in the supernatant of the cell cultures following 24

h exposure to LA-PP (orange line) and LA-AGG (blue line) suspensions. Data are expressed as means ± SD from three independent experiments performed in duplicates. Two way ANOVA followed by post-hoc tests were performed to determine statistical significances. p < 0.05 (*), p < 0.01 (**) and p < 0.001 (***)   represents significant difference compared to control.

A

B

C
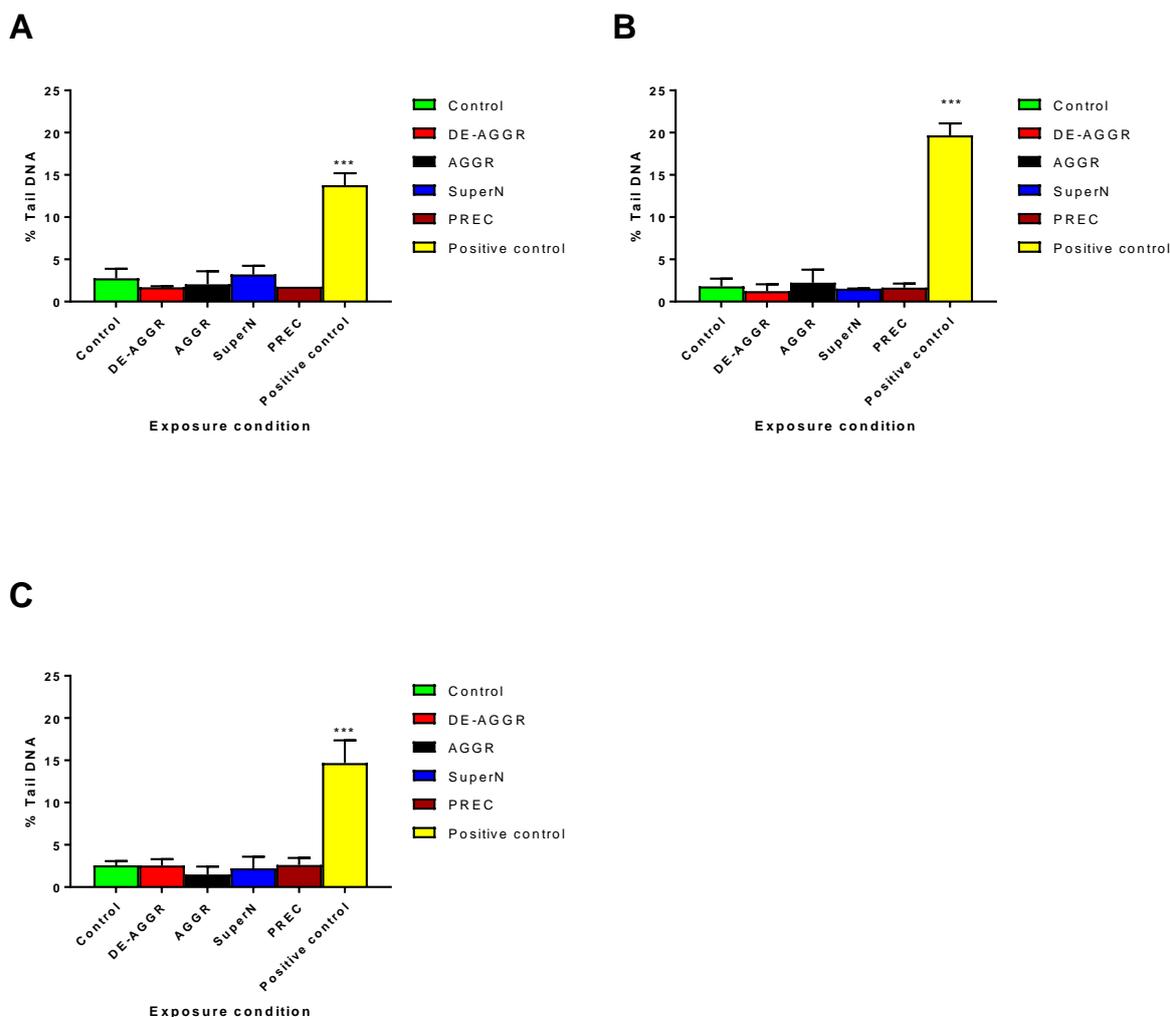
Figure 7: Effect of SiO$_2$ dispersions on Genotoxicity. DNA strand breaks measured using comet assay (A-HBE; B-Caco2; C-THP-1) after 24 h exposure to LC 30 of different particle suspensions. Data are expressed as means ± SD from three independent experiments performed in duplicates. Two way ANOVA followed by post-hoc tests were performed to determine statistical significances. Significance was considered at p < 0.001 (***).
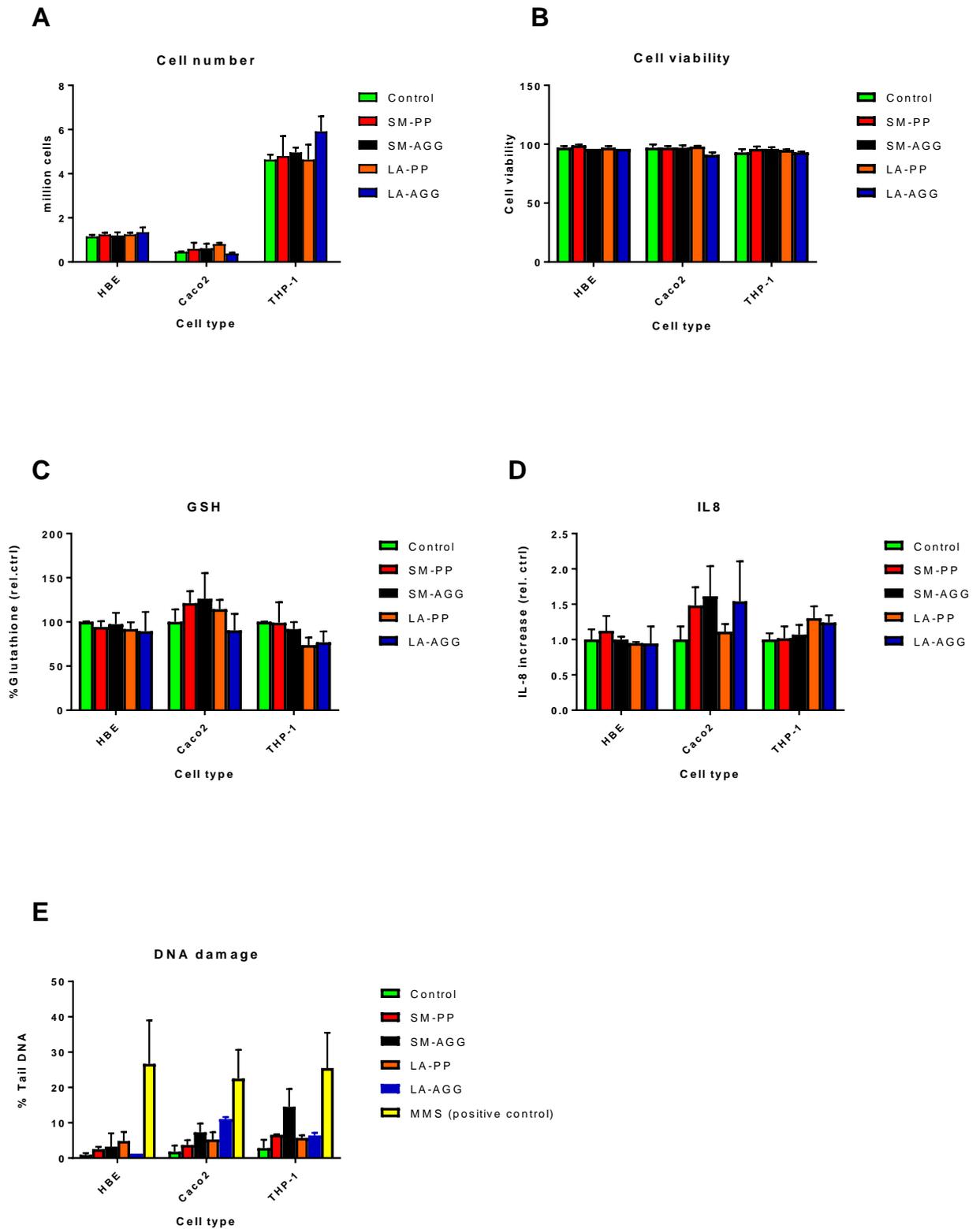
Figure 8: Toxic and biological effects induced by repeated exposure to a low concentration of TiO$_2$ dispersions. Cell number (A), cell viability (B), GSH levels (C), IL-8 release (D) and DNA damage (E) evaluated in different cell lines after three weeks of repeated exposure to 2 µg/ml of SM and LA dispersions.
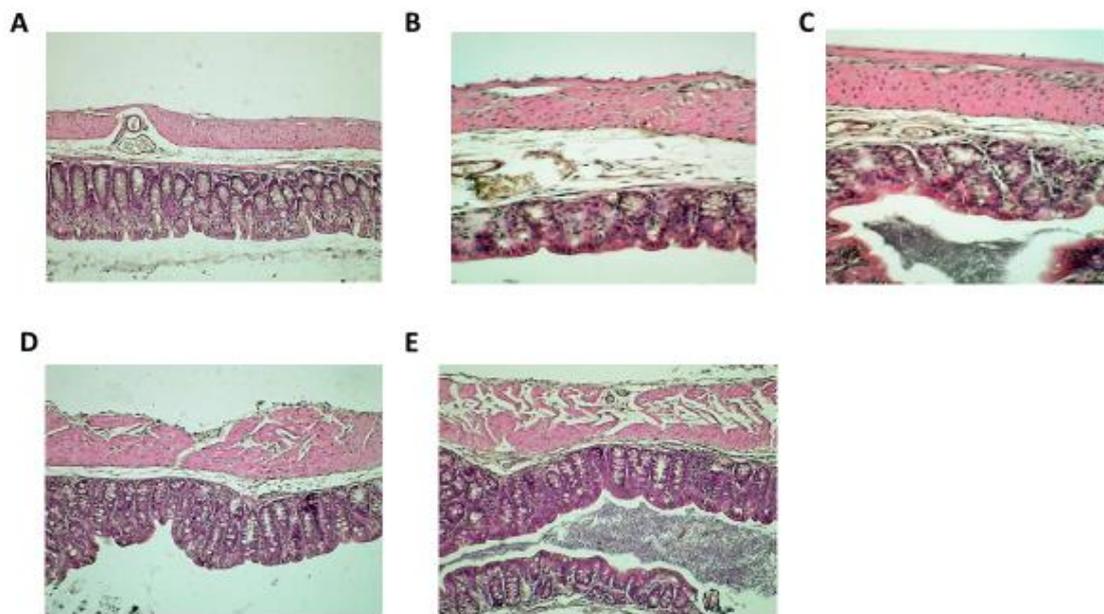
Figure 9: Effect on colon in mice gavaged 3d before with 500 µg of different TiO$_2$ dispersions. Control (A), SM-PP (B), SM-AGG (C), LA-PP (D) and LA-AGG (E).
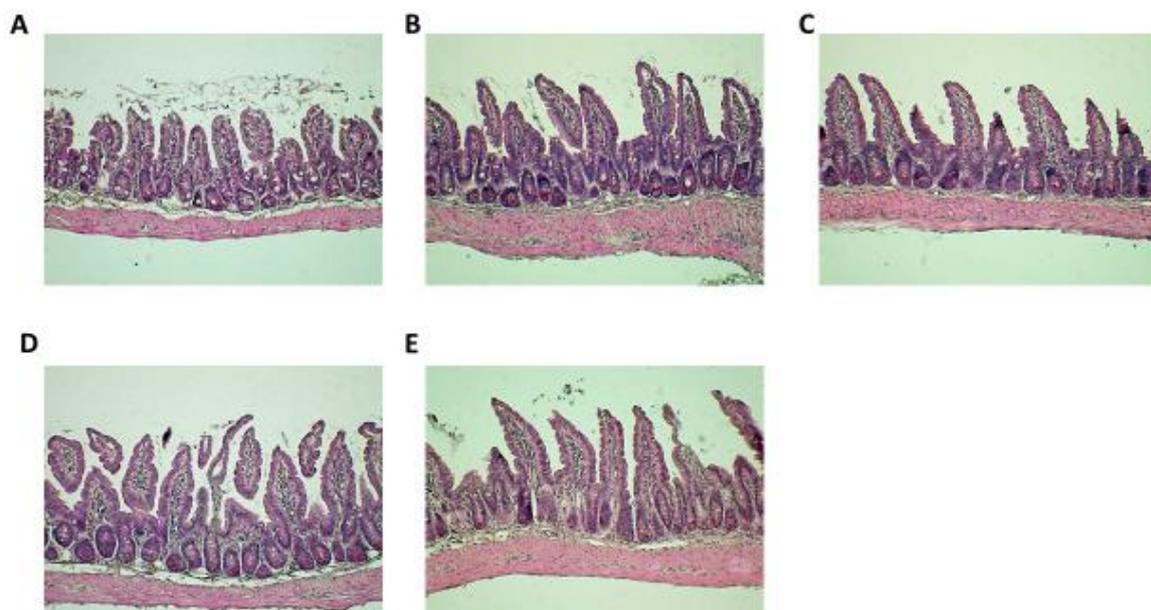
Figure 10: Effect on ileum in mice gavaged 3d before with 500 µg of different TiO$_2$ dispersions. Control (A), SM-PP (B), SM-AGG (C), LA-PP (D) and LA-AGG (E).

Table 1: Overview of the measurands determined by the different methods

Level	Method	Type of measurand	Measurand
Primary particle (PP)		1D size	Maximal enscribed circular ϕ
		Shape	Aspect ratio
Aggregate / agglomerate	BF-TEM	1D size	Feret Min ϕ
			Feret Max ϕ
			Feret Mean ϕ
			Minimal ϕ
			Mean ϕ
			Maximal ϕ
			Equivalent circular ϕ
			Maximal enscribed circular ϕ
			Perimeter
			Convex perimeter
		2D size	Area
			Convex Area
			Rectangle Min
			Rectangle Mean
			Rectangle Max
		Shape	Aspect Ratio
			Sphericity
			Elongation
		Surface topology	Convexity
			Shape Factor
		Fractal properties	Fractal pré-factor k_g
			Fractal dimension D_f
			Overlap coefficient
			VSSA
			Number of PP per aggregate/agglomerate
	AFM		Maximal height (Z)
	PTA		Hydrodynamic radius
	DLS		Hydrodynamic radius
Sample	Electron diffraction		Interplanar distances
			Phase / Crystal structure