Supplemental Material for

Metal Oxide Nanoparticles Induce Unique Inflammatory Footprints in the Lung; Important Implications for Nanoparticle Testing

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Figure 1. Effect of the panel of NP on LDH (A) and total protein (B) release in the BAL of rats after 24 h post-instillation. LDH and total protein concentration showed similar pattern and significantly increased by instillation of CeO$_2$NP, NiONP, ZnONP and CuONP. Instillation of ZnONP and CuONP induced severe responses in LDH and total protein levels in the BAL. Values are mean ± S.D. from four independent experiments. Each treatment group was compared with vehicle control for statistical significance whilst high-dose groups were compared with low-dose group to evaluate dose-dependency: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$. NS, not significant.
Figure 2. LDH (A) and total protein (B) levels in the BAL at 4 wks after instillation.

Values are expressed mean ± S.D. from four independent experiments. Significance versus vehicle control: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$. 
Figure 3. Immunohistochemical staining of B-cells (CD45RA) and T-cells (CD3), and negative control (-ve, without primary antibody) in the lungs of NiONP (150 cm$^2$/rat) at 4 wks after instillation. Organized lymphocytes consisted of both B- and T-cells. Abbreviations: al, alveolus; v, blood vessel. Bars = 100 µm.

Figure 4. Rare granulomas of lungs at 4 wks after intratracheal instillation of CeO$_2$NP at 150 cm$^2$/rat. About 2 granulomas were found in any entire lung section at sites showed high deposition of NP. Tissues were stained with H&E. Bars = 100 µm.
Figure 5. Inflammatory mediators in the BAL at 24 h after intratracheal instillation of NP. (A) IL-1β; (B) MIP-2; (C) eotaxin; (D) IL-13. Values are mean ± S.D. from four independent experiments. Each treatment group was compared with vehicle control for statistical significance while high-dose groups were compared with low-dose group to evaluate dose-dependency. Significance versus vehicle control: * p < 0.05, ** p < 0.01, *** p < 0.001. NS, not significant.
Figure 6. Inflammatory mediators in the BAL at 4 wks after intratracheal instillation of NP. (A) MIP-2; (B) IFN-γ. Values are mean ± S.D. from four independent experiments. Significance versus vehicle control: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$. 