

Supplemental Material for

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

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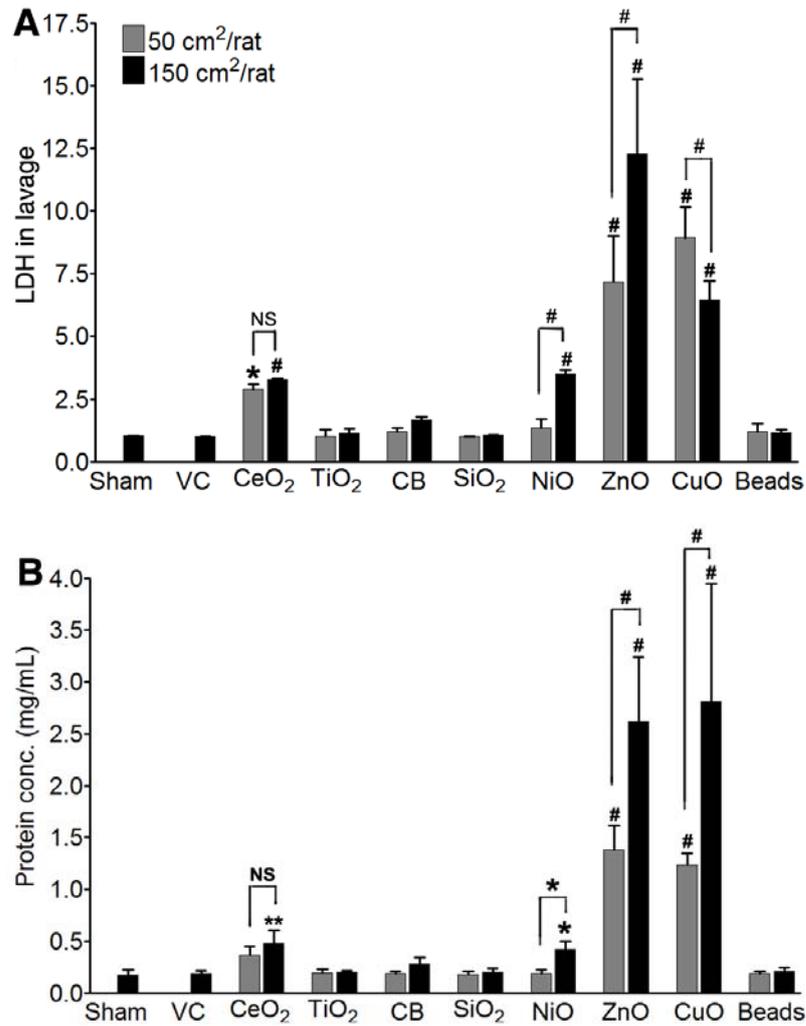


Figure 1. Effect of the panel of NP on LDH (A) and total protein (B) release in the BAL of rats after 24 h post-instillation. LDH and total protein concentration showed similar pattern and significantly increased by instillation of CeO₂NP, NiONP, ZnONP and CuONP. Instillation of ZnONP and CuONP induced severe responses in LDH and total protein levels in the BAL. Values are mean \pm 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.

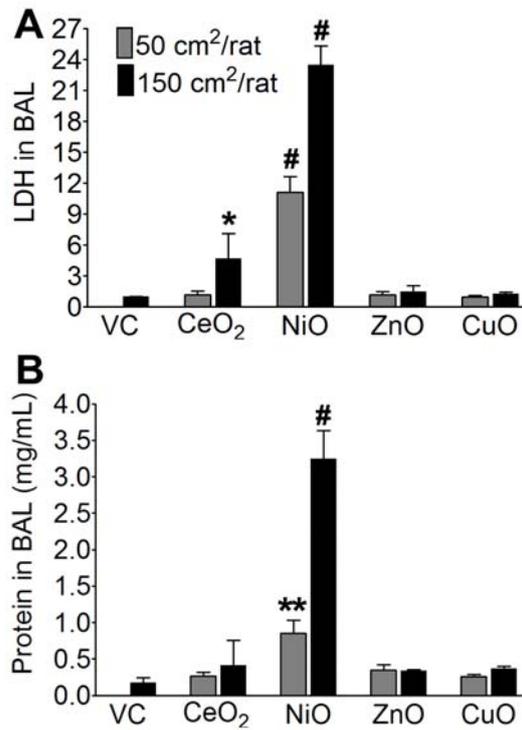


Figure 2. LDH (A) and total protein (B) levels in the BAL at 4 wks after instillation.

Values are expressed mean \pm S.D. from four independent experiments. Significance

versus vehicle control: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$.

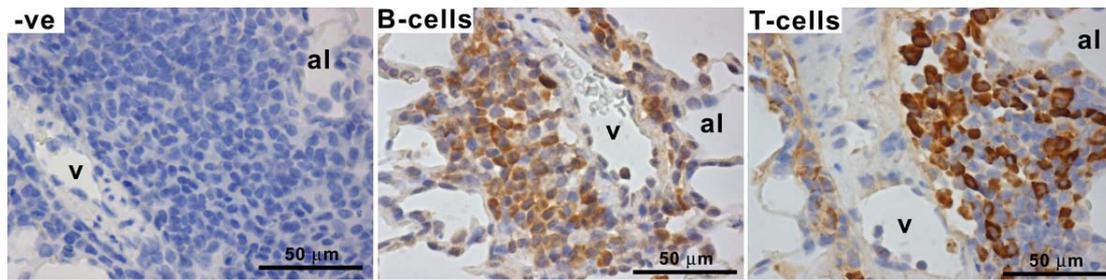


Figure 3. Immunohistochemical staining of B-cells (CD45RA) and T-cells (CD3), and negative control (-ve, without primary antibody) in the lungs of NiONP (150 cm²/rat) at 4 wks after instillation. Organized lymphocytes consisted of both B- and T-cells. Abbreviations: al, alveolus; v, blood vessel. Bars = 100 µm.

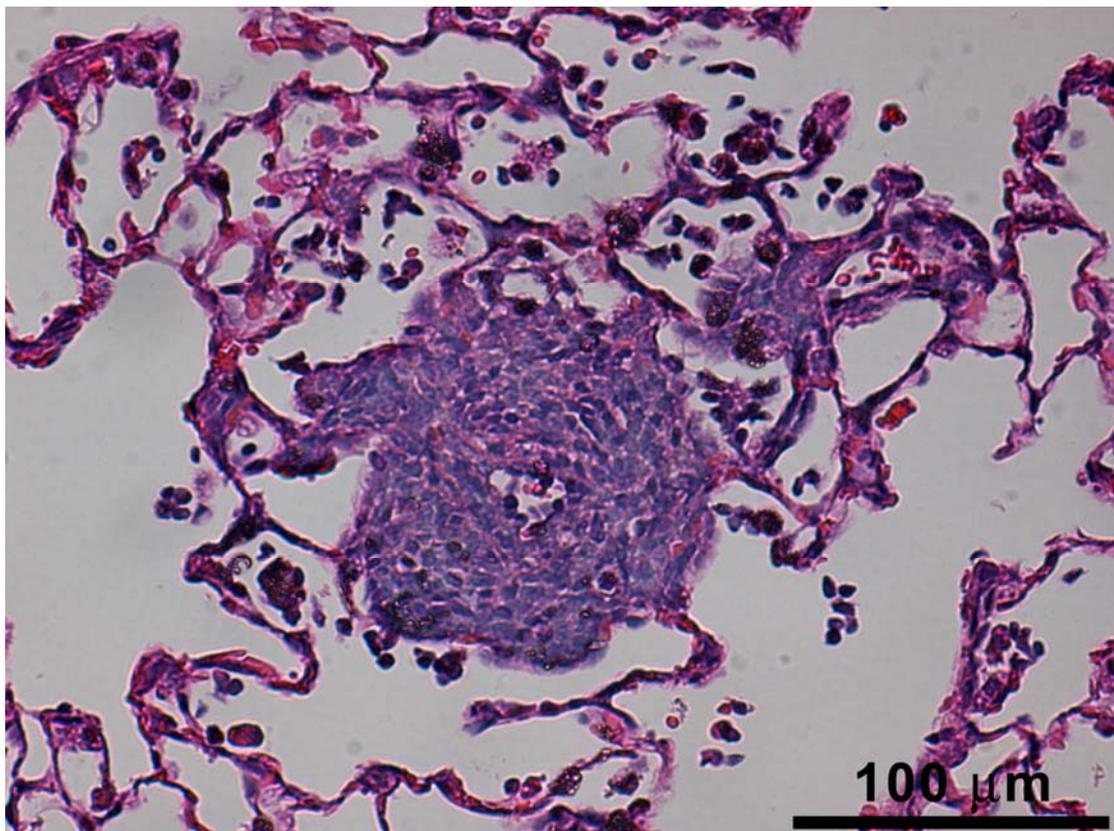


Figure 4. Rare granulomas of lungs at 4 wks after intratracheal instillation of CeO₂NP at 150 cm²/rat. About 2 granulomas were found any entire lung section at sites showed high deposition of NP. Tissues were stained with H&E. Bars = 100 µm.

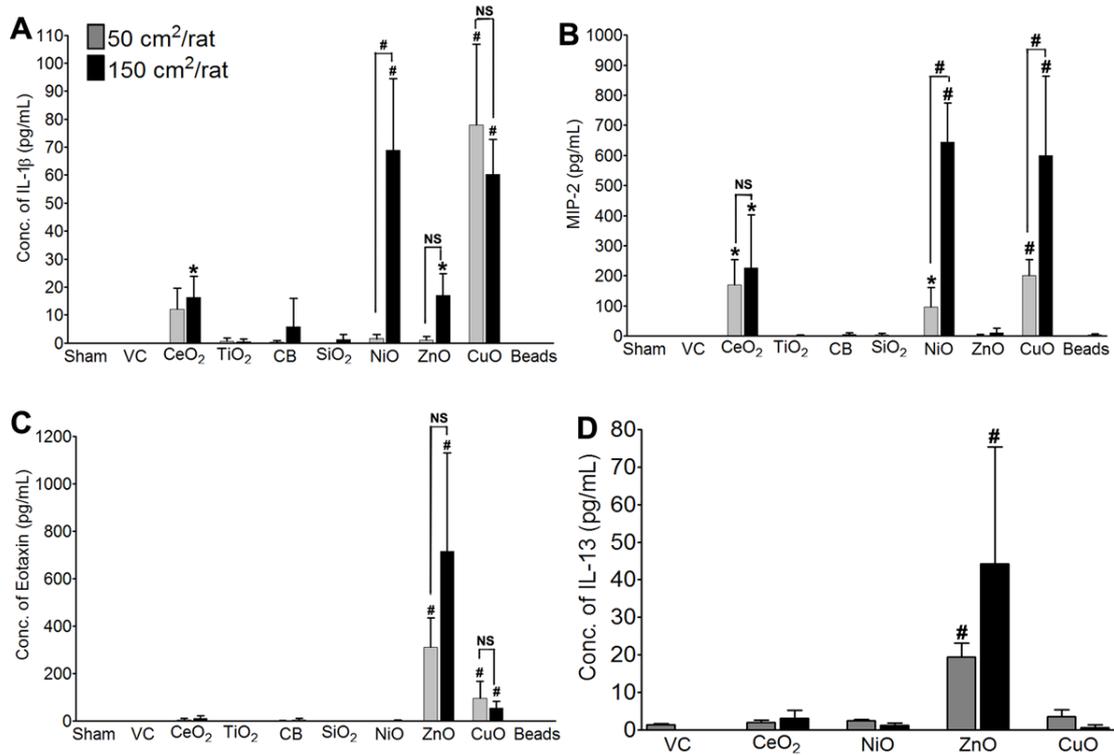


Figure 5. Inflammatory mediators in the BAL at 24 h after intratracheal instillation of NP. (A) IL-1 β ; (B) MIP-2; (C) eotaxin; (D) IL-13. Values are mean \pm S.D. from four independent experiments. Each treatment group was compared with vehicle control for statistical significance whilst high-dose groups were compared with low-dose group to evaluate dose-dependency. Significance versus vehicle control: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$. NS, not significant.

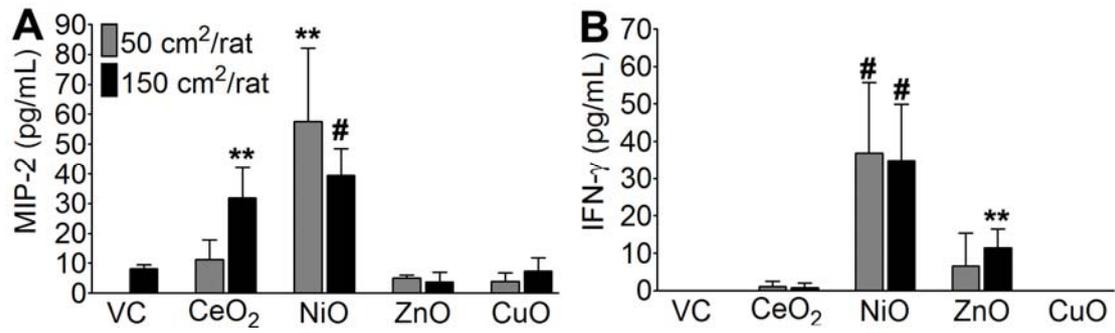


Figure 6. Inflammatory mediators in the BAL at 4 wks after intratracheal instillation of NP. (A) MIP-2; (B) IFN- γ . Values are mean \pm S.D. from four independent experiments. Significance versus vehicle control: * $p < 0.05$, ** $p < 0.01$, # $p < 0.001$.