# Evaluation of the Role of Oxidative Stress, Inflammation and Apoptosis in the Pulmonary and the Hepatic Toxicity Induced by the Intratracheal Instillation of Cerium Oxide Nanoparticles in Male Sprague-Dawley rats

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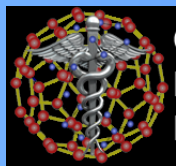

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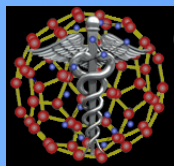
# Nanoparticles

Ultrafine particles with lengths in two or three dimensions greater than 1 nanometer (nm) and smaller than about 100 nm

## NIOSH: "Safety of nanoparticles"

"Nanotechnology is an emerging field. As such, there are many uncertainties as to whether the unique properties of engineered nanomaterials also pose occupational health risks"

Clayton Teague, NNI, 2005



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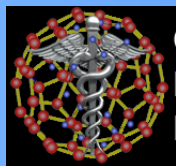
# Main types of manufactured nanomaterials

- Fullerenes (C60)
- Single-walled carbon nanotubes (SWCNTs)
- Multi-walled carbon nanotubes (MWCNTs)
- Silver nanoparticles
- Iron nanoparticles
- Carbon black
- Titanium dioxide
- Aluminium oxide
- Zinc oxide
- Silicon dioxide
- Cerium oxide
- Polystyrene
- Dendrimers
- Nano clays

Organization for Economic Cooperation and Development Working Party on Manufactured Nanomaterials, 2008



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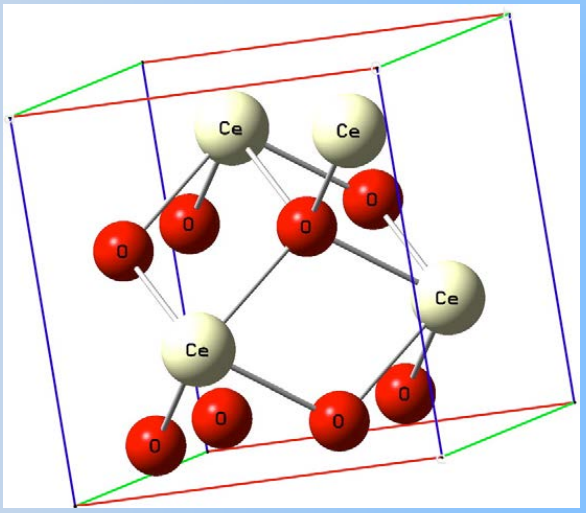
# Cerium oxide ($CeO_2$) nanoparticles

## Cerium

- Rare earth metal
- Strong oxidizing agent
- Very reactive and can undergo redox cycling

## Identified as a material of potential concern

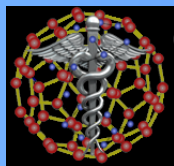



American Society for Testing and Materials (ASTM 2006)

# $CeO_2$ nanoparticles

- Used as catalysts to improve diesel fuel efficiency and reduce toxic emissions
- Cerium based diesel fuel additives
  - Envirox ™
  - Rhodia
- Widely used as polishing agents
- Potential biomedical applications as antioxidants
- Several industrial applications

Cassandra Korsvik et al., 2007 & Flemming R. Cassee et al 2008



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# $CeO_2$ nanoparticles and cellular toxicity

- $CeO_2$ nanoparticles (20nm) can reduce cell viability and can induce oxidative stress in human bronchoalveolar carcinoma (A549) and lung epithelial cell lines (BEAS2B) (Park et al., 2008)

- *In vivo* studies using male Sprague-Dawley rats showed that $CeO_2$ nanoparticles (20nm) can cause dose-dependent pulmonary inflammation and lung injury (Ma J Y et al., 2011)

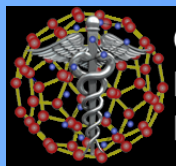
- $CeO_2$ nanoparticles (20nm) can cause inflammatory mediated oxidative stress and apoptosis in alveolar macrophages (Ma J Y et al., 2011)

*Mechanism(s) of $CeO_2$ toxicity is not well understood*

# Background

## Routes of Exposure

- Nanoscale $CeO_2$ (<100 nm) was detected in diesel exhaust emissions employing nanoscale cerium based fuel additive (HEI, 2001; Jung et al., 2005)
- Most common routes of exposure are
  - Inhalation
  - Ingestion
- Inhalation exposure is the greatest concern as little is absorbed through ingestion (Flemming R. Cassee et al., 2008)



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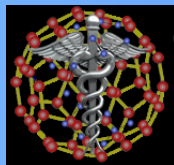
# Purpose

To improve our understanding of how exposure to $CeO_2$ nanoparticles may affect the lungs and other organ systems.

## Aim I

To investigate the role of stress responsive MAPKs and inflammatory protein signaling in the oxidative stress and apoptosis induced by $CeO_2$ nanoparticle exposure in the lungs



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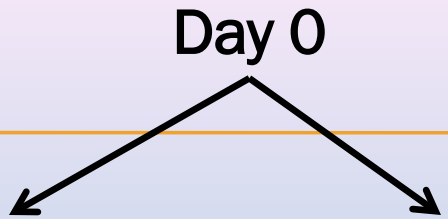
# Materials and Methods

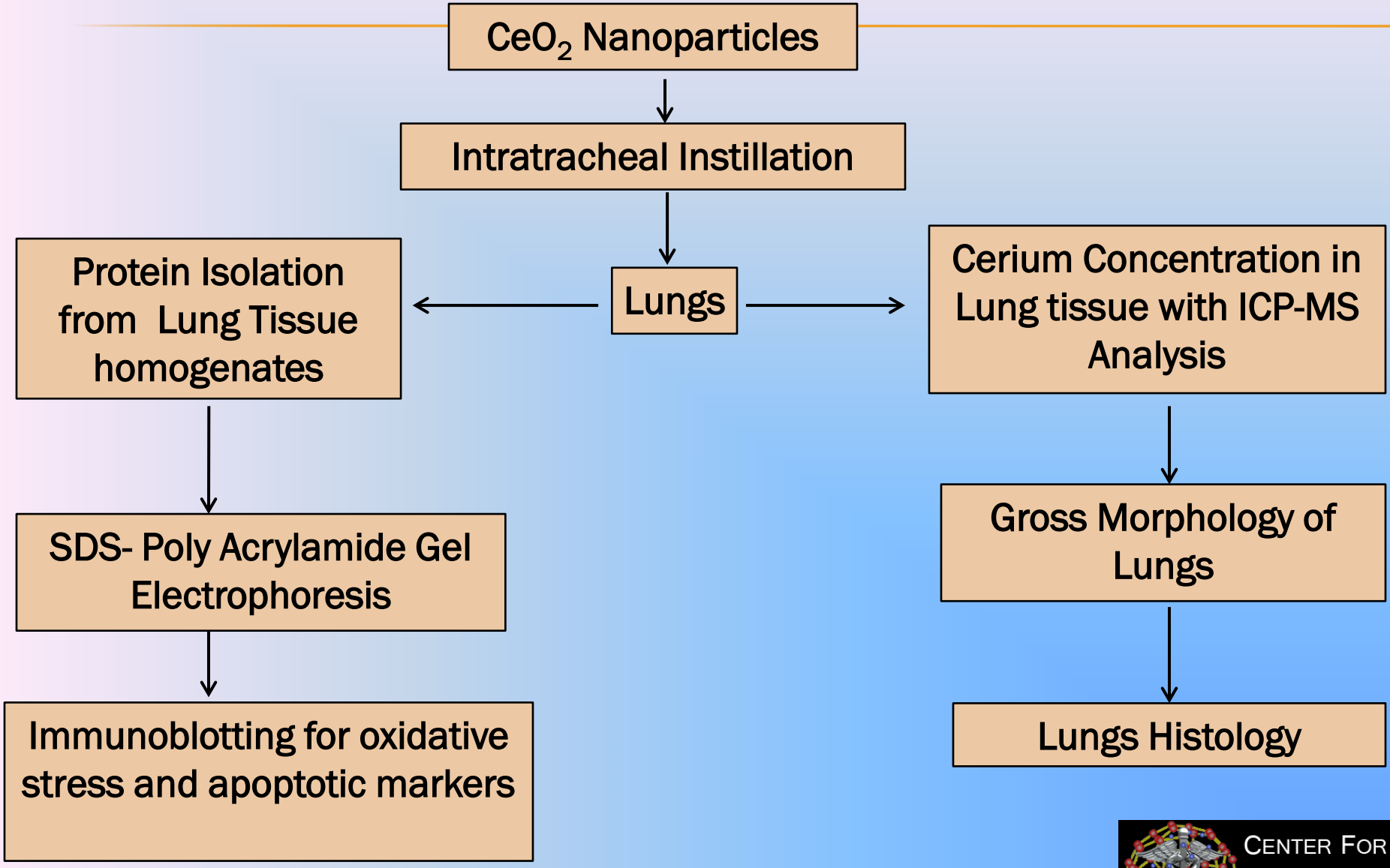
- Male Sprague-Dawley rats
- $CeO_2$ (20 nm) nanoparticles obtained from Sigma Aldrich and suspended in normal saline (Vehicle)
- Dose- 7.0 mg/kg
- Route of Exposure: single intratracheal instillation

# Study Design



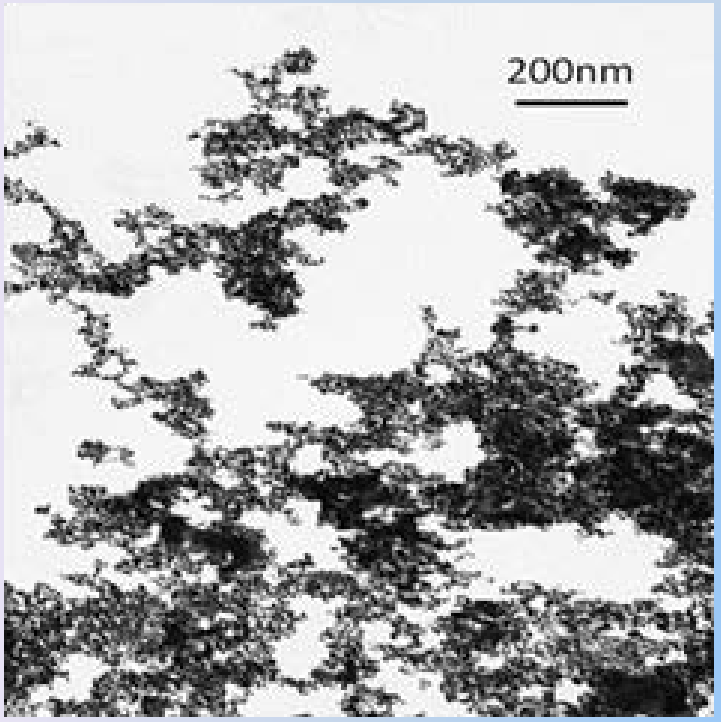

| | Normal Saline instillation | $CeO_2$ instillation |
|---|---|---|
| Sacrifice at day 1 | N=6 | N=6 |
| Sacrifice at day 3 | N=6 | N=6 |
| Sacrifice at day 14 | N=6 | N=6 |
| Sacrifice at day 28 | N=6 | N=6 |
| Sacrifice at day 56 | N=6 | N=6 |
| Sacrifice at day 90 | N=6 | N=6 |

# Methods

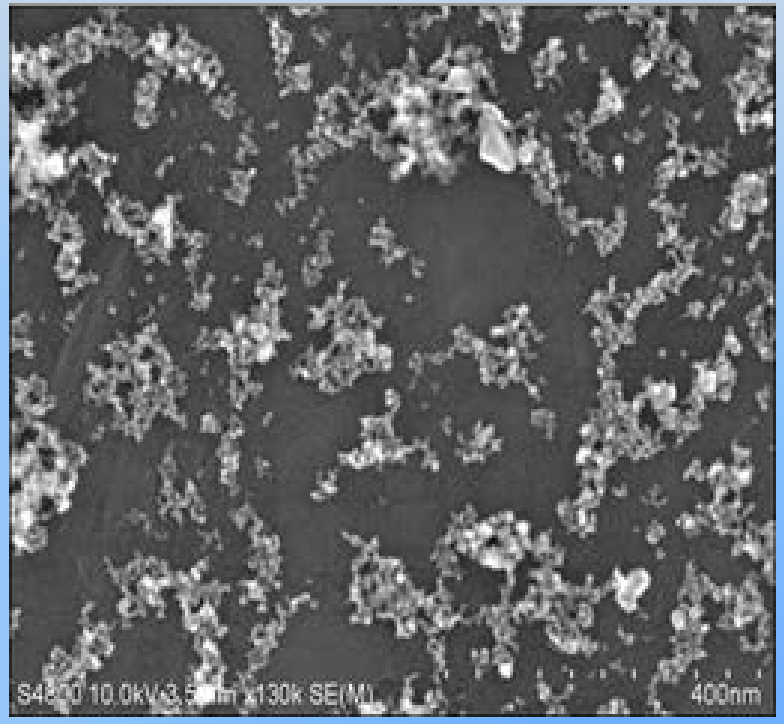


# Characterization of the cerium oxide nanoparticles by (a) TEM micrograph and (b) Field emission SEM of a dilute cerium oxide suspension

a




b

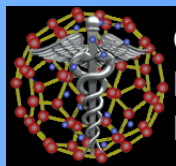



Scale bar = 200 nm

# $CeO_2$ nanoparticle exposure increases the lung weight to body weight ratio

| Days of exposure | Body weight (g) | | Lung weight (g) | | Coefficient of lung weight to bodyweight | |
|---|---|---|---|---|---|---|
| | Saline Control | CeO2-7.0mg/kg | Saline Control | CeO2-7.0mg/kg | Saline Control | CeO2-7.0mg/kg |
| 1 | 319.67±15.92 | 319.67±15.20 | 1.74±0.0.28 | 1.88±0.08 | 5.42±0.66 | 5.84 ±0.24 |
| 3 | 310.33±28.10 | 331.67±24 | 1.54±0.27 | 2.19±0.15 | 4.97± 0.68 | 6.64± 0.66$^{\dagger}$ |
| 14 | 345.67±27.11 | 332.33±21.07 | 1.90±0.31 | 2.12±0.23 | 5.55±0.66 | 6.40± 0.89$^{\dagger}$ |
| 28 | 411.33±29.2$^{*\mu}$ | 403.67±28.94$^{*\mu}$ | 1.82±0.09 | 2.43±0.30 | 4.44 ±0.38 | 6.03 ±0.69$^{\dagger}$ |
| 56 | 451.67±26.21$^{*\alpha\mu}$ | 451.33±34.6$^{*\alpha\mu\P}$ | 1.56±0.24 | 2.84±0.58 | 3.50± 0.57$^{*\alpha\mu}$ | 6.30±1.19$^{\dagger}$ |
| 90 | 523.33±60.87$^{*\alpha\mu\P\#}$ | 519.33±44.84$^{*\alpha\mu\P\#}$ | 1.62±0.11 | 2.75±0.51 | 3.11±0.27$^{*\alpha\mu\P}$ | 5.27±0.64$^{\dagger\alpha}$ |

† Significant different from the control in each day of exposure
*Significant different from the 1 Day exposure group in each condition
$^{\alpha}$ Significant different from the 3 Day exposure group in each condition
μ Significant different from the 14 Day exposure group in each condition
¶ Significant different from the 28days exposure group
# Significant different from the 56days exposure group



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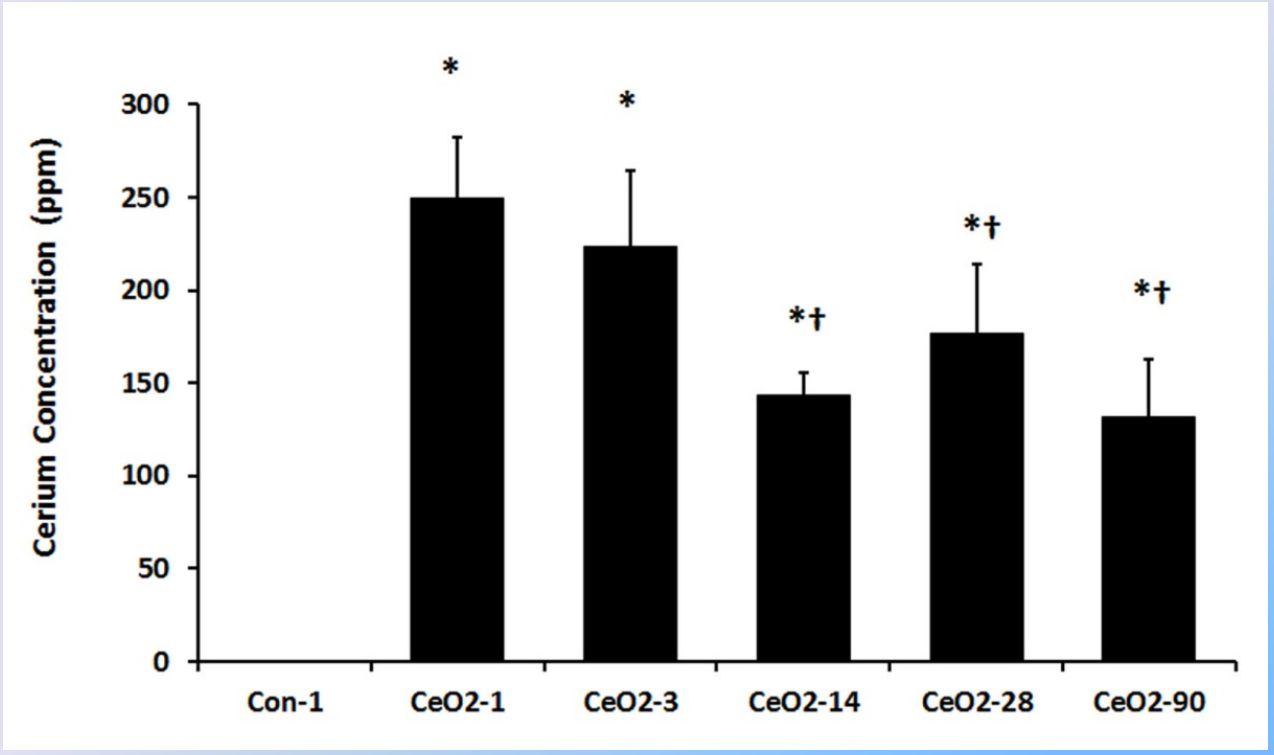
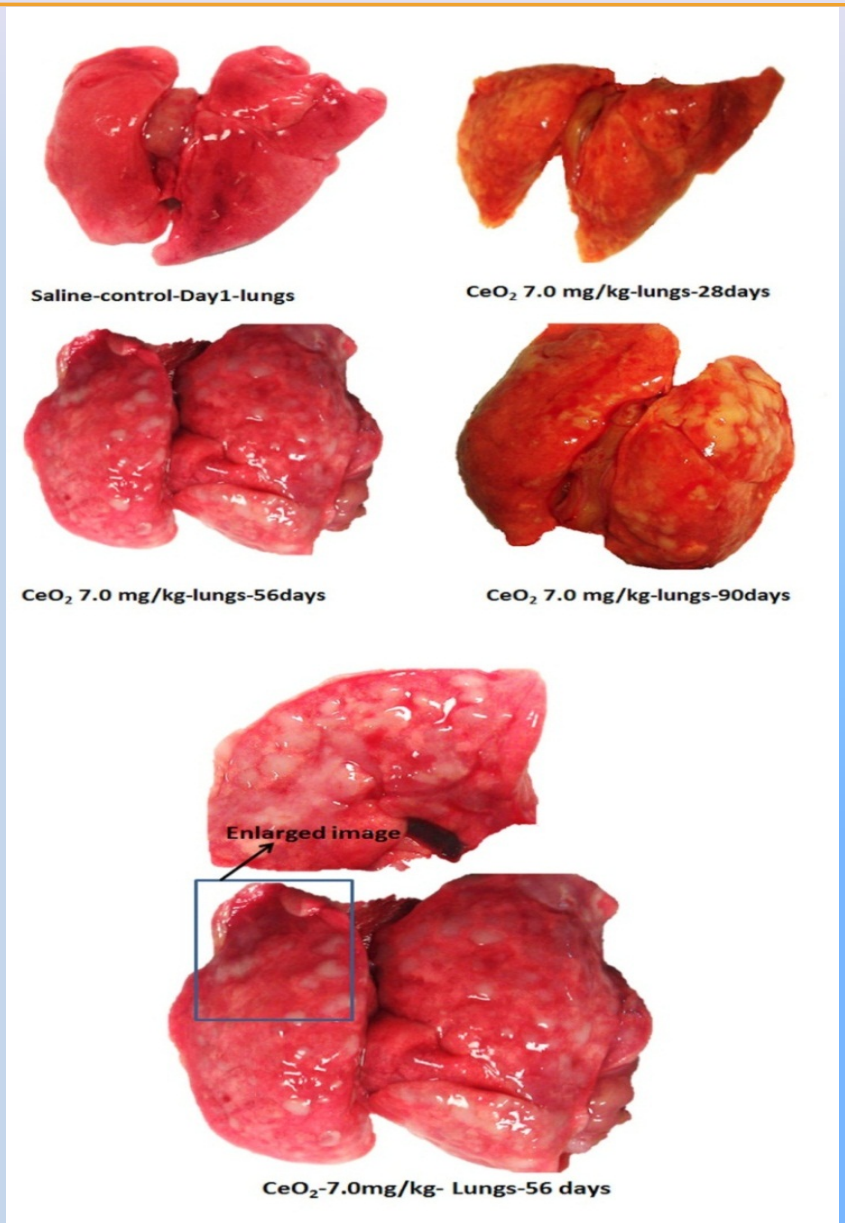
# Cerium deposition in the lung appears to diminish over time




∗ Significantly different from the saline control day-1
† Significantly different from the $CeO_2$-day-1

# Gross morphological alterations in the lungs following $CeO_2$ nanoparticle instillation

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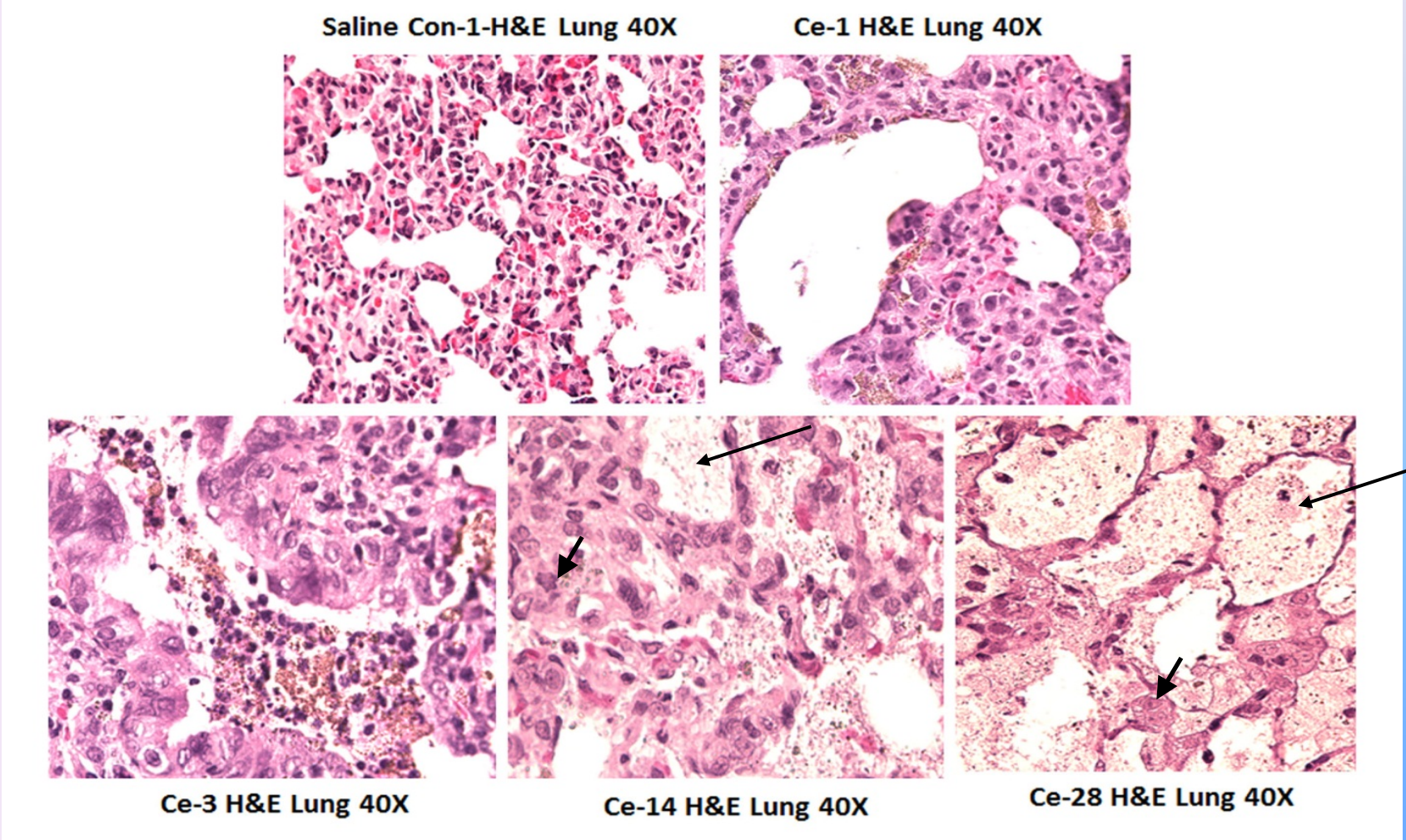
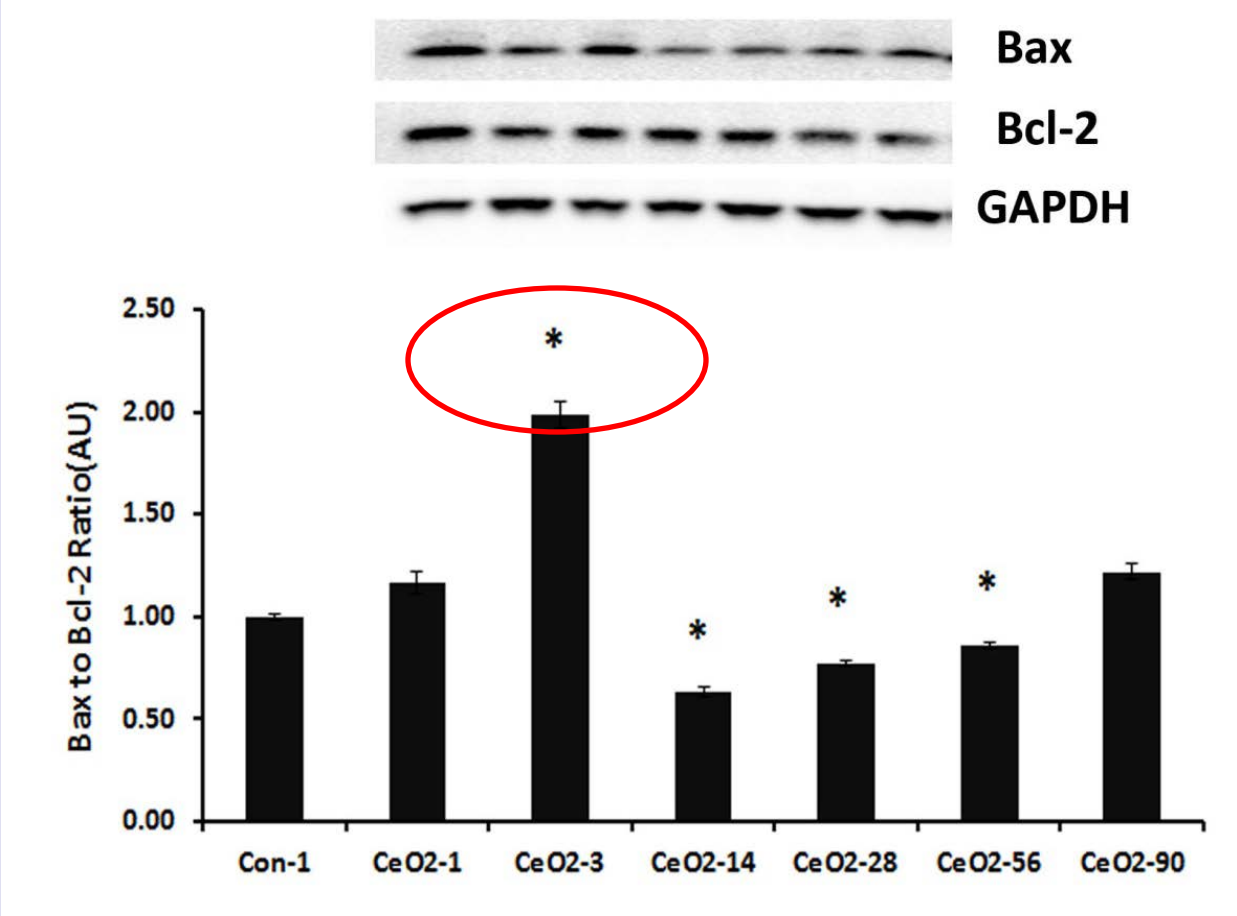
# Alterations in histological appearance of the lungs following $CeO_2$ nanoparticle instillation

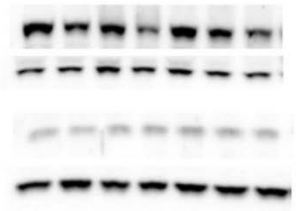


# $CeO_2$ nanoparticle exposure increases proapoptotic signaling in the lungs




* Significantly different from the saline control day-1

# $CeO_2$ nanoparticle exposure increases caspase -3 cleavage

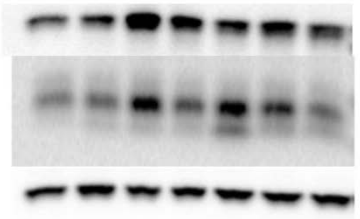


Caspase-9

Cleaved Caspase-9 39kDa

Cleaved Caspase-9 17kDa

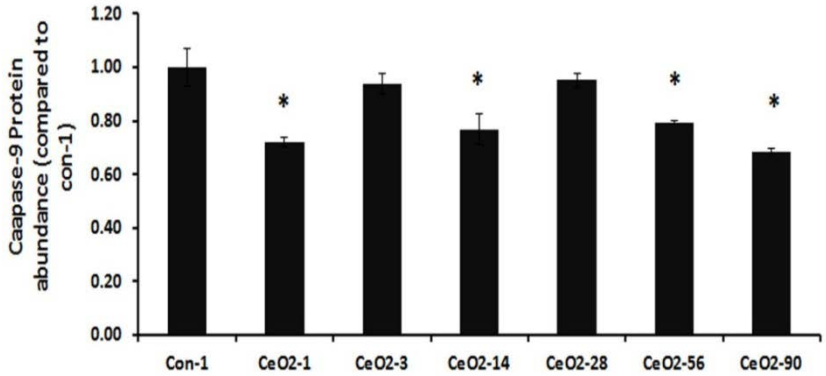
GAPDH

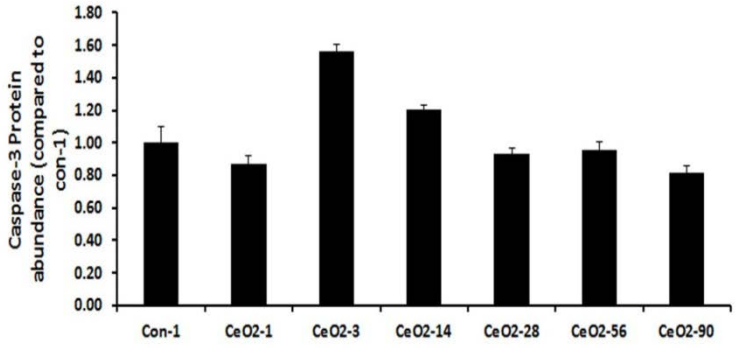


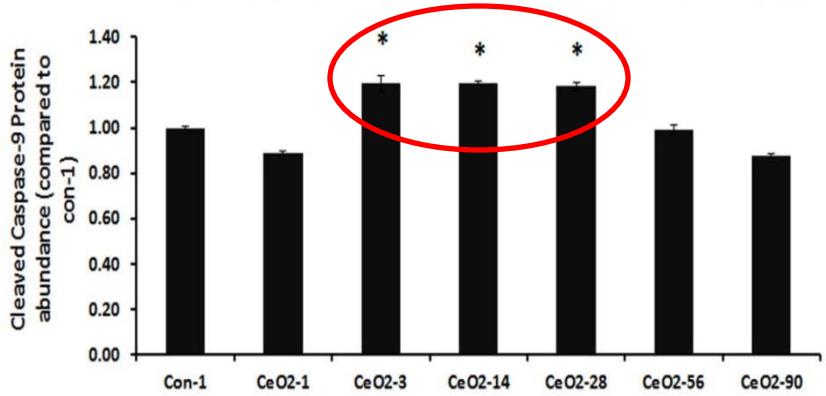
Caspase-3

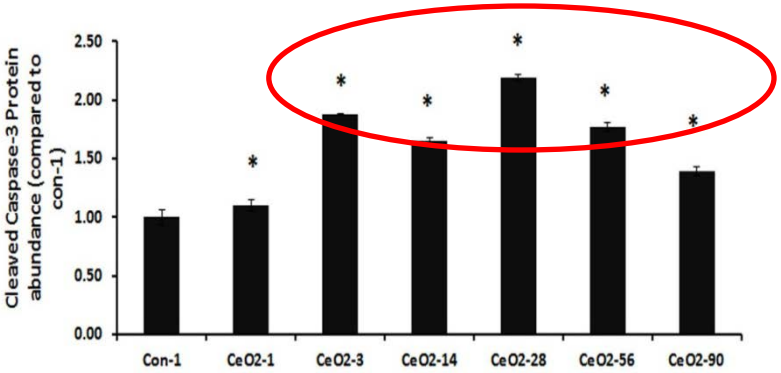
Cleaved Caspase-3

GAPDH

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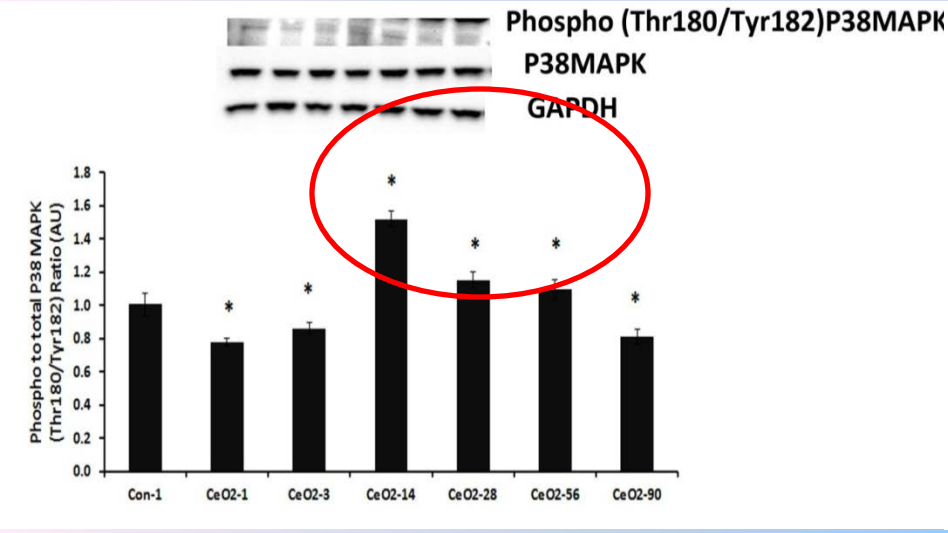
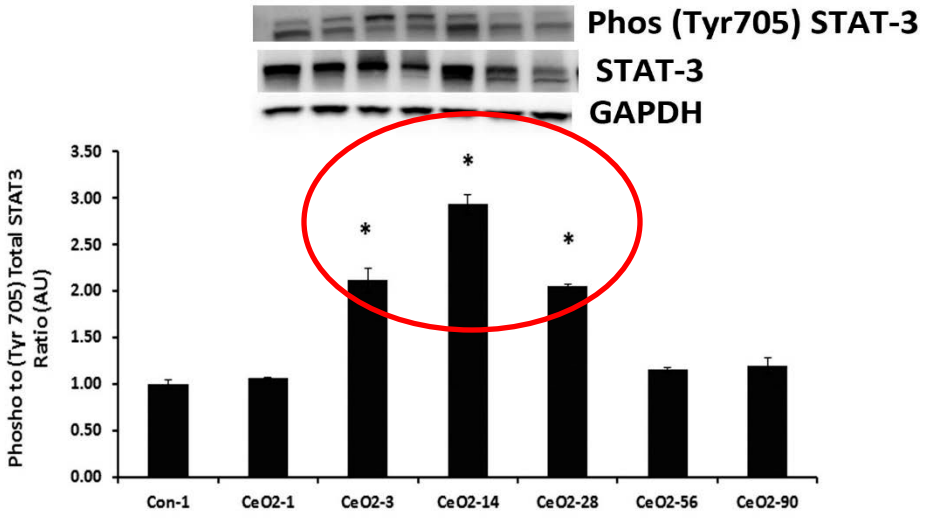
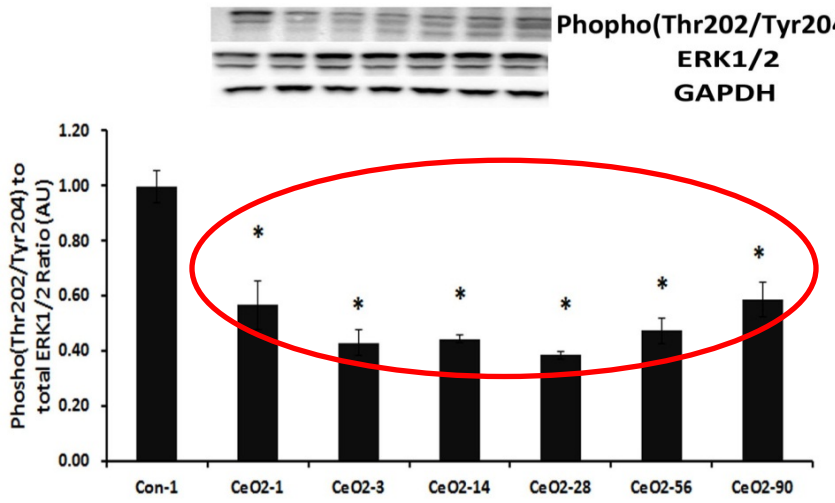
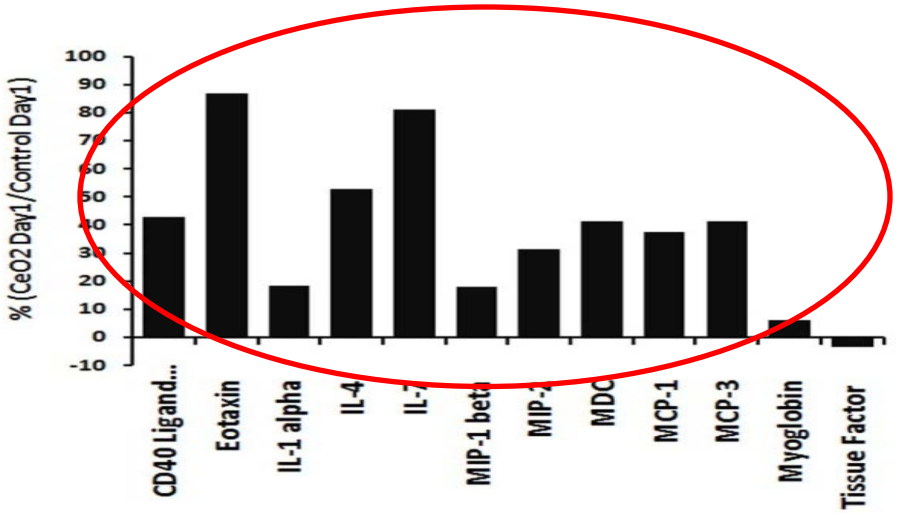
# $CeO_2$ nanoparticle exposure increases serum inflammatory cytokines, p38 MAPK and STAT-3 phosphorylation but diminishes p-ERK1/2

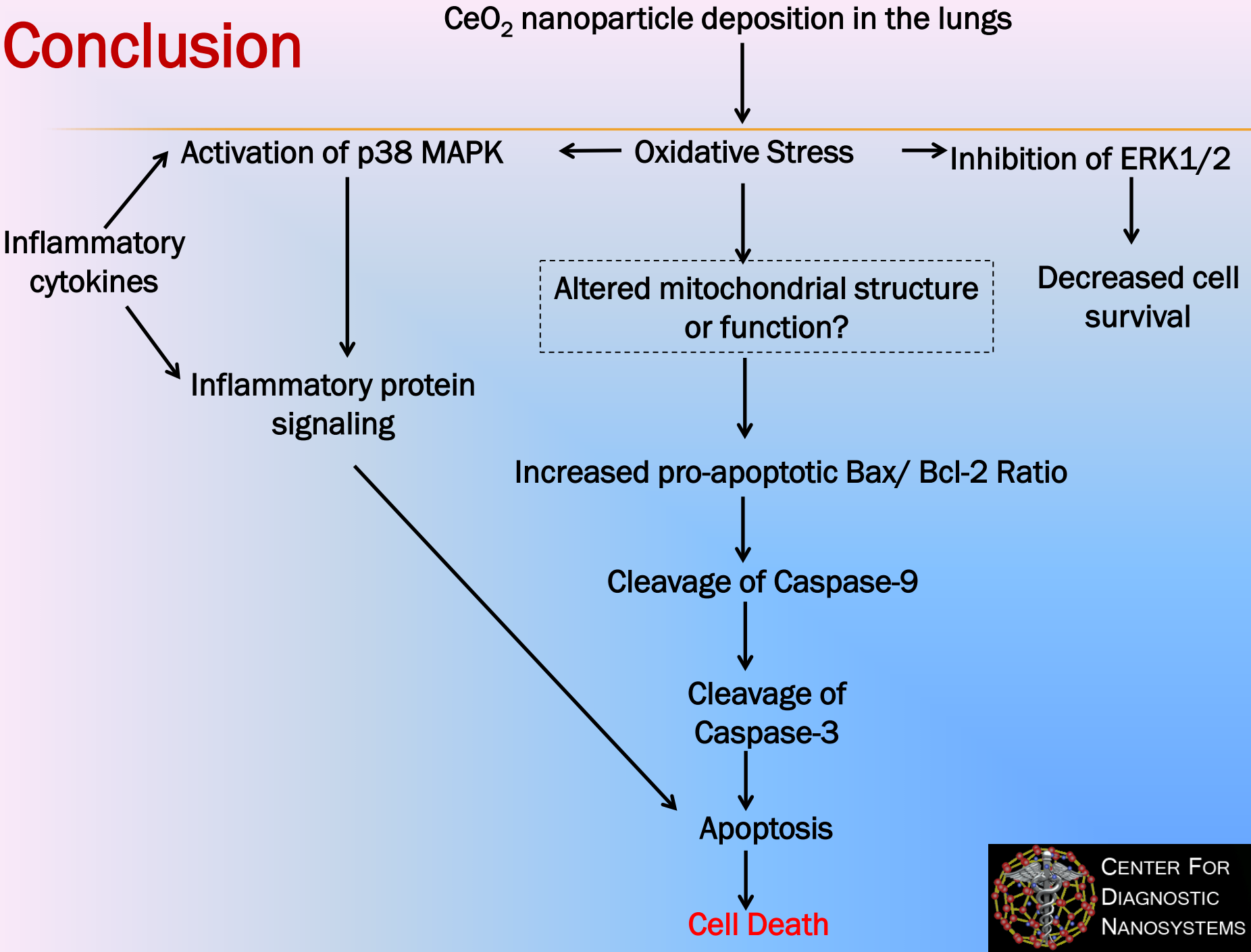


# Conclusion

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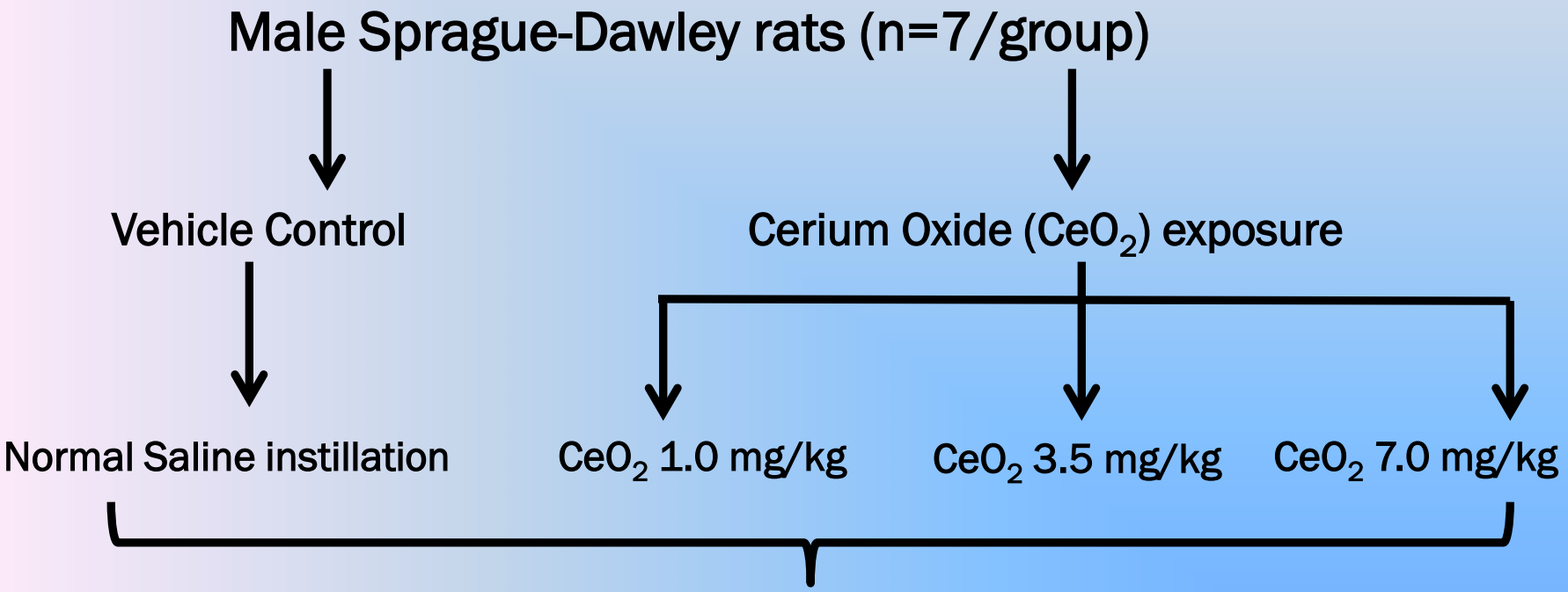
To investigate if the intratracheal instillation of $CeO_2$ nanoparticles has any toxic effects on the liver, kidney, spleen and hearts of rats

This paper has been previously published

Nalabotu SK, Kolli MB, Triest WE, Ma JY, Manne ND, Katta A, Addagarla HS, Rice KM, Blough ER. Int J Nanomedicine. 2011; 6: 2327-35. Epub 2011 Oct 14

# Study Design

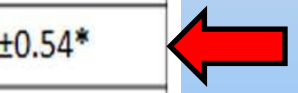


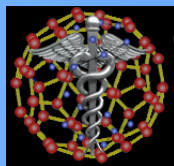

Sacrifice animals at 28 days post exposure

Examined liver and serum for biochemical changes

# Alterations in absolute organ wet weight 28 days after intratracheal instillation of cerium oxide nanoparticles

| Organ weight (g) | Saline Control (n=7) | $CeO_2$ 1.0 mg/kg (n=7) | $CeO_2$ 3.5 mg/kg (n=7) | $CeO_2$ 7.0 mg/kg (n=7) |
|---|---|---|---|---|
| Heart (g) | 1.52±0.15 | 1.35±0.05 | 1.27±0.07 | 1.23±0.05 |
| Liver (g) | 14.55±0.27 | 14.30±1.04 | 14.78±0.57 | 12.50±0.54* |
| Kidney (g) | 2.67±0.31 | 2.55±0.21 | 2.54±0.33 | 2.43±0.31 |
| Spleen (g) | 0.58±0.06 | 0.65±0.10 | 0.56±0.08 | 0.64±0.04 |





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# Changes in serum biochemical parameters 28 days post intratracheal instillation of $CeO_2$ nanoparticles

| Analyte | Saline Control (N=7) | $CeO_2$ 1.0mg/kg (N=7) | $CeO_2$ 3.5mg/kg (N=7) | $CeO_2$ 7.0mg/kg (N=7) |
|---|---|---|---|---|
| Glucose | 186.4±25.7 | 208±43.0 | 197.6±40.2 | 231±93.5 |
| ALP | 276.1±53.7 | 263±55.4 | 242±35.3 | 222.23±81.9 |
| ALT | 58.3±10.7 | 83.4±28.5 | 88.3±31.4 | 130.5±94.5* |
| Amylase | 974.7±97.4 | 1055.1±124.2 | 991.4±116 | 908.4±277.0 |
| Total Protein | 6.0±0.1 | 5.9±0.6 | 6.2±0.5 | 5.4±1.3 |
| Albumin | 4.2±0.2 | 4.1±0.5 | 4.5±0.4 | 3.5±1.1* |
| Globulin | 1.8±0.2 | 1.8±0.2 | 2.0±0.2 | 1.8±0.2 |
| ALB-GLOB Ratio | 2.3±0.3 | 2.3±0.3 | 2.2±0.3 | 1.9±0.6 |
| BUN | 15.4±1.1 | 15±3.1 | 15.7±1.9 | 14.4±4.2 |
| Creatinine | 0.3±0.1 | 0.27±0.1 | 0.23±0.1 | 0.28±0.1 |
| $Ca^{2+}$ | 11.4±0.7 | 10.7±1.3 | 11.5±1.1 | 10.4±2.4 |
| Phosphorus | 8.6±0.9 | 7.9±1.2 | 8.7±1.0 | 8.2±1.9 |
| $Na^+$ | 142.3±0.9 | 138±10.7 | 138.1±10.7 | 132.1±16.3 |
| $K^+$ | 5.5±0.4 | 6.0±0.5 | 6.5±0.6 | 5.8±0.9 |
| $Na^+$ - $K^+$ Ratio | 25.8±2.0 | 22.9±1.7* | 21.2±1.4* | 22.8±2.5* |

| Analyte | Saline Control (N=7) | $CeO_2$ 1.0mg/kg (N=7) | $CeO_2$ 3.5mg/kg (N=7) | $CeO_2$ 7.0mg/kg (N=7) |
|---|---|---|---|---|
| Total Cholesterol | 100.7±1.9 | 100±0 | 100±0 | 103.1±8.3 |
| Triglycerides | 143±53. | 109.6±50.9 | 190.3±83.7 | 93.1±22.3* |
| HDL | 21±6.0 | 19.4±5.4 | 20±6.4 | 19±5.1 |

Instillation is associated with alterations in ALT, albumin, Na/K ratio, and triglyceride levels

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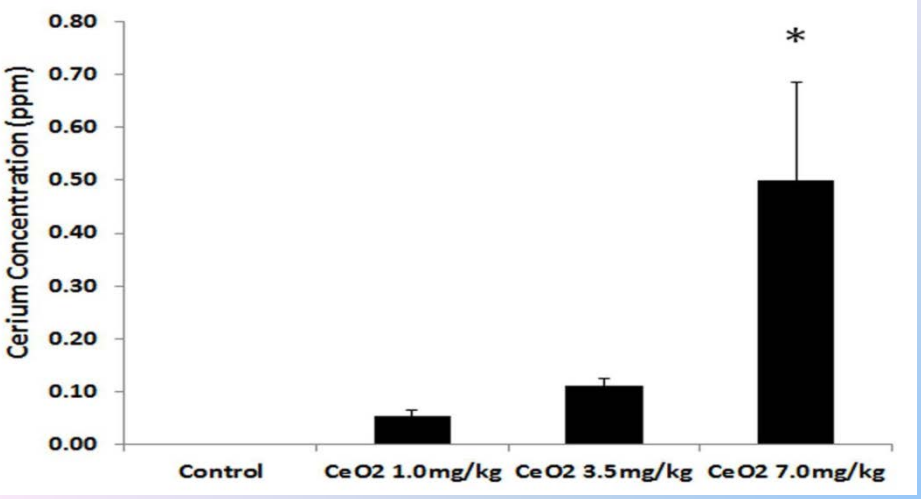
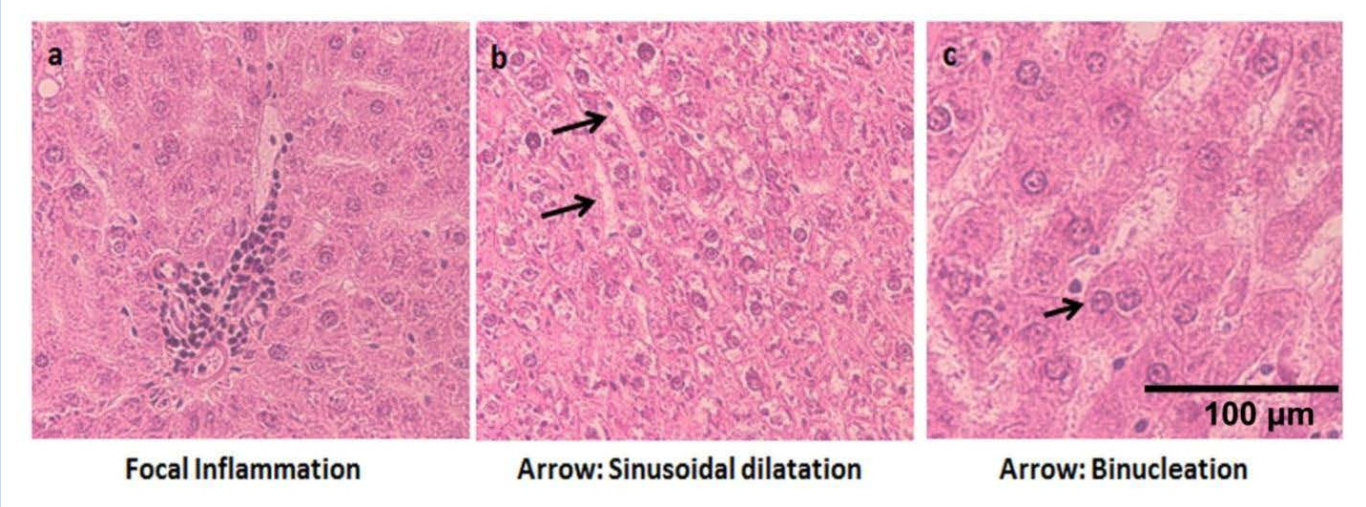
# Instillation of $CeO_2$ nanoparticles, ceria deposition and liver histology




Liver cerium concentration




$CeO_2$ nanoparticle exposure alters histopathological architecture of the liver

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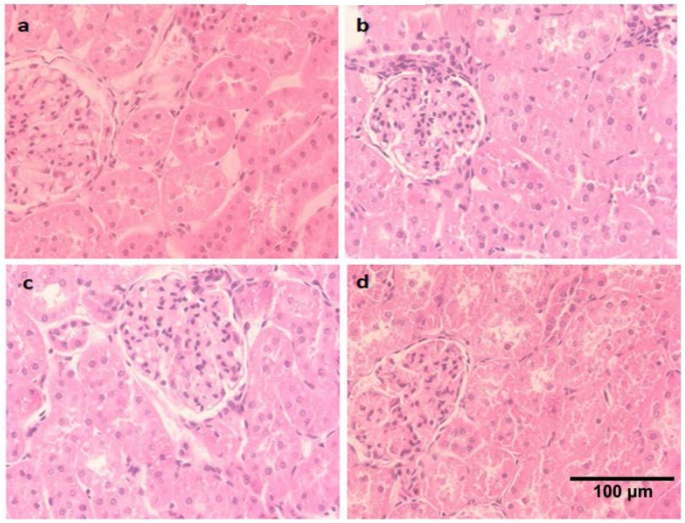
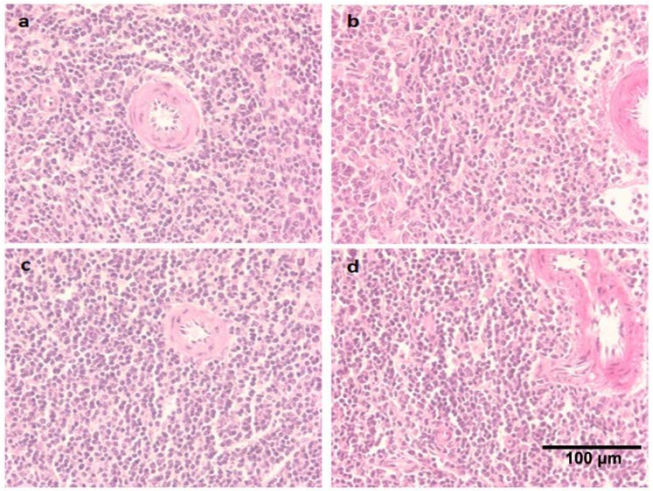
# $CeO_2$ nanoparticle exposure has no effect on the histological appearance of the kidney, spleen, or heart

## Kidney



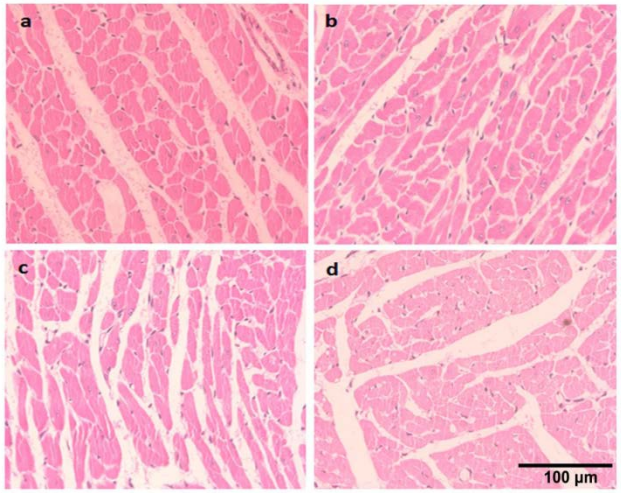

a. Saline Control
b. $CeO_2$ 1.0mg/kg
c. $CeO_2$ 3.5mg/kg
d. $CeO_2$ 7.0mg/kg

## Spleen




a. Saline Control
b. $CeO_2$ 1.0mg/kg
c. $CeO_2$ 3.5mg/kg
d. $CeO_2$ 7.0mg/kg

## Heart




a. Saline Control
b. $CeO_2$ 1.0mg/kg
c. $CeO_2$ 3.5mg/kg
d. $CeO_2$ 7.0mg/kg

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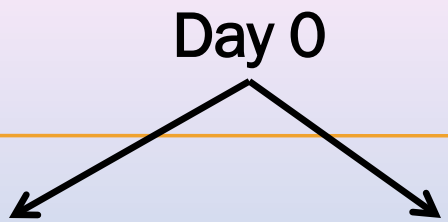
## Aim III

To investigate the role of oxidative stress and apoptosis in the hepatic toxicity induced by $CeO_2$ nanoparticles following intratracheal instillation

# Study Design




| | Normal Saline instillation | $CeO_2$ instillation |
|---|---|---|
| Sacrifice day 1 | N=6 | N=6 |
| Sacrifice day 3 | N=6 | N=6 |
| Sacrifice day 14 | N=6 | N=6 |
| Sacrifice day 28 | N=6 | N=6 |
| Sacrifice day 56 | N=6 | N=6 |
| Sacrifice day 90 | N=6 | N=6 |

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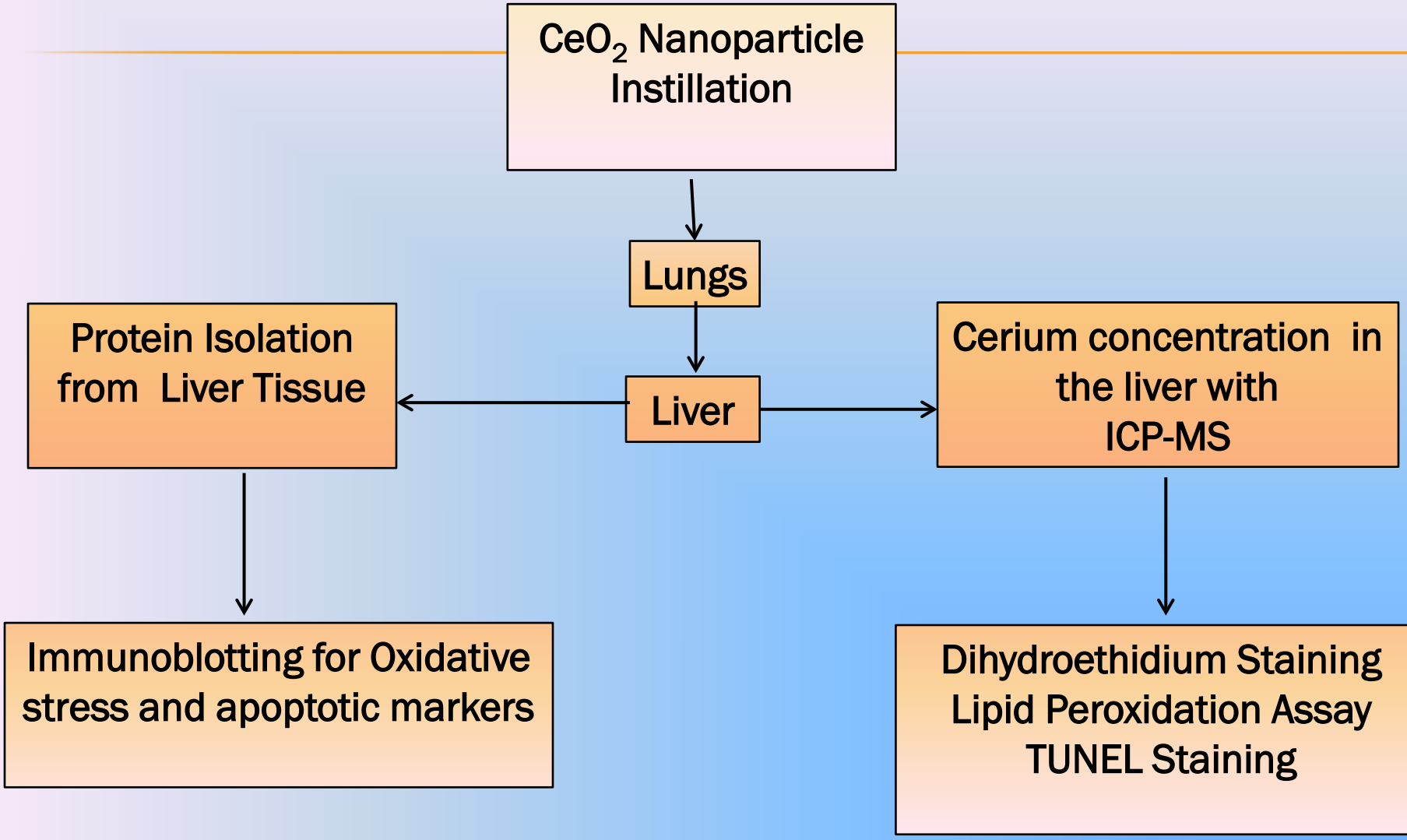
# Methods

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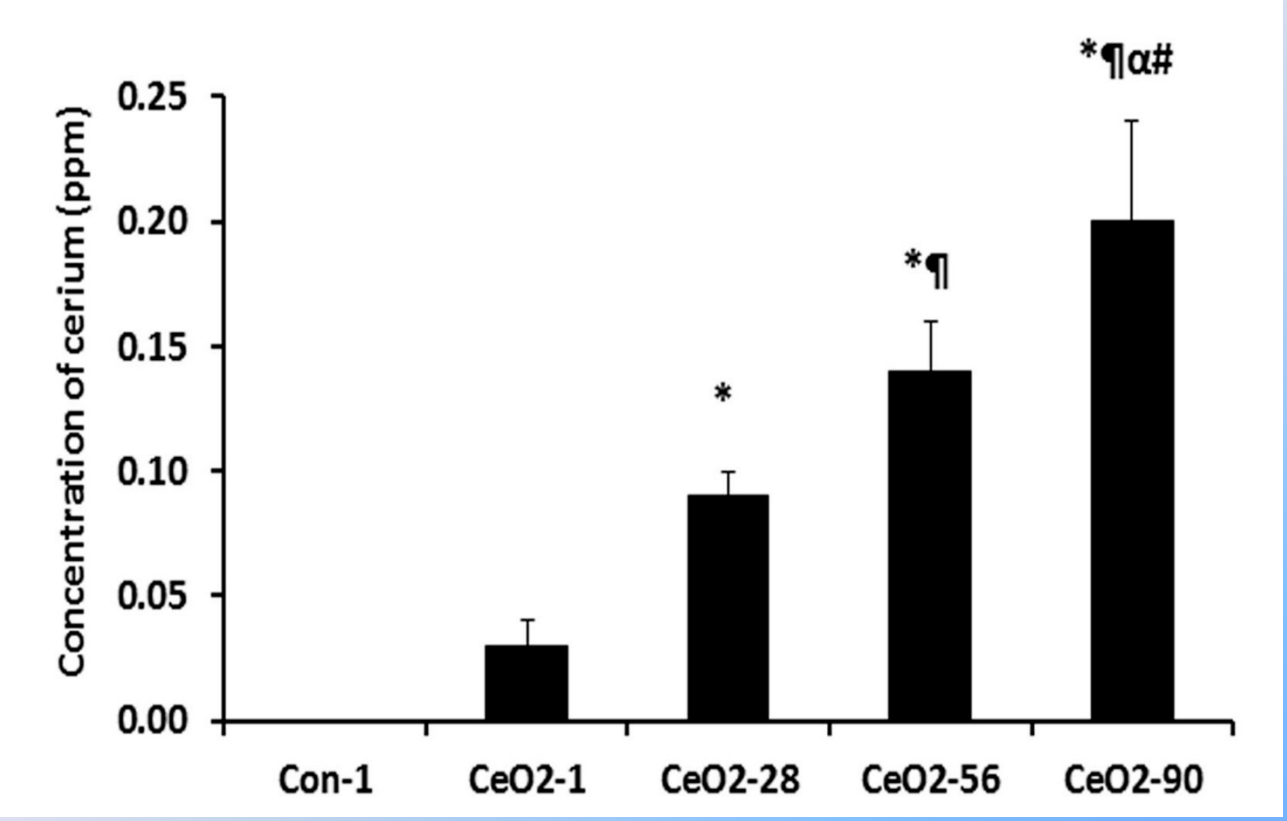
# Cerium accumulation in the liver over time




* Significant difference from control-1
¶ Significant difference from Day-1 exposure
α Significant difference from Day-28 exposure
# Significant difference from Day-56 exposure

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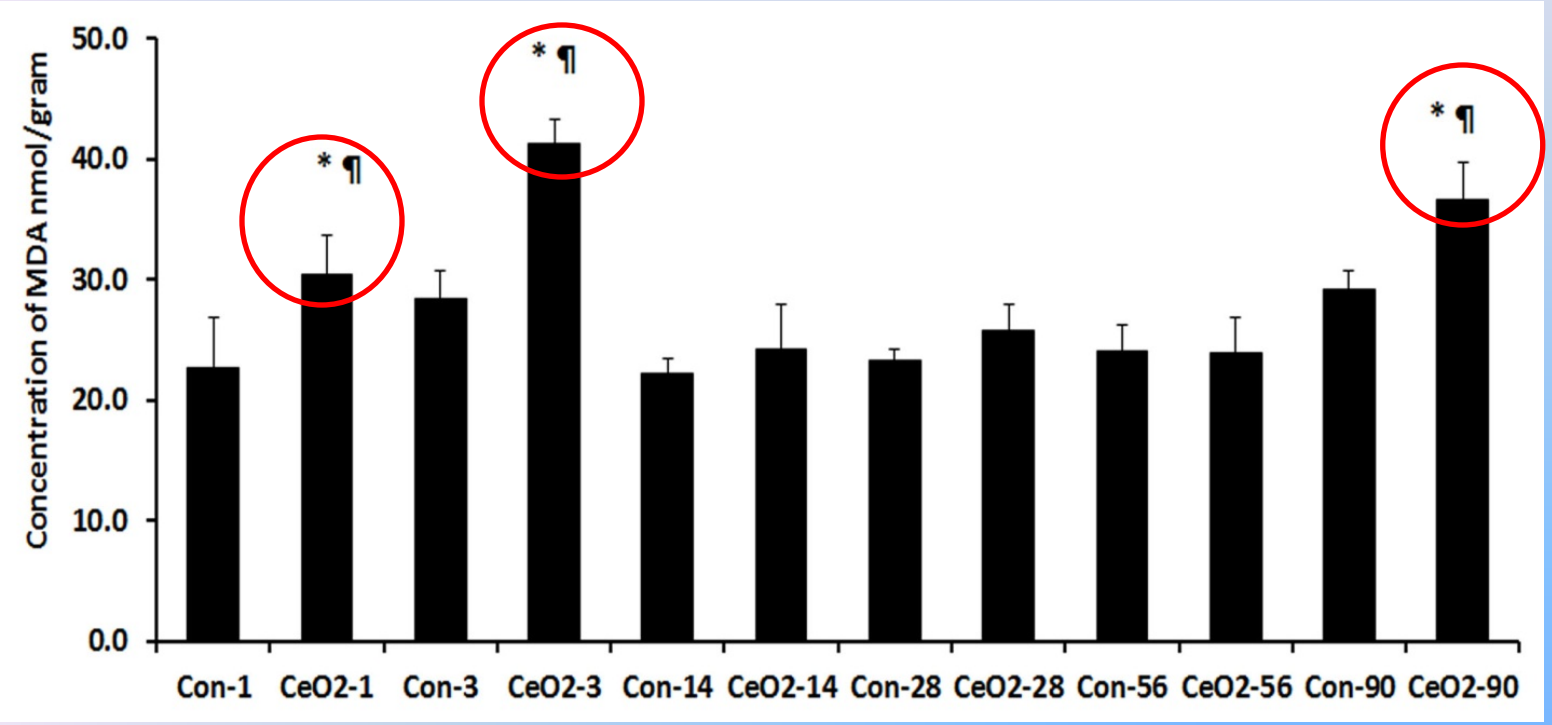
# $CeO_2$ nanoparticle exposure is associated with lipid peroxidation of the hepatic cell membrane




* Significant difference from the controls in each group

¶ Significant difference from the 14, 28 and 56 days $CeO_2$ exposure group

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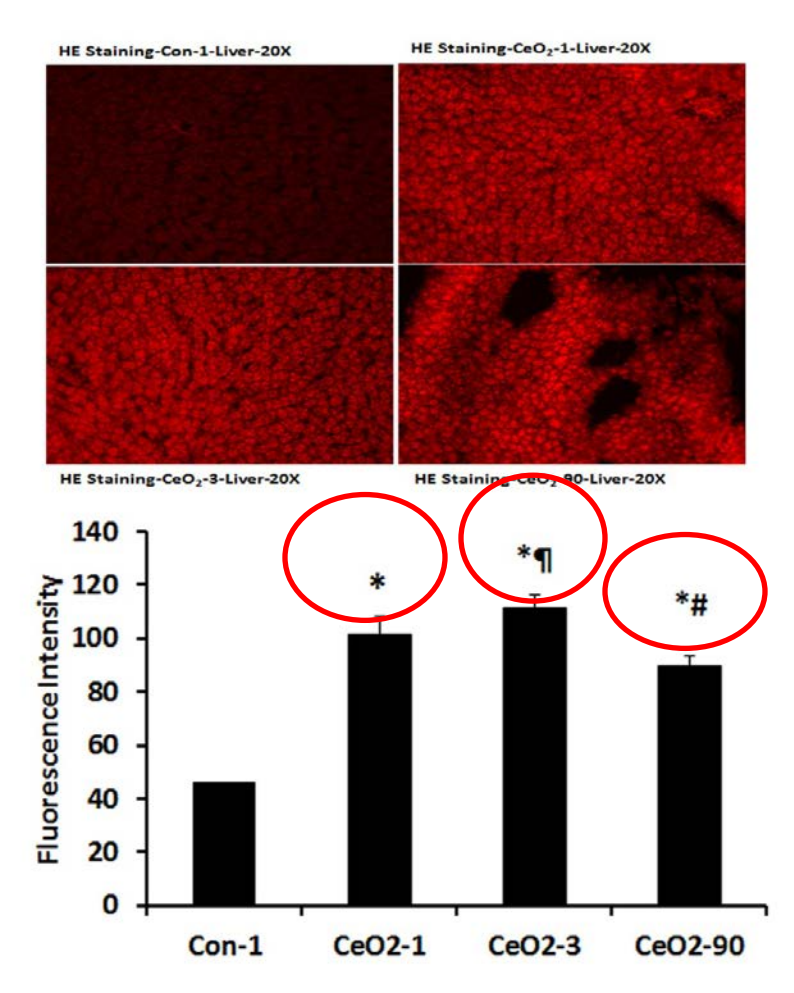
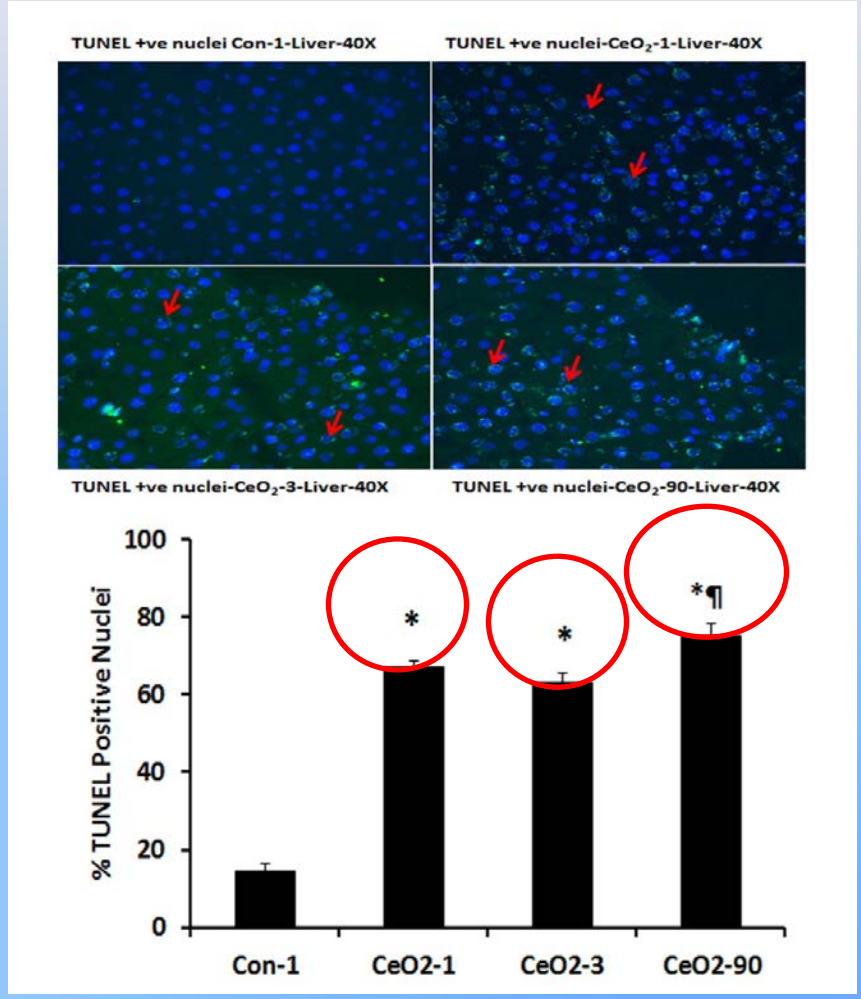
# $CeO_2$ nanoparticle exposure is associated with increased superoxide and TUNEL positive nuclei




*Significant difference from control-1
¶ Significant difference from Day-1 exposure
# Significant difference from Day-3 exposure

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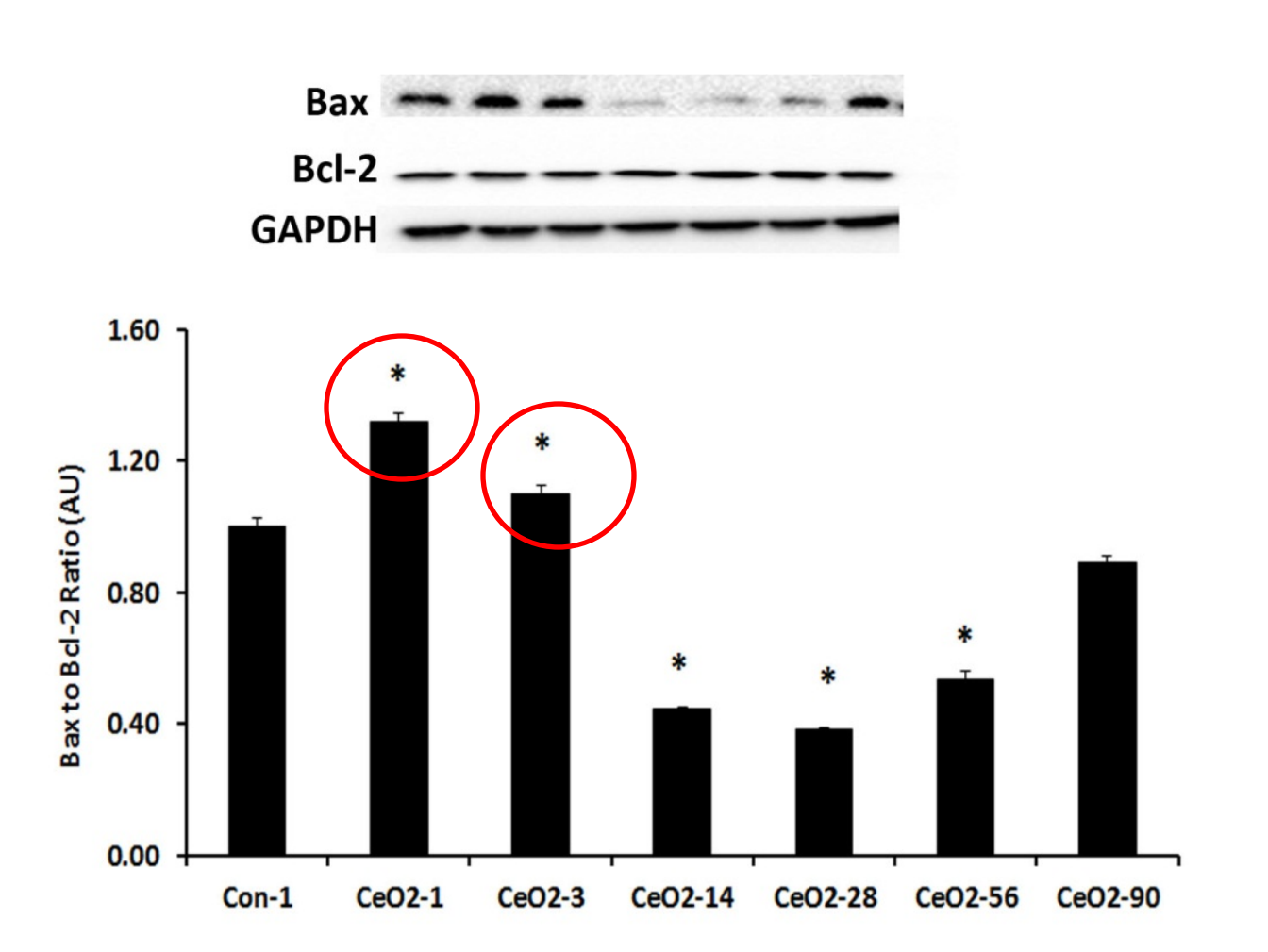
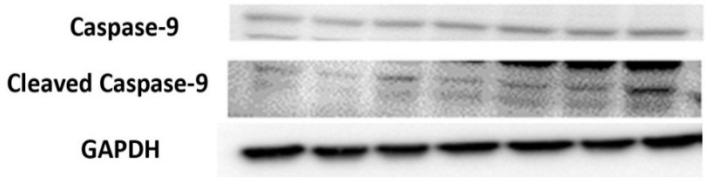
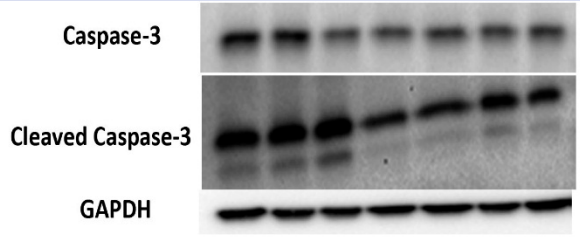
# $CeO_2$ nanoparticle exposure is associated with increased Bax/Bcl-2 ratio

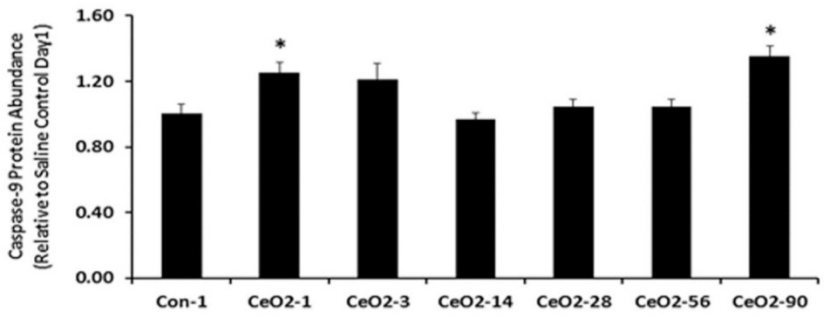


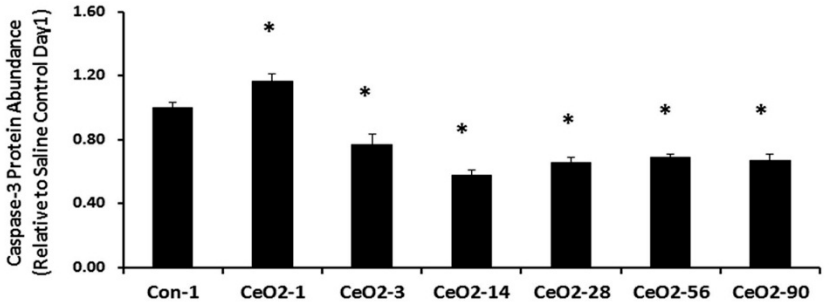

* Significant difference from control-1

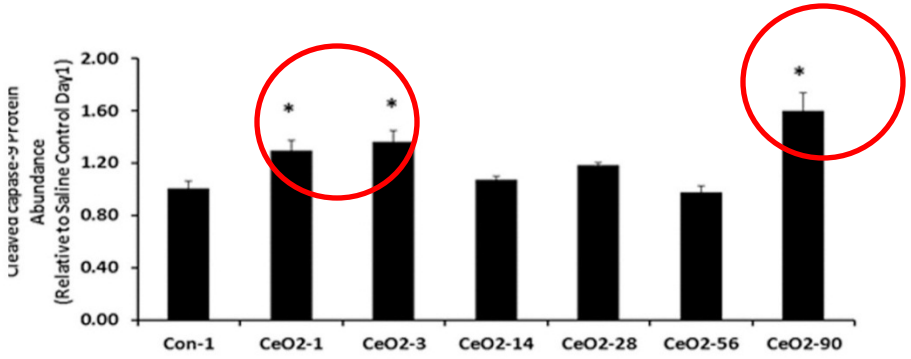
# $CeO_2$ nanoparticle exposure appears to activate caspase-3

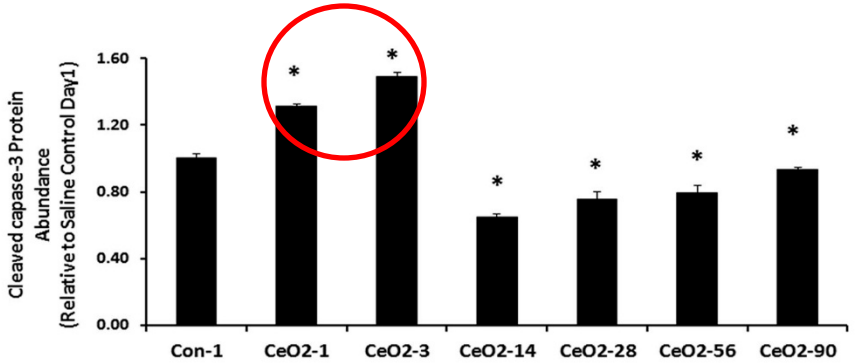
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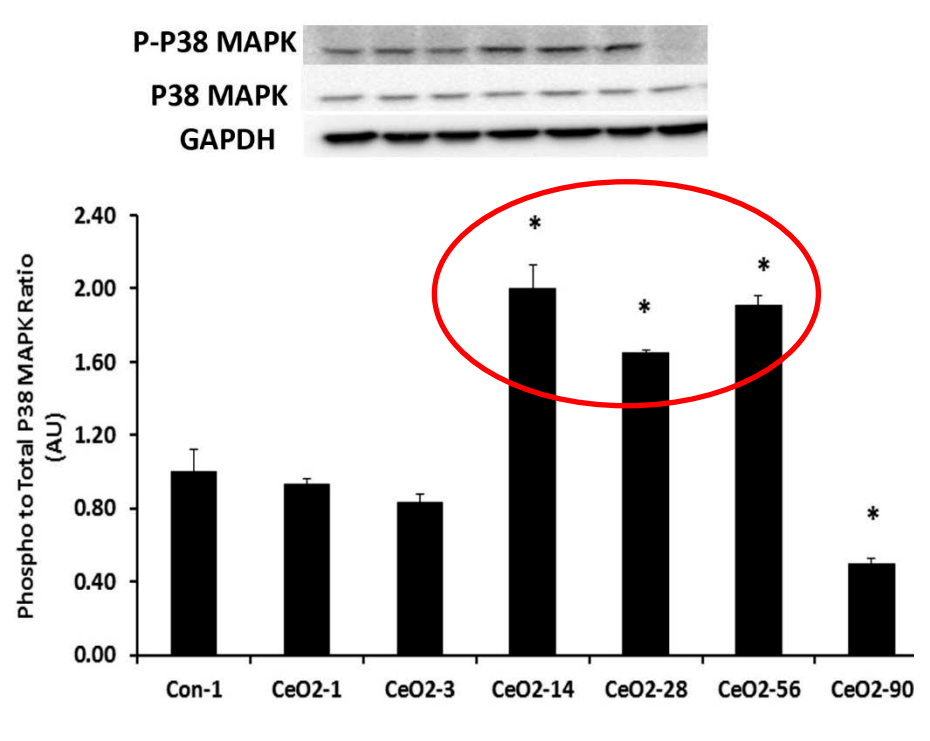
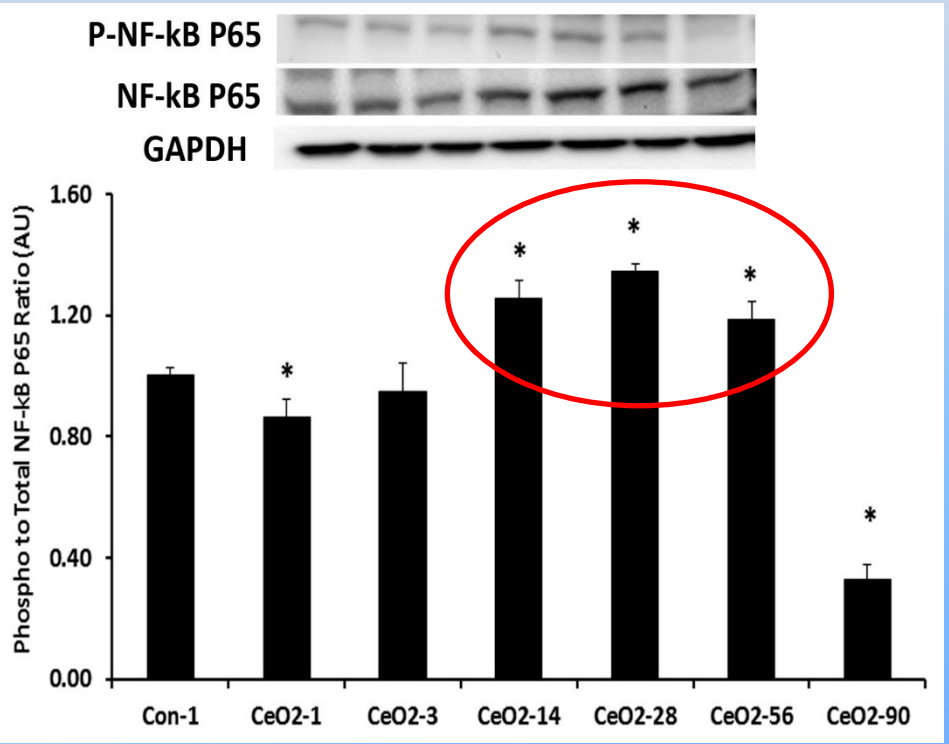
# $CeO_2$ nanoparticle exposure is associated with phosphorylation (activation) of p38 MAPK and Nf-kβ p65

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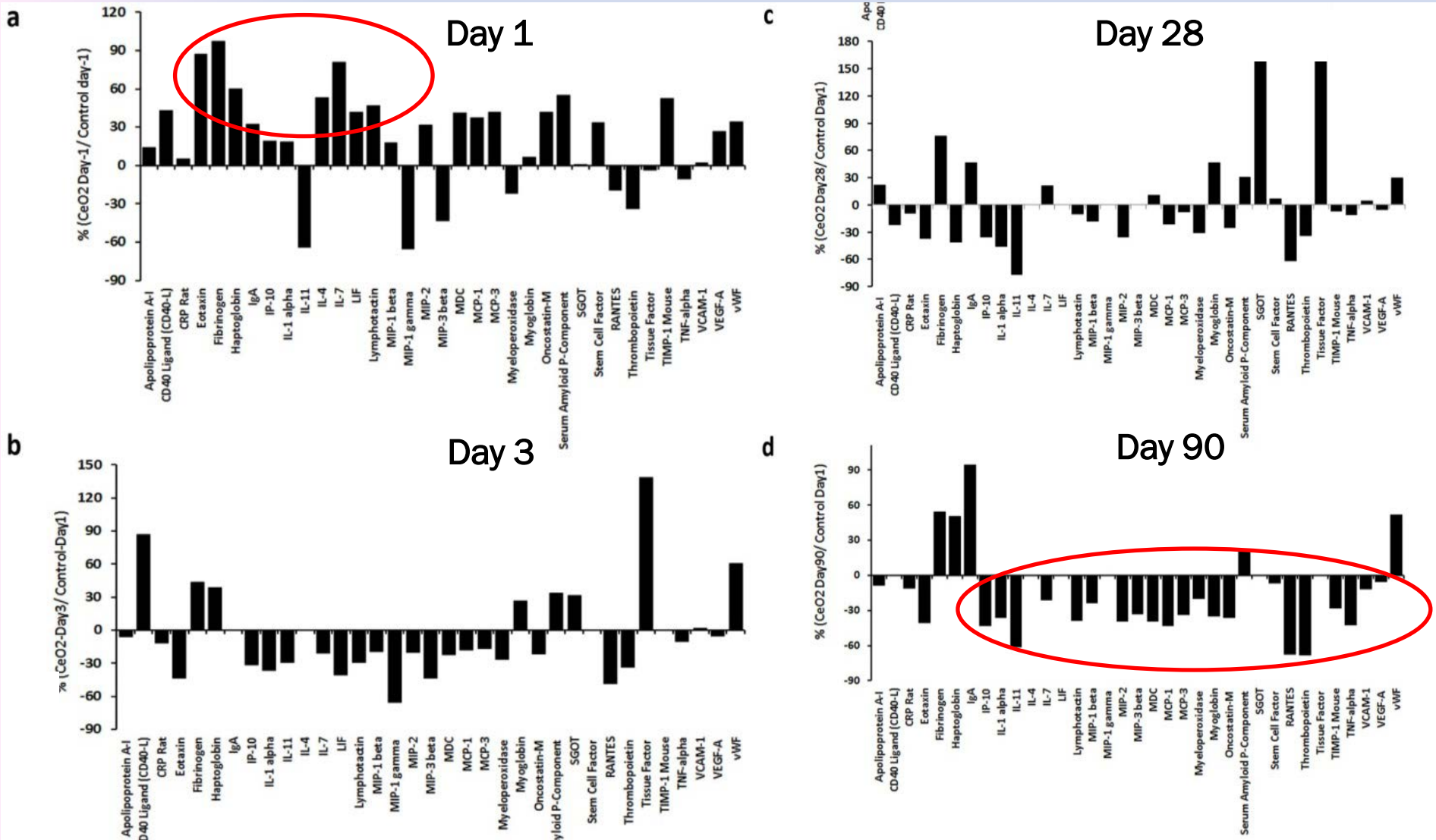
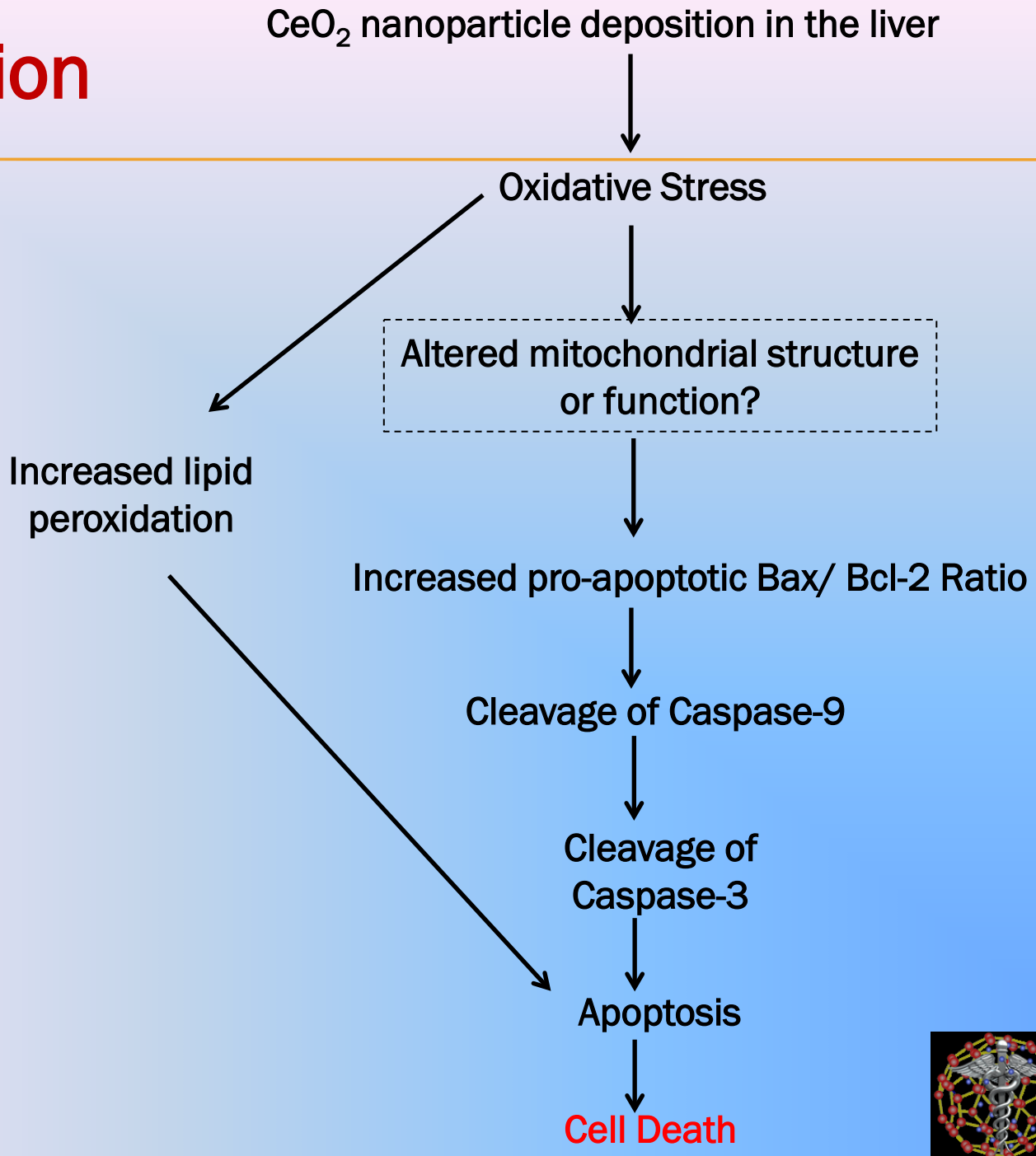
# $CeO_2$ nanoparticle exposure affects serum biomarkers that may play a role in inflammation



# Conclusion



# Summary of Findings

Intratracheal instillation of $CeO_2$ nanoparticles is associated with oxidative stress and apoptosis in the lungs

$CeO_2$ nanoparticles can translocate from the lungs to the liver where they appear to bioaccumulate over time.

$CeO_2$ nanoparticle deposition in the liver is associated with histological alterations (hydropic degeneration, hepatocyte enlargement, sinusoidal dilatation and the accumulation of granular material inside the hepatocytes), increases in oxidative stress and apoptosis.

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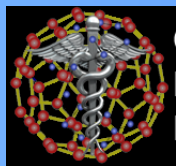

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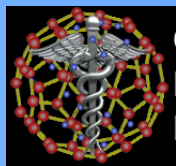

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