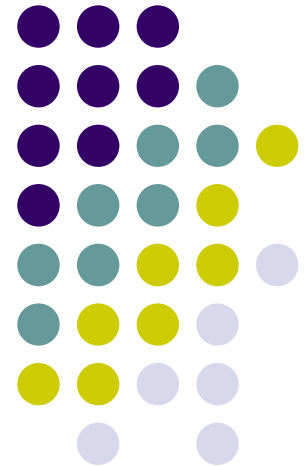


Development and characterisation of a murine model of diesel exhaust particle exposure and its effects on lung function.

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Diesel Exhaust Particles (DEP)



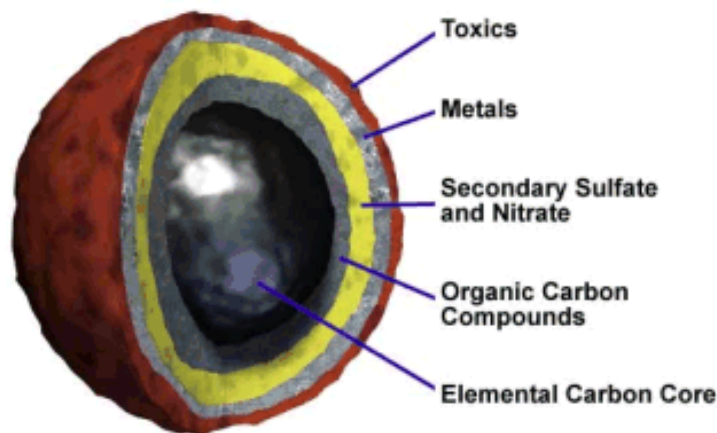
- Rapid industrialisation and urbanisation occurring in many parts of the world
- More people are being exposed to air pollutants e.g. fossil fuels
- Significant increase in the incidence of various respiratory diseases in many developed countries
- Exposure to fossil fuel products may play a role in the exacerbation of respiratory illness

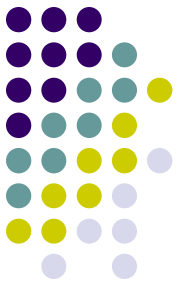


Diesel Exhaust Particles (DEP)



- DEP, produced by diesel powered motor vehicles, are the major contributor to particulate pollution in urban areas
- Particles consist of ultrafine carbon nuclei and adsorbed organic substances and trace amounts of heavy metals
- Diesel Exhaust Particles range from 50nm-10 μ m in diameter
 - remain suspended for long periods of time
 - easily inhaled
 - able to penetrate deeply into the lungs.

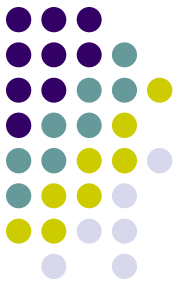




AIM:

To create and characterise a mouse model of acute Diesel Exhaust Particle (DEP) exposure

Model



- US Environmental Protection Agency standard C-DEP
- C-DEP prepared in saline (+ 0.05% Tween-80) and sonicated for 30 min
- Male and female 8 week old BALB/c mice
- Single dose of C-DEP solution (or saline + Tween alone) delivered intranasally to BALB/c mice under light methoxyflurane anaesthesia.



Research Plan



Part I: *Kinetics of inflammation in BAL following DEP exposure*

- 3 doses (10 μg , 30 μg , 100 μg)
- Bronchoalveolar lavage (BAL) taken 3, 6, 12 or 24 hr (+ 48 hr or 7 days for 100 μg) after exposure
 - Mice euthanized
 - Trachoestomised
 - 0.5 mL 0.9% saline gently washed in and out of lung 3 times
 - Total cell count and differential cell counts obtained

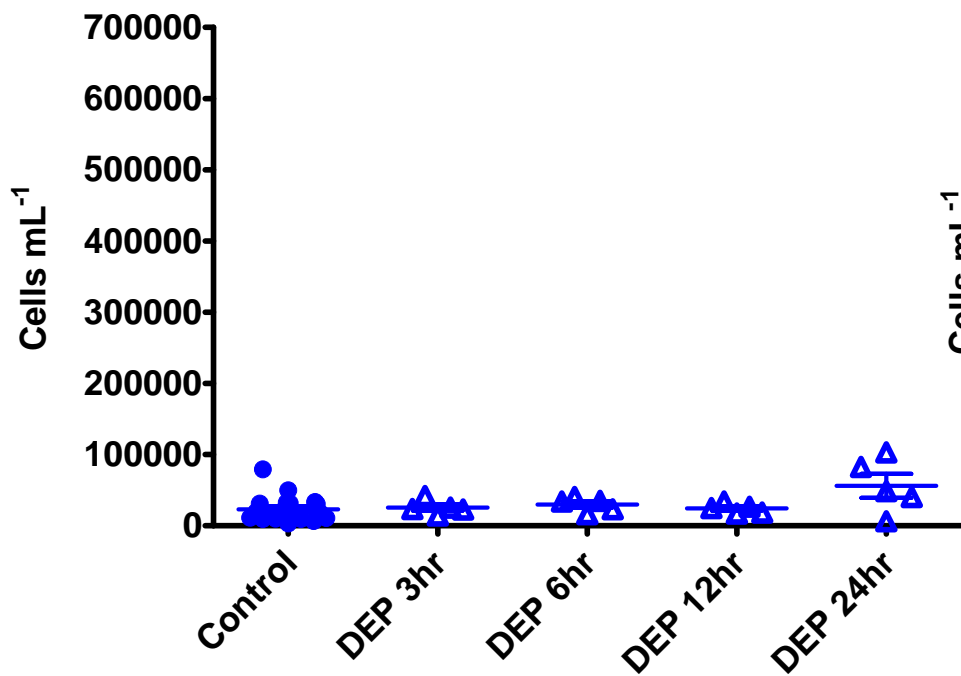
Part II: *Uptake of DEP by alveolar macrophages*

- 100 μg
- Macrophages from BALs taken at 12, 24 or 48 hours examined under light microscopy for carbon content

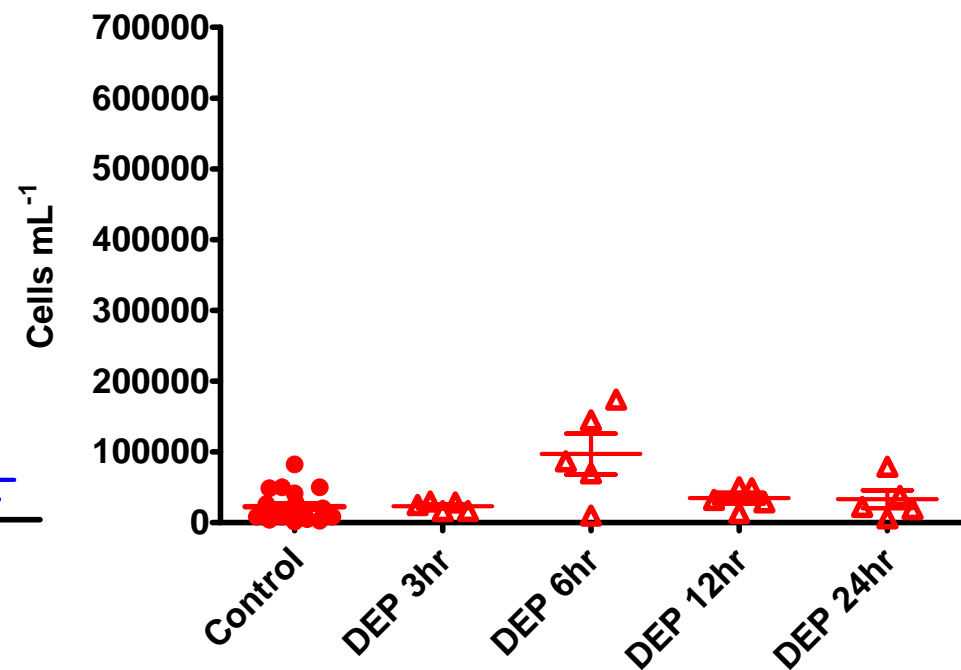
Total Cell Counts



10ug DEP Male



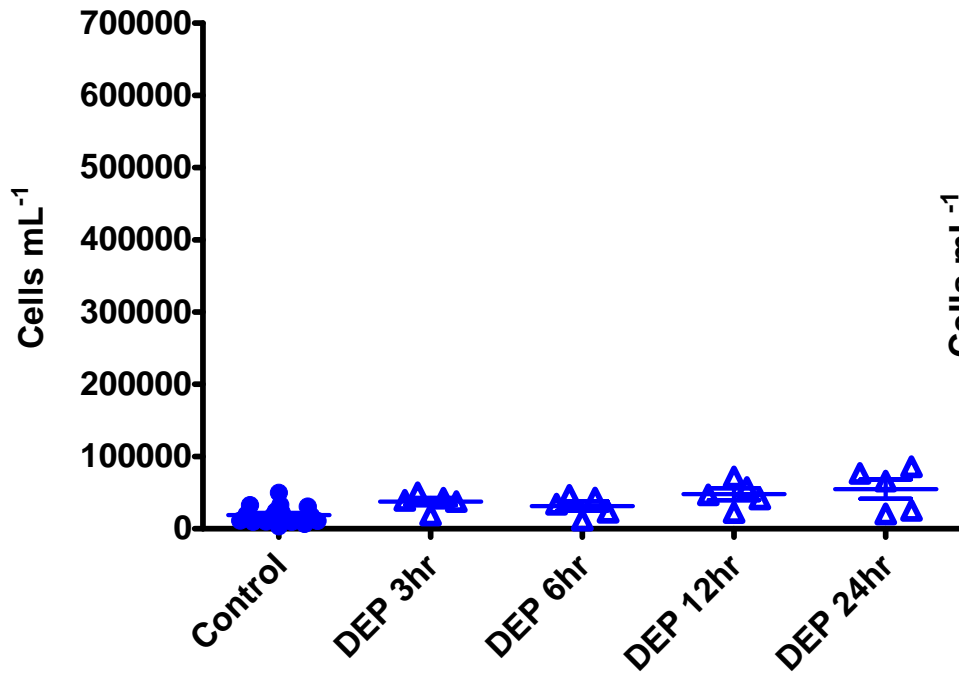
10ug DEP Female



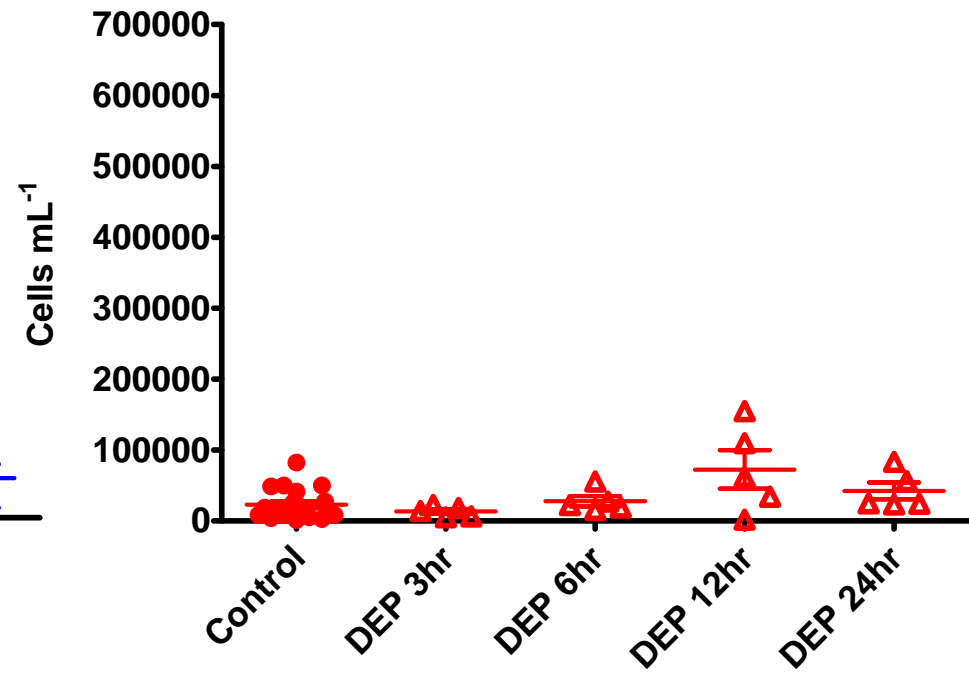
Total Cell Counts



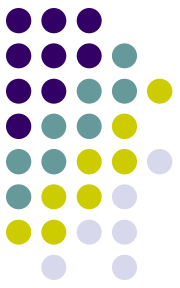
30ug DEP Male



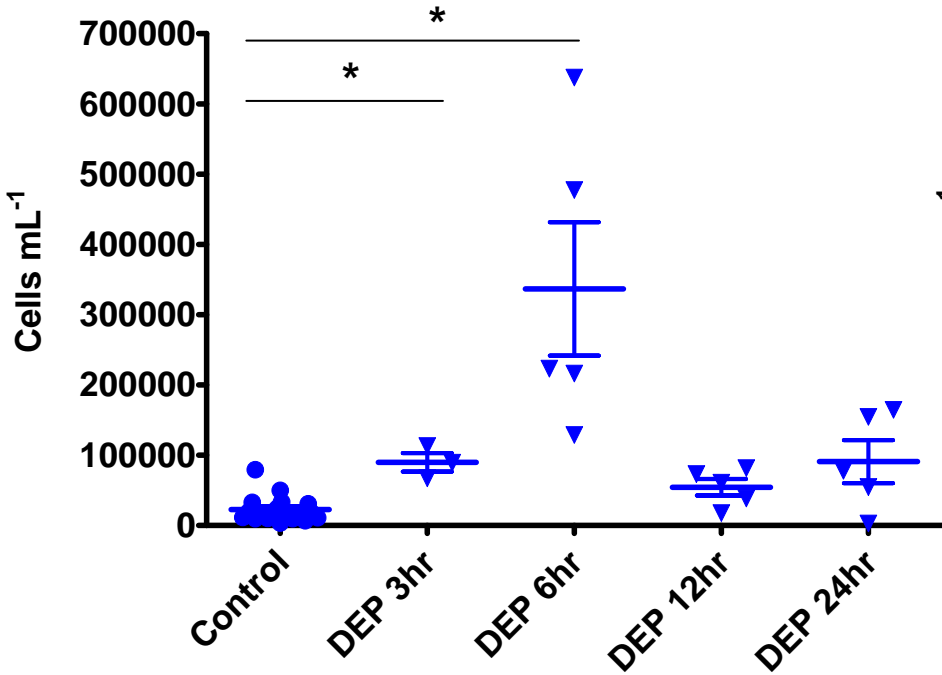
30ug DEP Female



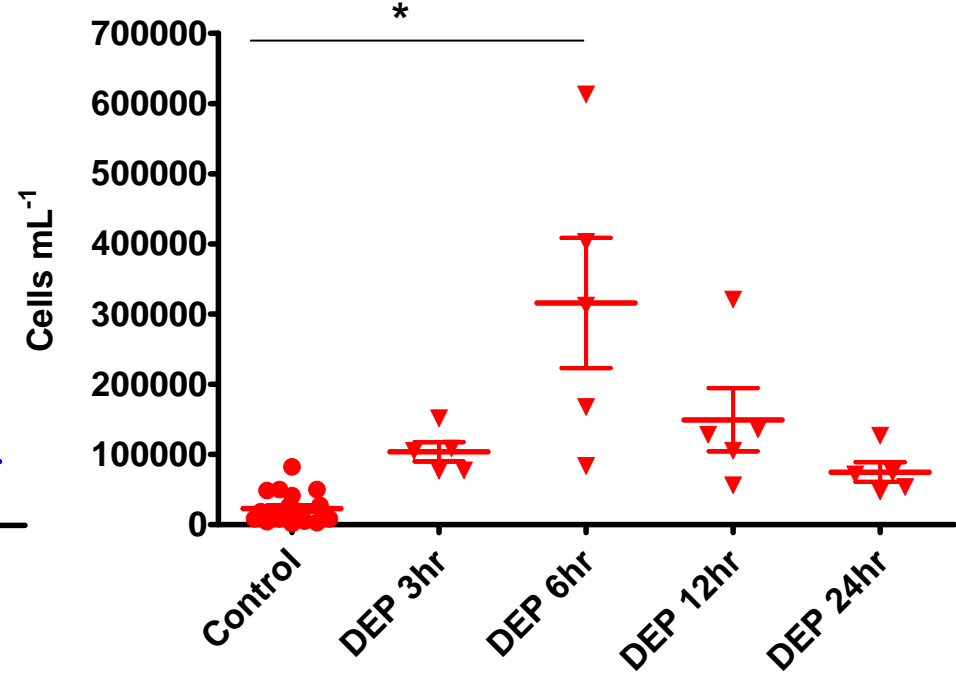
Total Cell Counts



100ug DEP Male

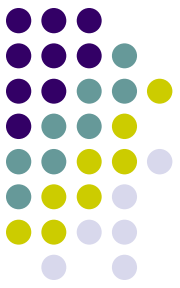


100ug DEP Female



* p < 0.05

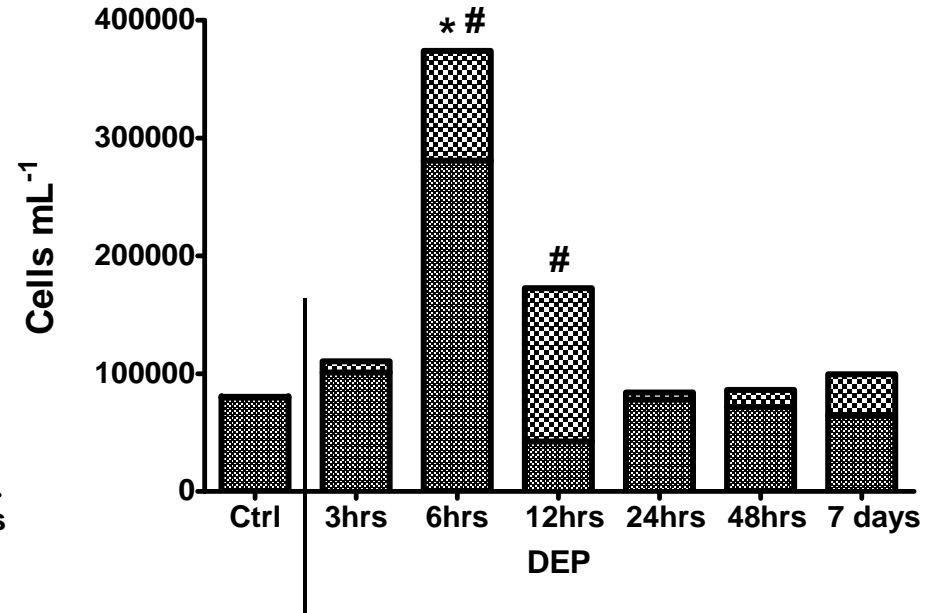
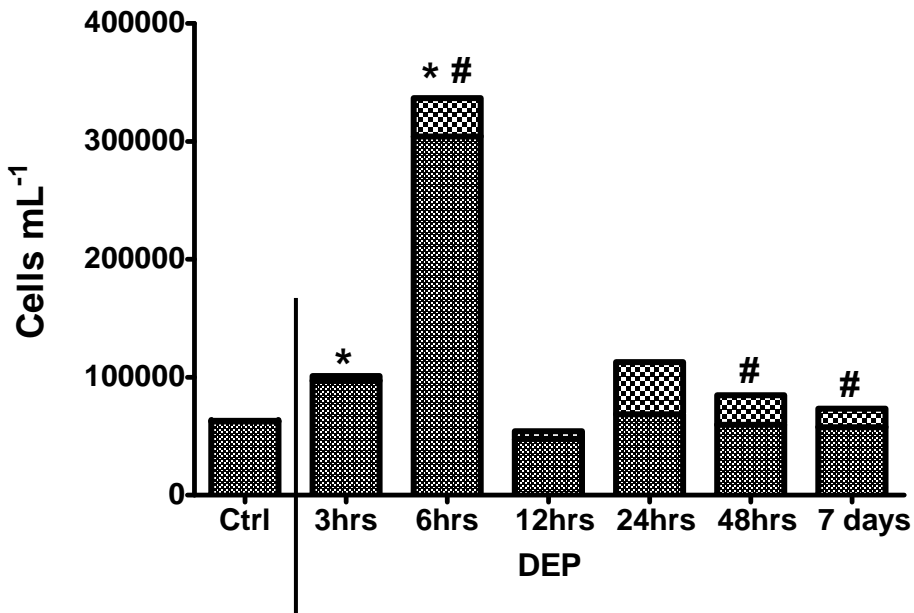
Differential Cell Count Results



▨ Macrophages
▨ Neutrophils

100ug DEP Male

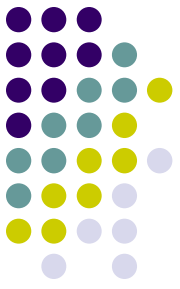
100ug DEP Female



* $p < 0.05$ vs control (Mac)

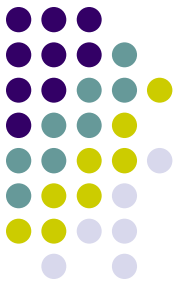
$p < 0.05$ vs control (Neut)

Part 1 Summary



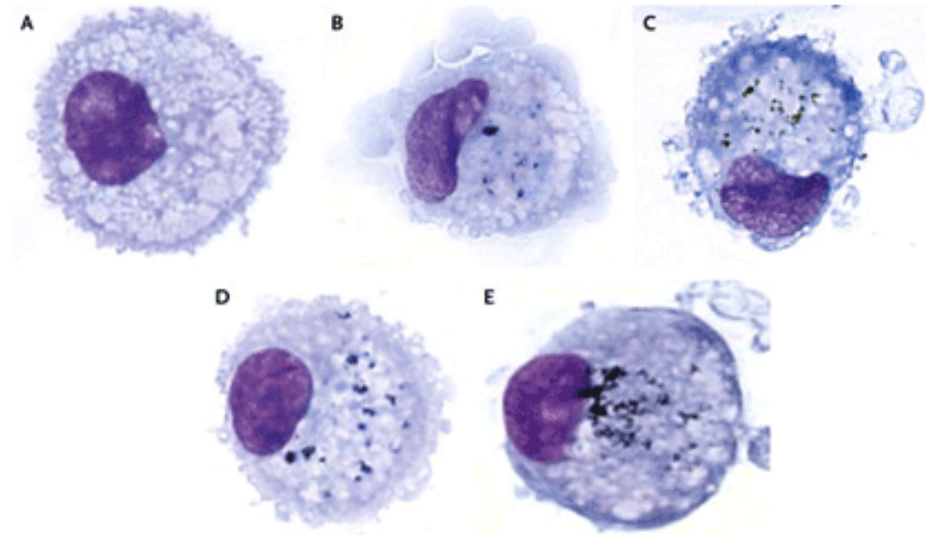
- Total cell counts showed a dose dependent response
 - 100 μ g showed greatest response
- Differential cell count
 - predominantly macrophages
 - also included an influx of neutrophils
- Pattern of response similar between males and females
- How does this model relate to human exposure?

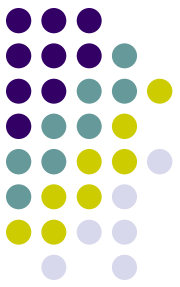
Research Plan



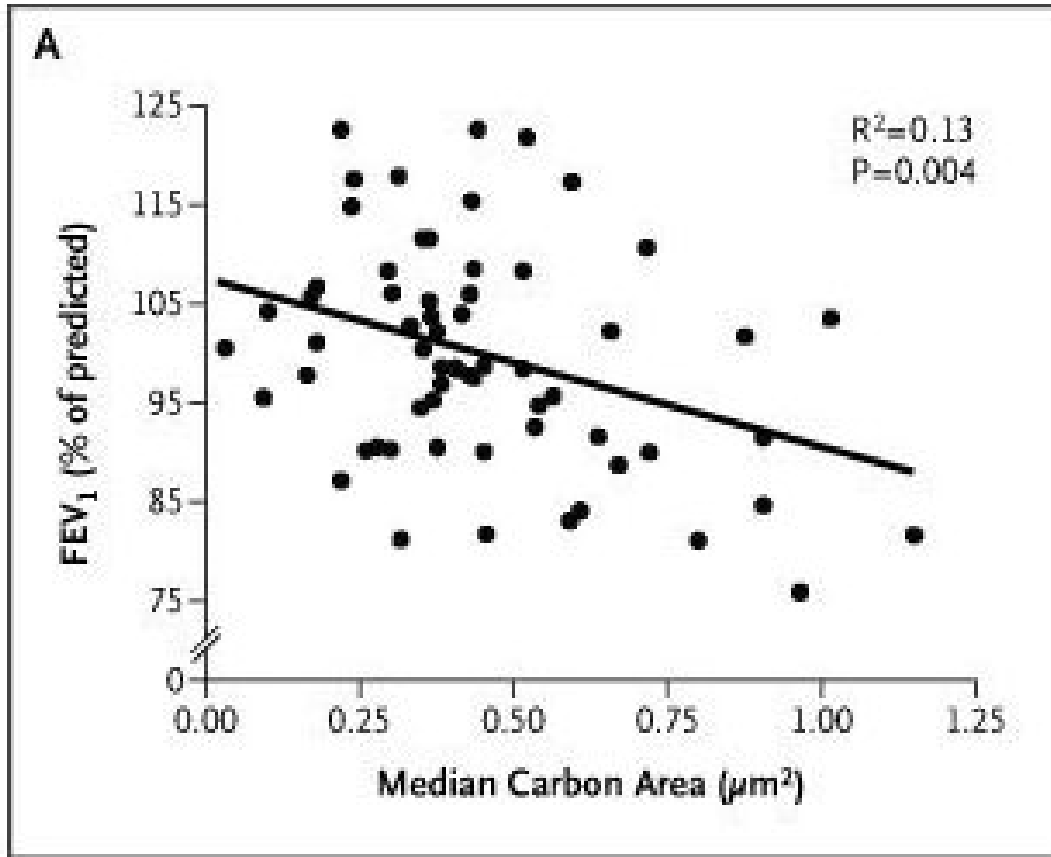
Part II: *Uptake of DEP by alveolar macrophages*

- Method used from Kulkarni et al.
- 64 Children in Leicester city, UK
- Sputum induction performed from Nov 2002 – Dec 2003
- Area occupied by carbon in each macrophage was assessed in 100 randomly selected cells
- Median area per cell calculated
- Related lung function to carbon content in airway macrophages





Associations between Carbon in Airway Macrophages and Lung Function in Healthy Children



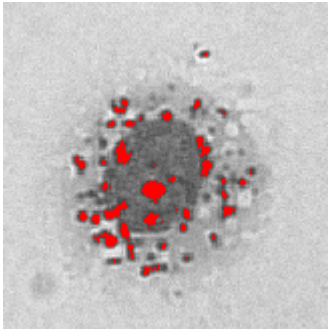
Each increase of $1\mu\text{m}^2$ in carbon content in the alveolar macrophage:

17%↓ in forced expiratory volume in 1 second (FEV₁)

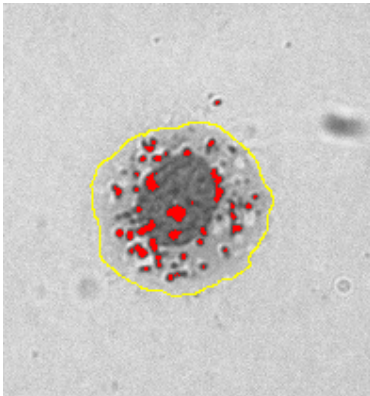
8 wk Female Balb/c 100ug DEP at 48 hours



Images taken of alveolar macrophages containing carbon



ImageJ software used to calculate carbon content of cell



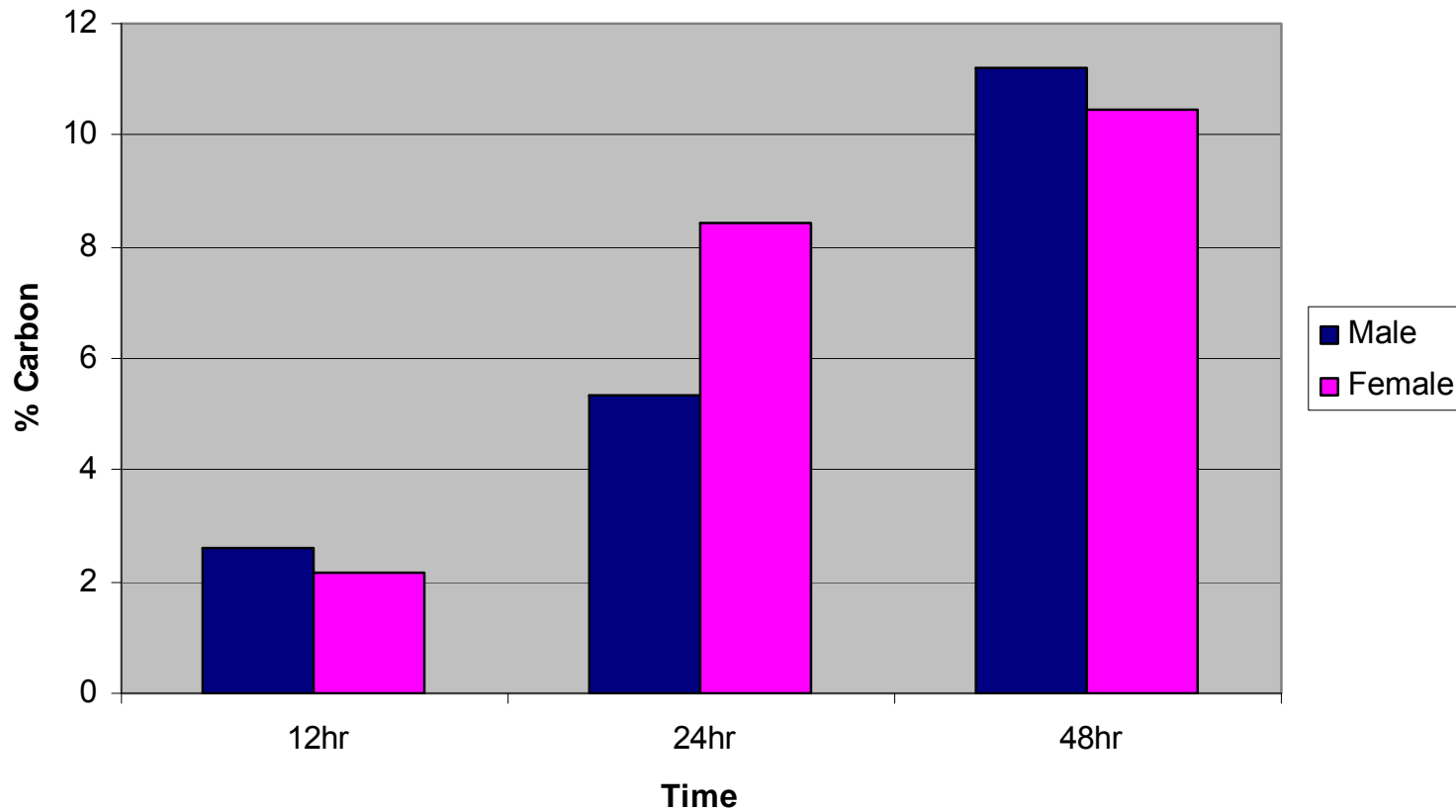
	Label	Area	Mean	Min	Max	%Area
1	9.jpg:Blue	1669	50.792	0	69	6.195

Area of interest identified
Carbon content calculated

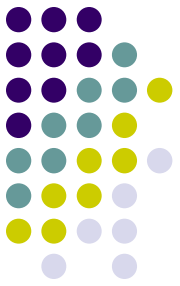
100 μg DEP



Median Value of Carbon Black in Alveolar Macrophages



Research Plan



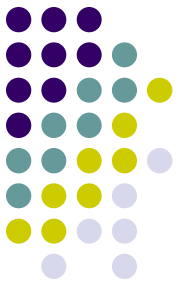
Future Work:

Cytokine responses: MIP-2, TNF α , MCP-1

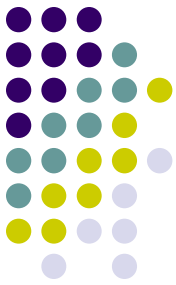
Lung function: measured at peak of DEP inflammation to determine the physiological consequences of a single acute exposure to DEP.

Time course of DEP uptake: examine longer time periods after exposure in order to determine when or if the carbon disappears from within alveolar macrophages.

Find optimum dose that matches human exposure based on macrophage uptake and use this dose for chronic exposure model.



This work will allow us to assess whether acute exposure to DEP exacerbates respiratory illnesses. This is important as with the increasing prevalence of DEP in urban areas throughout the world strategies will need to be established for intervention to prevent and improve life-long changes in respiratory health.



National Health and Medical Research Council (Australia)