Development of an Innovative in Vitro Inhalation Model for Studying the Effects of Diesel Exhaust

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Diesel exposure and lung diseases

- Diesel engines are the major source of pollution in urban areas

- Diesel exposure is possibly associated with:
  - onset of childhood asthma
  - asthma exacerbations
  - COPD exacerbations
  - respiratory infections
  - respiratory symptoms not related to asthma
  - impaired lung function
  - lung cancer

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Biological mechanisms still unclear
No data available on the relative importance of exposure concentration and duration
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Aim

Study the sensitivity of differentiated primary bronchial epithelial cells (PBEC) from COPD and asthma patients compared to cells from healthy subjects and to explore underlying mechanisms.

- Value effect of different concentration of diesel exhaust versus different exposure duration.
- Compare response from continuous and intermittent exposure.
Integrated approach

- State of art exposure facilities to generate well defined and **realistic** emissions
- Air liquid interface (ALI) cell exposure system
- Primary bronchial epithelial cells cultured at the ALI obtained from COPD and asthma patients from the LUMC
Powertrain test center at TNO represents **realistic** diesel engine exposure

- Lack of data of primary cells response to diesel
- Logistic and cost issues

- Lab scale set up to mature experience
Lab scale engine exposure

Exhaust produced in situ
Modulation of engine load

Triplicate of each condition

Dose-control by using 4 modules

Air Low Mid High
Chemical characterization

**Mixture characterization**

*Independently from exposure*

- PM mass
- EC
- PAH
- Oxy-PAH
- Nitro-PAH
- CO
- CO₂
- NO/NO₂
- TCH
- Oxidative potential

Measured at five engine loads points

**Mixture characterization**

*During exposure*

- Rel.hum./temperature
- [CO₂]
- [O₂]
- SMPS (particle size distribution)
- [PM] for each dose from gravimetric filter deposition
Exposure duration

- Cells exposed to diluted diesel exhaust (DE) mixtures:
  - High (9-fold diluted DE mixture)
  - Mid (27-fold diluted DE mixture)
  - Low (81-fold diluted DE mixture)

Cells were harvested at 6 and 24hr post exposure

- **Epithelial barrier function** (TEER measurement)
- **Cytotoxicity** (LDH release)
- **Oxidative stress induction** (HMOX1 and NQO1 mRNA expression)
Exposure duration: *barrier function and cytotoxicity*

**Membrane function - TEER meas.**
- 6hr

**Cytotoxicity - LDH release**
- 6hr

**Membrane function - TEER meas.**
- 24hr

**Cytotoxicity - LDH release**
- 24hr

No cytotoxic effect after 1:00 hr exposure; time-dependent increase at 2:30hr and 6:15hr.
Exposure duration: oxidative stress

DE dose-dependent activation of oxidative stress response for all exposure durations
Donor variation (n=3)

**HMOX1 - Ox. Stress response -**

<table>
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<th>6hr</th>
<th>Air</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
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**NQO1 - Ox. Stress response -**

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**IL-8 - Inflammation -**

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**GADD34 - ER Stress response -**

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DE dose-dependent activation of oxidative stress response, inflammation and ER stress response
Effect of engine load

Chemical characterization

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Cell exposure conditions

- n=2 donors
- Air (Air D1, Air D2)
- High DE (High D1, High D2)
- 2:30hr exposure
- Analyses 6hr post exposure
Effect of engine load

Membrane function - TEER meas. -

Cytotoxicity - LDH release -

Increasing the engine load lowers the cytotoxic effect
Conclusions

- We are able to study biological effects of diesel exhaust from differentiated primary bronchial epithelial cells at the air-liquid interface using the air exposure route.

- We have optimized our testing system for diesel exhaust exposures using a diesel generator at lab scale conditions.
- Use of (at least) three donors is recommended.
- A clear oxidative and ER stress response was found, but also in activation of the inflammation.
- Increasing engine load lowers the cytotoxicity.
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