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

M.C. Zarcone, G. Amatngalim, E. Duistermaat, P.S. Hiemstra, I.M. Kooter

18th ETH-Conference on Combustion Generated Nanoparticles
Session 6 B-Health Effects

25th June 2014

Maria C Zarcone, PhD student

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

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


TNO innovation for life

LUMC

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

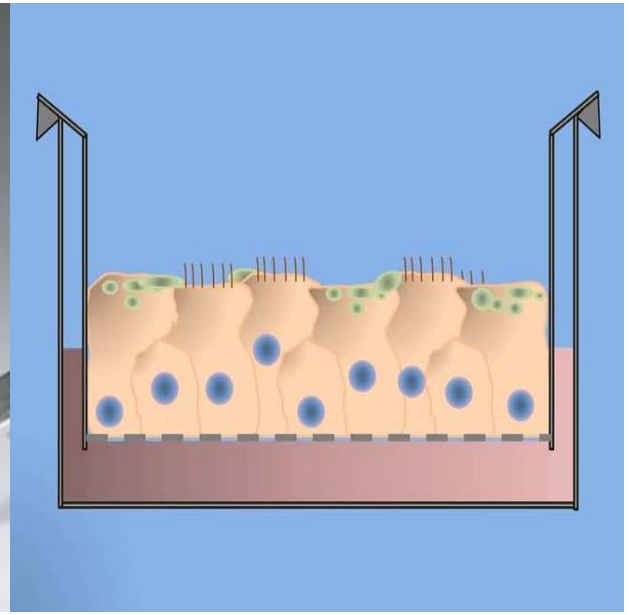
  - Value effect of different concentration of diesel exhaust versus different exposure duration

  - 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





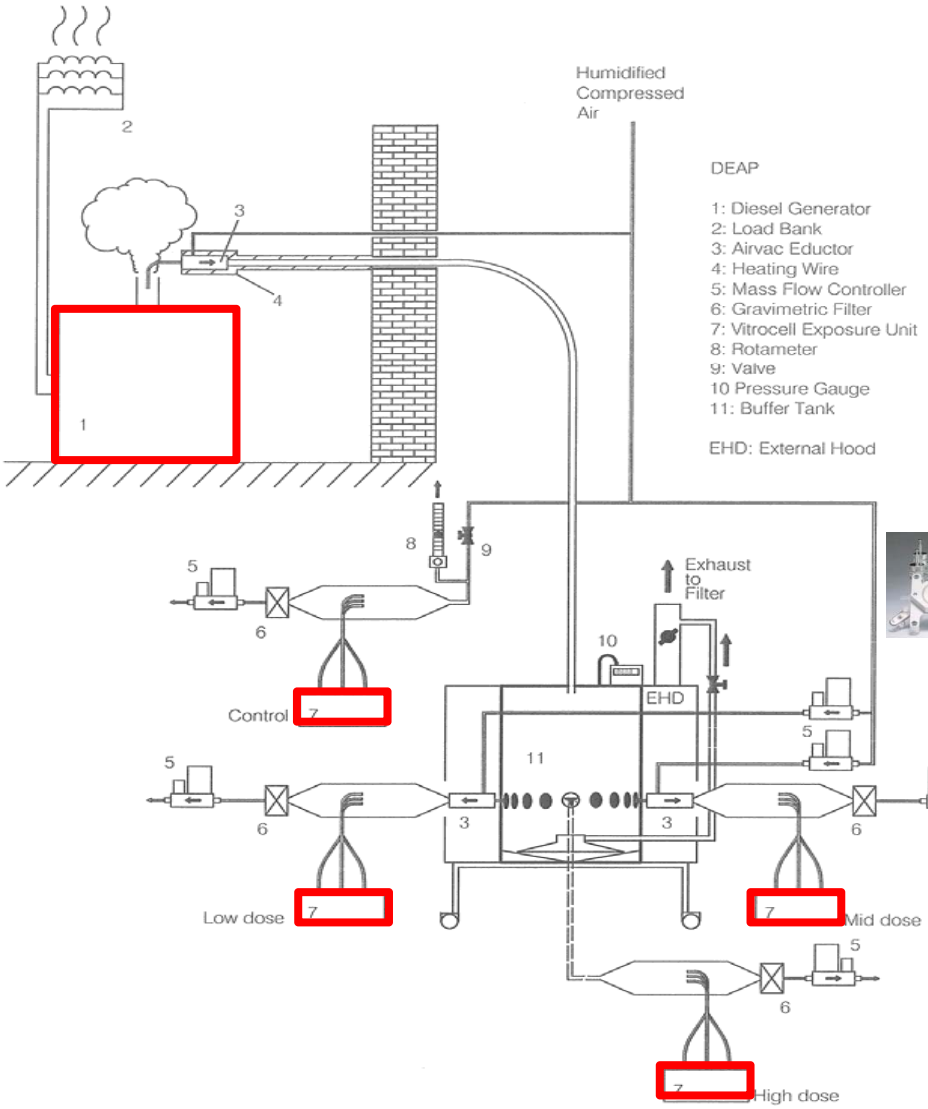

TNO innovation for life

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


TNO innovation for life

LUMC

Chemical characterization

Mixture characterization
Independently from exposure

- PM mass
- EC
- PAH
- Oxy-PAH
- Nitro-PAH
- CO
- CO_2
- NO/NO_2
- TCH
- Oxidative potential

Measured at five engine loads points

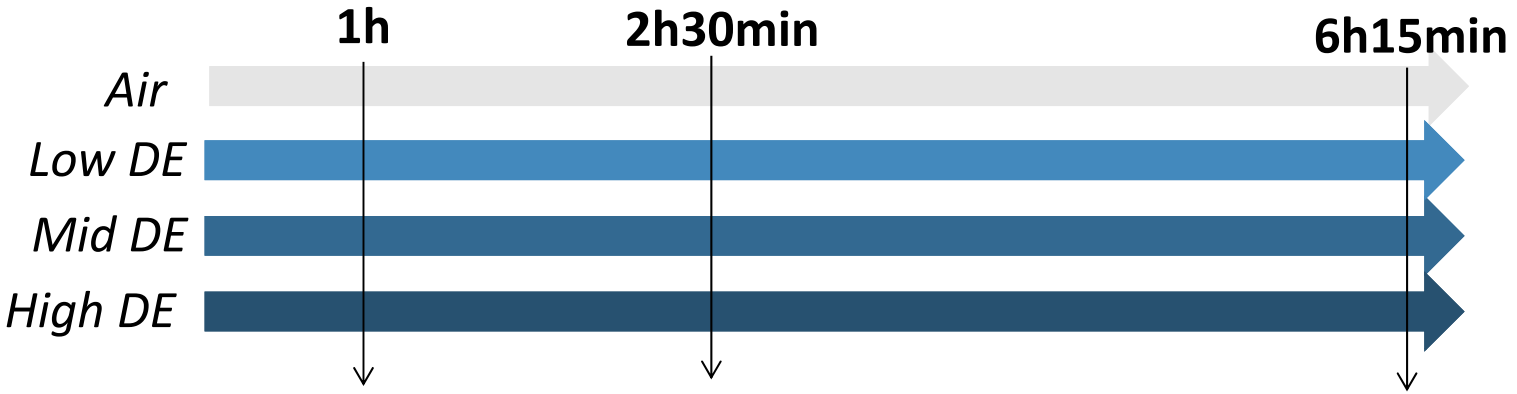
Mixture characterization
During exposure

- Rel.hum./temperature
- $[CO_2]$
- $[O_2]$
- 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

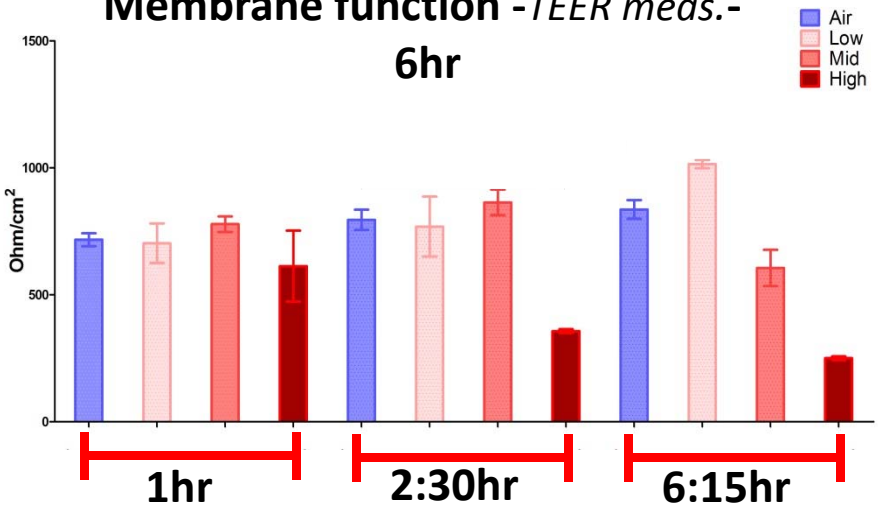
Epithelial barrier function (TEER measurement)

Cytotoxicity (LDH release)

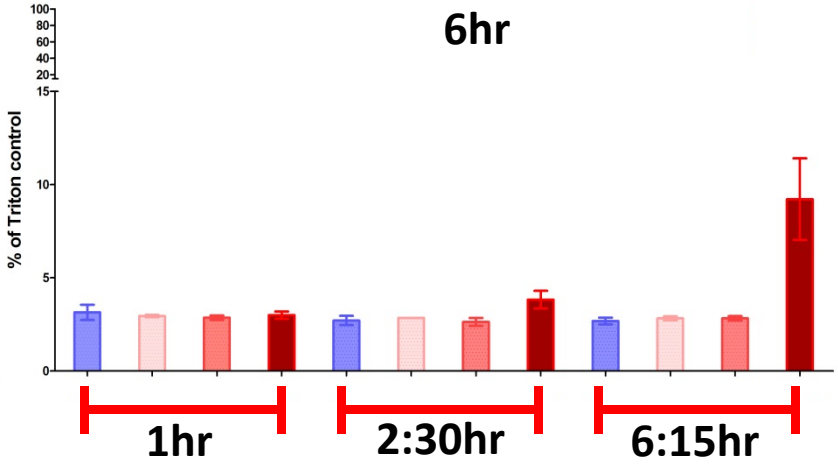
Oxidative stress induction (HMOX1 and NQO1 mRNA expression)

Exposure duration: *barrier function and cytotoxicity*

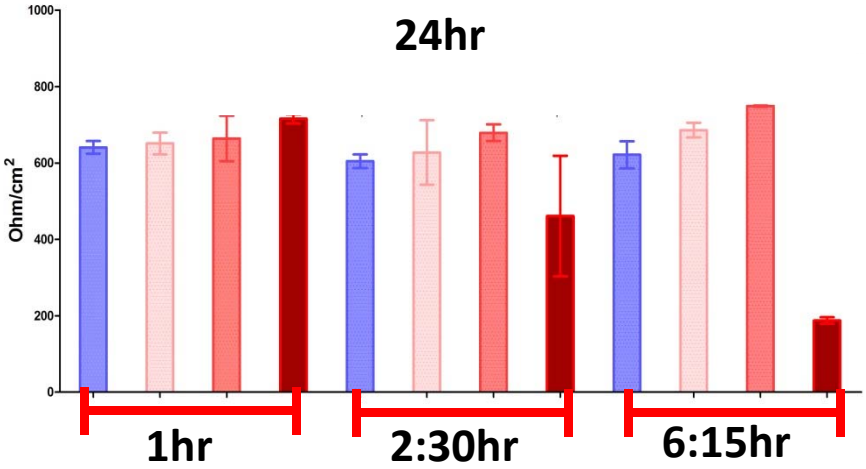
Membrane function -*TEER meas.*-
6hr



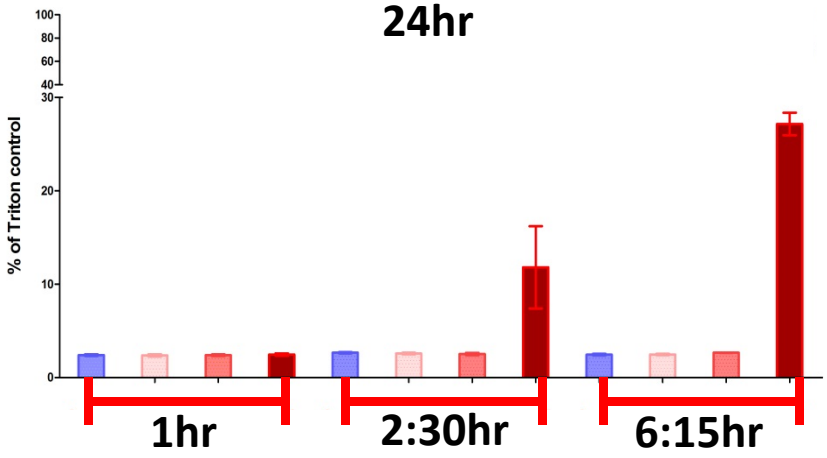
Cytotoxicity -*LDH release*-
6hr



Membrane function -*TEER meas.*-
24hr

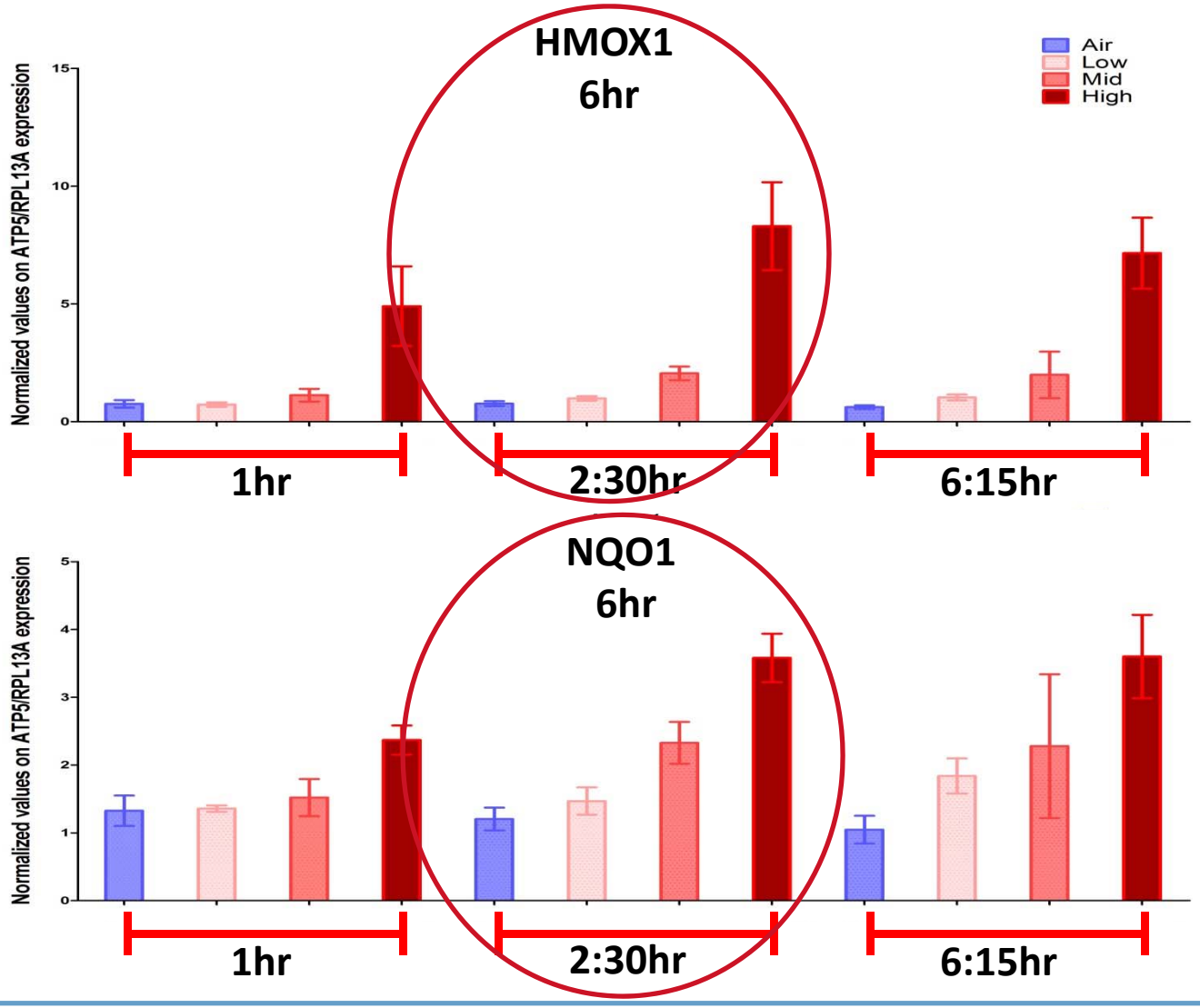


Cytotoxicity -*LDH release*-
24hr



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

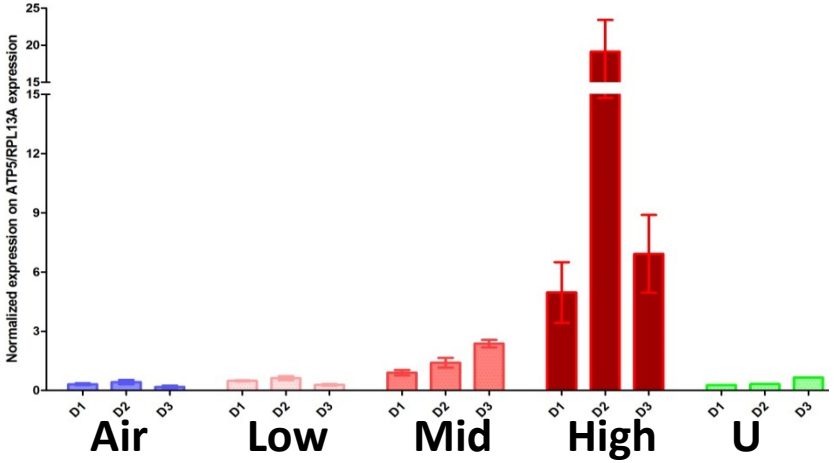
Exposure duration: *oxidative stress*



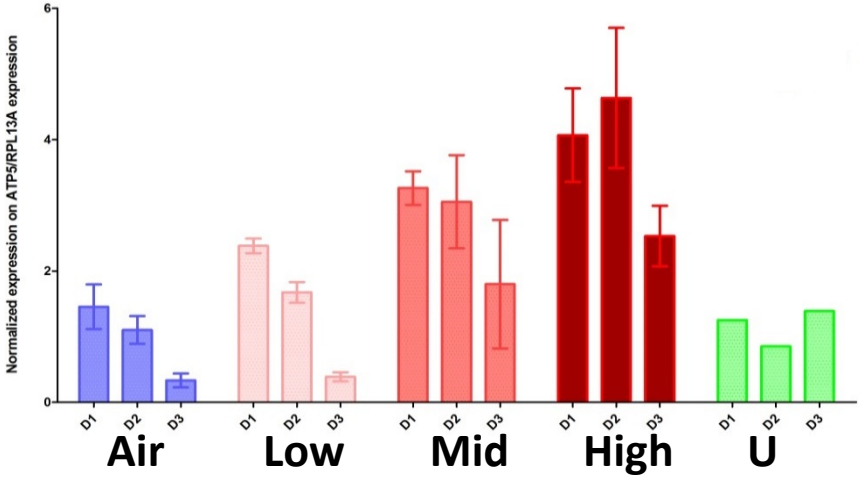
DE dose-dependent activation of oxidative stress response for all exposure durations

Donor variation (n=3)

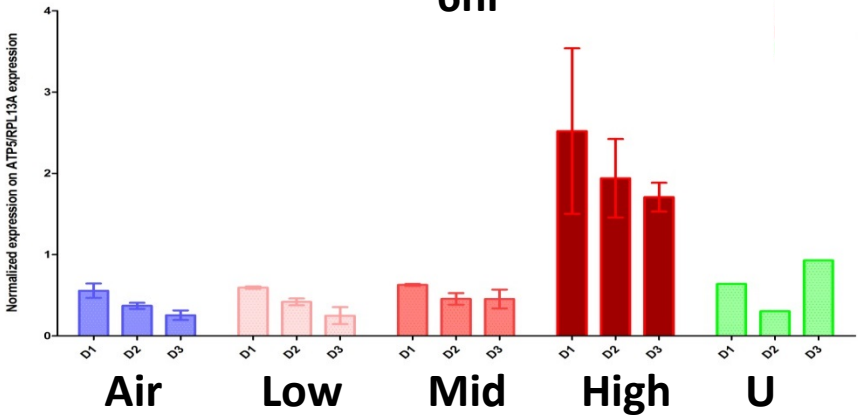
HMOX1 *-Ox. Stress response-*
6hr



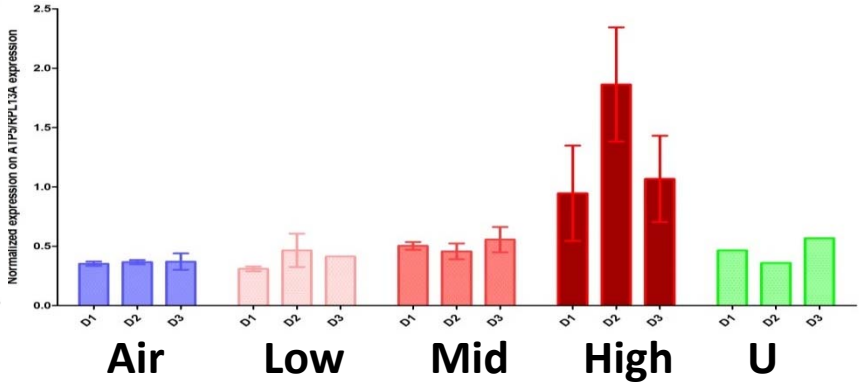
NQO1 *-Ox. Stress response-*
6hr



IL-8 *-Inflammation-*
6hr



GADD34 *-ER Stress response-*
6hr



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

Effect of engine load

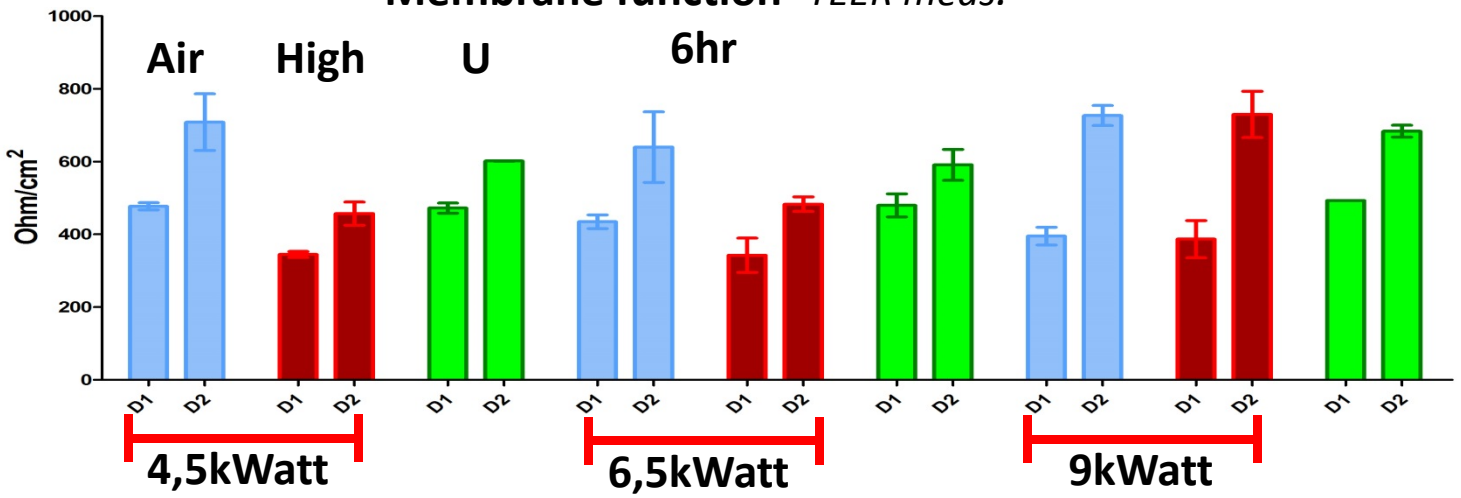
- Chemical characterization

kWatt	PM	CO	NO	NO_2	NO_x
	mg/m^3	ppm	ppm	ppm	ppm
4,5	32,7	252	94	62	156
6,5	17,8	251	120	42	162
9	17,7	202	189	23	212

- Cell exposure conditions
  - n=2 donors
  - Air (Air D1, Air D2)
  - High DE (High D1, High D2)
  - 2:30hr exposure
  - Analyses 6hr post exposure

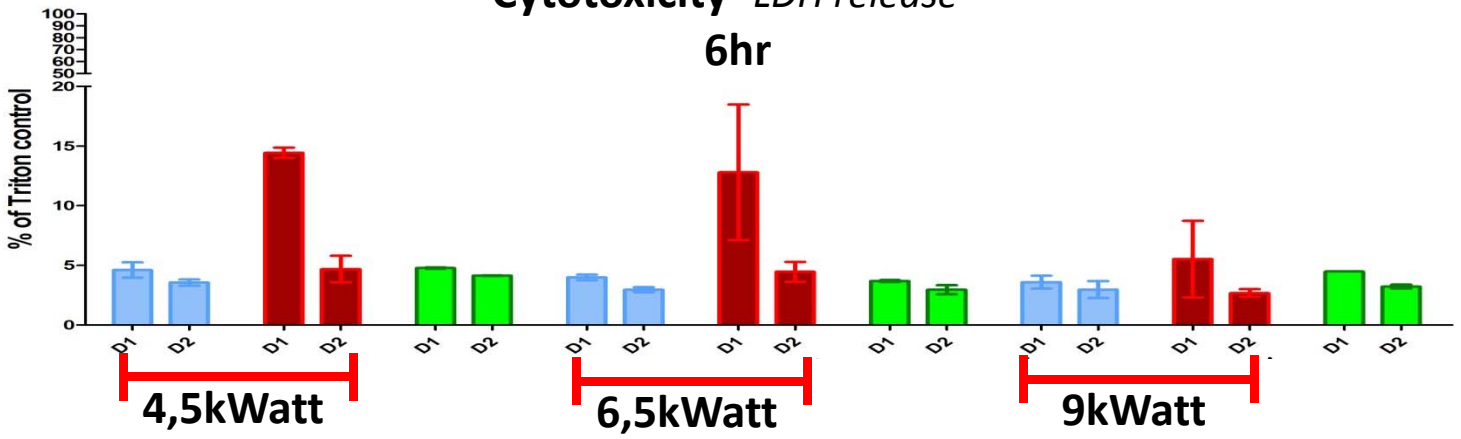
Effect of engine load

Membrane function -*TEER meas.*-



Cytotoxicity -*LDH release*-

6hr



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

Acknowledgements

Lung Foundation Netherlands
(Longfonds)

LUMC
Pieter Hiemstra
Gimano Amatngalim
Renate Verhoosel

TNO Triskelion
Evert Duistermaat
Birol Usta

TNO
Ingeborg Kooter
Aleksandra Jedynska
Marc Houtzager
Thomas Ivens
Gertjan Koornneef