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in vitro inhalation model

TNO innovation
for life



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

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Session 6 B-Health Effects

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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



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



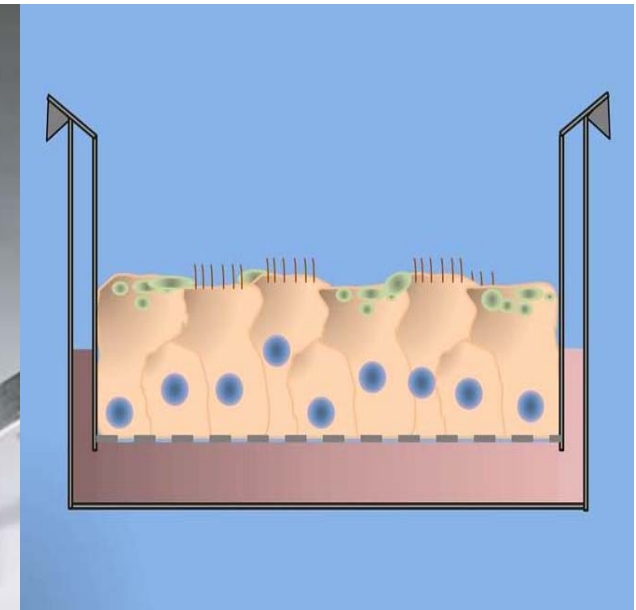
Aim

- › 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



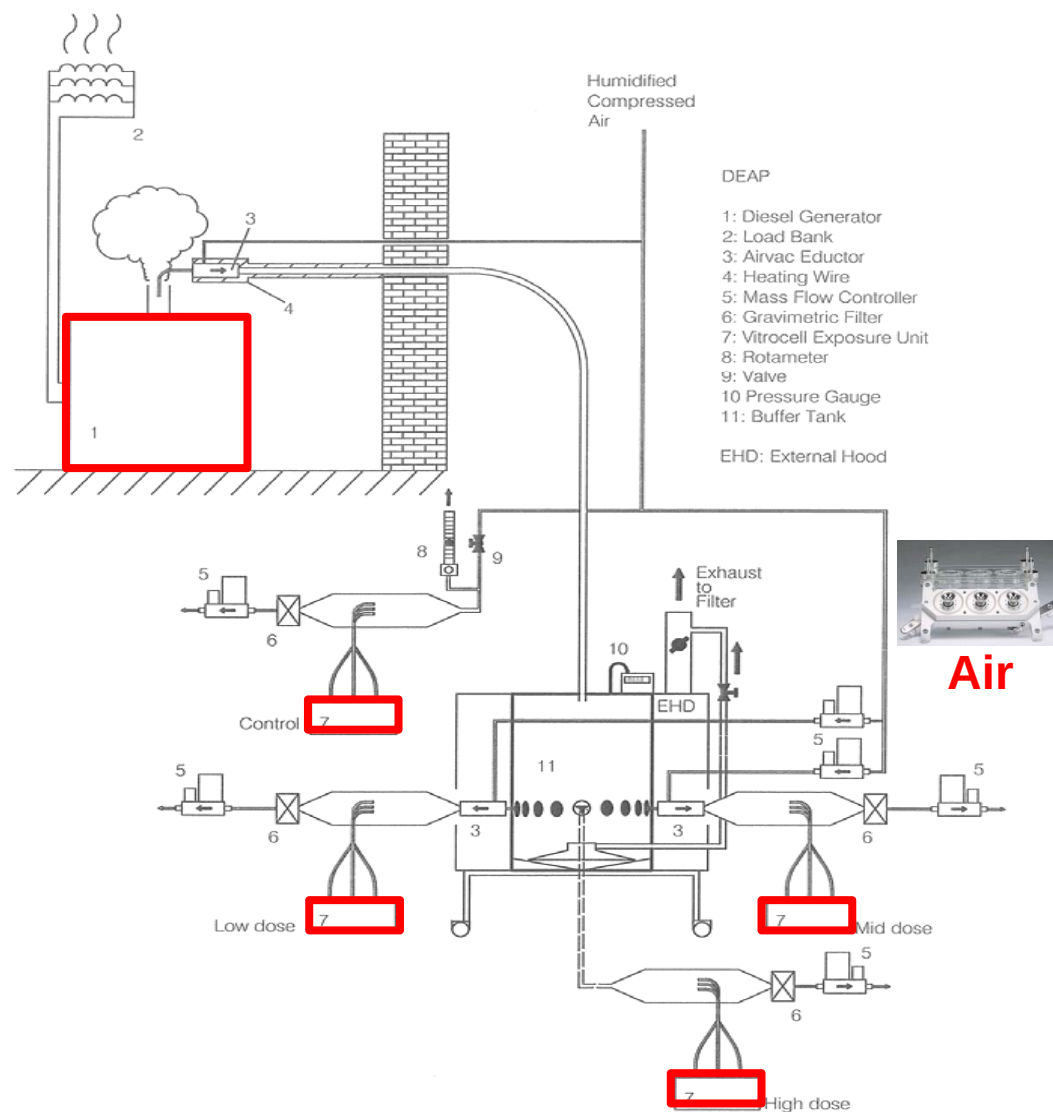


Powertrain test center at TNO represents **realistic** diesel engine exposure

- › 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

Mixture characterization

During exposure

- Rel.hum./temperature
- [CO₂]
- [O₂]
- SMPS (particle size distribution)

- [PM] for each dose from gravimetric filter deposition

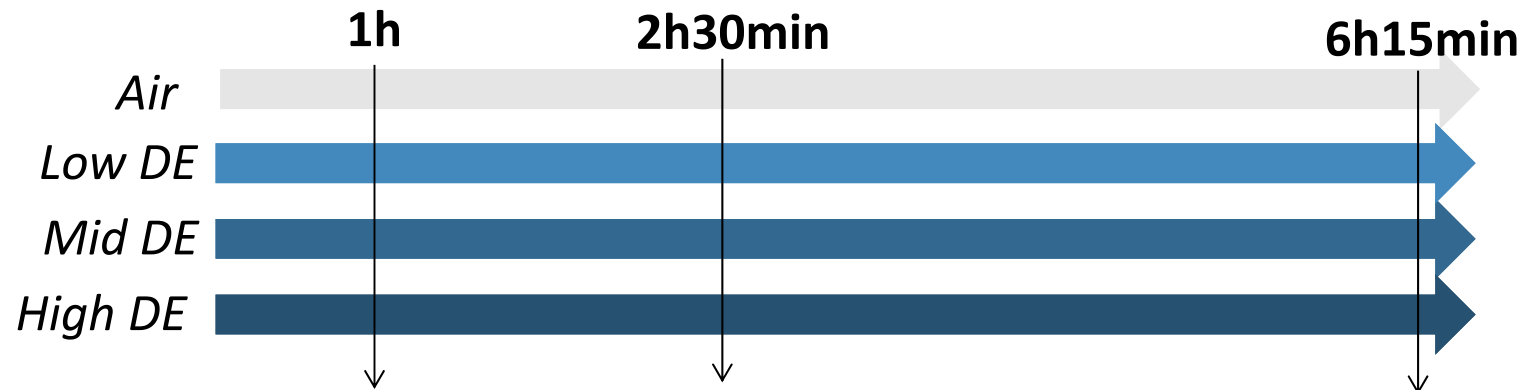
Measured at five engine loads points



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

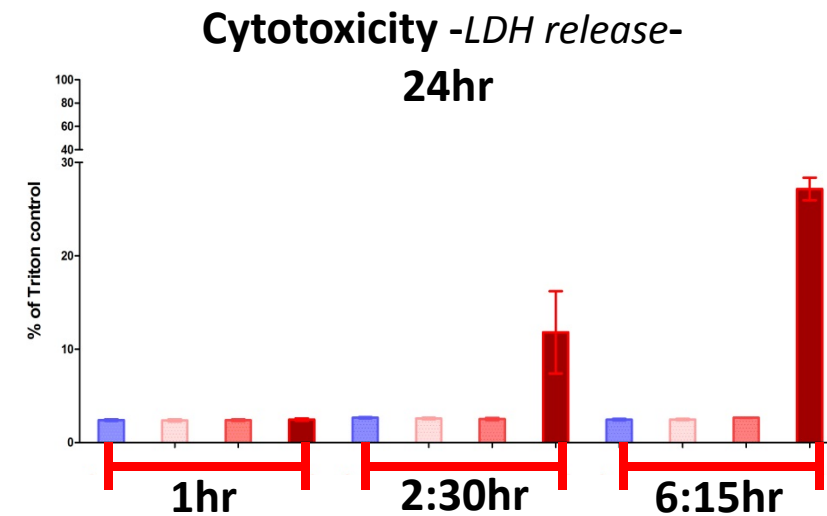
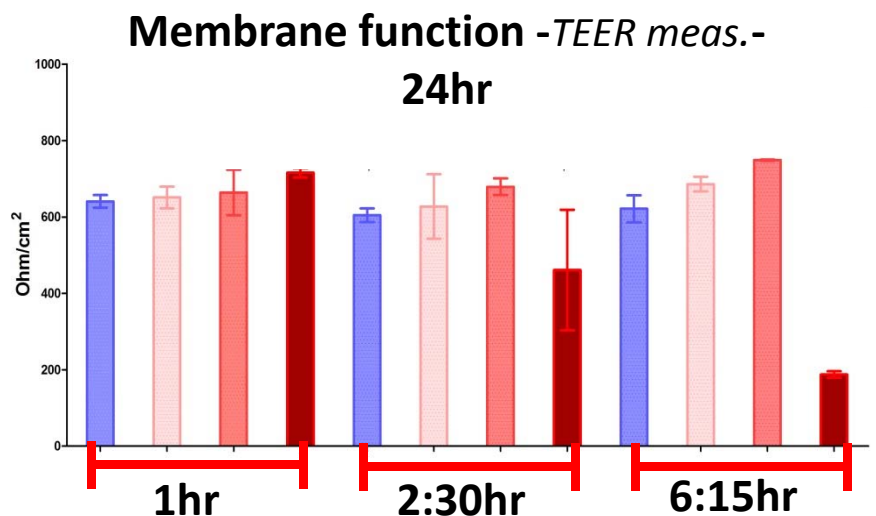
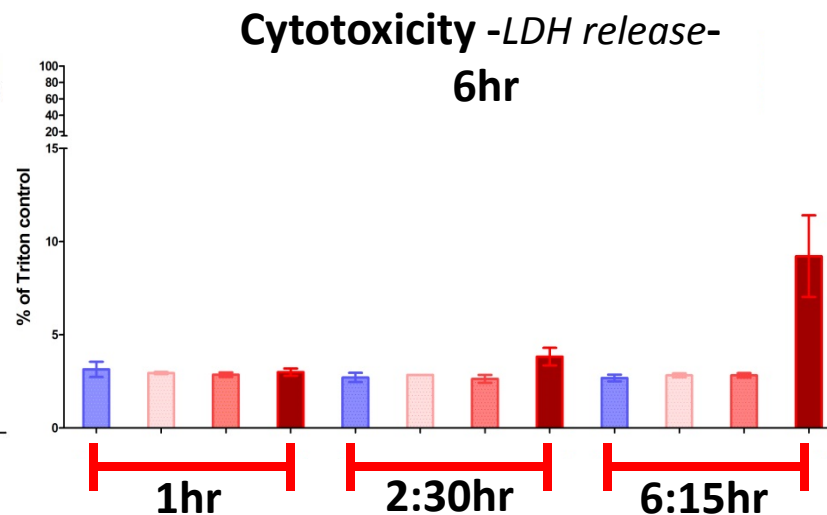
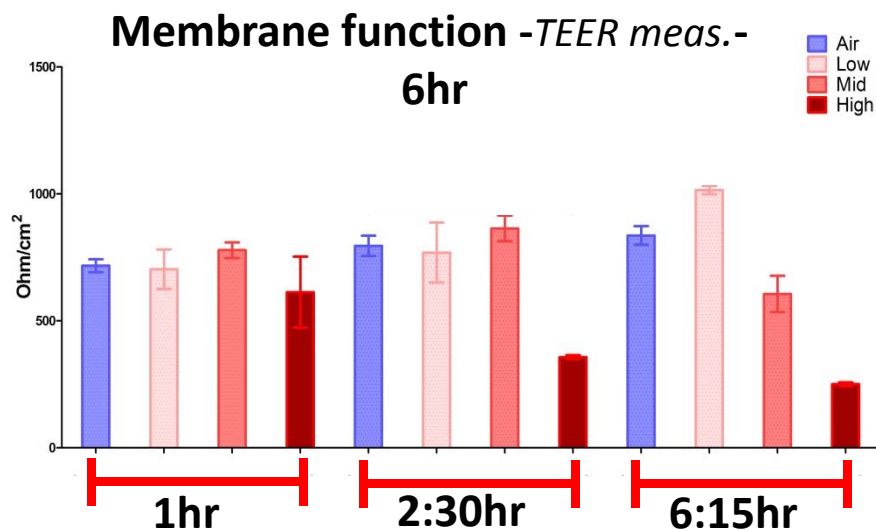
Epithelial barrier function (TEER measurement)

Cytotoxicity (LDH release)

Oxidative stress induction (HMOX1 and NQO1 mRNA expression)



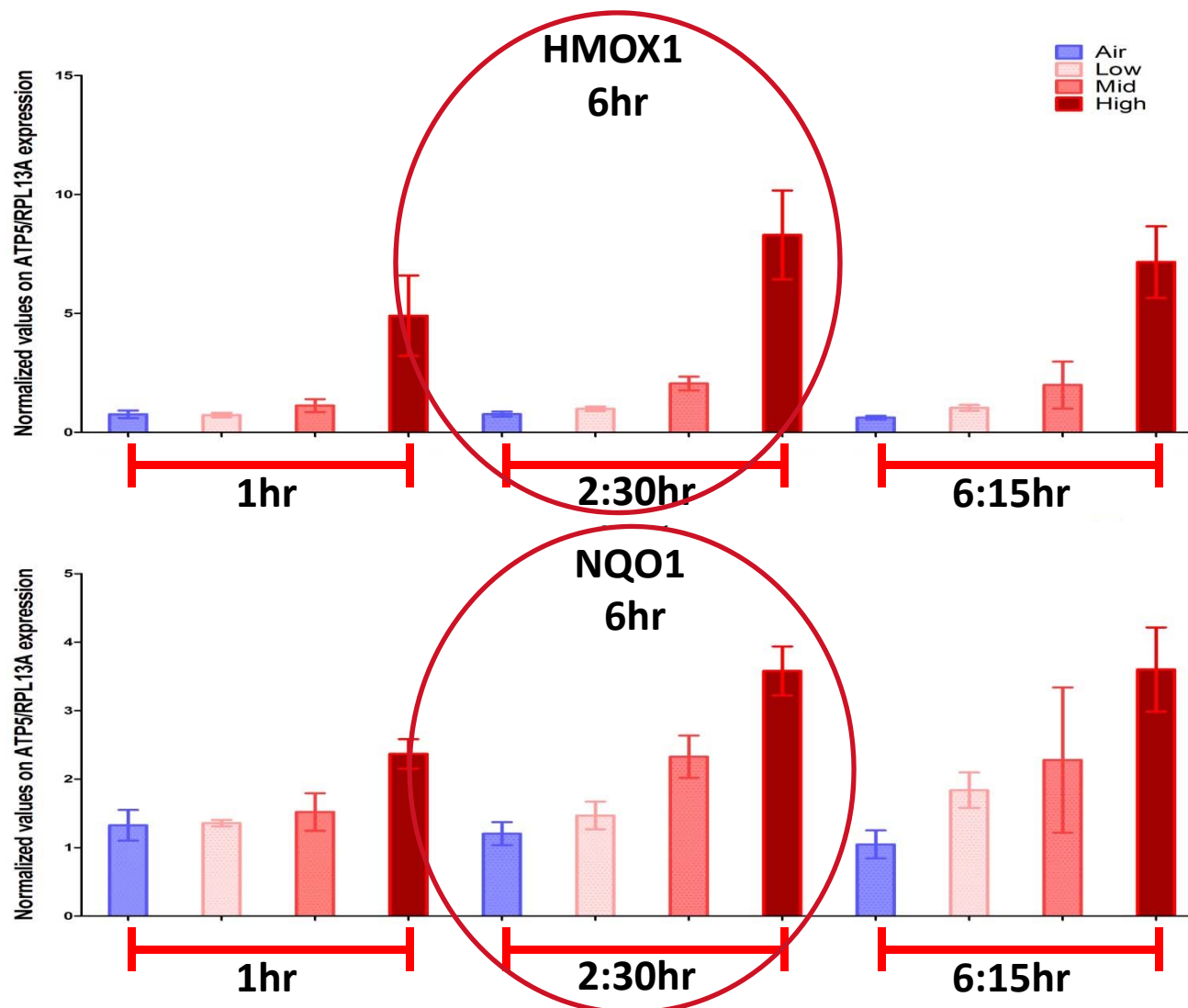
Exposure duration: *barrier function and cytotoxicity*



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



Exposure duration: *oxidative stress*

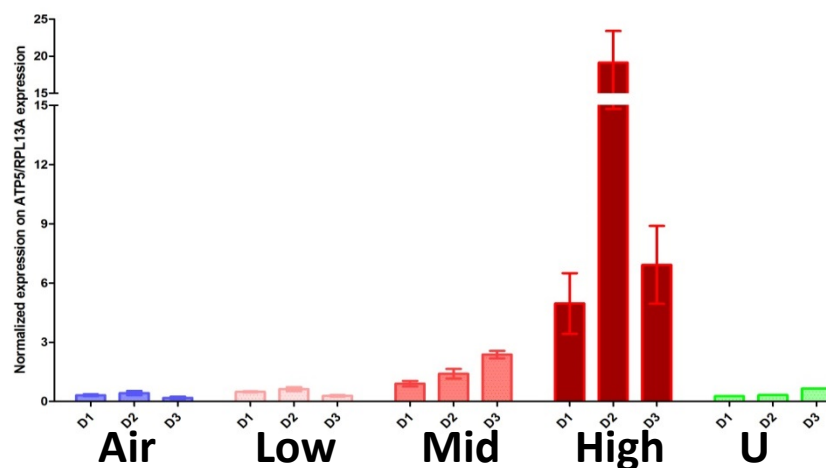


DE dose-dependent activation of oxidative stress response for all exposure durations

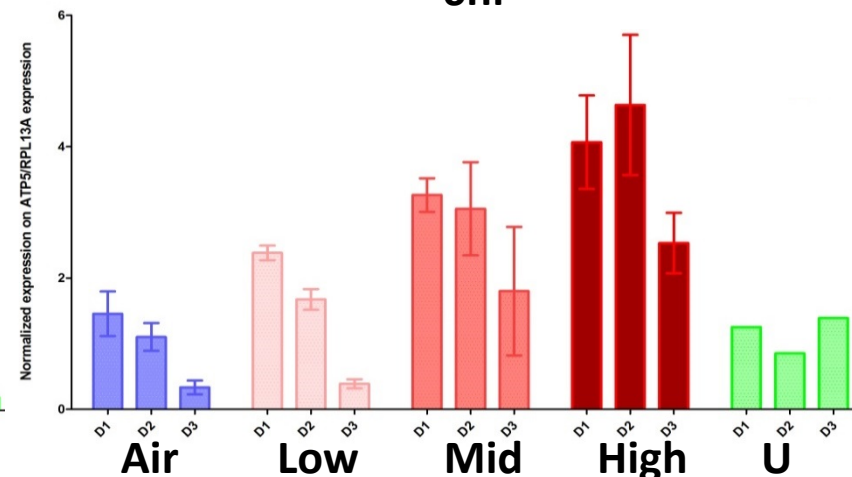


Donor variation (n=3)

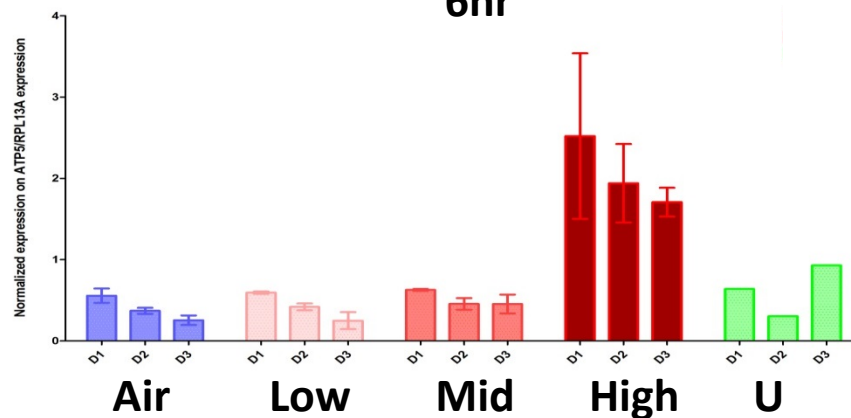
HMOX1 -Ox. Stress response-
6hr



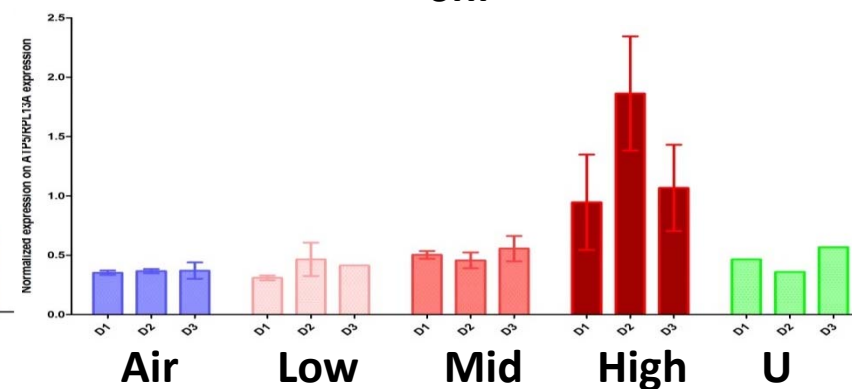
NQO1 -Ox. Stress response-
6hr



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

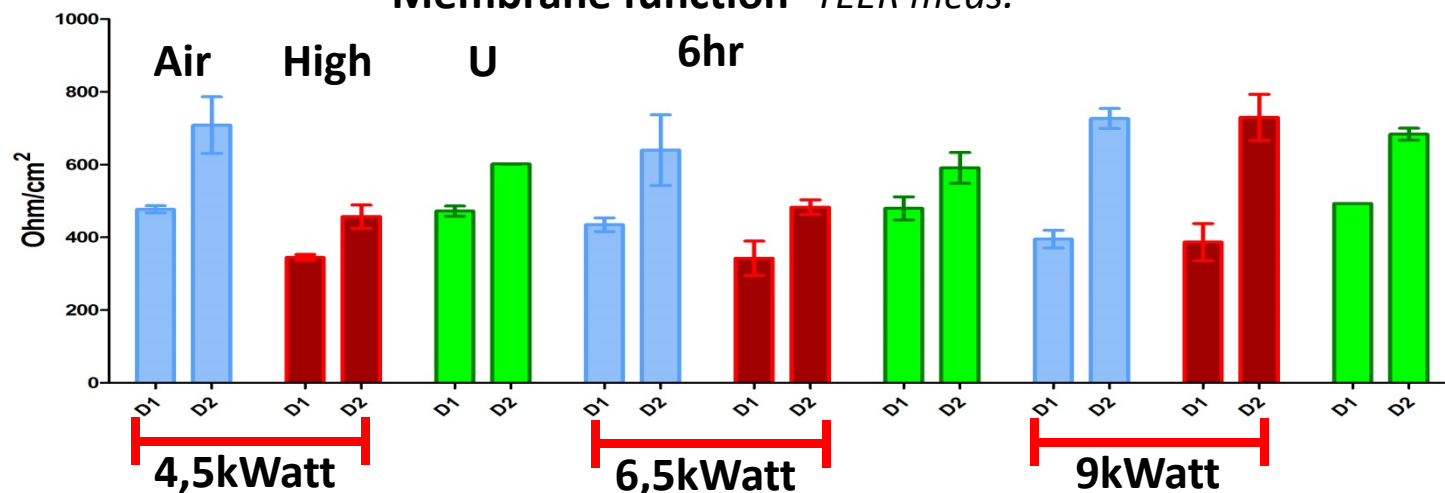
› Cell exposure conditions

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

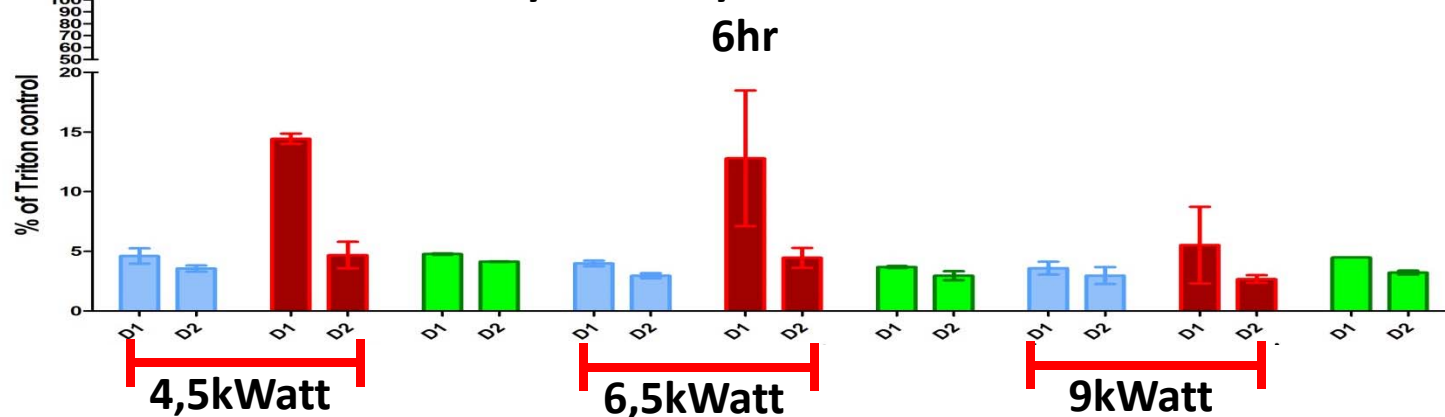


Effect of engine load

Membrane function -*TEER meas.*-



Cytotoxicity -*LDH release*-



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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