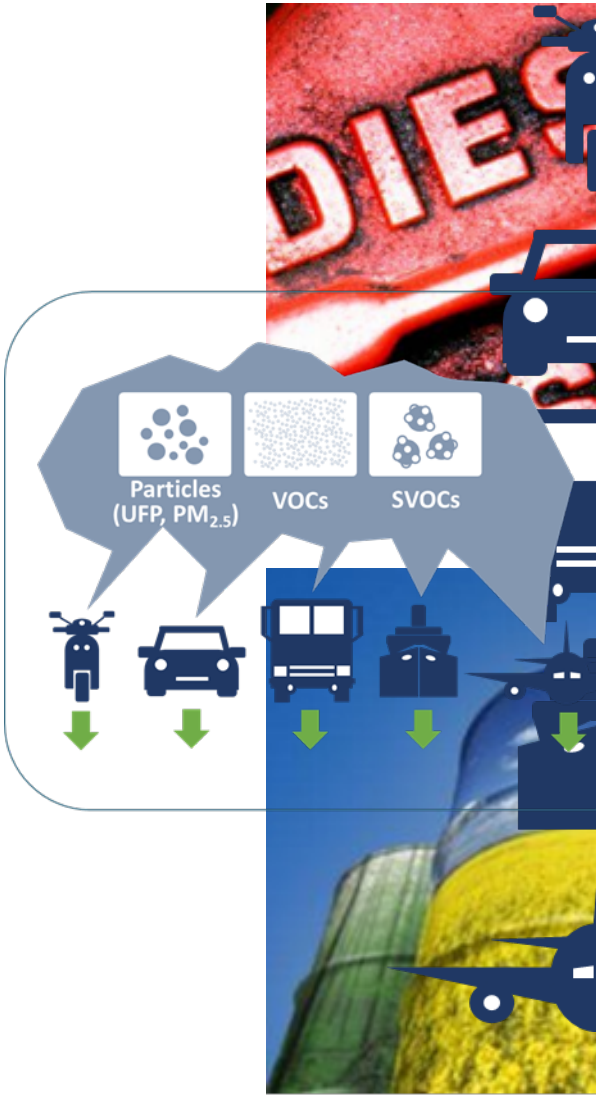
OLE FOR IN VITRO MODELS IN THE OLOGICAL EVALUATION OF COMPLEX RES, SUCH AS EMISSIONS OF USTION PROCESSES

ORG M.KOOTER

on of new emission standards (PM mass) lowers the engine
from several transport modes

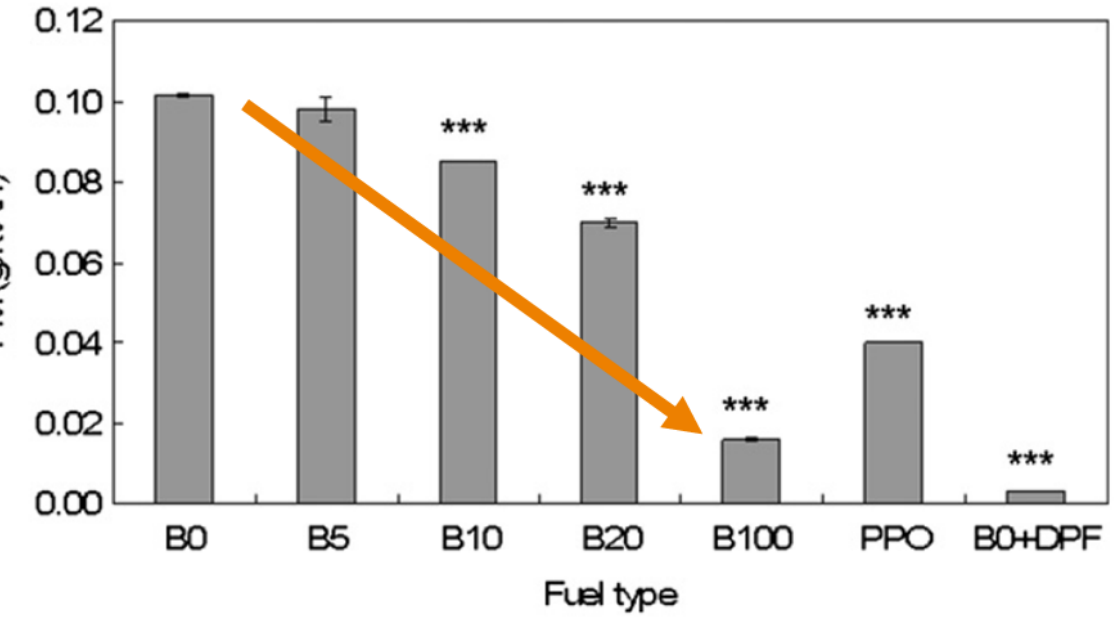
tation of new engine/emission control technologies and renewable
eet the standards can lead to changes in the chemical mixture of
ollutants

regulatory requirements are met the actual health effects of these
s often are untested



toxicity of combustion of biodiesel at lower PM emission vs regular diesel

PM mass



1. PM mass ($g\,kWh^{-1}$) emissions for the fuel types and blends tested (6 ETC, ept B10, 5 ETC and PPO 4 ETC). Error bars indicate the standard error of the mean. *, *** significantly different from B0 at $P < 0.05$, <0.01, <0.001 respectively.

PM cytotoxicity

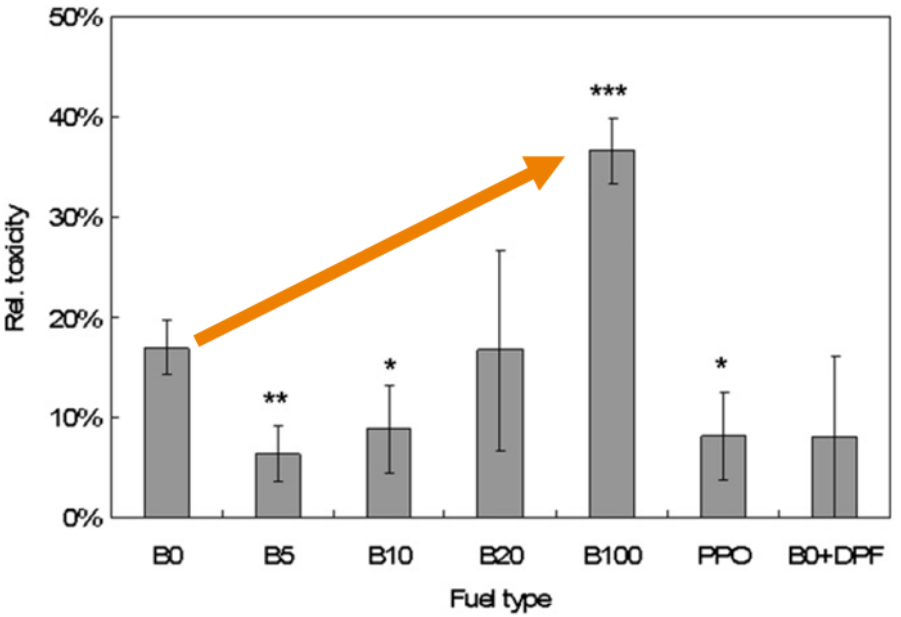


Fig. 5. Relative cytotoxicity for the fuel types and blends tested, in % compared to positive control, 1% Triton-X100. Each bar represents 3 ETC. Error bars indicate the standard error of the mean. *, **, *** significantly different from the mean of B0 at $P < 0.05$, <0.01, <0.001 respectively.

Atm.Env. 2011

biological responses at lower mass concentrations of nvPM from aircraft turbine engines

PM mass

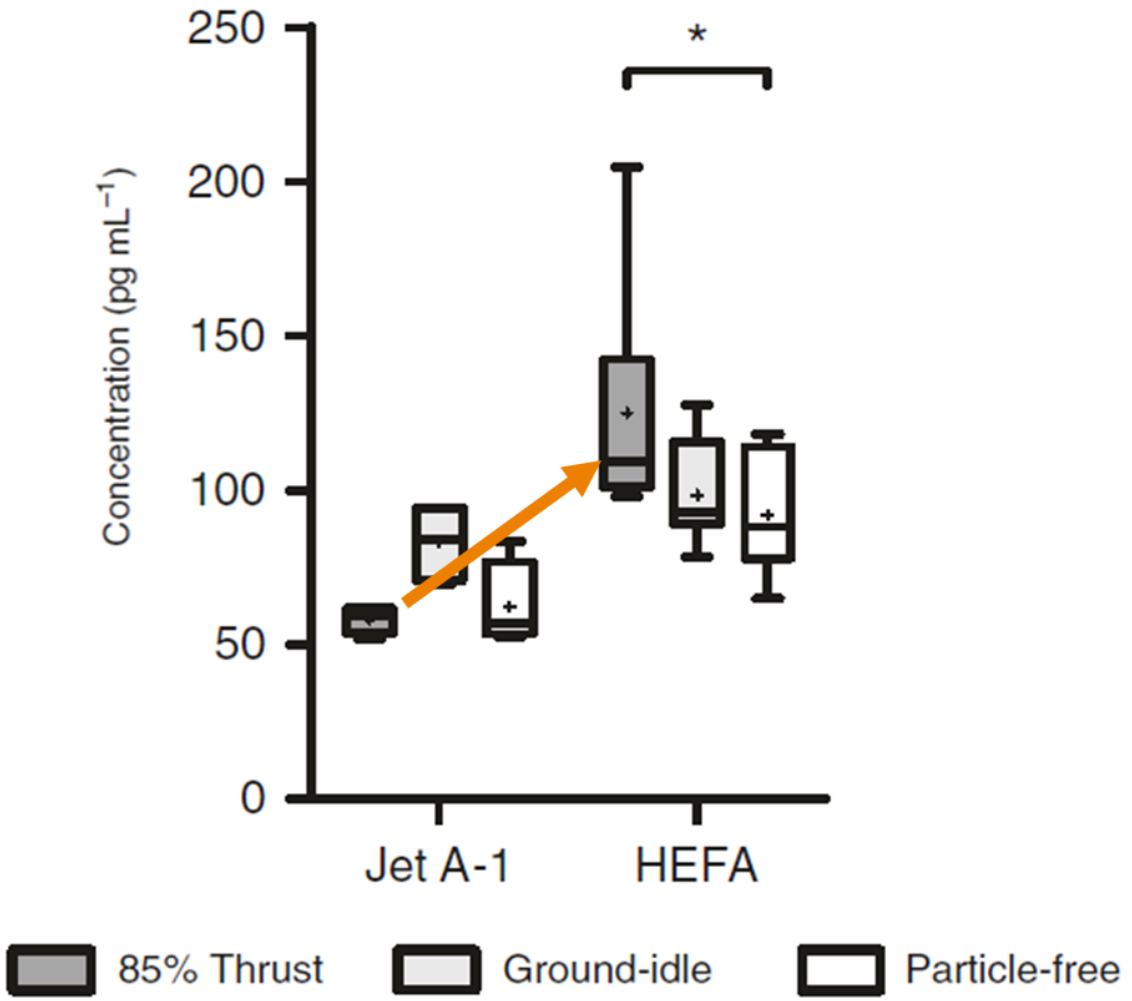
Table 2 Physical properties of non-volatile exhaust particles

Fuels and thrust levels	Non-volatile particles		
	Mass conc. (μg m^{-3}) (SD)	Number conc. (×10^6 cm^{-3}) (SD)	CMD (nm
Jet A-1			
85% thrust	485 (14)	2.30 (0.03)	50
Ground-idle	4.6 (0.7)	1.14 (0.04)	18
HEFA blend			
85% thrust	335 (8)	2.04 (0.02)	47
Ground-idle	1.3 (0.5)	0.29 (0.01)	17

CMD count median diameter, *HEFA* hydroprocessed esters and fatty acid, *SD* standard deviation, *est*M_{dep} estimated particle mass
cultures
[a]Estimated per hour exposure according to Jeannet et al.[38]

ttir et al. Communications Biology (2019) 2:90

PM inflammatory response (IL-8)



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from several transport modes

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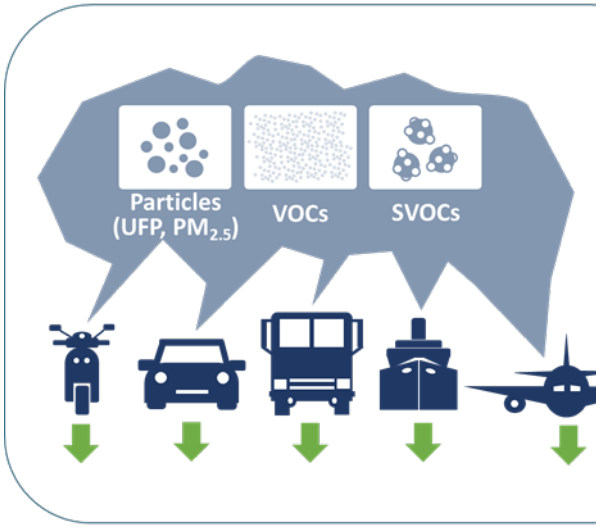


regulatory requirements are met the actual health effects of these
s often are untested

**hensive evaluation of the actual toxicity of emissions is
y needed**

**uld result into additional metrics to evaluate the health risks of
s**

easurements linking PM and complex exhaust mixtures from engines operating under realistic c
comes

automotive, as well aircraft turbine, ship engines etc.

commonly used fuel, but also new fuels

ical characterisation

ed as well non-regulated compounds

cal characterisation

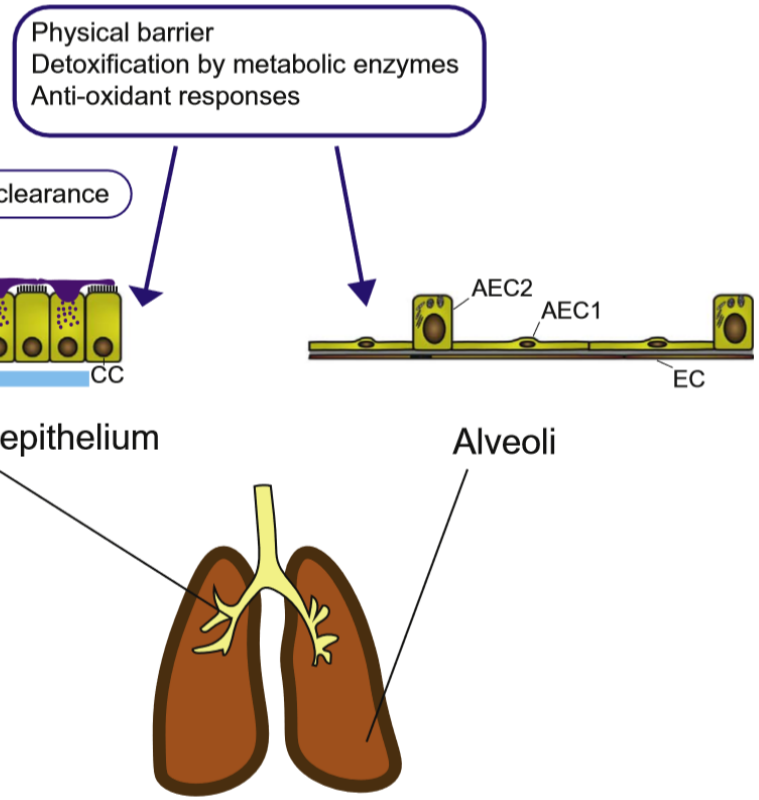
o models relevant for human exposure be the basis for such an evaluation to test for adverse h
specific emissions?

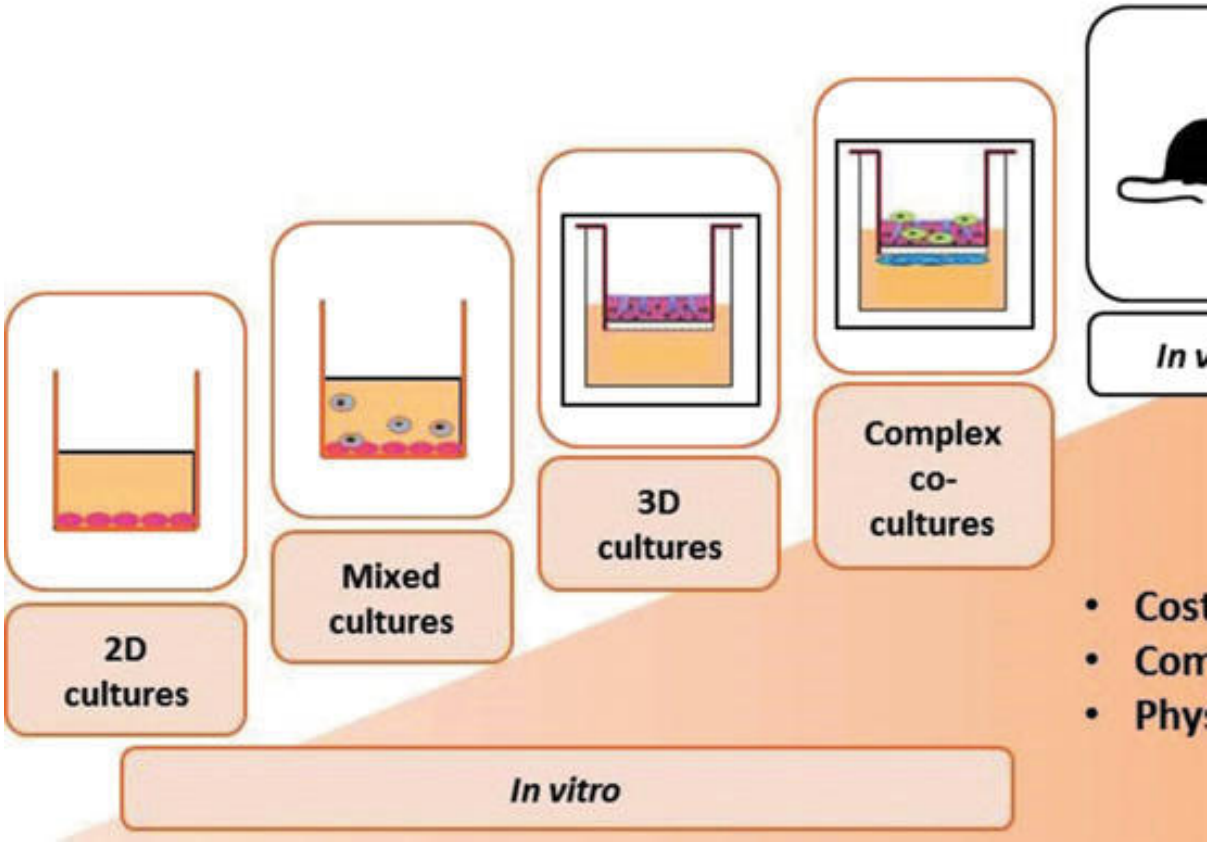
ituation is mimicked as much as possible

ex as needed, as simple as possible

cells cover airways and alveoli

no easy to use testing system for inhalation route





Hiemstra et al. TIV 47 (2018)

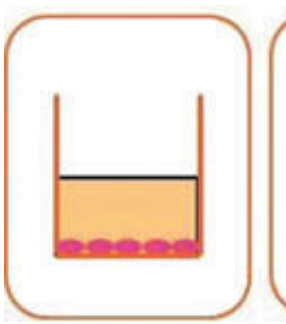
of cells

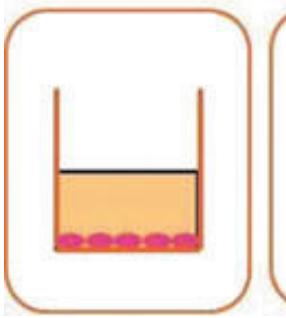
es

human lung epithelials cells

l pluripotent stem cells

› Mono or co-cultured





of cells

es

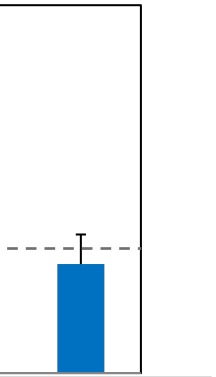
human lung epithelials cells

l pluripotent stem cells

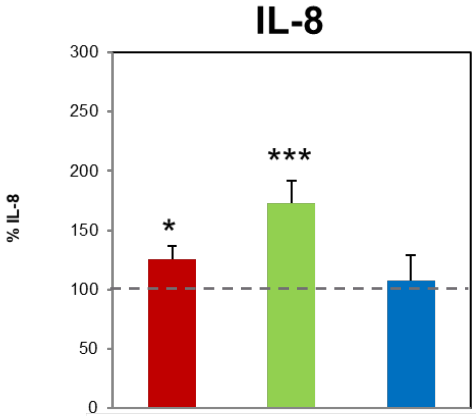
› Mono or co-cultured

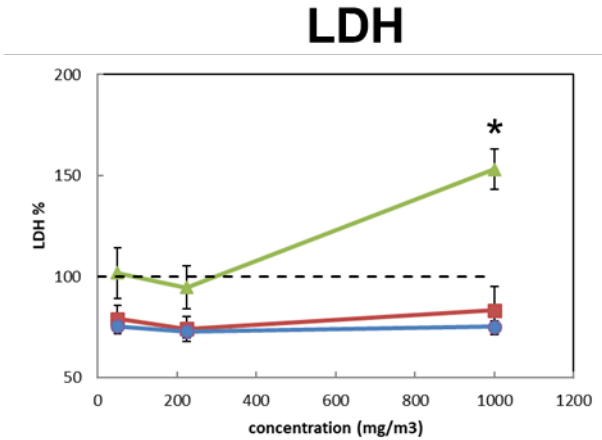
rison study between A549, Beas-2B and primary epithelial cells (MucilAir) using CeO_2 nanoparti

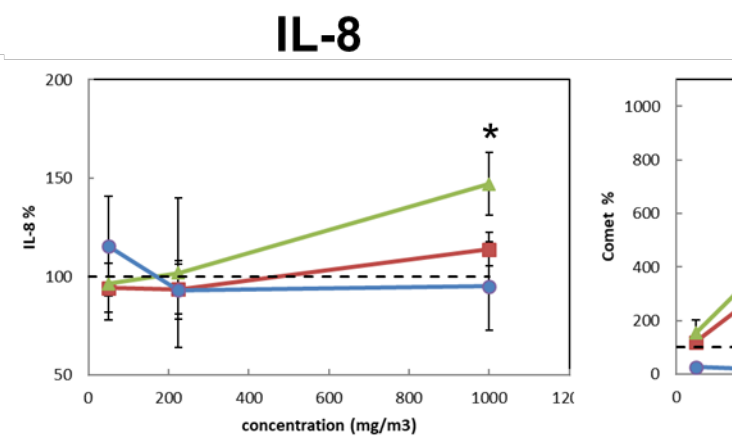
re:



2B MucilAir







r are less affected by air stream then cell lines

› Cell lines showed higher response for cytotoxici and genotoxic dose

IN VITRO SYSTEMS

of cells

es
› Mono or co-cultured

human lung epithelials cells

l pluripotent stem cells

l culture system used

d interface (ALI), organ-on-a-chip models and microfluidics

ids

on cut lung slices

e of exposure

n of compounds to the medium of submerged cell cultures

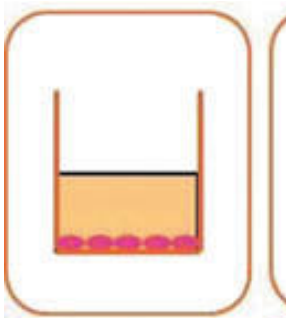
on ALI

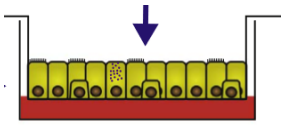
aerosol exposure on ALI

ibilities for **valid read-outs** to assess the effect of
re

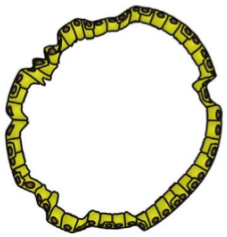
ath, DNA breaks, barrier function, cytokine release,
e stress

–omics techniques: protein and gene expression level

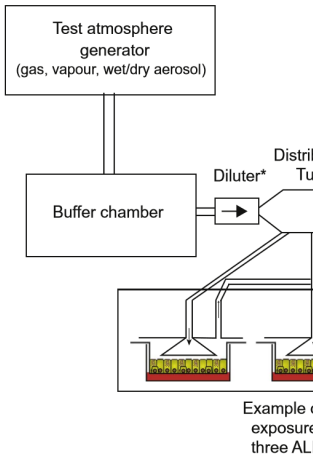




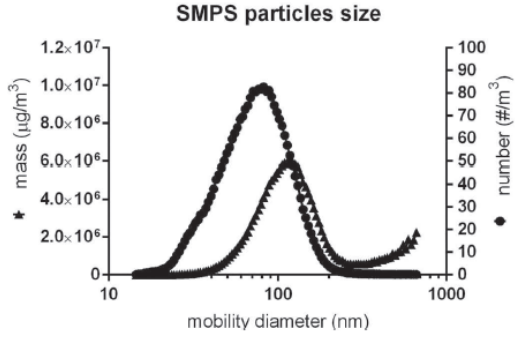
air-liquid interface

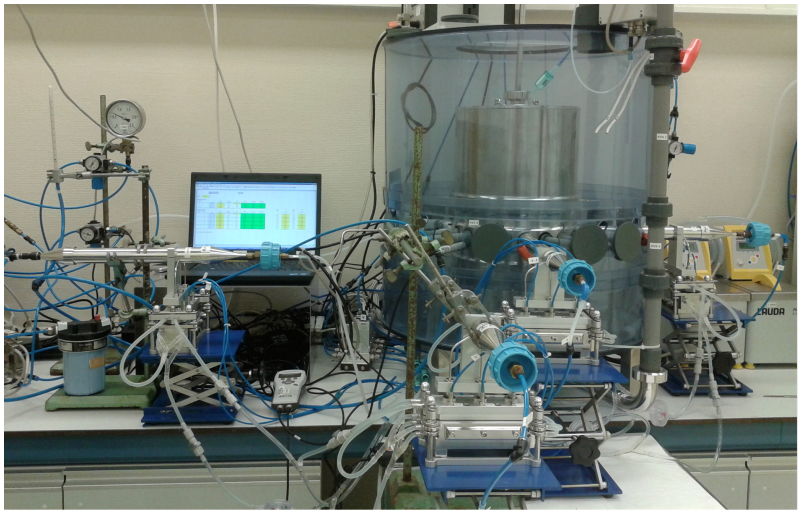


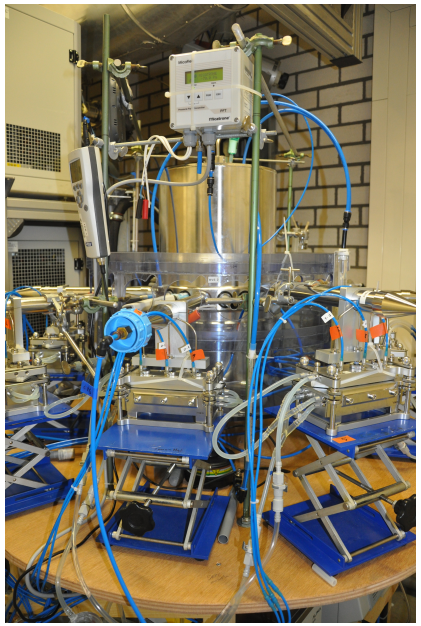
organoid

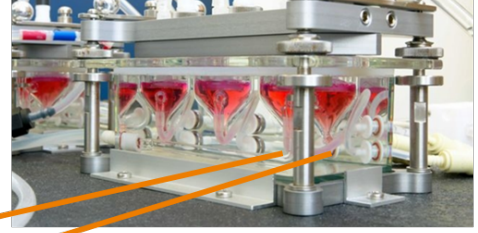


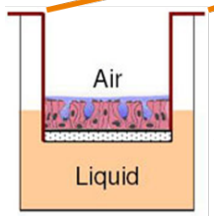
esponse of primary bronchial epithelial cells to diesel exhaust

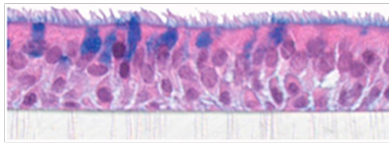












toxicology department

d generator (Kipor ID10)

a steady load (4.5 kW)

0.43/1.29 mg/m^3

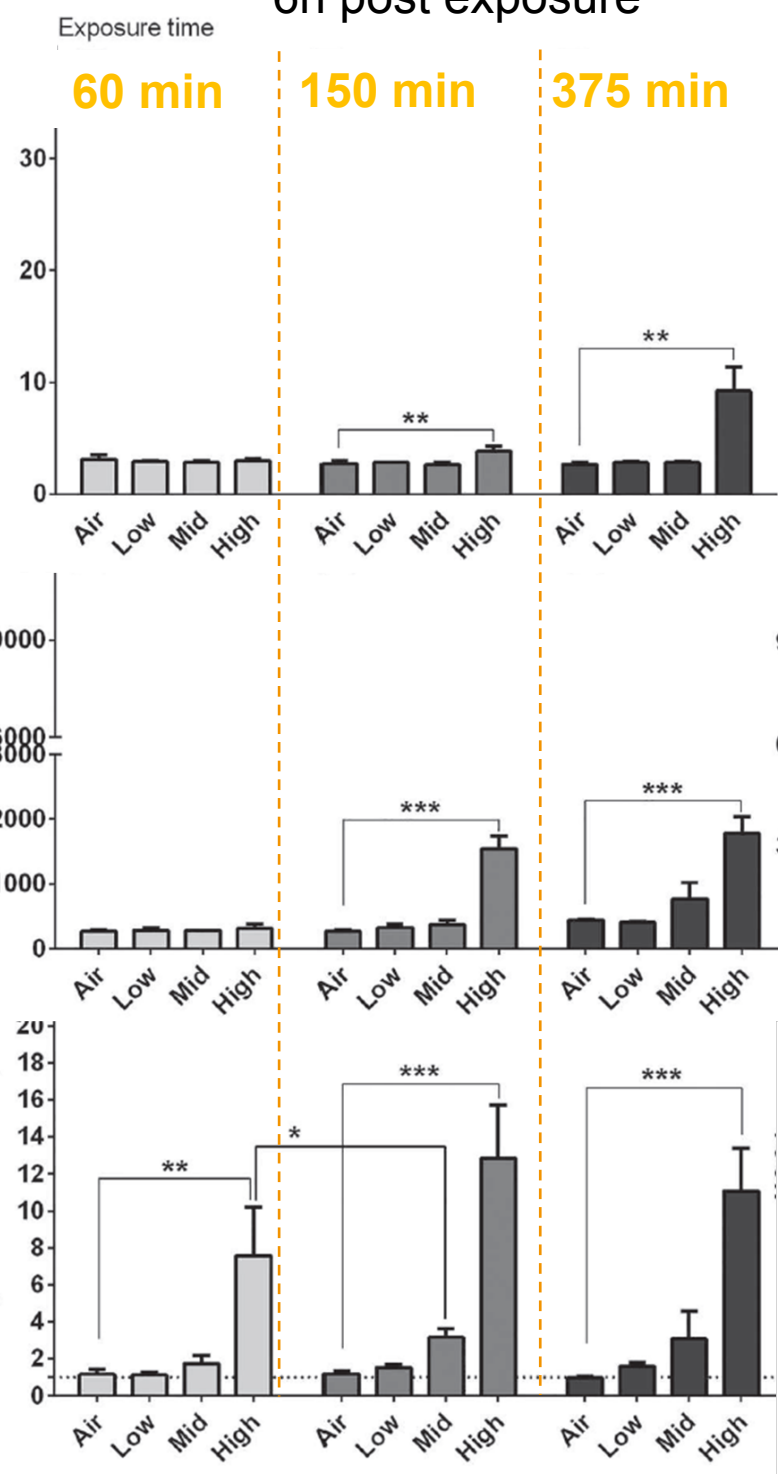
ition

- Helmond - TNO automotive PTC
- EuroV engine from city bus
- Braunschweig City Driving Cycle
- L/M/H: 34/82/206 µg/m^3
- 1.9% deposition

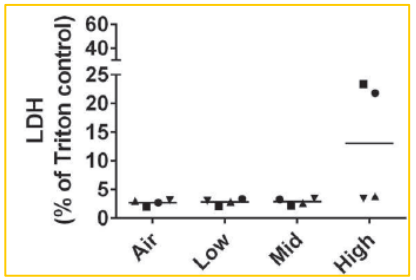
OF THE EXPOSURE DURATION

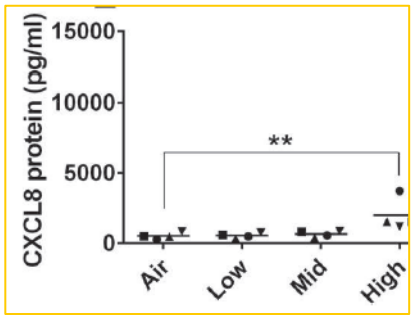
6h post exposure

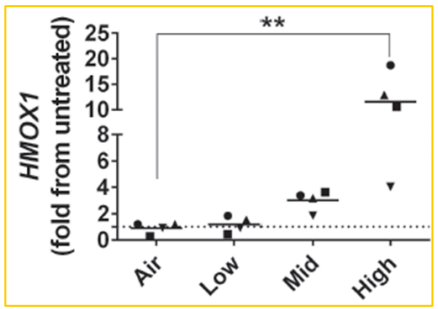
L/M/H: 0.14/0.43/1.29 mg/m^3



150 min

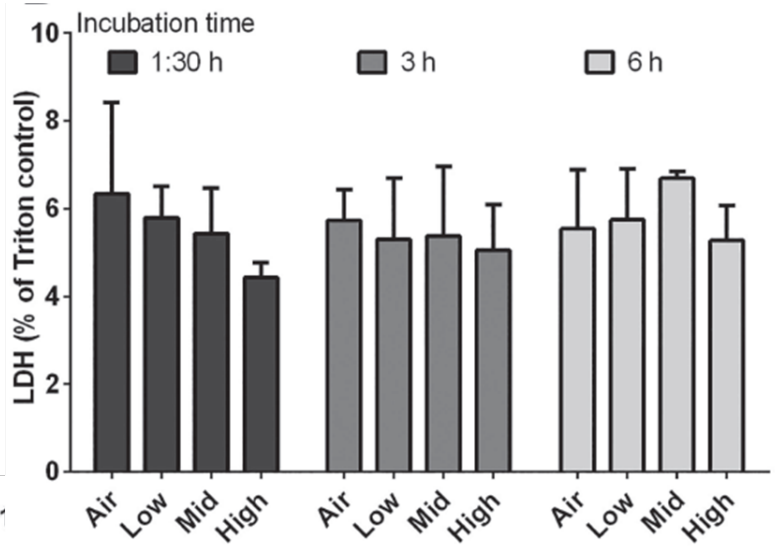


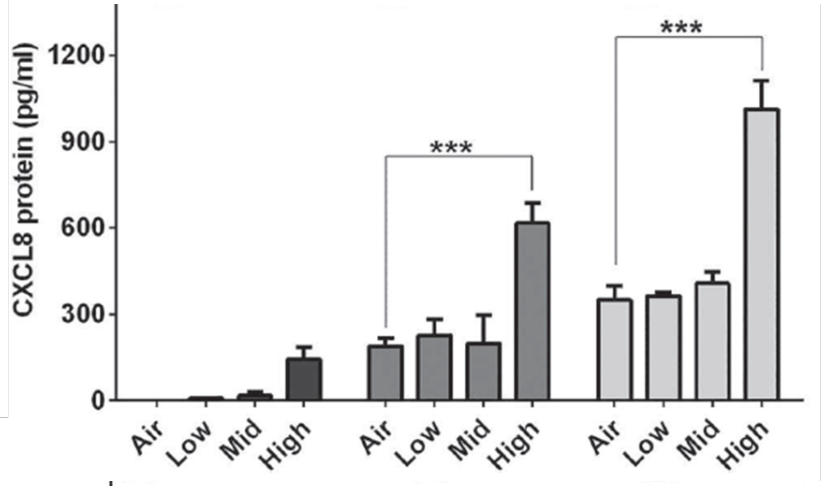


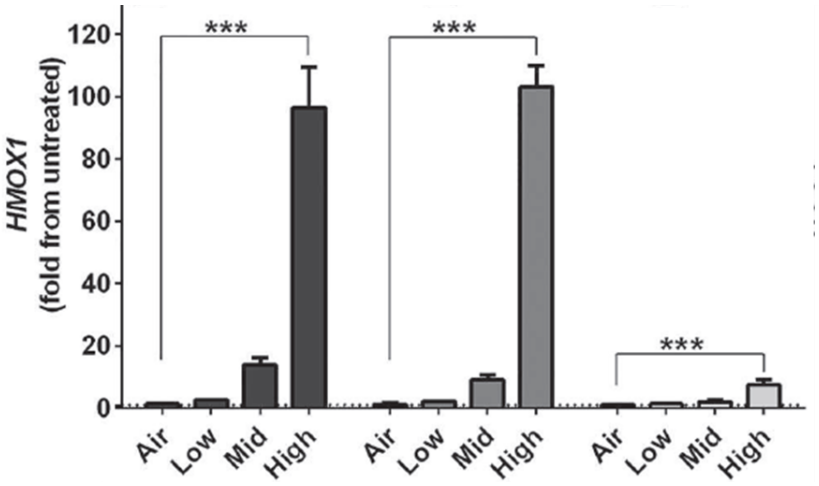


1h exposure

L/M/H: 0.14/0.43/1.29 mg/m^3



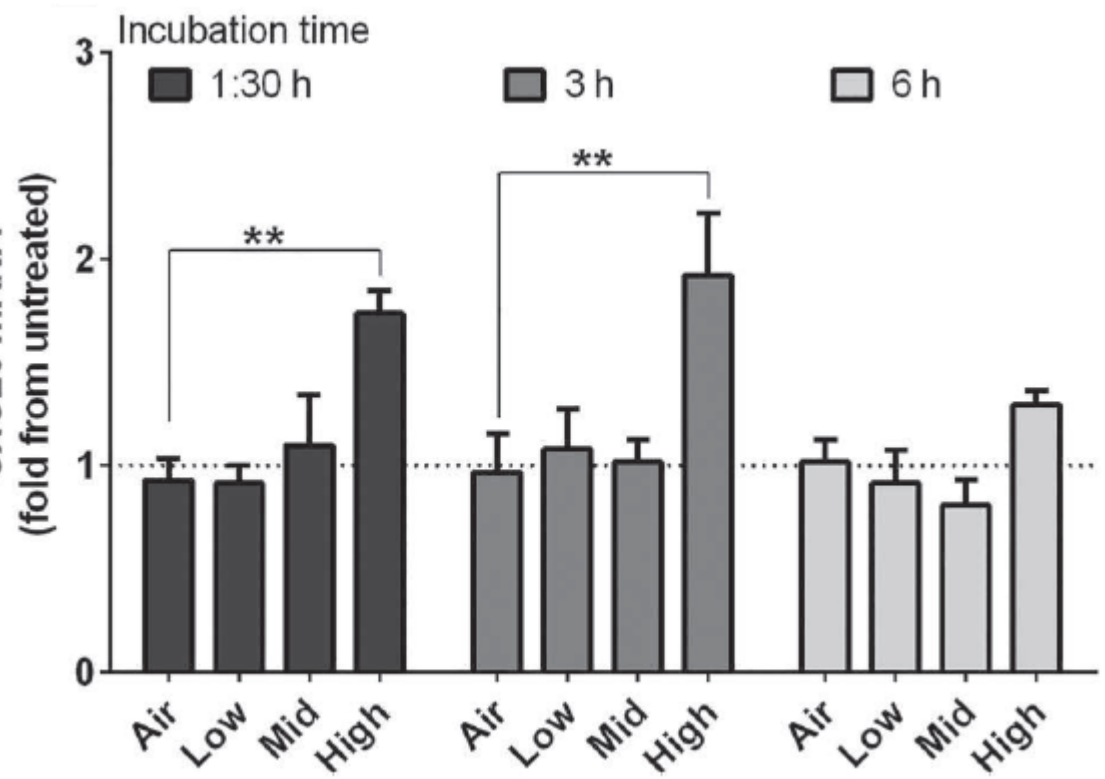




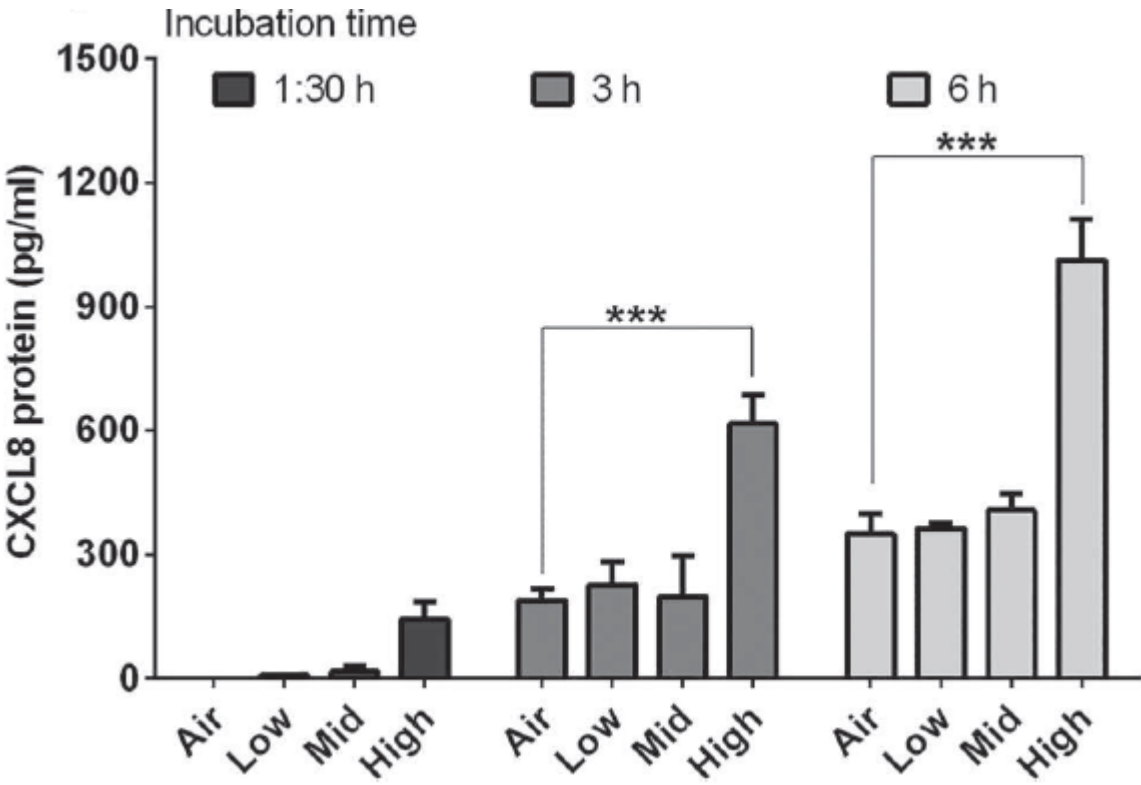
L/M/H: 0.14/0.43/1.29 mg/m^3

1h exposure

IL-8 gene expr



IL-8 protein

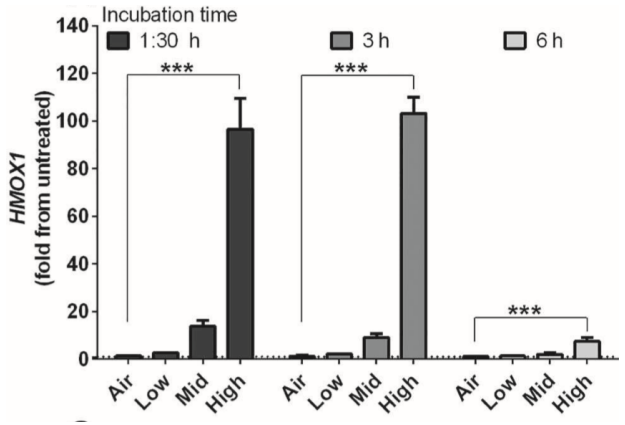


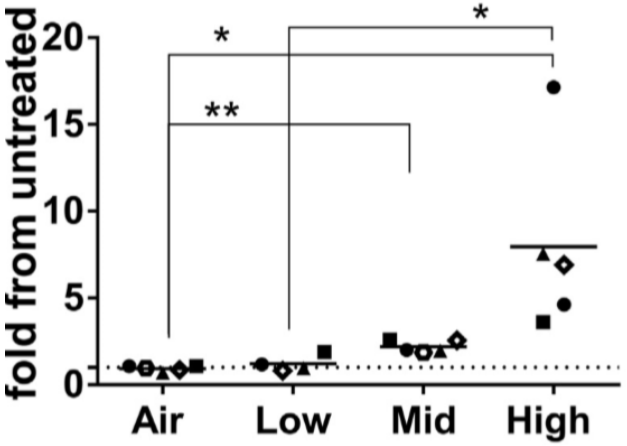
DIESEL EMISSION - OXIDATIVE STRESS RESPONSE

L/M/H: 0.14/0.43/1.29 mg/m^3

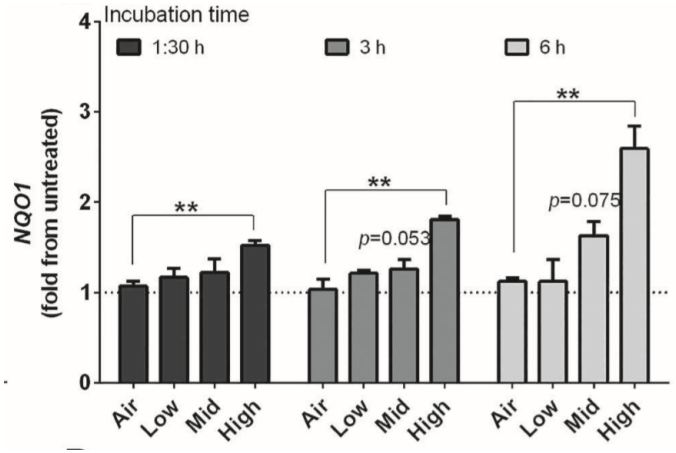
L/M/H: 34/82/206 μg/m^3

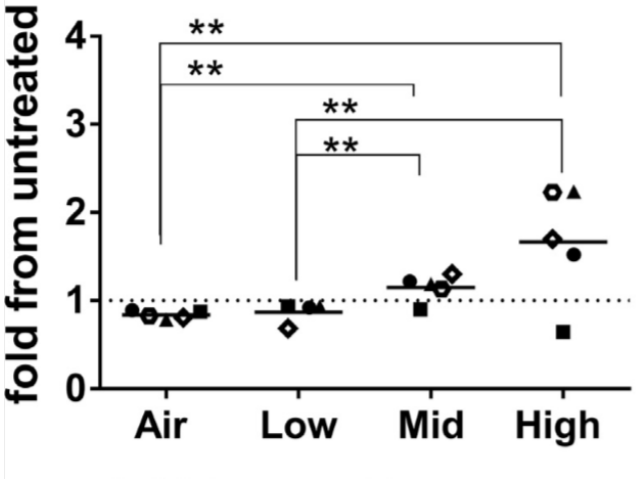
se (HMOX1)





drogenase,
O1)





1 hrs exposure; 1:30/3/6h post exp

6 hrs exposure; 1:30h post exp

diesel exposures using a EuroV engine show even at **non-cytotoxic concentrations**

on of oxidative stress response (HO-1, NQO1)

vitro inhalation models could be valuable for evaluation of the toxicity of combustion emissions

that data from (future) studies can be easily compared and that conclusions are more robust
l for regulation; harmonization and validation is needed!

Gröllers-
omp
oen
van Someren
uper
rul
erbeek
adijk

Mulderij

LUMC
Pieter Hiemstra
Maria Zarcone

ON
uistermaat
que van Acker

LIST
Arno Gutleb
INERIS
Ghislaine Lacroix
University of Finland
Harri Alenius


IK YOU FOR YOUR
NTION!
Amsterdam
Den Haag
A13
E19
900 m

of current regulatory emission standards: PM mass

ty evaluation based on in vitro models be a solution?

xicity screening of Euro V emissions

n

IN VITRO SYSTEMS

of cells

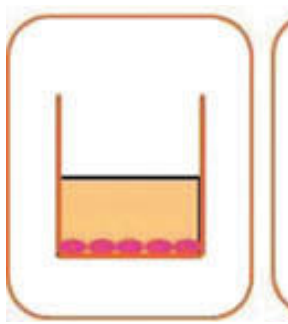
es

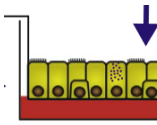
› Mono or co-cultured

human lung epithelials cells

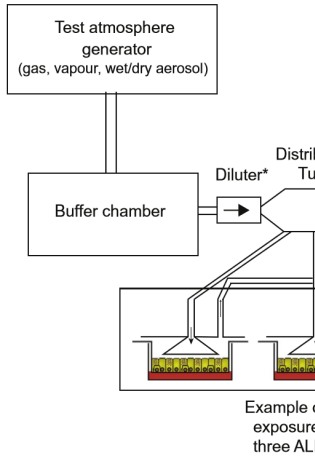
l pluripotent stem cells

l **culture system used**

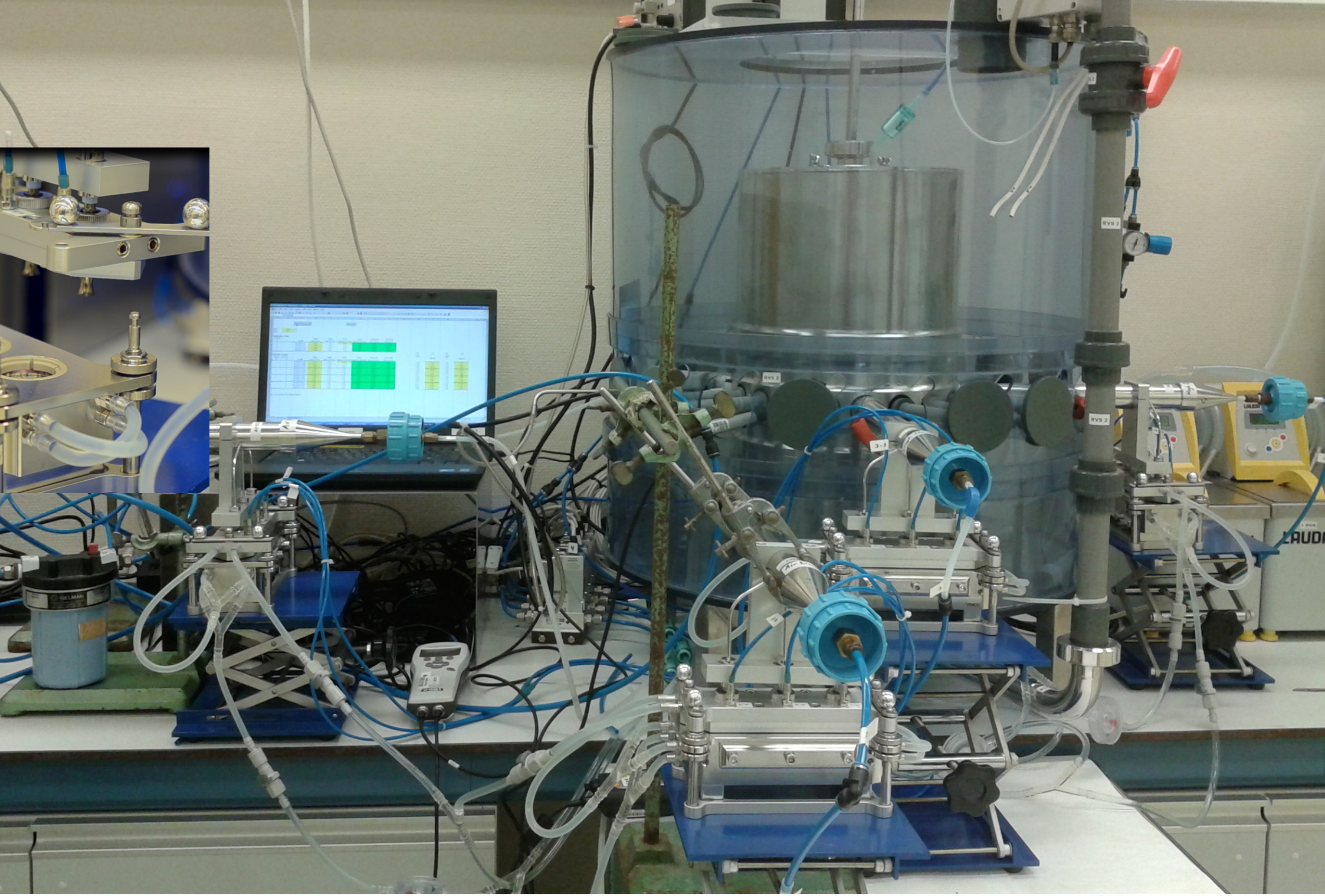




e of exposure



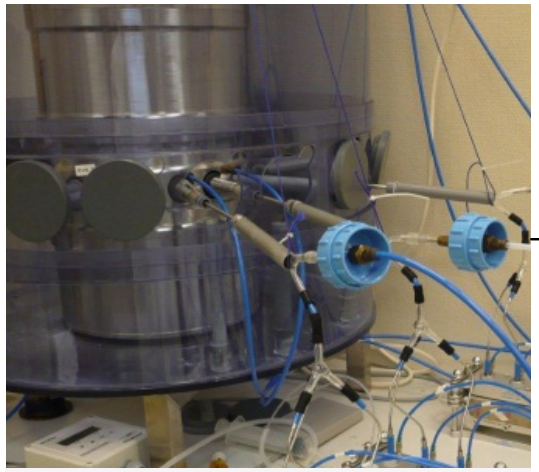
ibilities for **valid read-outs** to assess the effect of
re



lizing

Dilution & Exposure (1h)

Biologi
(24h po



Buffer chamber

SEM analyses
MMAD (APS)
gravimetric
analysis

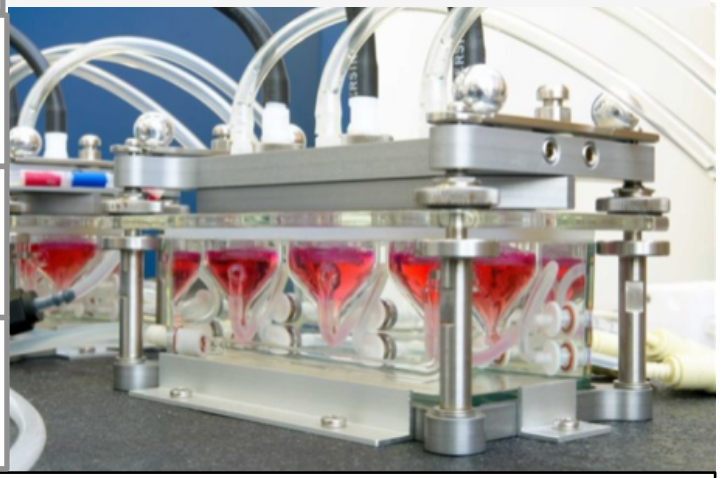
Air control

Dilution 1: L

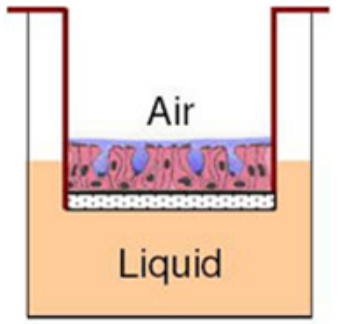
Dilution 2: M

Dilution 3: H

Vitrocell system



cell types:
A549
Beas-2B
MucilAir



Oxidative
HO-1

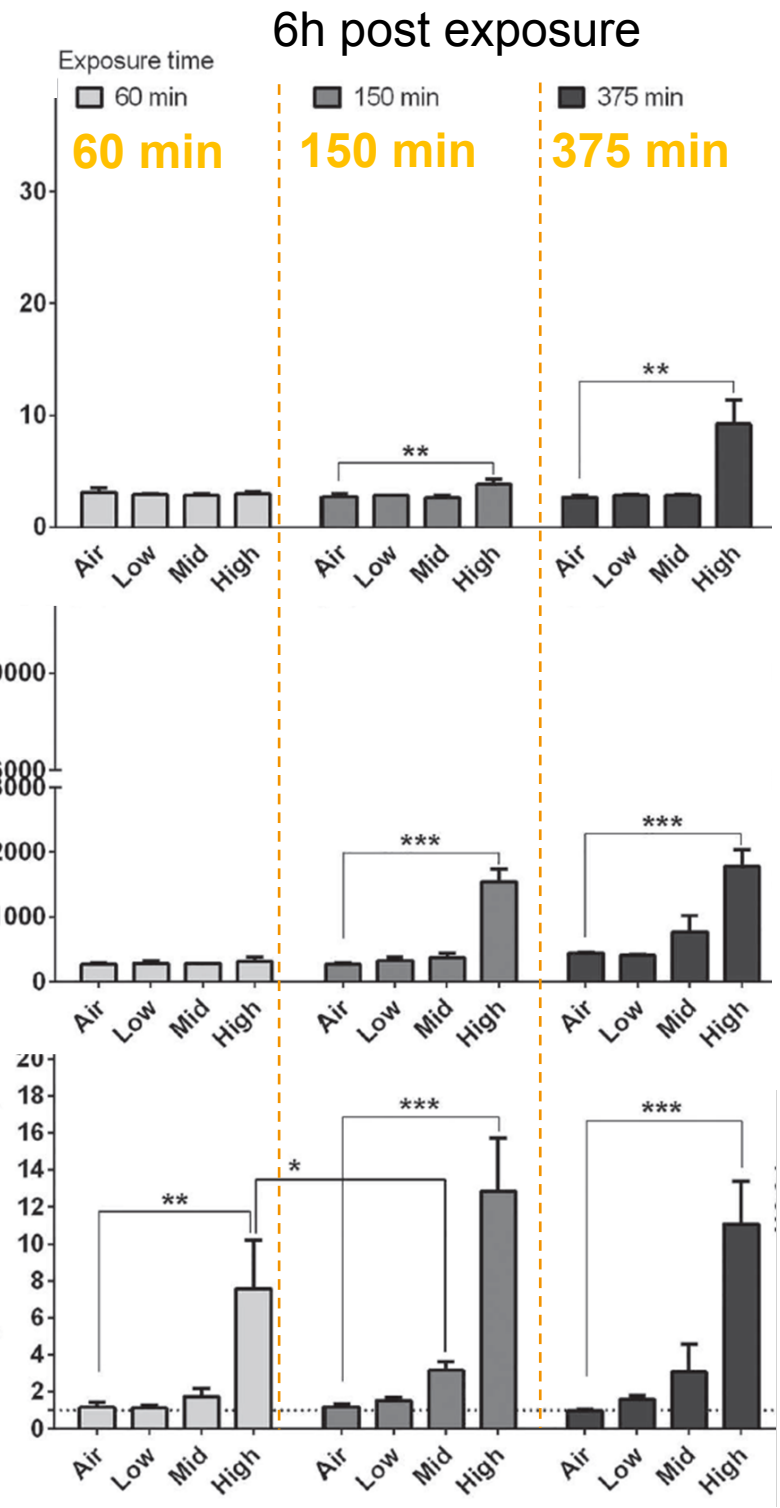
Inflamma
ICAM-1, IF
IL-13, IP-1
analyses

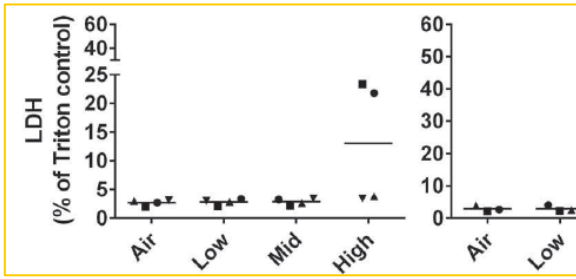
Cytotoxic
LDH, TEER

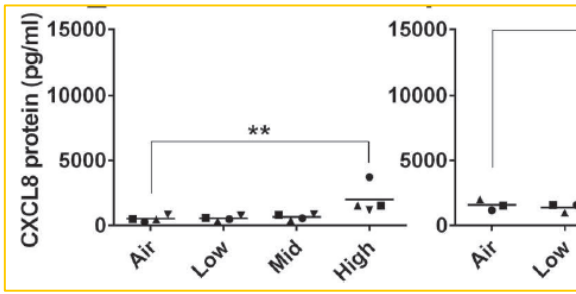
Genotoxi
Comet ass

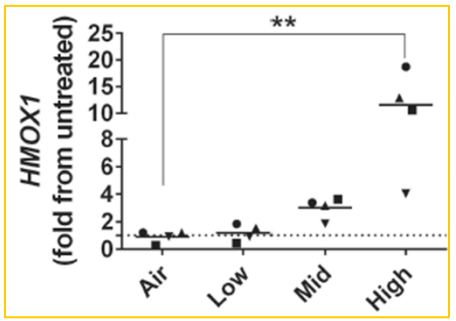
Gene arra
Illumina be

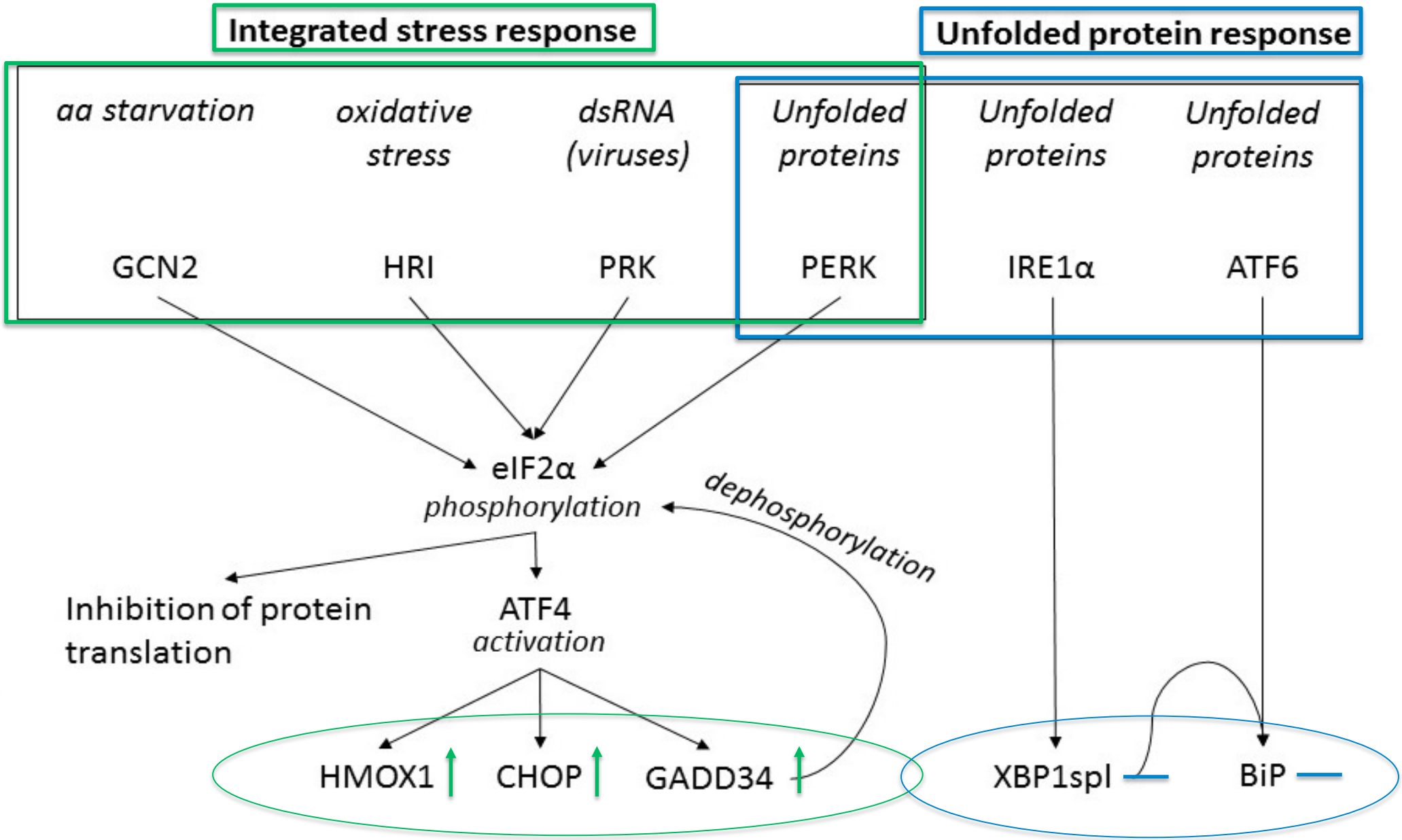
OF THE EXPOSURE DURATION





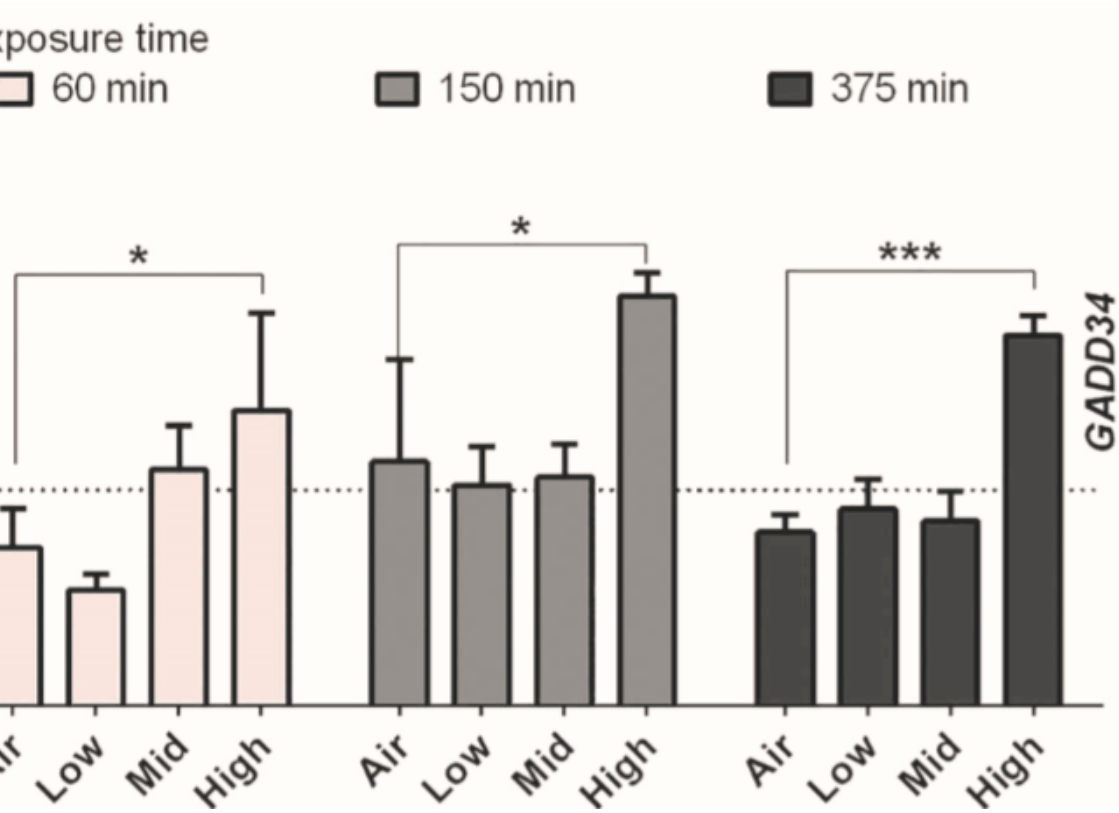


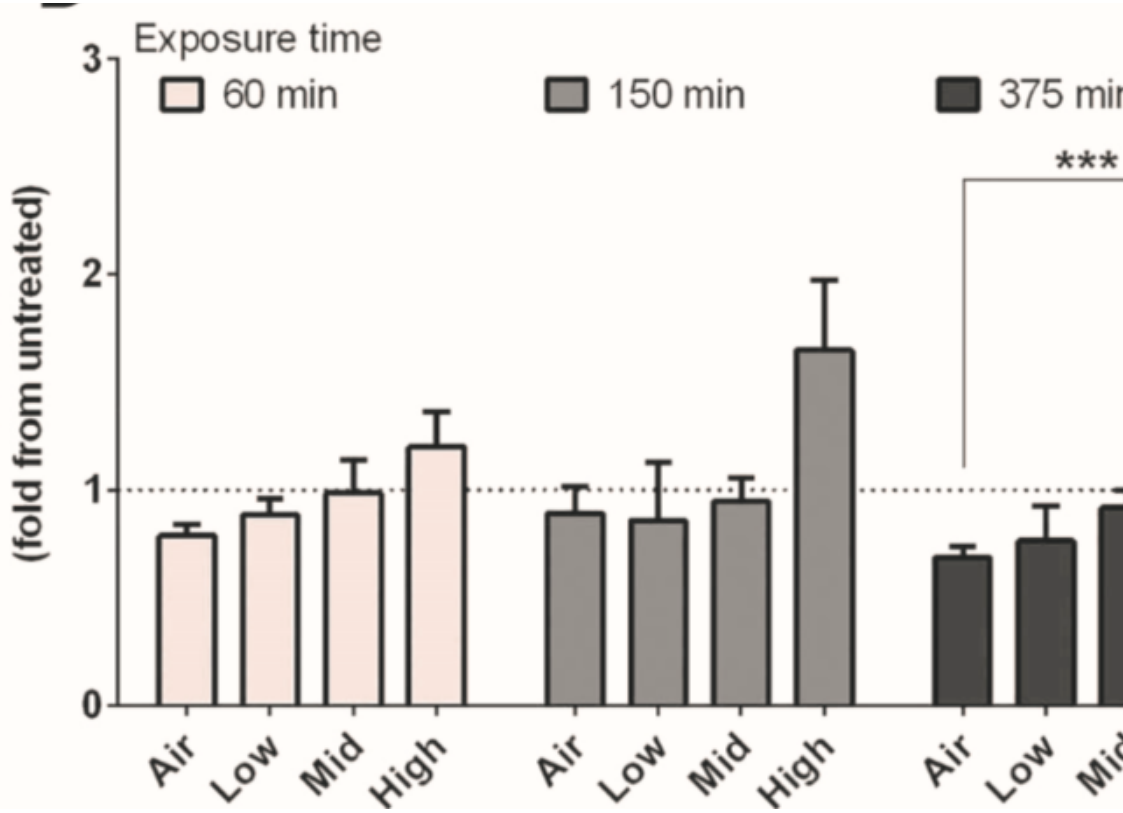



Integrated stress response
Unfolded protein response
aa starvation
oxidative stress
dsRNA (viruses)
Unfolded proteins
Unfolded proteins
Unfolded proteins
GCN2
HRI
PRK
PERK
IRE1α
ATF6
eIF2α
phosphorylation
dephosphorylation
Inhibition of protein translation
ATF4
activation
HMOX1
CHOP
GADD34
XBP1spl
BiP

CHOP

GADD34





exposure; 6h post exposure

Zarcone

ALI in vitro models for respiratory toxicology – Paris 2016

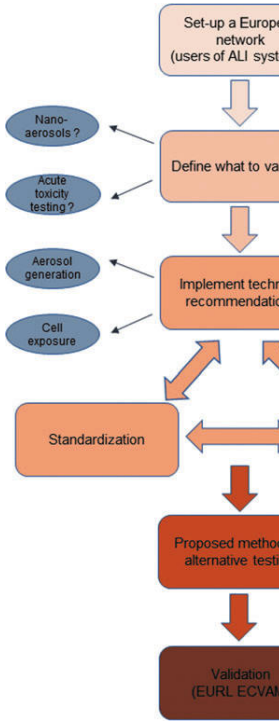
that data from (future) studies can be easily compared and that conclusions are more robust ar
; harmonization and validation is needed!

es to overcome:

hould be validated?

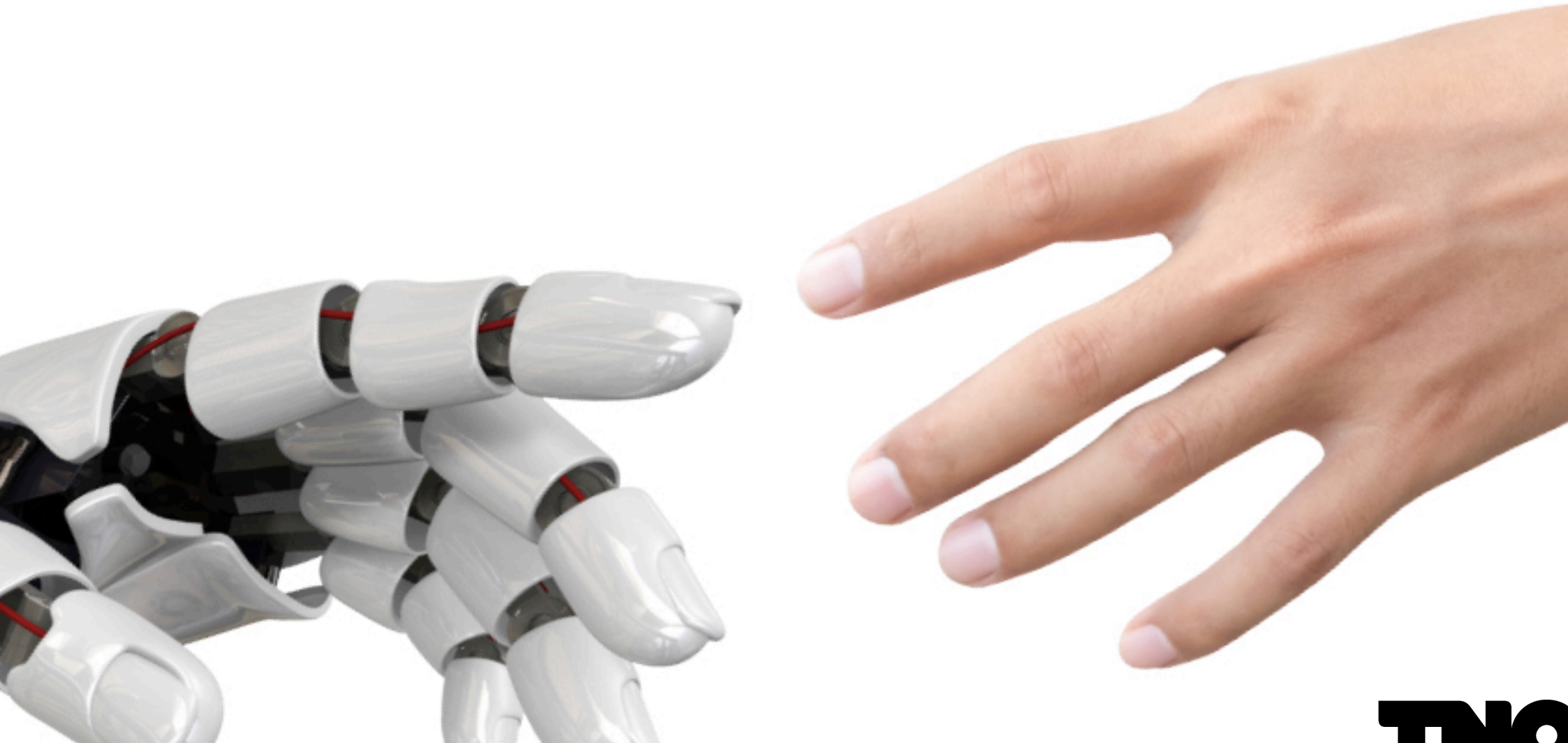
standardization between groups

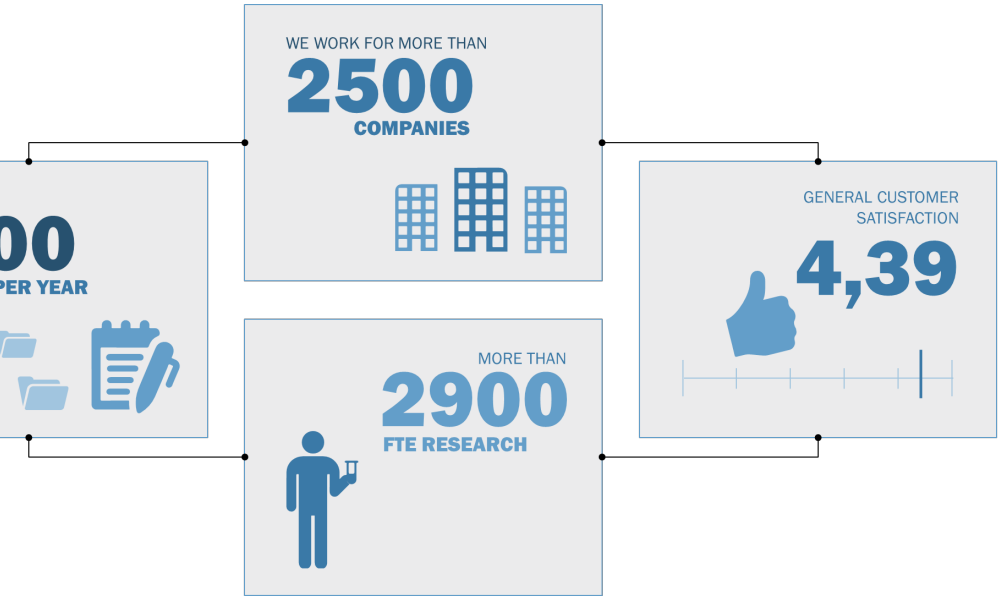
human relevance

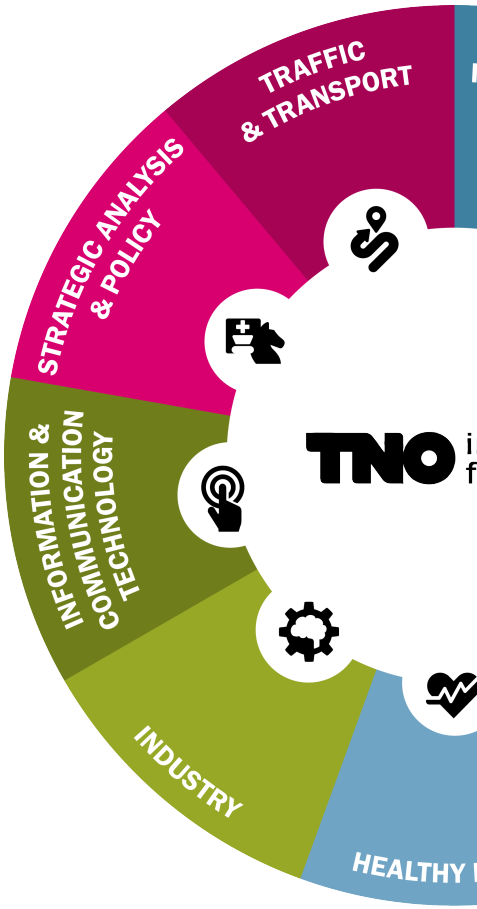


ects people and knowledge to create innovations
t the competitive strength of industry and the
of society in a sustainable way.

TION FOR LIFE’







TION PROCESS

DEVELOPING KNOWLEDGE

In public-private collaboration with partners from the golden triangle

APPLYING KNOWLEDGE

Contract research for and with customers

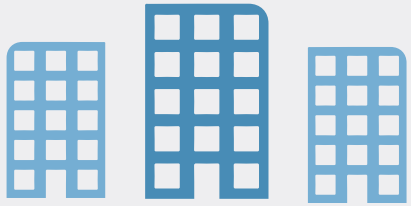
TRANSFERRING KNOWLEDGE

Exploiting knowledge through spin-offs, licences, etc together with other companies

WE WORK FOR MORE THAN

2500

COMPANIES



ORE THAN

4800

ROJECTS PER YEAR

MORE THAN

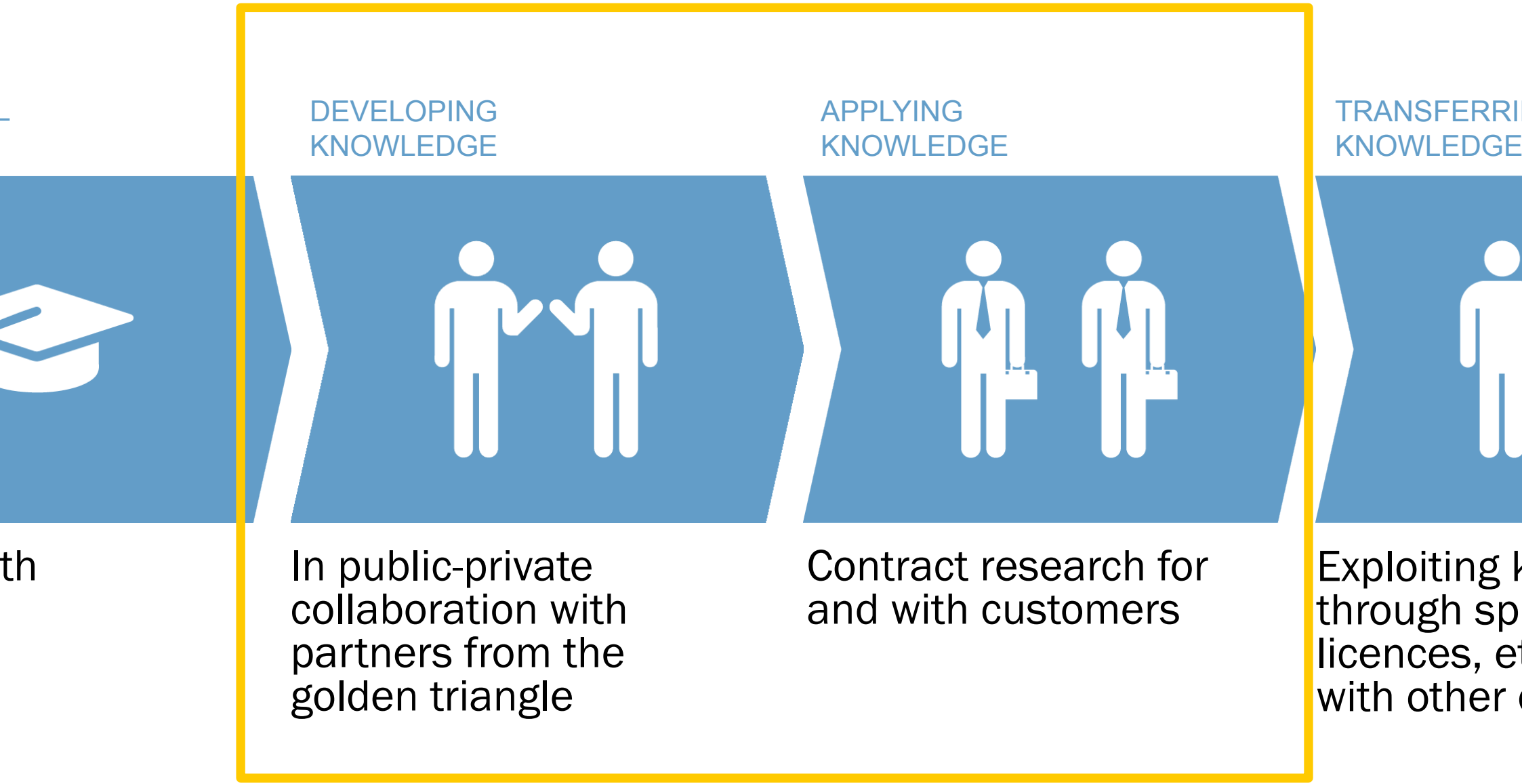
2900

FTE RESEARCH

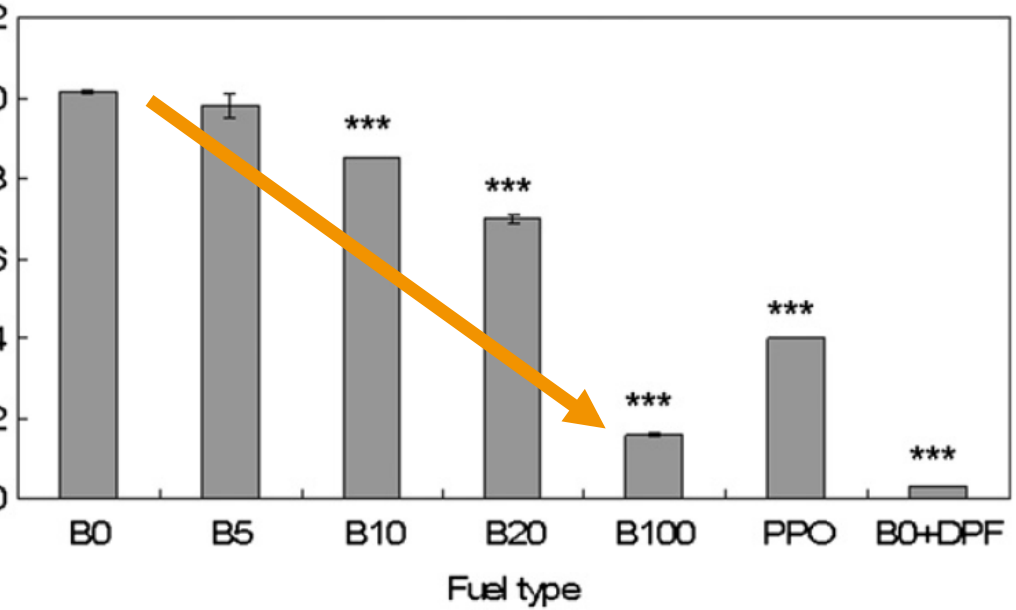
GENERAL

S

4,


DEVELOPING KNOWLEDGE
APPLYING KNOWLEDGE
th
In public-private collaboration with partners from the golden triangle
Contract research for and with customers
Exploiting k
through sp
licences, et
with other

PM mass



mass (g kWh^{-1}) emissions for the fuel types and blends tested (6 ETC, , 5 ETC and PPO 4 ETC). Error bars indicate the standard error of the mean. *, ificantly different from B0 at $P < 0.05$, <0.01, <0.001 respectively.

PM cytotoxicity

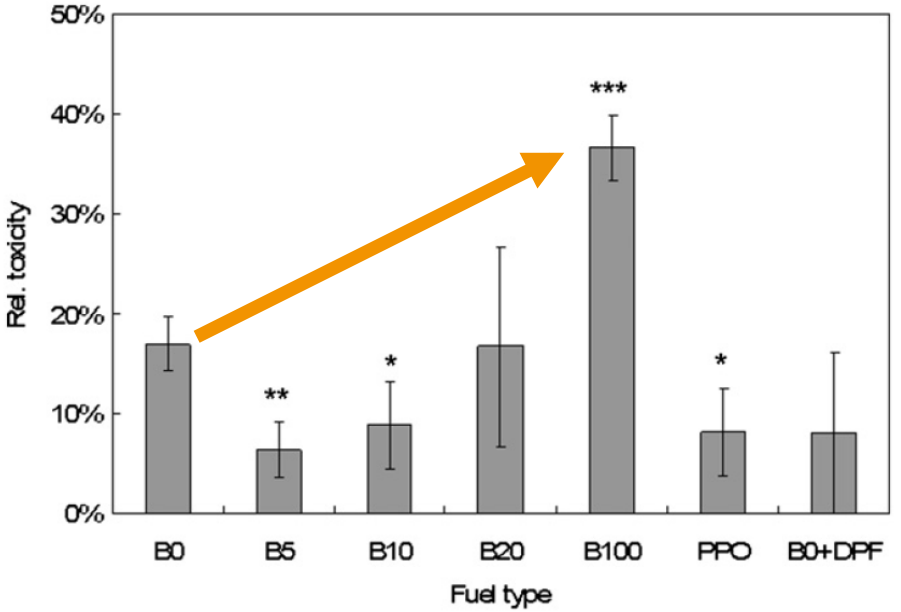


Fig. 5. Relative cytotoxicity for the fuel types and blends tested, in % compared to positive control, 1% Triton-X100. Each bar represents 3 ETC. Error bars indicate the standard error of the mean. *, **, *** significantly different from the mean of B0 at $P < 0.05$, <0.01, <0.001 respectively.

PM mass

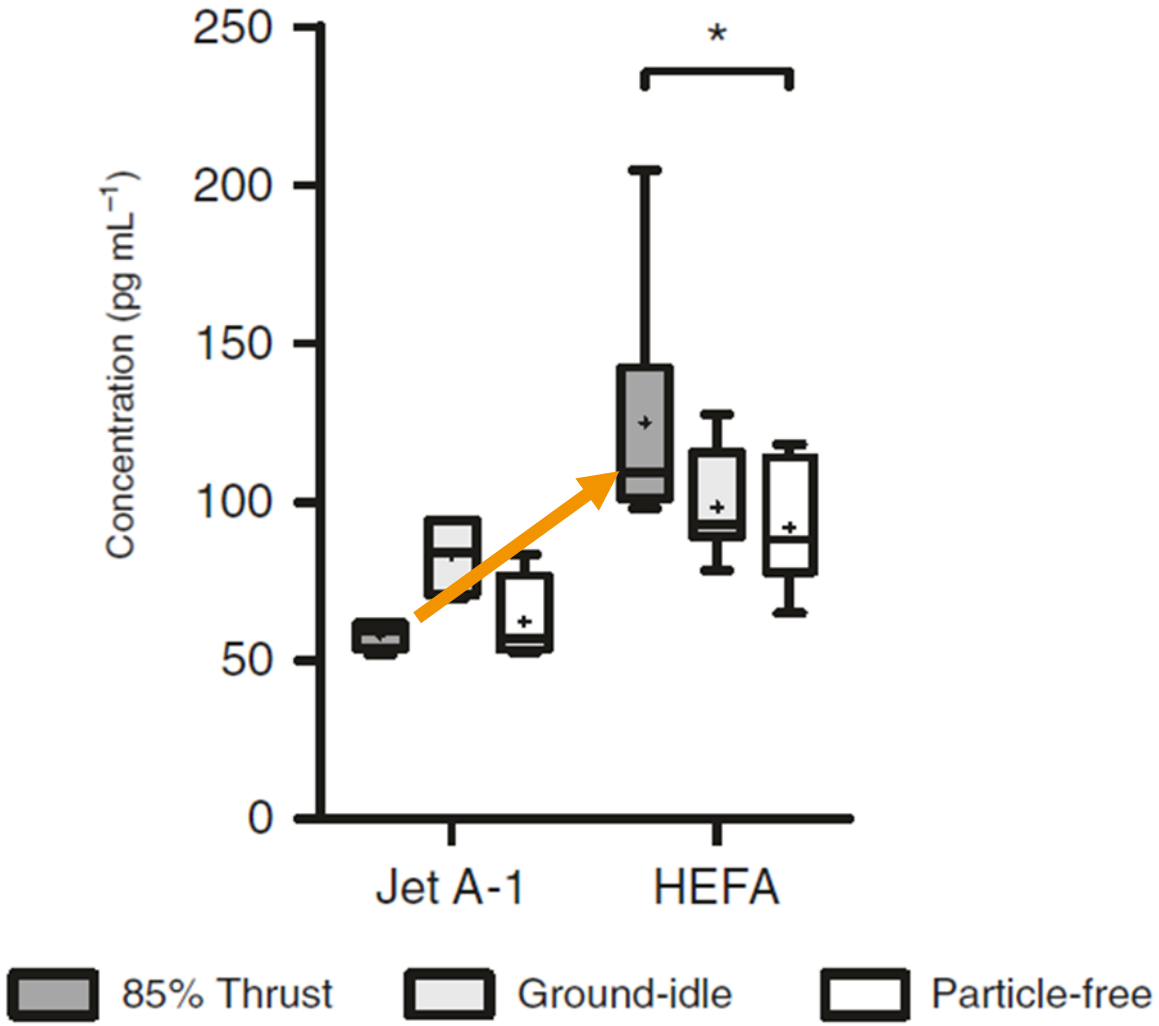
perties of non-volatile exhaust particles

	Non-volatile particles		
	Mass conc. (µg m^{-3}) (SD)	Number conc. (×10^6 cm^{-3}) (SD)	CMD (nm
	485 (14)	2.30 (0.03)	50
	4.6 (0.7)	1.14 (0.04)	18
	335 (8)	2.04 (0.02)	47
	1.3 (0.5)	0.29 (0.01)	17

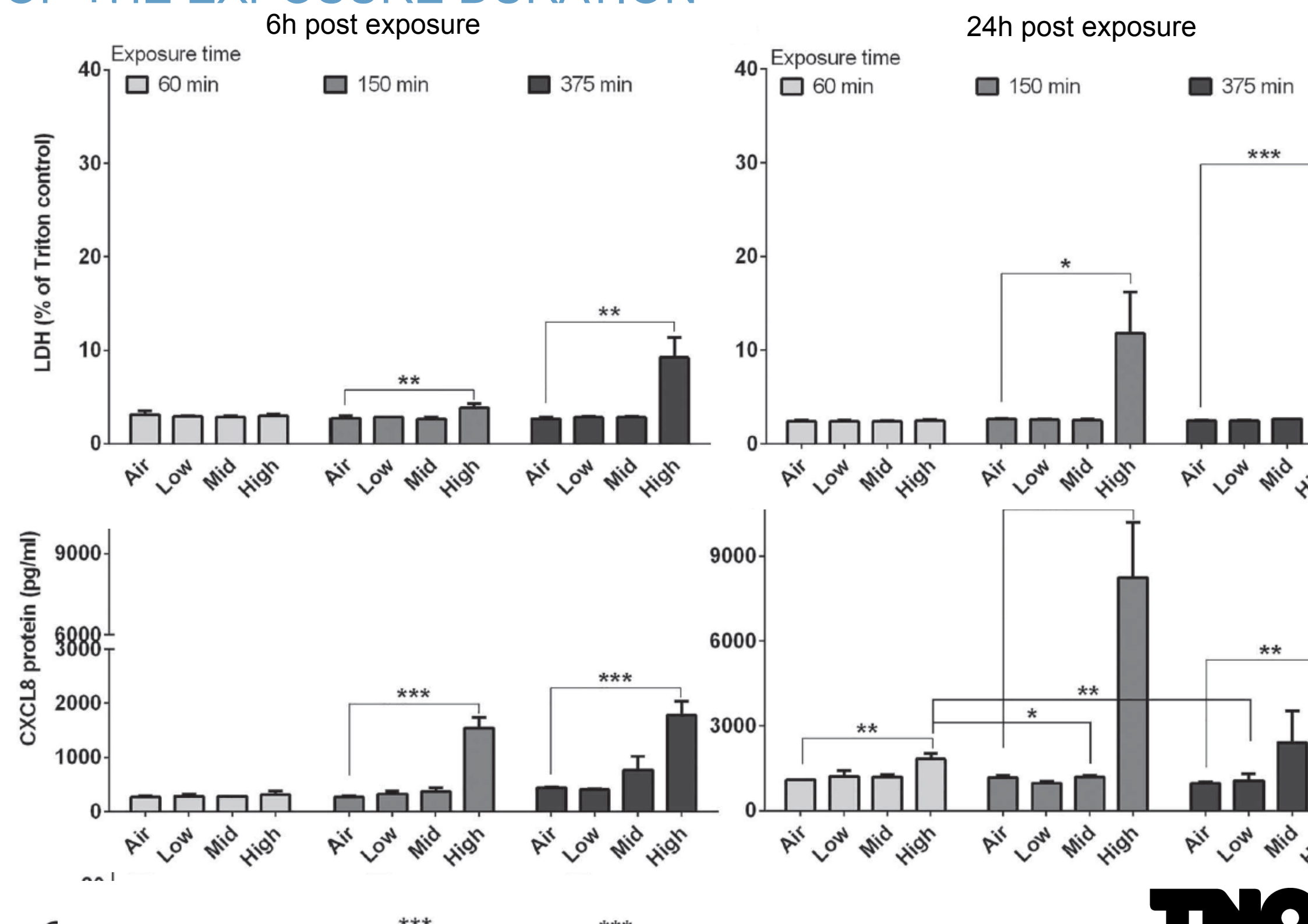
FA hydroprocessed esters and fatty acid, *SD* standard deviation, *estM*$_{dep}$ estimated particle mass

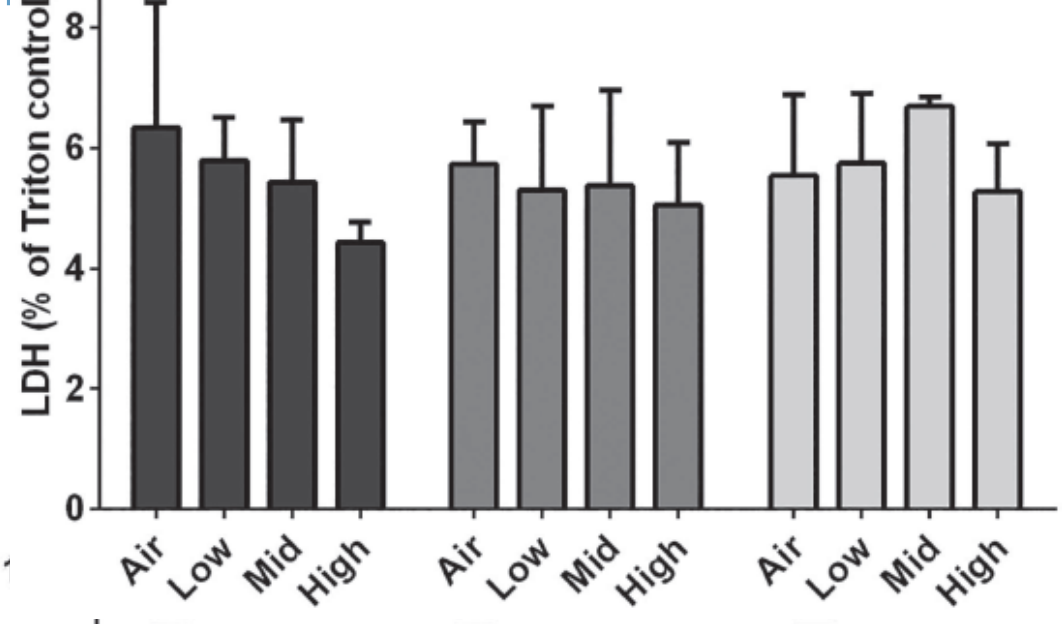
cording to Jeannet et al.[38]

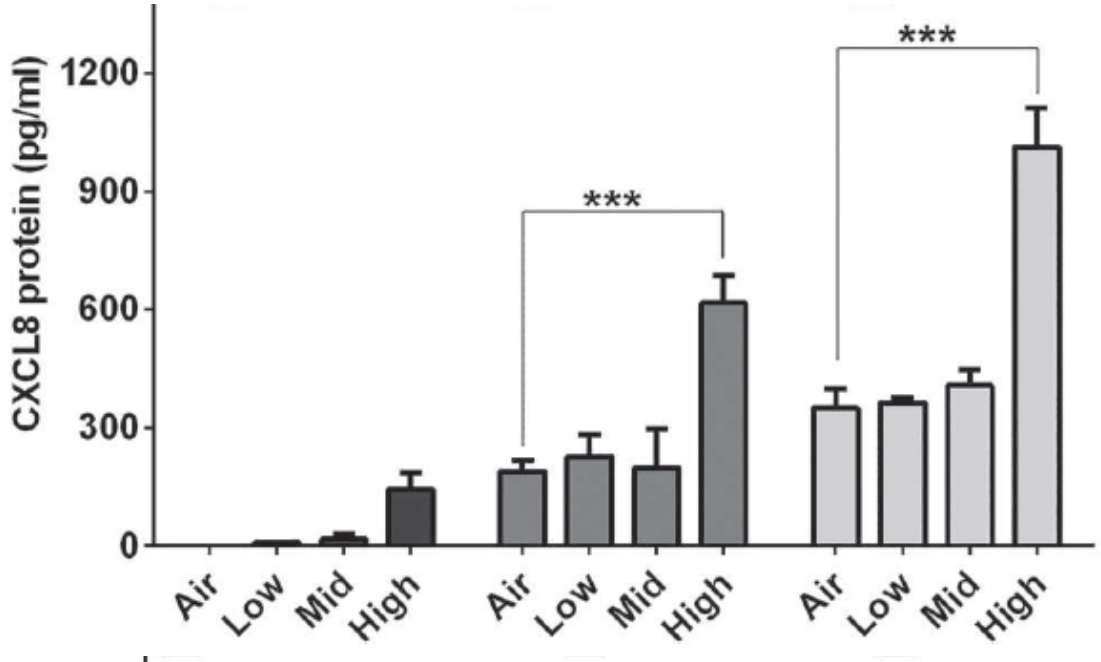
PM inflammatory response (IL-8)

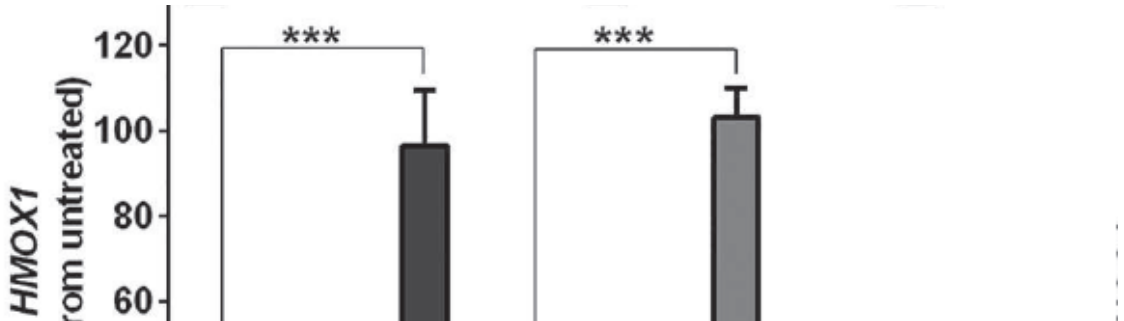


OF THE EXPOSURE DURATION




LDH (% of Triton control
0
2
4
6
8
Air
Low
Mid
High


CXCL8 protein (pg/ml)
0
300
600
900
1200
***
***
Air
Low
Mid
High


HMOX1
rom untreated)
60
80
100
120
***
***