Revisiting the Trojan Horse Effect

On the role of lipophilic chemicals in the toxicity of fine and ultrafine combustion particles and implications for regulatory needs

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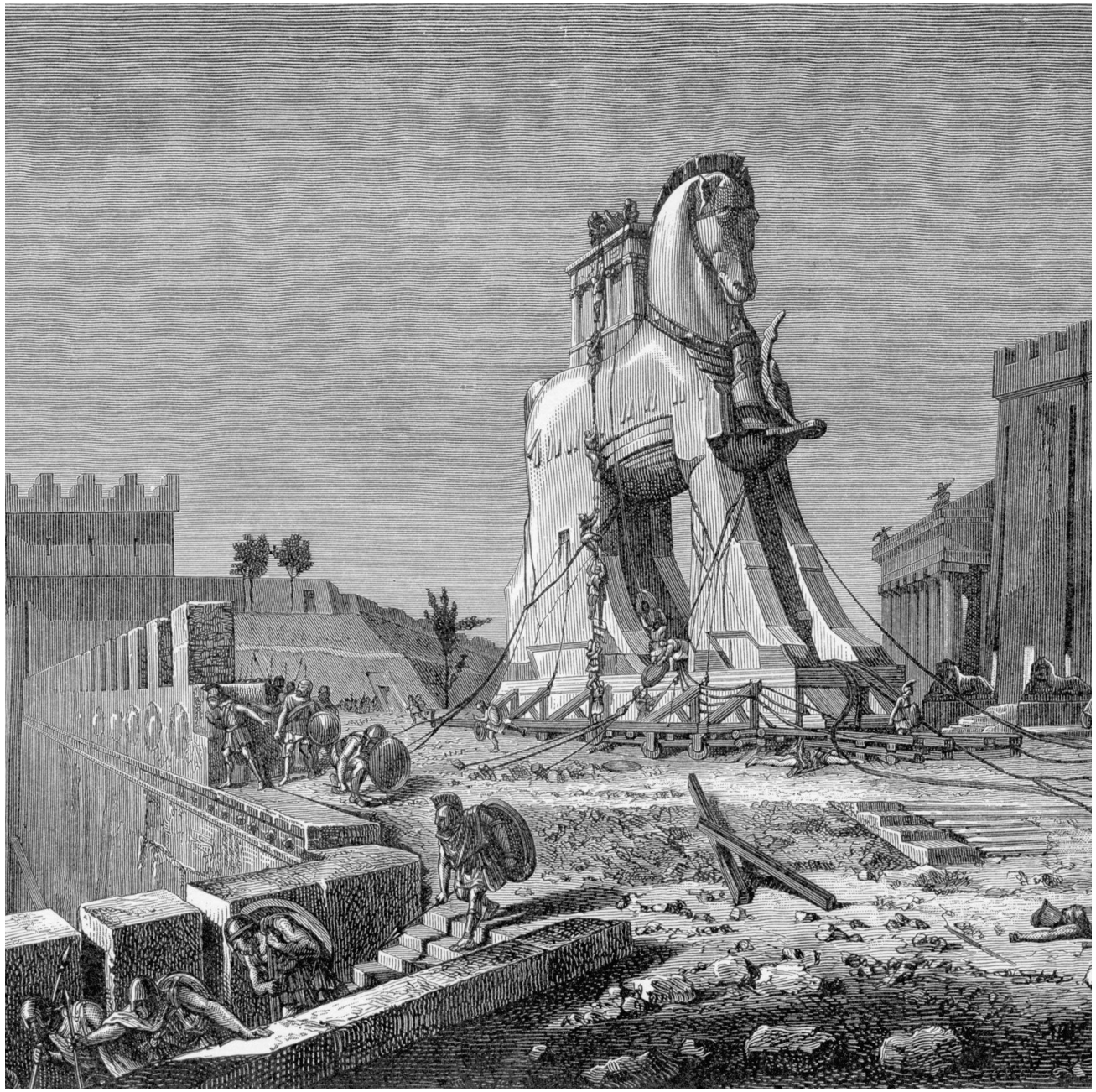
June 2024



The Trojan Horse

Homer's Iliad:

Odysseus and a troop of Greek soldiers hid inside the Trojan Horse to enter the walled city of Troy

Won the Trojan War after a 10-year siege.

Metaphor

Tricks to invite or smuggle something harmful into a protected area.

Particle toxicology

Particles mediate much of their effects by acting as carriers of harmful soluble components such as organic chemicals and transition metals.

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Science of The Total Environment
Volume 788, 20 September 2021, 147854

Are standardized diesel exhaust particles (DEP) representative of ambient particles in air pollution toxicological studies?

V.J. Farahani, M. Pirhadi and C. Sioutas

Science of the Total Environment 788 (2021) 147854

Combustion PM - complex soluble mixture of organic chemicals on a tiny carbon core

Mass fraction of EC and OC in PM2.5 sampled at different US urban areas (Los Angeles, Houston, Pittsburgh, and New York)

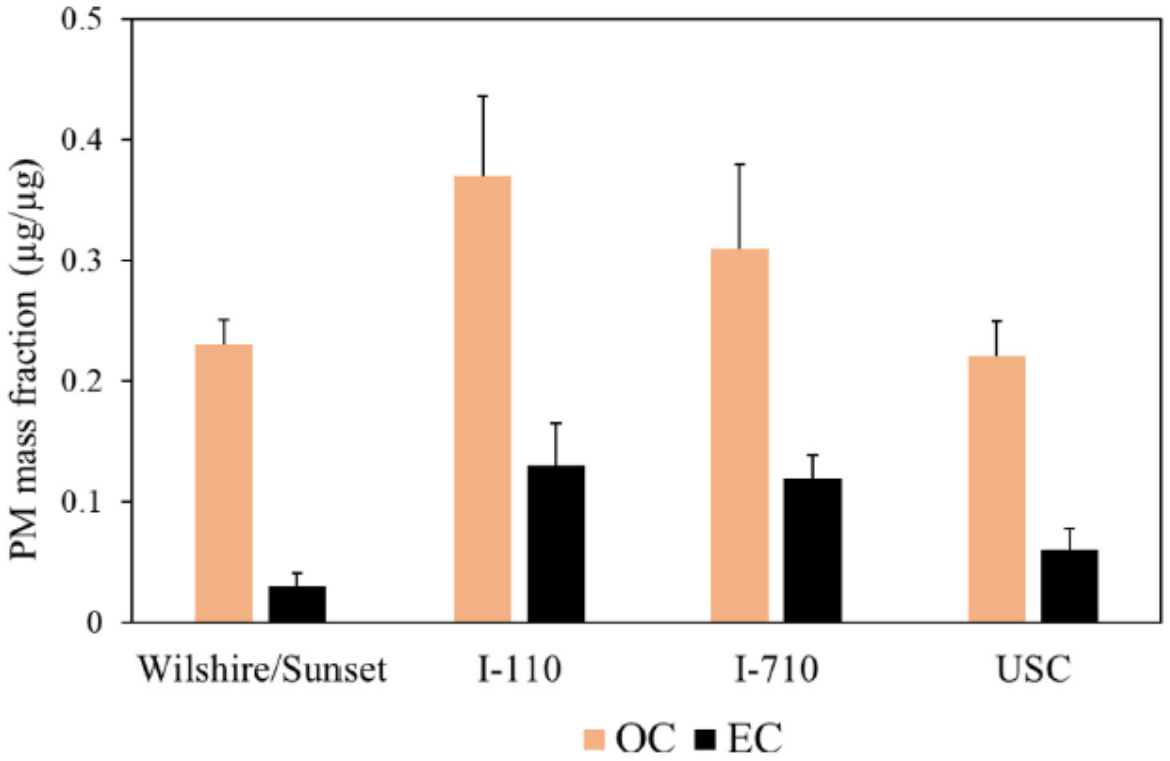
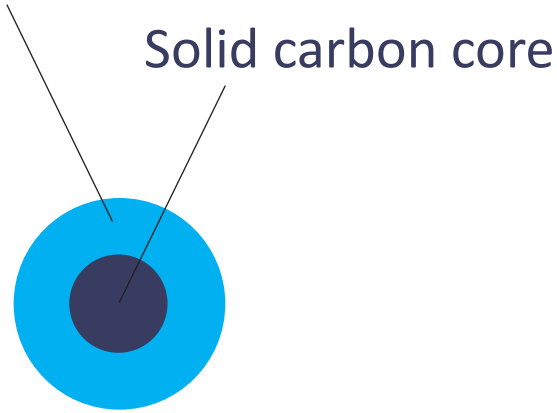


Fig. 5. PM mass fraction of EC and OC at Interstate 110 (I-110), Interstate 710 (I-710), Wilshire/Sunset Blvd., and USC sampling site.

Soluble organic material



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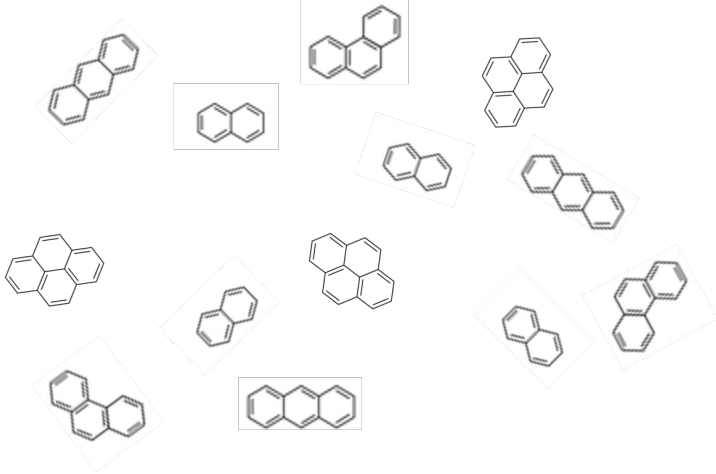
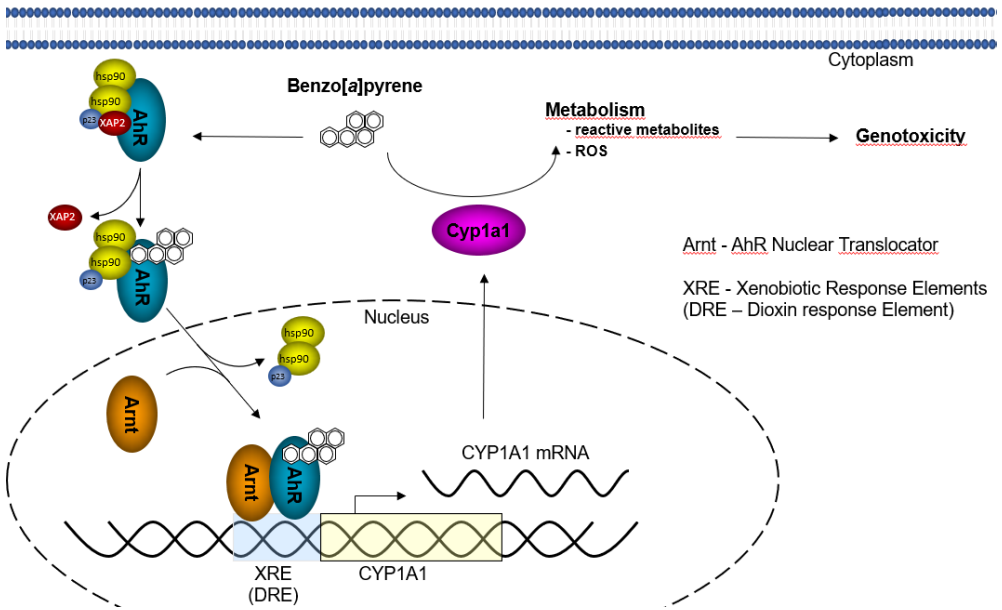
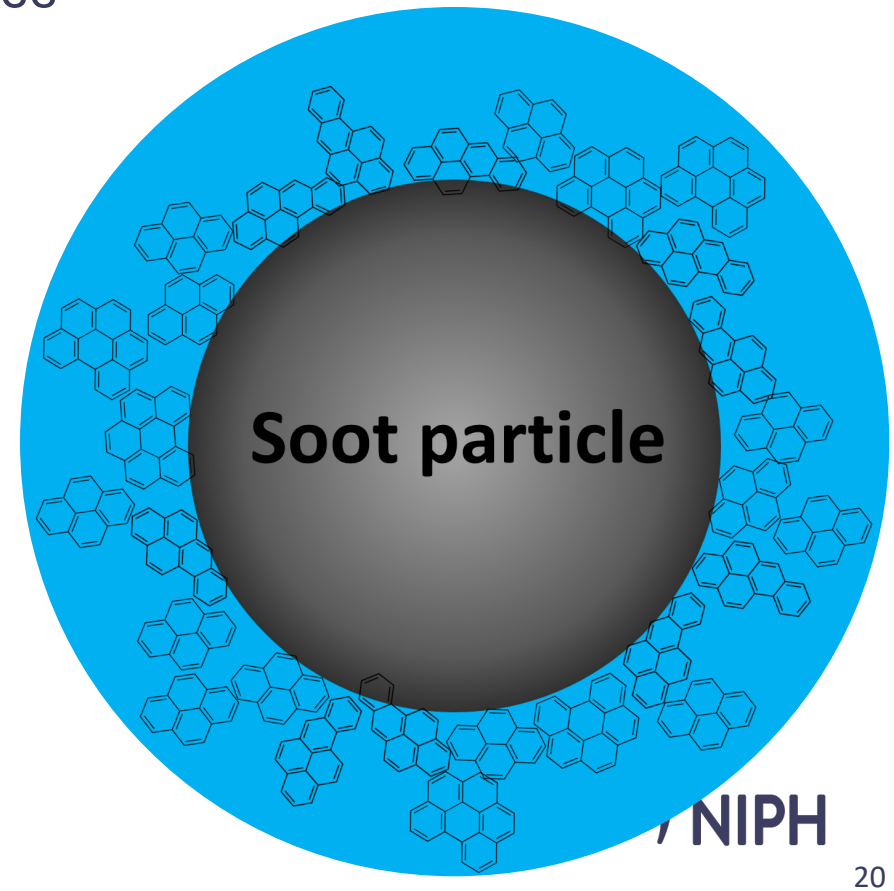
Polycyclic aromatic hydrocarbons (PAHs)

- Lipophilic compounds formed during combustion processes
- Many well-known carcinogens (benzo[*a*]pyrene)
- Associated with atherosclerosis and cardiovascular effects
- Bind and activate the aryl hydrocarbon receptor (AhR) and induce phase-1 metabolic enzymes like CYP1A1 and CYP1B1







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Combustion-Derived Ultrafine Particles Transport Organic Toxicants to Target Respiratory Cells

Arthur Penn,[1] Gleeson Murphy,[1] Steven Barker,[1] William Henk,[1] and Lynn Penn[2]

VOLUME 113 | NUMBER 8 | August 2005 • Environmental Health Perspectives

Combustion-Derived Hydrocarbons Localize to Lipid Droplets in Respiratory Cells

Gleeson Murphy, Jr.[1], Rodney L. Rouse[1], William W. Polk[1], William G. Henk[1], Steven A. Barker[1], Marc J. Boudreaux[2], Z. Elizabeth Floyd[3], and Arthur L. Penn[1]

AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY VOL 38 2008

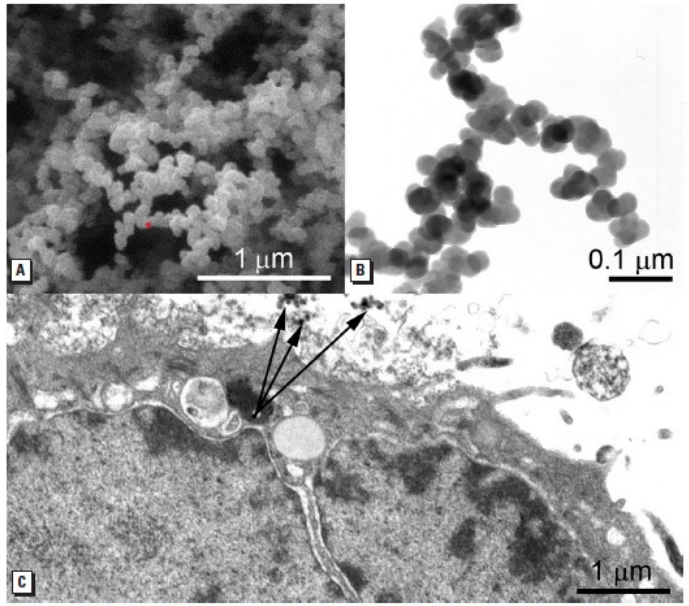


Figure 1. (*A*) An SEM image illustrating the lacy openwork character typical of the BDS aggregates; individual, solid, spherical particles, 50–70 nm in diameter, are the fundamental structural units of the aggregates. (*B*) A TEM image of BDS showing individual spheres, 30–50 nm in diameter, arranged in branching clusters. The difference in diameter of the spheres in the SEM versus TEM images results from the 10–20 nm gold/palladium conductive coating that was applied to the SEM samples. (*C*) A TEM image of a portion of the surface of a BEAS-2B cell with individual spherical particles, 30–50 nm in diameter, and small aggregates (arrows) immediately adjacent to the cell membrane. Cells were photographed after 42 hr exposure.

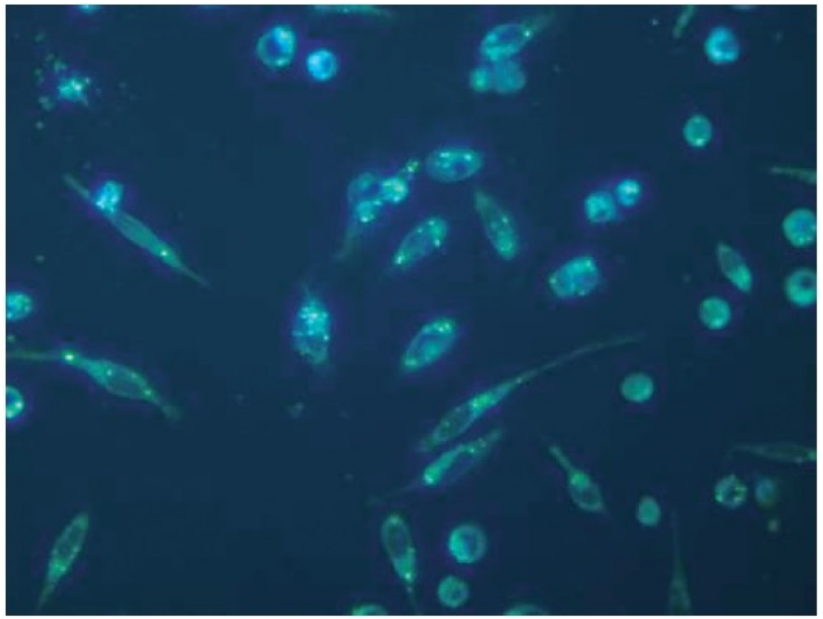


Figure 2. Fluorescence localized in punctate cytoplasmic vesicles of BEAS-2B cells. Cells were photographed 4 hr after BDS, without carrier, was sprinkled onto the surface of the BEGM overlying the cells. Excitation/emission wavelengths = 360/420 nm. Magnification, 400×.

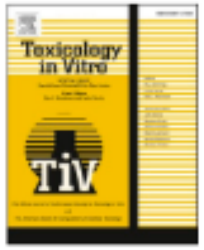
BEAS-2B cells exposed to UFP butadiene soot particles from CAST burner

Utilized fluorescence of PAHs to study uptake/transfer into cells

Fluorescent PAHs were transferred from the particle surface to the cell membrane, cross the membrane into the cytosol and accumulate in lipid vesicles.

There was no evidence of particle uptake in the cells

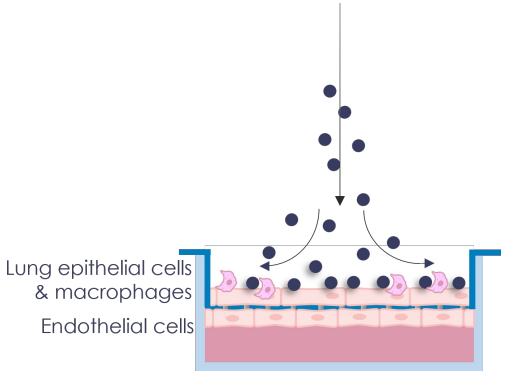
PAHs detach from particles and enter cells independent of the particle core



The effects of fine particulate matter (SRM 2786) on three different 3D lung models exposed at the air-liquid interface – A comparative study

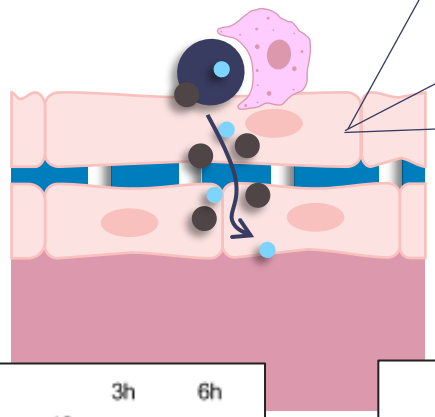
Vegard Sæter Grytting[a,*], Tonje Skuland[a], Jarle Ballangby[a], Magne Refsnes[a], Marit Låg[a], Johan Øvrevik[a,b], Espen Mariussen[a,*]

Toxicology in Vitro 98 (2024) 105841



Epithelial cells

Endothelial cells (EA.hy293)



Calu-3 cells

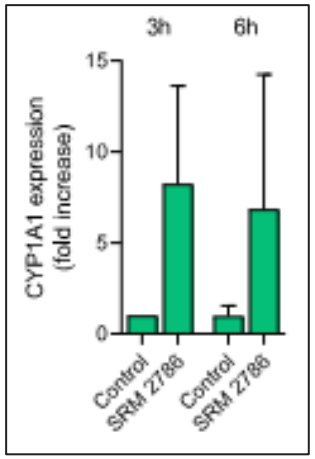
High barrier integrity
High CYP1A1 expression

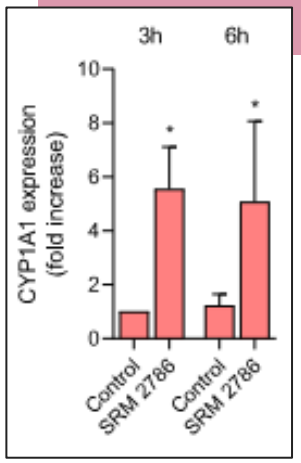
HBEC3-KT

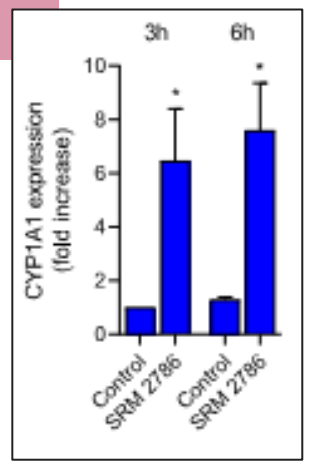
Low barrier integrity
High CYP1A1 expression

A549

Low barrier integrity
Low CYP1A1 expression







PAHs are transported to endothelial cells…

- through the epithelial cells irrespective of barrier integrity
- relatively fast, preceeding metabolism in the epithelial layer

Barrier integrity and expression of PAH-metabolizing enzymes like CYP1A1 in apical epithelial cells did not affect the CYP1A1 responses in the basolateral endothelial EA.hy926 cells

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Inhalation exposure PAH (B[a]P) coated particles

P.Gerde[3], B.A.Muggenburg[1], M.Lundborg and A.R.Dahl[2]
Carcinogenesis vol.22 no.5 pp.741–749, 2001

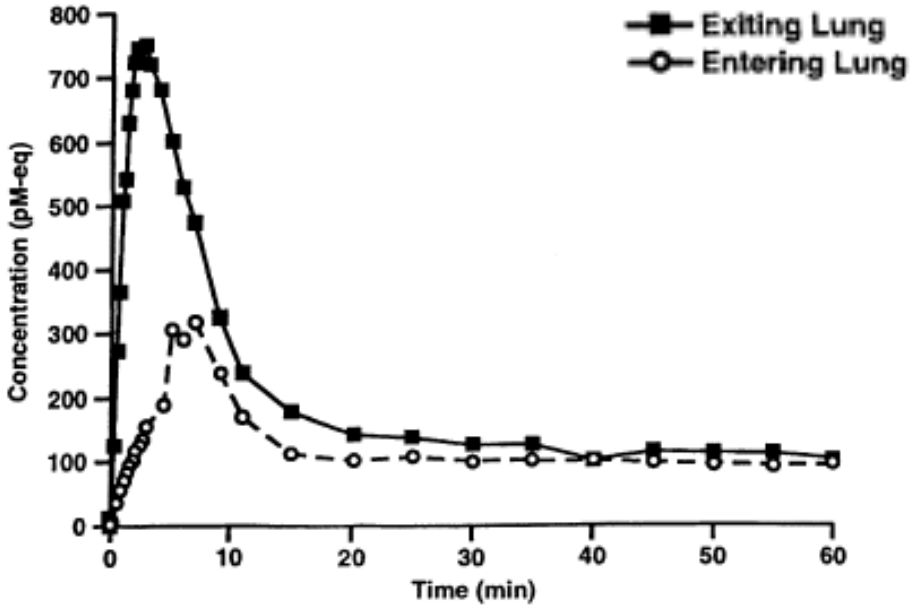

Fig. 2. The concentration of BaP-equivalent activity in blood as a function of time in dogs (**A**)–(**C**) following inhalation exposure of the alveolar region to an aerosol bolus of BaP adsorbed onto organic-denuded diesel soot. Samples from blood exiting and entering the lungs were drawn at the ascending aorta and the posterior vena cava, respectively. Note that the concentration scale in (C) is different from those in (A) and (B).

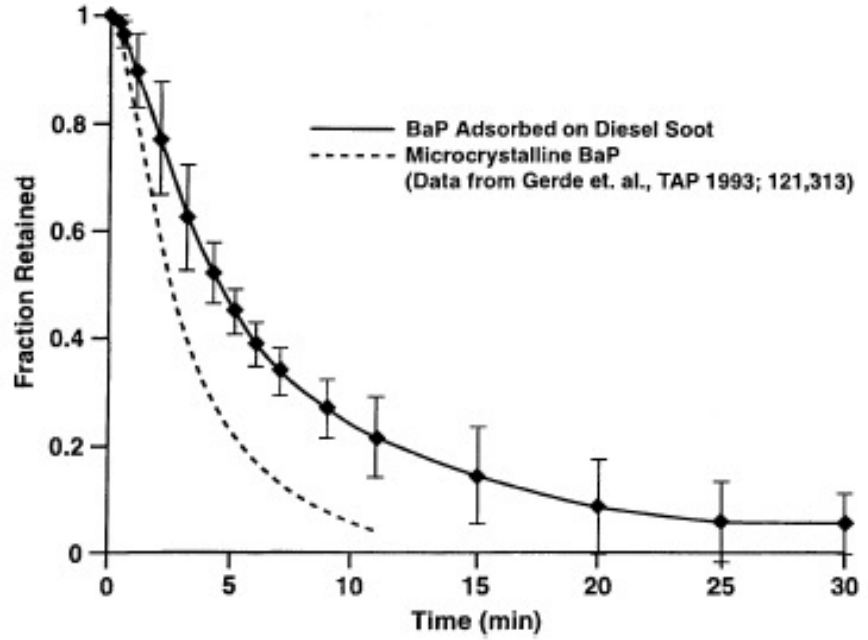

Fig. 3. Fractional retention of the readily bioavailable BaP-equivalent adsorbed onto denuded diesel soot in the dogs' lungs as a function of time. The dashed curve shows the corresponding fractional retention of microcrystalline BaP (data from ref. 43). Error bars show SD ($n = 3$).

During inhalation exposure to combustion aerosols, about 80% of the bioavailable PAH fraction will deposit in the alveoli and the majority of this will rapidly become systemic (within minutes)

Fraction released corresponded to fraction extractable by solvents (octanol at 37°C)

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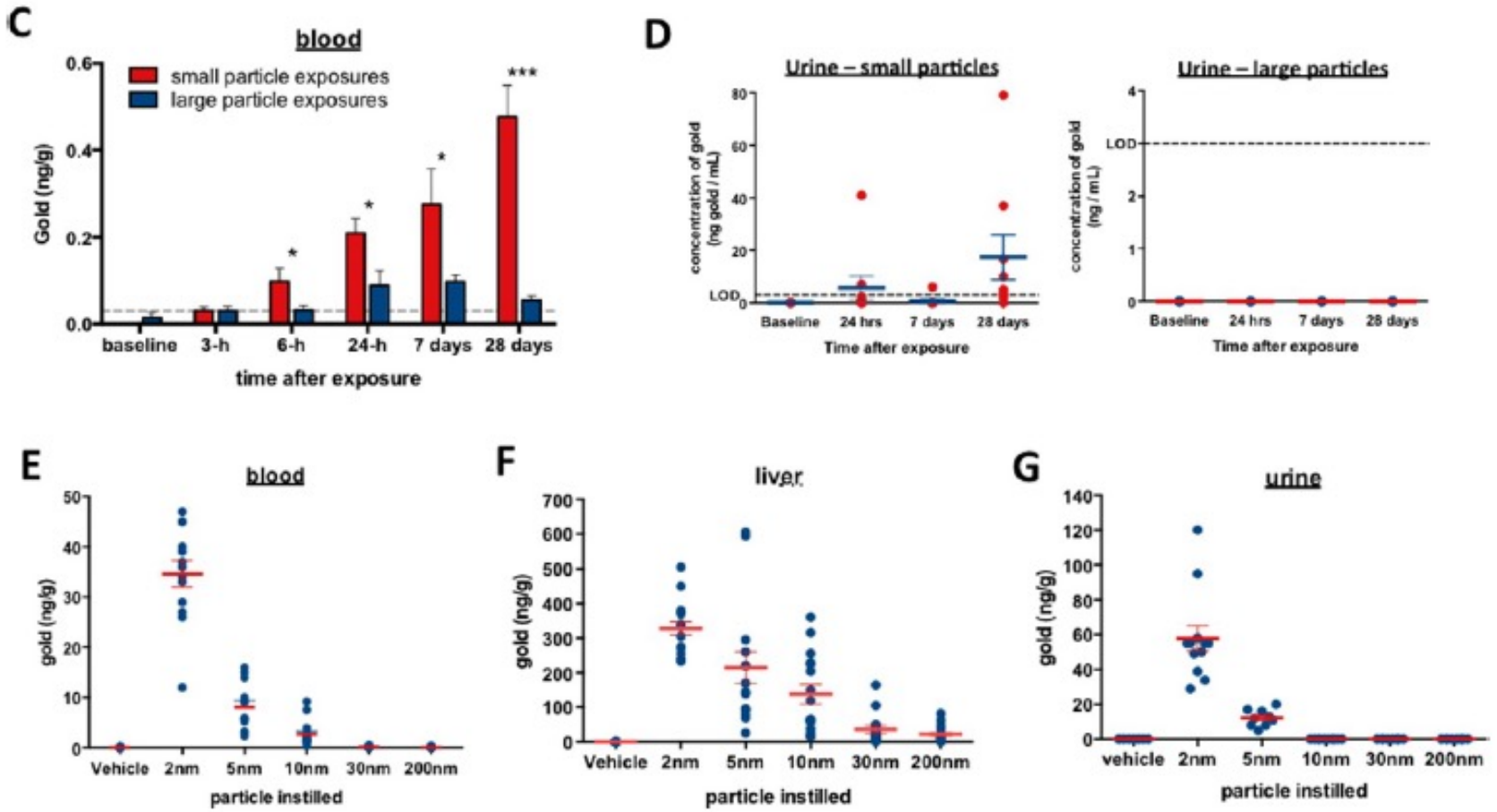
ACSNANO www.acsnano.org

Inhaled Nanoparticles Accumulate at Sites of Vascular Disease

Mark R. Miller,[*,†,∞,▽] Jennifer B. Raftis,[‡,∞,▽] Jeremy P. Langrish,[†] Steven G. McLean,[†] Pawitrabhorn Samutrtai,[§] Shea P. Connell,[†] Simon Wilson,[†] Alex T. Vesey,[†] Paul H. B. Fokkens,[∥] A. John F. Boere,[∥] Petra Krystek,[⊥] Colin J. Campbell,[§] Patrick W. F. Hadoke,[†] Ken Donaldson,[‡] Flemming R. Cassee,[∥,#] David E. Newby,[†] Rodger Duffin,[‡,▽] and Nicholas L. Mills[†,▽]

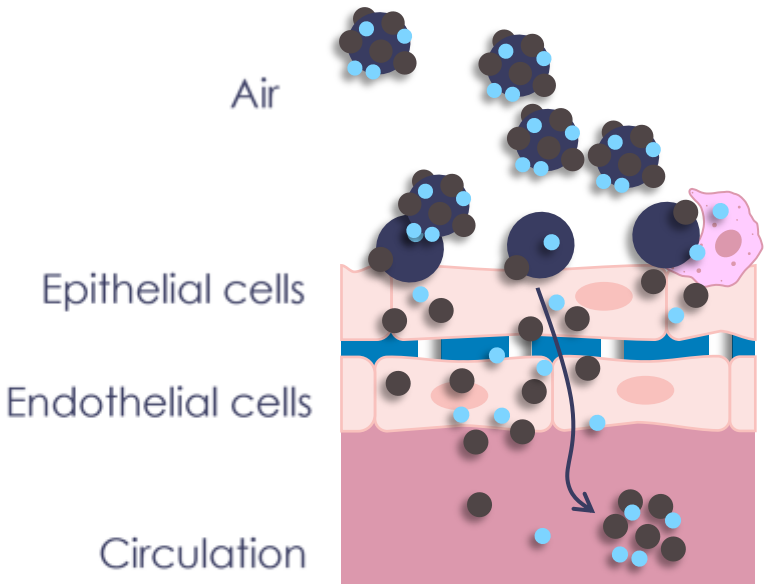


0.02% of inhaled gold nanoparticles translocated from the lung into the circulation 24 post-exposure in humans.

Experiments in mice suggests that particles larger than 10 nm translocate to a very limited extent

Earlier estimates suggest that 0.01-10% of NPs <50 nm may translocate from the lung into circulation, with most estimates around 0.3% or less for a given tissue at 24 h post-exposure.

Miller et al. ACS Nano 2017, 11(5),4542-4552



Translocation of adhered chemicals

When combustion PM deposits in the alveoli…

- the majority of lipophilic fraction detach and translocate into lung cells and further into circulation within minutes
- the majority of particles are retained in the airways - only a small fraction translocate over hours and days

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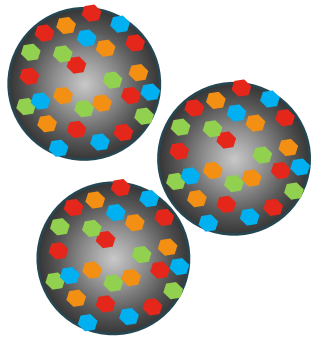
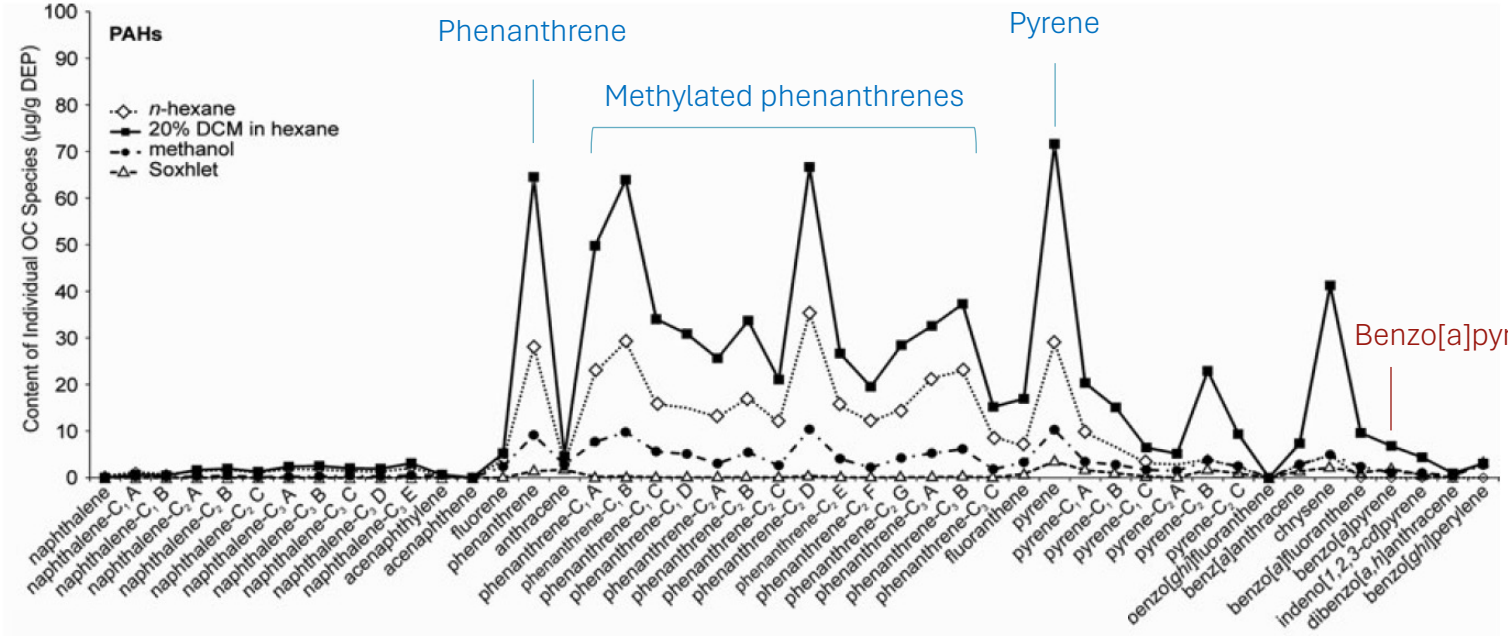
Diesel exhaust particle (DEP) from unloaded diesel engine (Deutz, 4 cylinder, 2.2 L, 500 rpm)

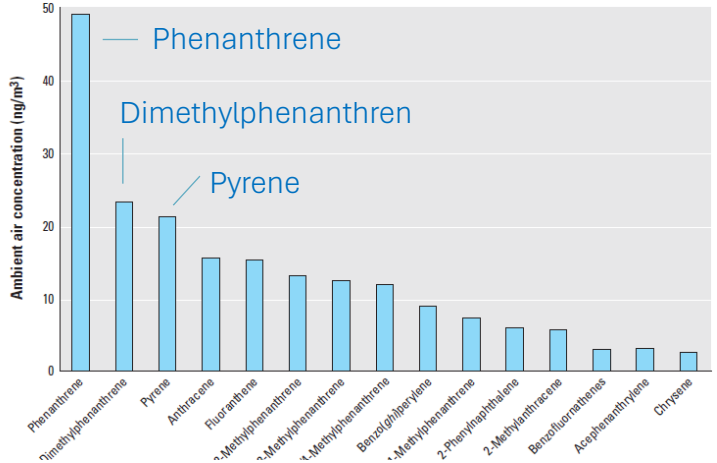
OC: 60% of total mass
OC:EC ~ 5:1

PAH content dominated by 3- and 4-ring species: phenanthrene, methylated phenanthrene, pyrene

(Resembles traffick particles outdoors)







The 15 most abundant PAHs at Hornsgatan (Stockholm city) from April to June 1996.

Boström et al., Environ Health Perspect 2002: 110(3)

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Totlandsdal *et al. Particle and Fibre Toxicology* 2010, 7:41
http://www.particleandfibretoxicology.com/content/7/1/41

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells

Annike I Totlandsdal[1*], Flemming R Cassee[2], Per Schwarze[1], Magne Refsnes[1], Marit Låg[1]

Totlandsdal *et al. Particle and Fibre Toxicology* 2010, 7:41
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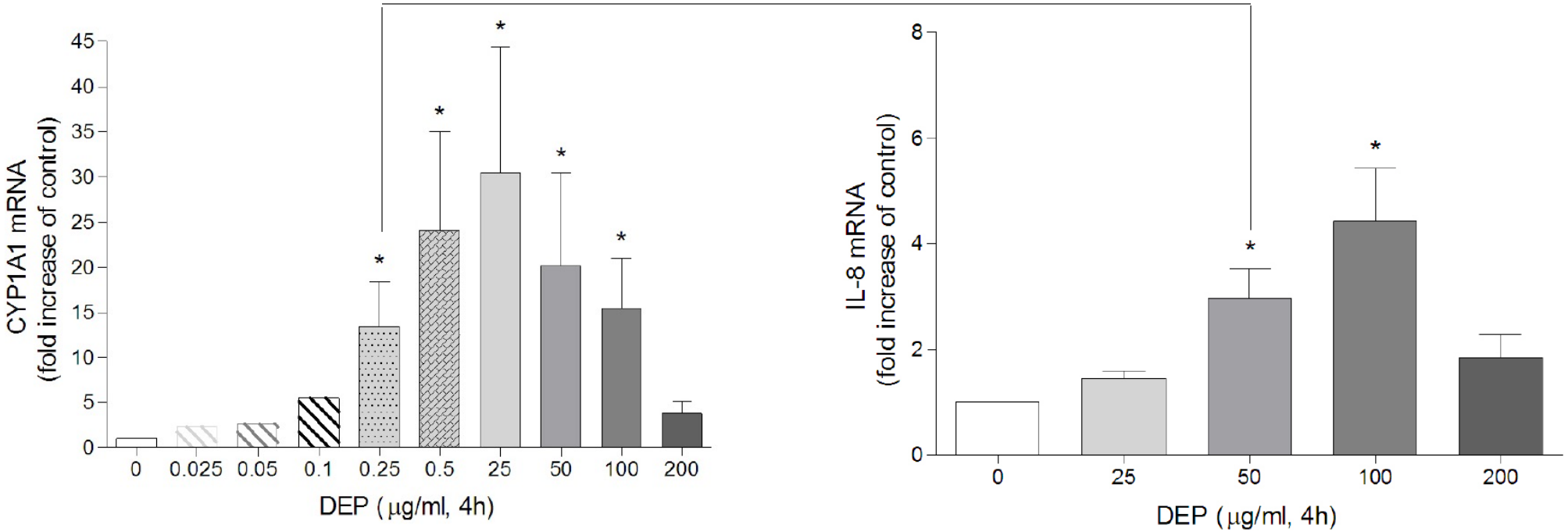

Aryl hydrocarbon receptor (AhR) activation among the most sensitive endpoints induced by diesel exhaust particles (DEP) in human bronchial BEAS-2B cells

CYP1A1 induced from 0.25 µg/ml (40 ng/cm^2) - possibly as low as 0.025 µg/ml (4-16 ng/cm^2)

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Totlandsdal *et al. Particle and Fibre Toxicology* 2010, 7:41
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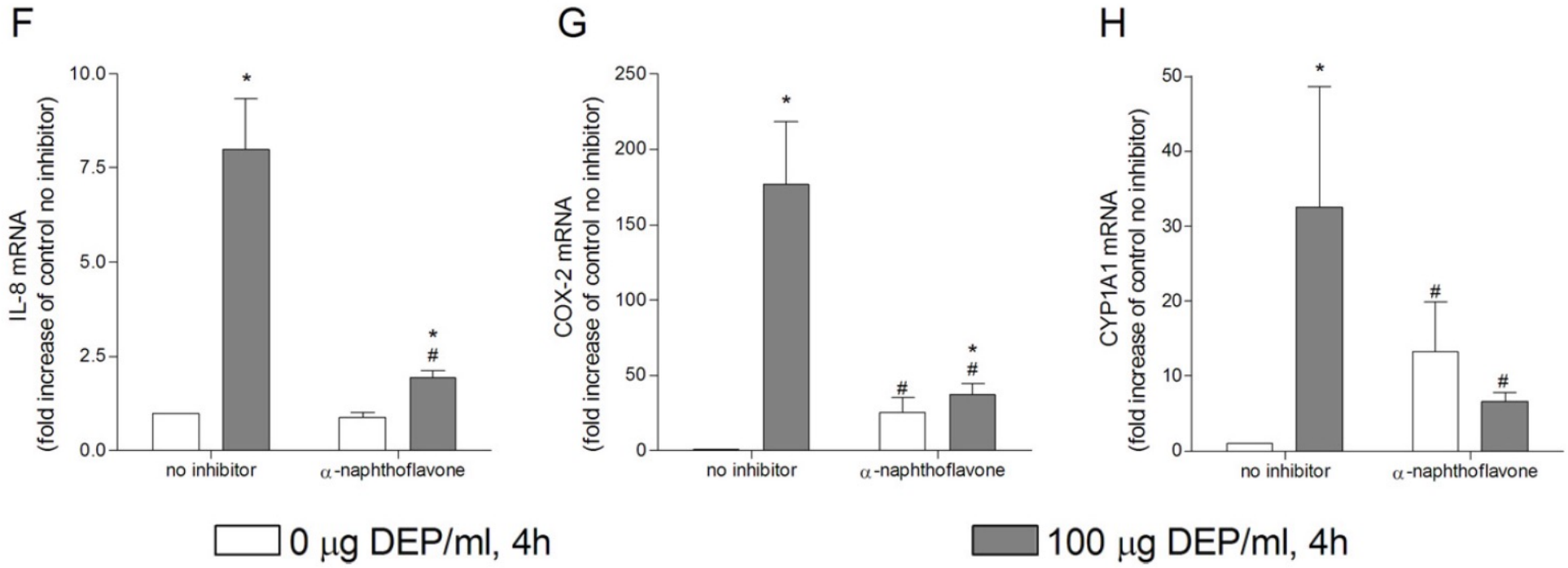
Proinflammatory effects (IL-8/CXCL8 and COX2) were strongly attenuated by AhR inhibition with α-napthtoflavone



Human bronchial BEAS-2B cells exposed to DEO in presence or absence of α-napthtoflavone (25 µM)

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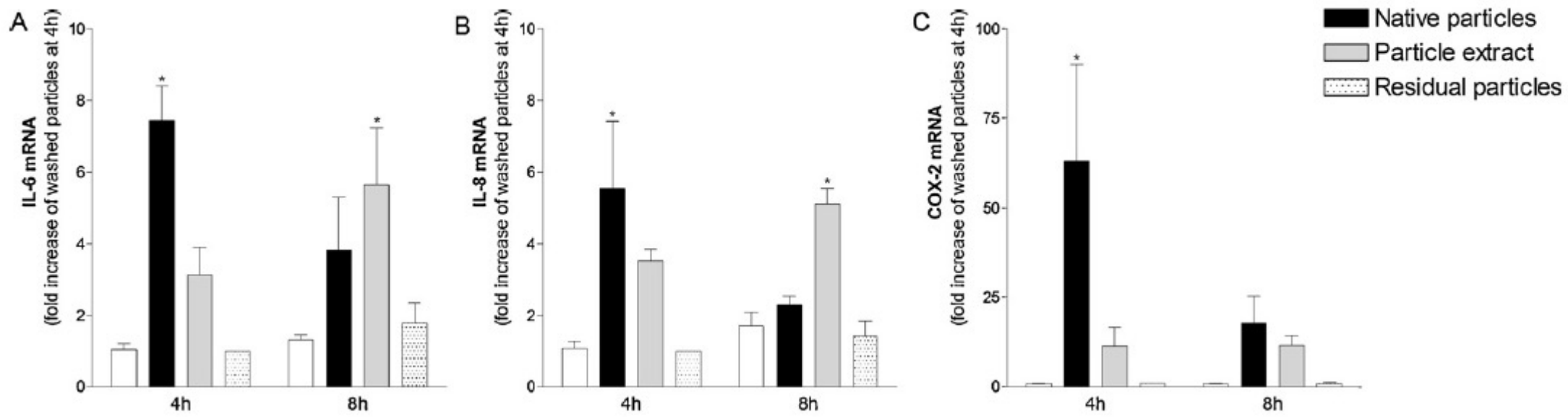
Extractable organic fraction is driving proinflamatory effects of DEP in human bronchial epithelial cells



mRNA expression of selected inflammation-related genes induced by exposure to native DEP and corresponding DEP extract and residual DEP (“washed” particles).

Human bronchial epithelial BEAS-2B cells were exposed to native DEPs (100 g/ml), or to corresponding concentrations of DEP methanol-extract or residual DEPs for 4 h and 8 h. Relative quantification of mRNA levels was performed by QRT-PCR. Bars represent means ± SEM of fold increase relative to unexposed cells detected in separate experiments (n = 3). *p < 0.05; exposed vs. unexposed cells.

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In vitro

DEP caused proinflammatory responses in human bronchial 16HBE cells. Effects largely driven by DEP-OE and less by the particle core (stripped DEP). *Bonvallot et al. 2001. Am J respir Cell Mol Biol.*

DEP with different organic content induce proinflammatory cytokines through different mechanisms in human airway epithelial cells *Tal et al. 2010 Toxicol Appl Pharmacol.*

DEP induced polarization of airway sensory nerves of humans and guinea pigs. Effects were due to DEP-OE and not the particle core. *Robison et al J. 2018. Allergy Clin Imunol.*

Increased surface coating of soot nanoparticles with secondary organic matter increase cytotoxicity, and proinflammatory effects in normal human bronchial epithelial cells. *Leni et al 2022 Environ Sci technol.*

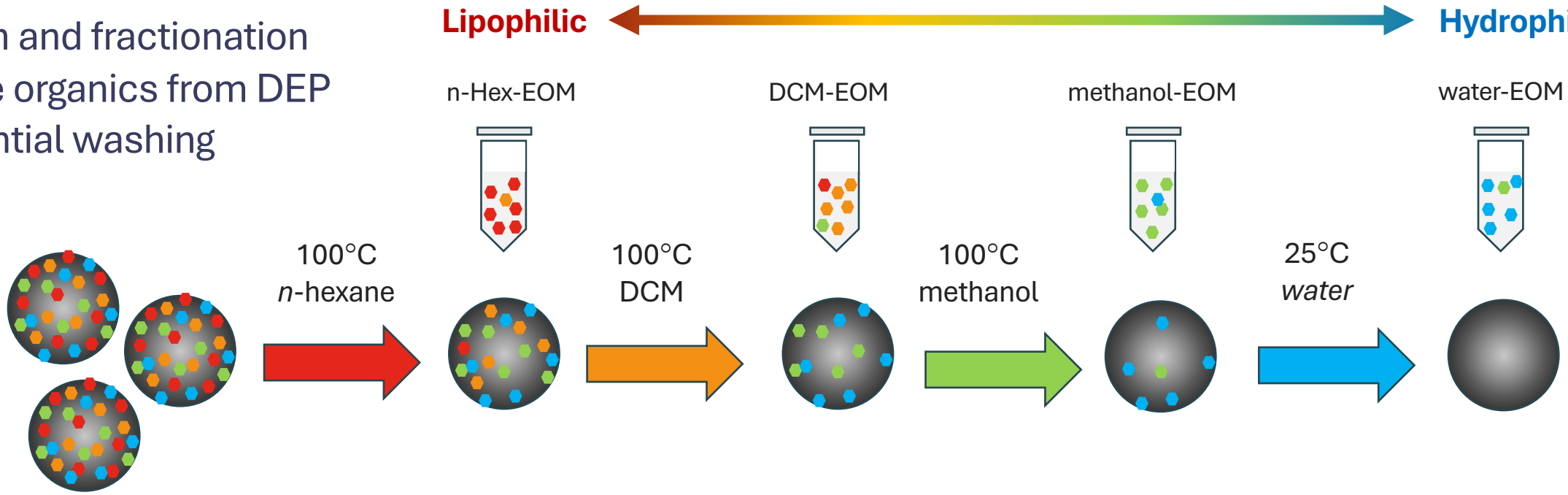
In vivo

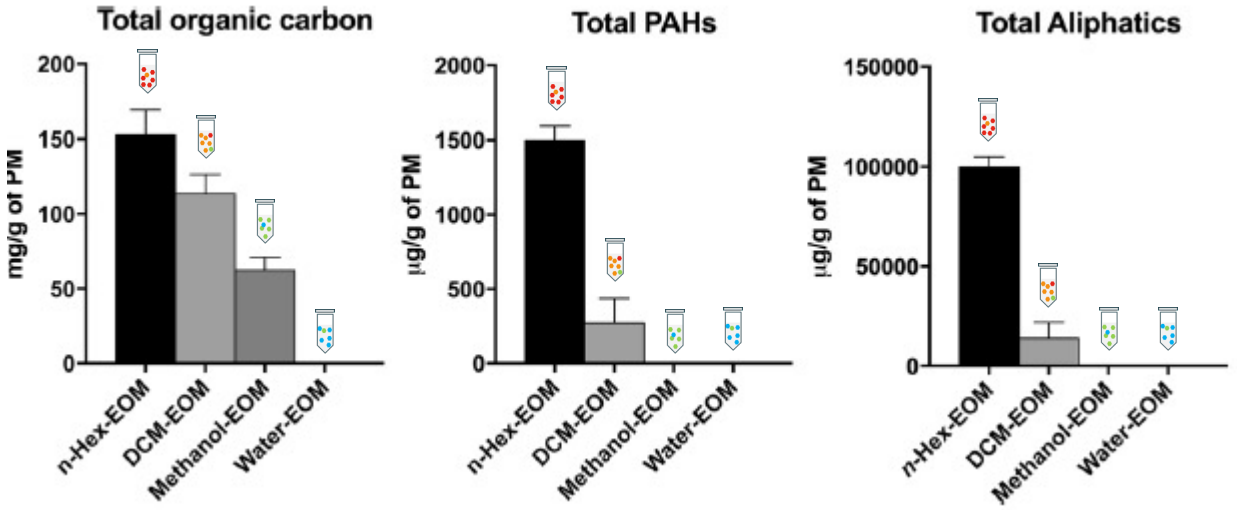
Inhalation of DEP but not pure carbon nanoparticles induce increased systolic blood presssure and attenuated pharmaceutical vasodilation in human volunteers and in isolated rat aorta rings. *Mills et al. 2011. Europ Heart J.*

Concentrated UFP accelerated atherosclerosis in ApoE-/- mice. Effects was abolished by denuding UFPs (removing organics by evaporation). *Keebaugh et al. 2015.Sci Tot Environ.*

Inhalation of graphene nanosheets did not affect heart rate, blood pressure, lung function or inflammatory markers in human volunteers. *Andrews et al. 2024. Nat Nanotechnol.*

Extraction and fractionation of soluble organics from DEP by sequential washing





	n-Hex-EOM	DCM-EOM	
Phenanthrene	124	23	µg/g PM
Methylated phen.	997	286	
Fluoranthene	38	24	
Pyrene	118	9	
Benzo(a)pyrene	n.d.	n.d.	
1-nitropyrene	65	n.d.	

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Brinchmann et al. Particle and Fibre Toxicology (2018) 15:21
https://doi.org/10.1186/s12989-018-0257-1

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Lipophilic components of diesel exhaust particles induce pro-inflammatory responses in human endothelial cells through AhR dependent pathway(s)

Bendik C. Brinchmann[1,2], Tonje Skuland[1], Mia H. Rambøl[3], Krisztina Szoke[3], Jan E. Brinchmann[3], Arno C. Gutleb[4], Elisa Moschini[4], Alena Kubátová[5], Klara Kukowski[5], Eric Le Ferrec[6,7], Dominique Lagadic-Gossmann[6,7], Per E. Schwarze[1], Marit Låg[1], Magne Refsnes[1], Johan Øvrevik[1] and Jørn A. Holme[1*]

Concentration in exposure media

	n-Hex-EOM	*DCM-EOM*	
	1 / 5	1 / 5	
Phen	124 / 620	23 / 115	pg/ml
Pyr	118 / 590	9 / 45	pg/ml

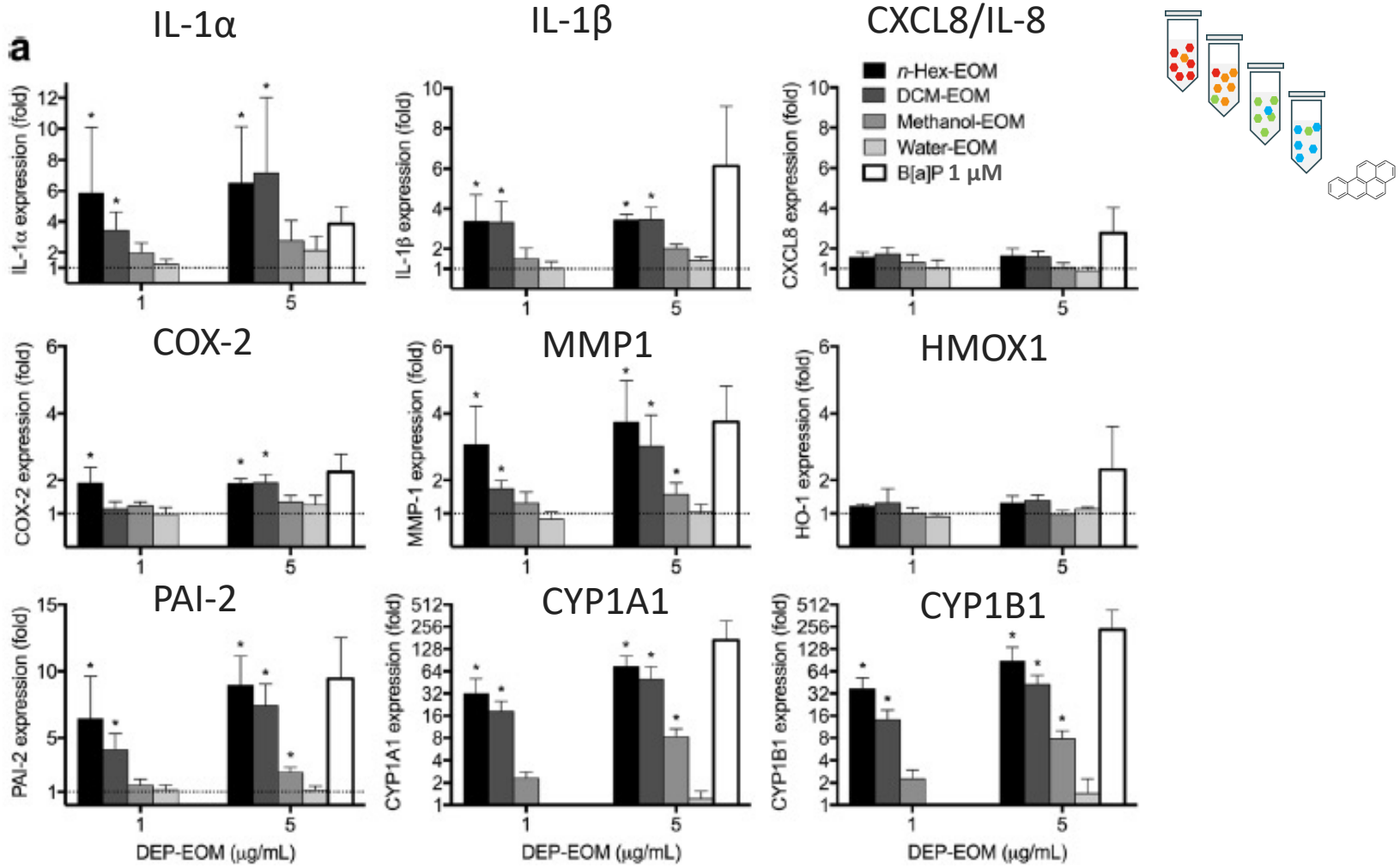
Brinchmann et al. Int. J. Mol. Sci. 2018, 19, 1429

Concentration in human plasma

Phen	19-1793	pg/ml
Pyr	7-392	pg/ml

Pleil et al. 2010. J Chromatogr B 878, 1753-1760

Lipophilic DEP-EOM induce proinflammatory and AhR-response genes in primary human endothelial cells at PAH conc. representative of human plasma



Gene expression in primary human endothelial cells exposed to organic extracts of diesel exhaust particles (DEP-EOM) or benzo[*a*]pyrene (B[a]P) for 24 h.

Brinchmann et al. Particle and Fibre Toxicology (2018) 15:21
https://doi.org/10.1186/s12989-018-0257-1

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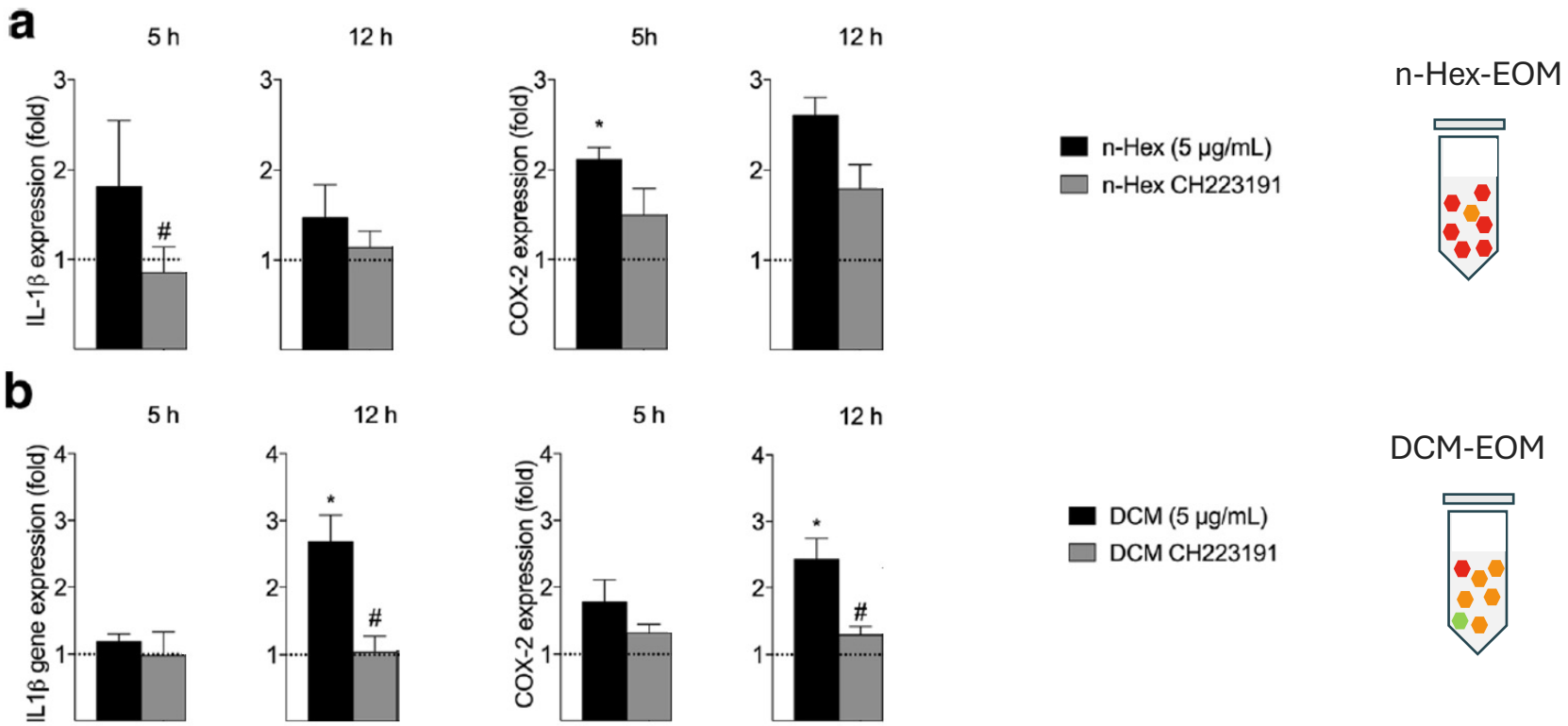
AhR inhibition attenuate expression of proinflammatory genes (IL-1β and COX-2) in primary human endothelial cells exposed to lipophilic organic extracts of DEP



Gene expression in primary human endothelial cells exposed to lipophilic organic extracts of diesel exhaust particles alone or in combination with the AhR inhibitor CH223191.

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International Journal of Molecular Sciences

MDPI

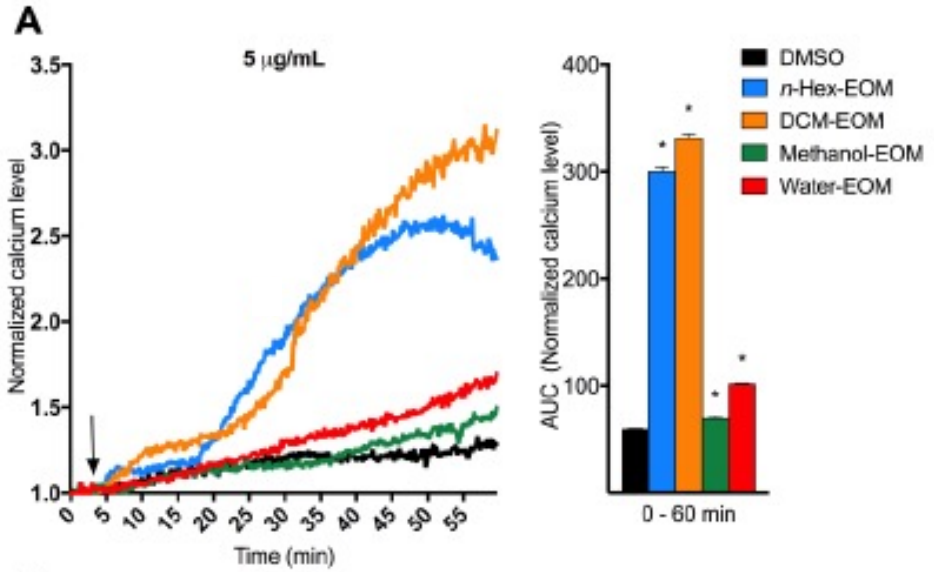
Article

Lipophilic Chemicals from Diesel Exhaust Particles Trigger Calcium Response in Human Endothelial Cells via Aryl Hydrocarbon Receptor Non-Genomic Signalling

Bendik C. Brinchmann [1,2,*], Eric Le Ferrec [3], Normand Podechard [3], Dominique Lagadic-Gossmann [3], Kenji F. Shoji [3], Aubin Penna [3], Klara Kukowski [4], Alena Kubátová [4], Jørn A. Holme [1] and Johan Øvrevik [1,*]

PAH-rich organic extracts of DEP induce intracellular Ca^{2+} influx in human endothelial cells through AhR dependent mechanisms

Effects induced at PAH concentrations reflecting PAH conc. in human plasma



Concentration in exposure media

	n-Hex-EOM	*DCM-EOM*	
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Brinchmann et al. Int. J. Mol. Sci. 2018, 19, 1429

Concentration in human plasma

Phen	19-1793	pg/ml
Pyr	7-392	pg/ml

Pleil et al. 2010. J Chromatogr B 878, 1753-1760

Human microvascular endothelial cells (HMEC-1) exposed to DEP-EOM of different polarity, corresponding to 1 or 5 ug/ml of original DEP mass (A and B). The figures also show the inhibitory effects of the AhR inhibitor CH223191 (C). Calcium measurements by micro-spectrofluorimetry with Fura2-AM.

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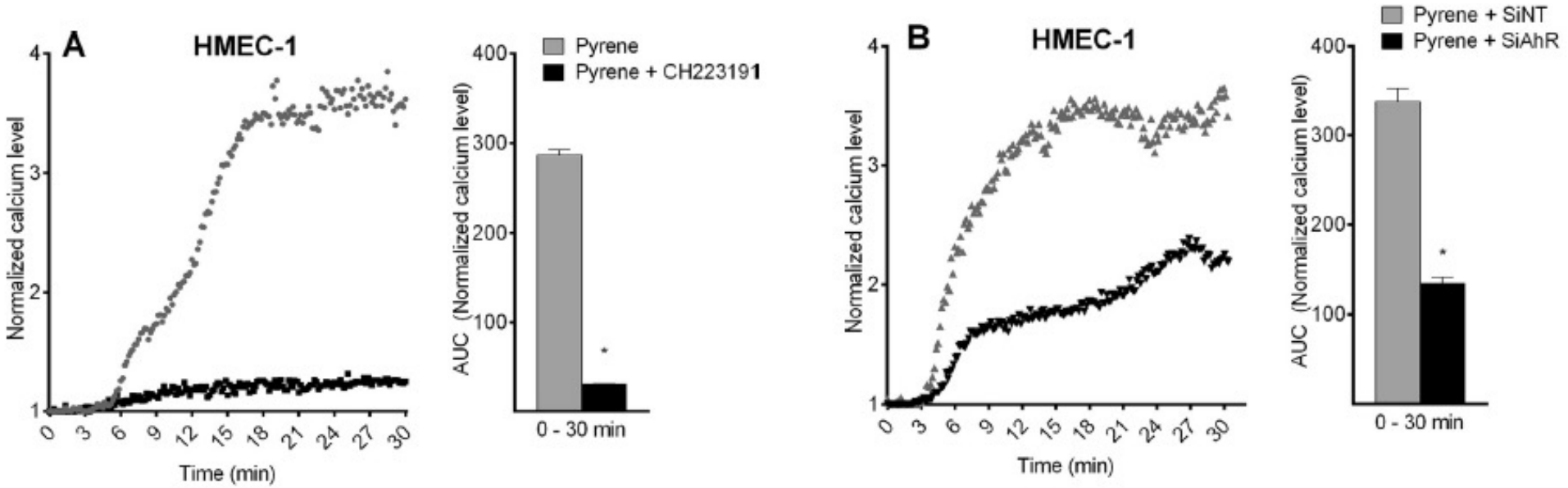
Evidence of selective activation of aryl hydrocarbon receptor nongenomic calcium signaling by pyrene

Bendik C. Brinchmann[a,b,1], Eric Le Ferrec[c,1], William H. Bisson[d], Normand Podechard[e], Henrik S. Huitfeldt[e], Isabelle Gallais[e], Odile Sergent[e], Jørn A. Holme[a,2], Dominique Lagadic-Gossmann[c,2], Johan Øvrevik[a,*,2]

Pyrene induced nongenomic AhR-dependent Ca^{2+} influx in endothelial cells



Human microvascular endothelial cells (HMEC-1) exposed to 1 µM pyrene. The figures show the inhibitory effects of the AhR inhibitor CH223191 or transient AhR knock-down by siRNA. Calcium measurements by micro-spectrofluorimetry with Fura2-AM.

Barath et al. Particle and Fibre Toxicology 2010, 7:19
http://www.particleandfibretoxicology.com/content/7/1/19

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Impaired vascular function after exposure to diesel exhaust generated at urban transient running conditions

Stefan Barath[1,2], Nicholas L Mills[3], Magnus Lundbäck[1,2], Håkan Törnqvist[1,2], Andrew J Lucking[3], Jeremy P Langrish[3], Stefan Söderberg[4], Christoffer Boman[5], Roger Westerholm[6], Jakob Löndahl[7], Ken Donaldson[8], Ian S Mudway[9], Thomas Sandström[1,2], David E Newby[3], Anders Blomberg[1,2*]

Circulation
Volume 112, Issue 25, 20 December 2005; Pages 3930-3936
https://doi.org/10.1161/CIRCULATIONAHA.105.588962

American Heart Association.

VASCULAR MEDICINE

Diesel Exhaust Inhalation Causes Vascular Dysfunction and Impaired Endogenous Fibrinolysis

Nicholas L. Mills, MRCP, Håkan Törnqvist, MD, Simon D. Robinson, MRCP, Manuel Gonzalez, MD, Kareen Darnley, RN, William MacNee, MD, Nicholas A. Boon, MD, Ken Donaldson, PhD, Anders Blomberg, MD, PhD, Thomas Sandstrom, MD, PhD, and David E. Newby, DM, PhD

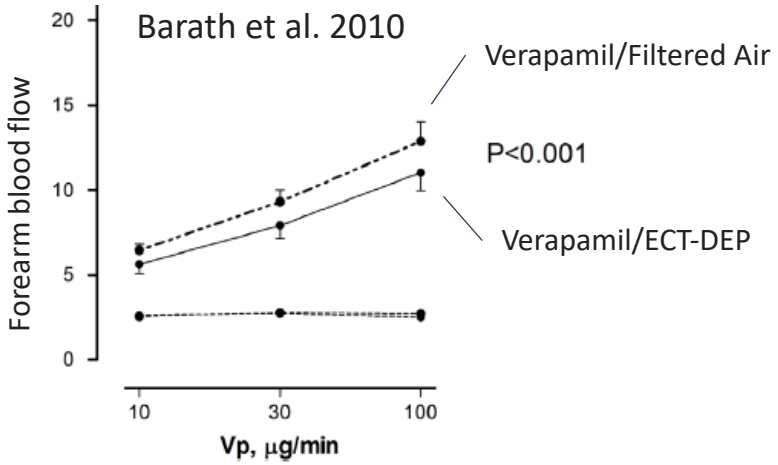
Barath *et al. Particle and Fibre Toxicology* 2010, **7**:19

Mills et al. *Circulation* 2005, 112:3930-3936

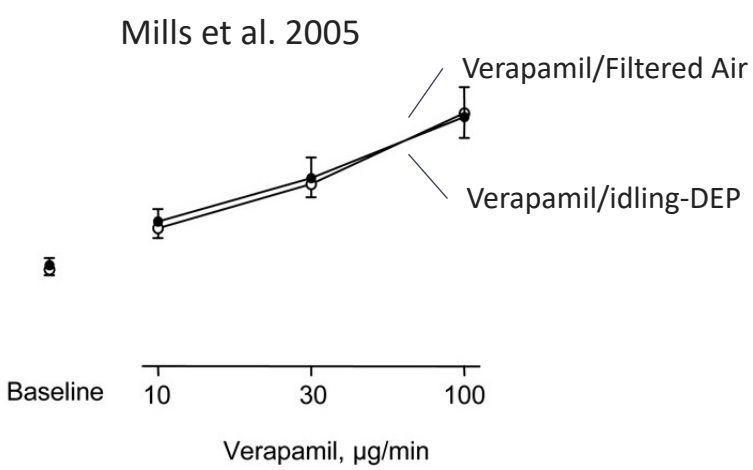
Exposure of human volunteers to diesel exhaust (DE) from idling (without engine load) and transient running condition (ETC: variable engine load) showed that:

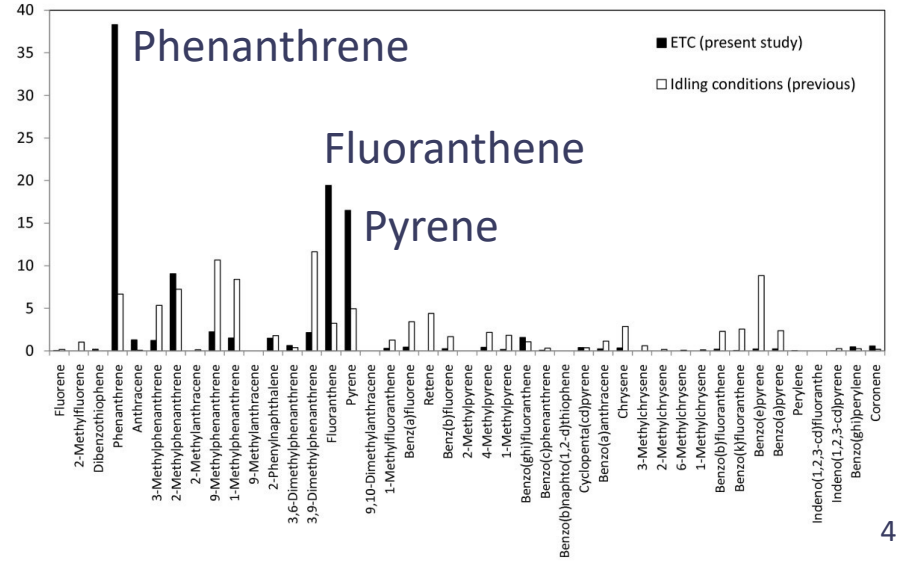
- ETC-DE impaired a novel calcium-channel vasomotor function, not seen with idling exhaust.
- ETC-DE contained higher levels of low-molecular species: phenanthrene, fluoranthene and pyrene

ECT Exhaust

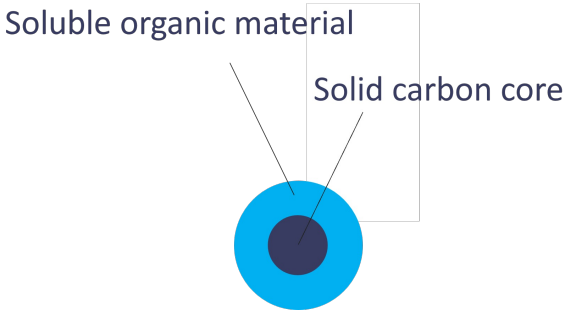


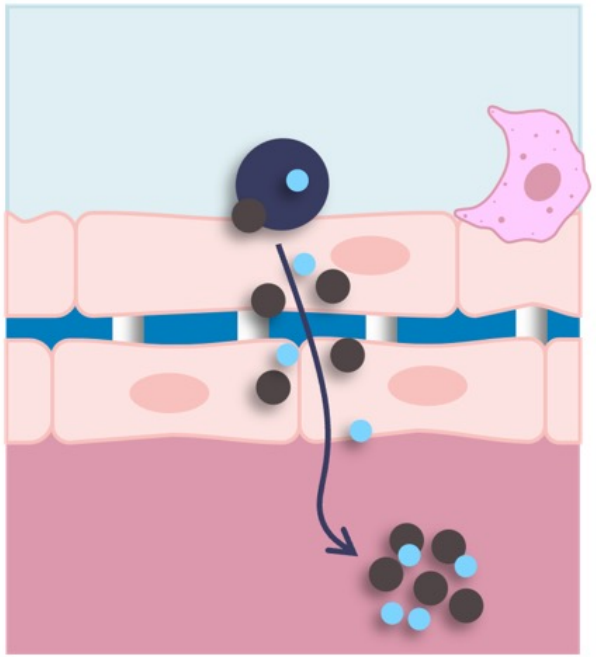
Idling Exhaust





Take-home





Translocation of adhered chemicals

Combustion PM from traffic - soluble organic material on a tiny carbon core

The majority of bioavailable lipophilic organic material rapidly detach upon deposition, enter lung cells, and transfer into circulation within minutes

Studies in cells, animals and humans suggest that the soluble organic fraction is central for biological effects of combustion PM, especially cardiovascular effects

AhR is a regulator of PM-induced effects pointing towards a role of PAHs, and the most sensitive endpoint induced by combustion PM in vitro

PAH-rich combustion PM extracts induce AhR dependent activation of calcium and proinflammatory cytokines in human endothelial cells at concentrations found in human blood

Data from our lab and others point towards a central role of 3- and 4-ring PAHs (phenanthrene and pyrene) with low mutagenicity

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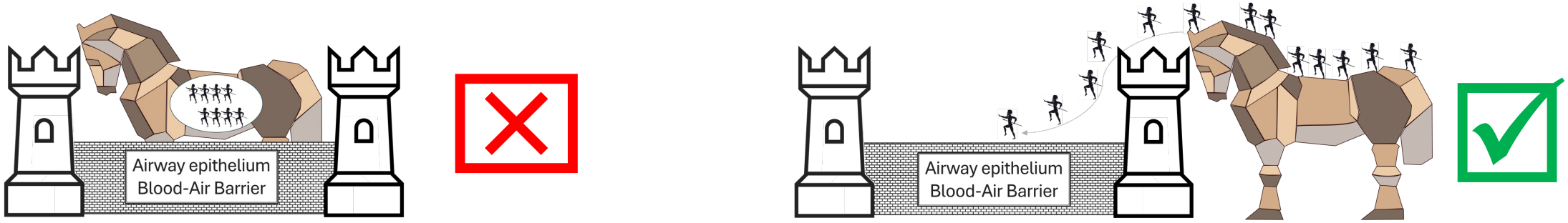
The Trojan Horse effects

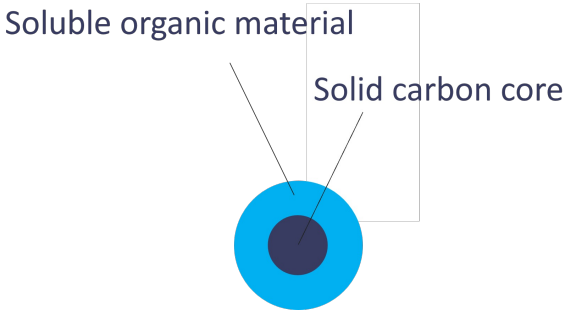
Central to combustion PM toxicity…

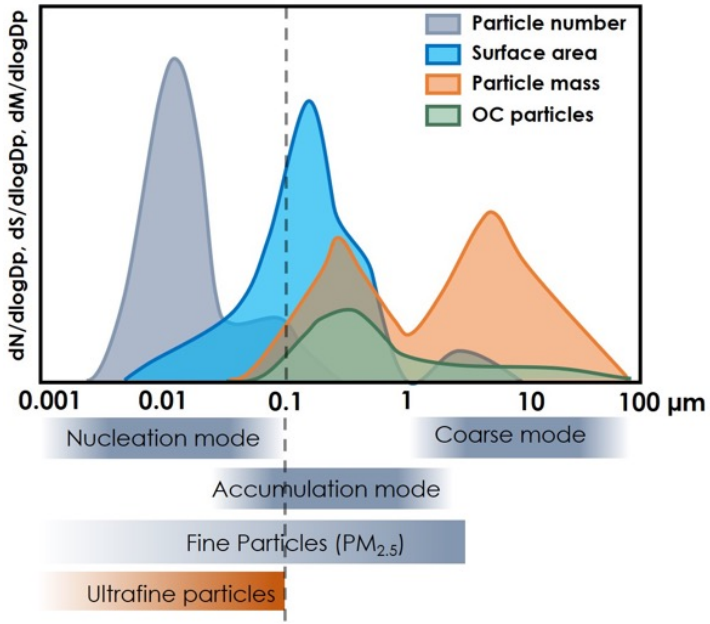
…but perhaps not the best analogy



Trojan horse or conventional horse?

Implications for regulation of UFPs





The need to regulate UFP has been discussed for more than a decade

UFPs constitute an insignificant amount of PM2.5 mass

A main argument for additional regulation of UFPs is that particle surface area and number concentrations are not captured by the PM2.5 mass regulation

Surface area is only a relevant metric for non-soluble particles

For soluble particles (droplets) – Mass Matters the Most

The majority of organic chemical mass occur in the accumulation mode, and is found in the fraction between 0.1 and 1 µm

For combustion emissions, regulation targeting organic carbon, VOC/SVOC or PM1 and secondary aerosols may be more relevant

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www.ultrhas.eu

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