folkehelseinstituttet

# Hvordan kan dieseleksospartikler medvirke til hjerte- og karsykdom?

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# Disposition

- Air pollution, a global health challenge

  Diesel exhaust particles (DEP), composition

  Cardiovascular disease (CVD)

- How may inhaled DEP trigger vascular effects?

  1. Pulmonary inflammation (systemic spill over)

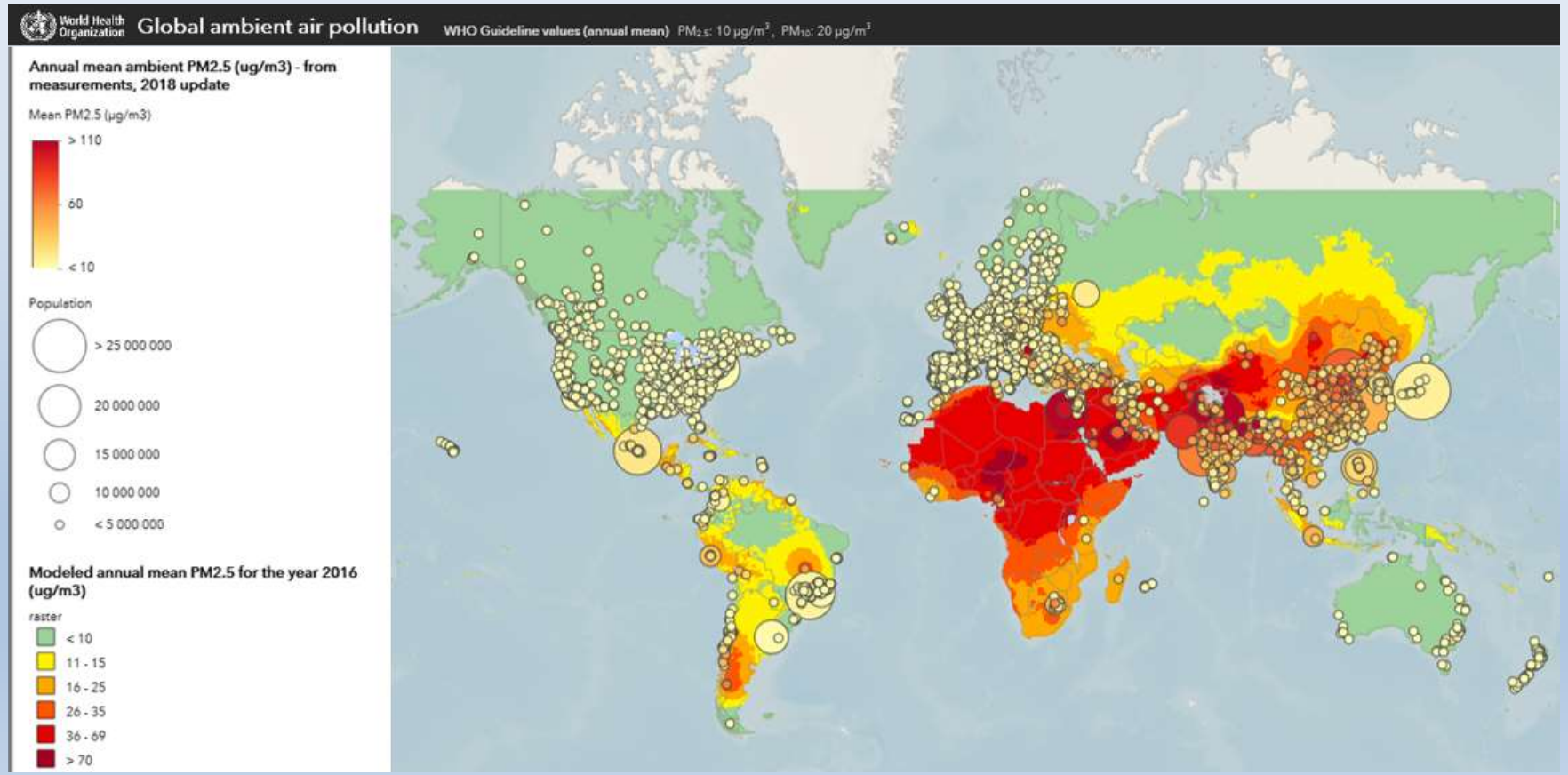
  2. Autonomic dysfunction

  3. Direct vascular effects of particles or particle-associated components

- Summary

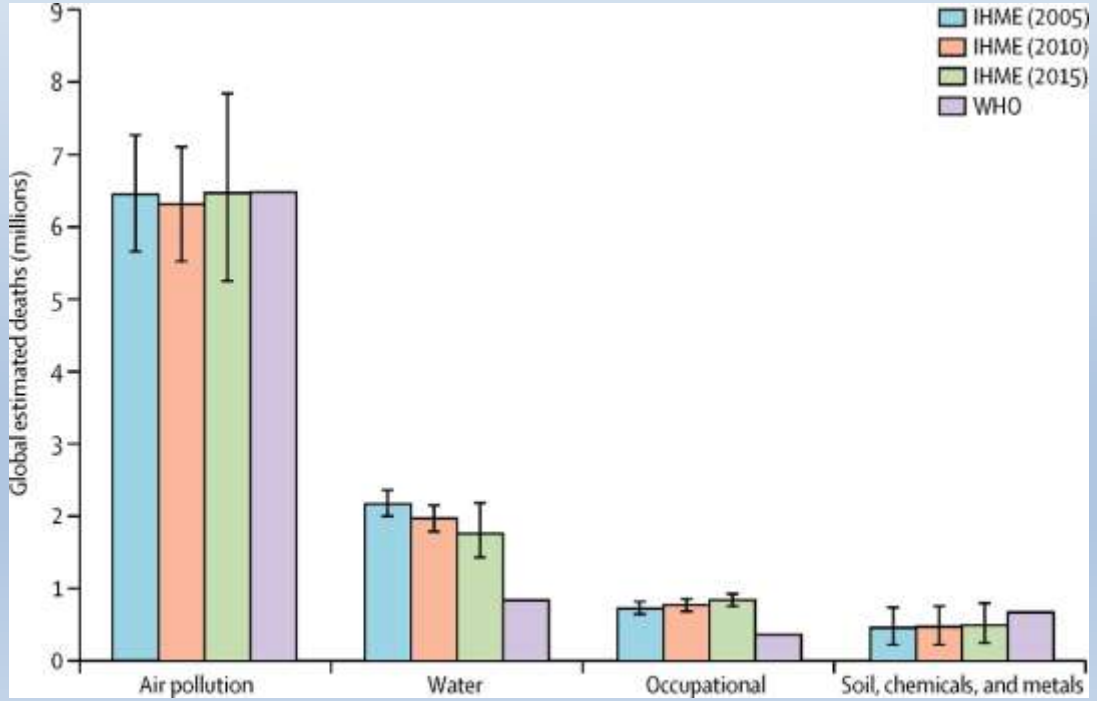
# Air pollution, a global health challenge

- 91% of the worlds population live in areas with unhealthy air
- Urbanization = majority of population now live in cities



# Air pollution, a global health challenge

- 6.5 million premature deaths annually
- Luftforurensing i Norge ca 1200 for tidlige dødsfall (røyking ca 6000)*
- Biggest environmental risk factor
- Ambient (outdoor) and household air pollution both contributes



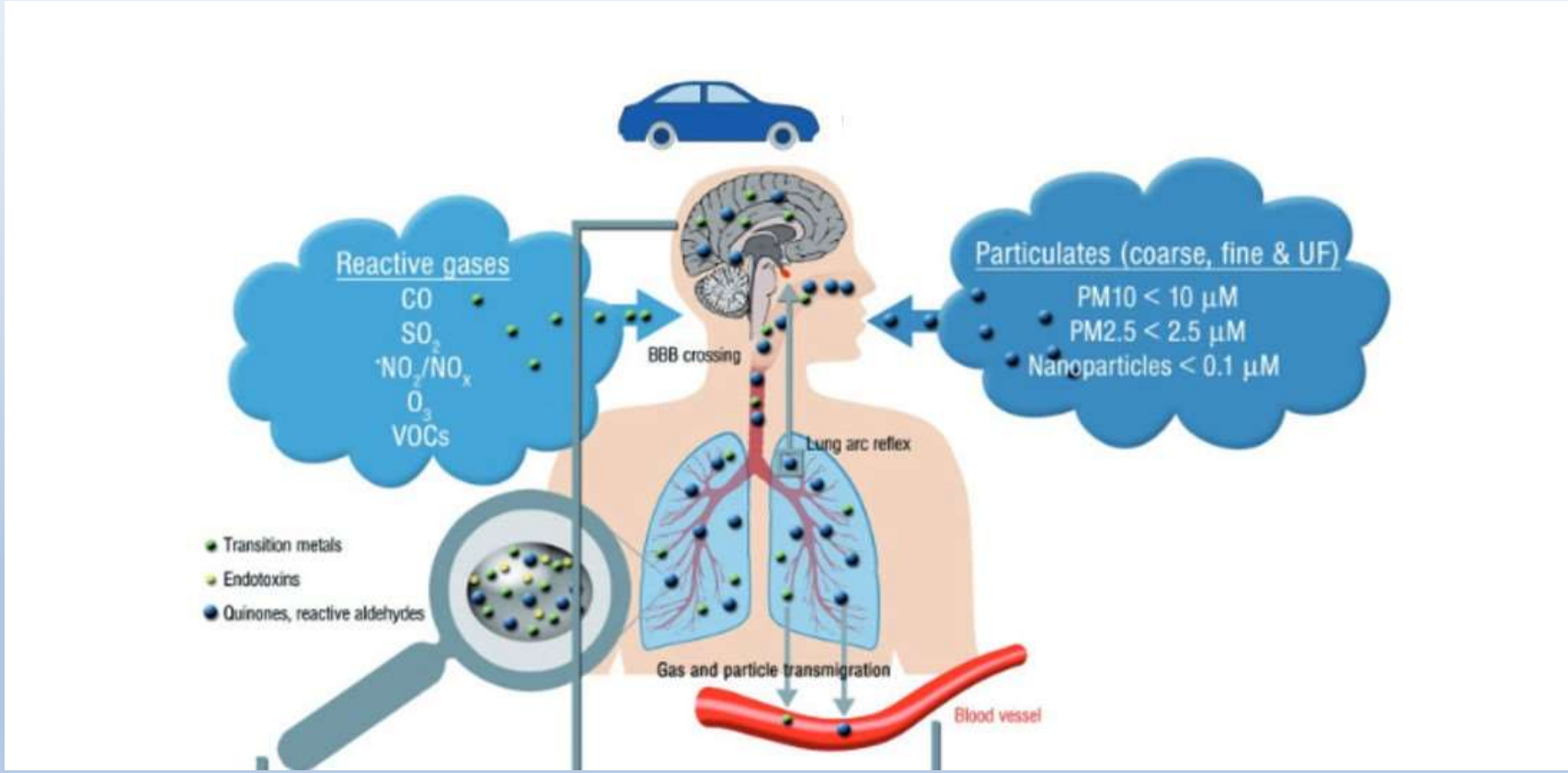

*Sykdomsbyrden i Norge 2016
Øverland et al 2018

Landrigan et al 2017

# Diesel exhaust contributes majorly to ambient air pollution

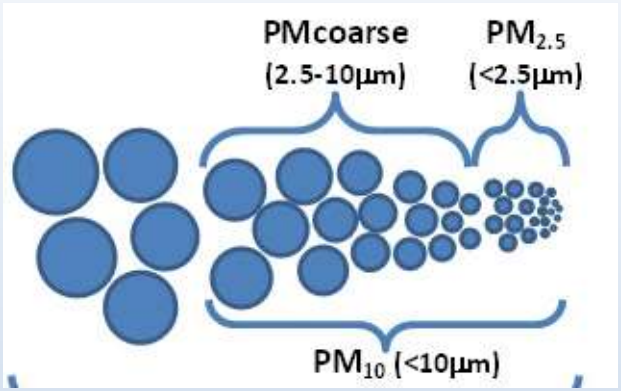

# Diesel exhaust: Particulates and gases

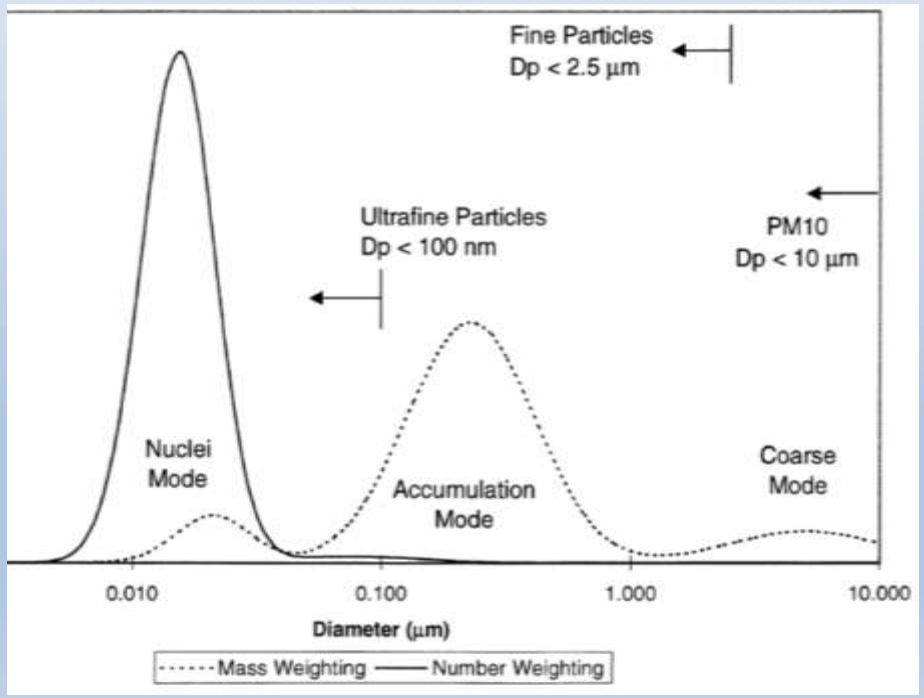
- Particulates and reactive gases linked to vascular effects
- Focus on particulates




Modified from Münzel et al 2018

# Particulate matter (PM)




Kittelson 1998

PM measured according to size
- $PM_{10}$ (<10 µm): thoracic PM
- $PM_{10-2.5}$ (10 -2.5 µm): coarse PM
- $PM_{2.5}$ (<2.5 µm): fine PM
- $PM_{0.1}$ (<0.1 µm): ultrafine PM (UFP)

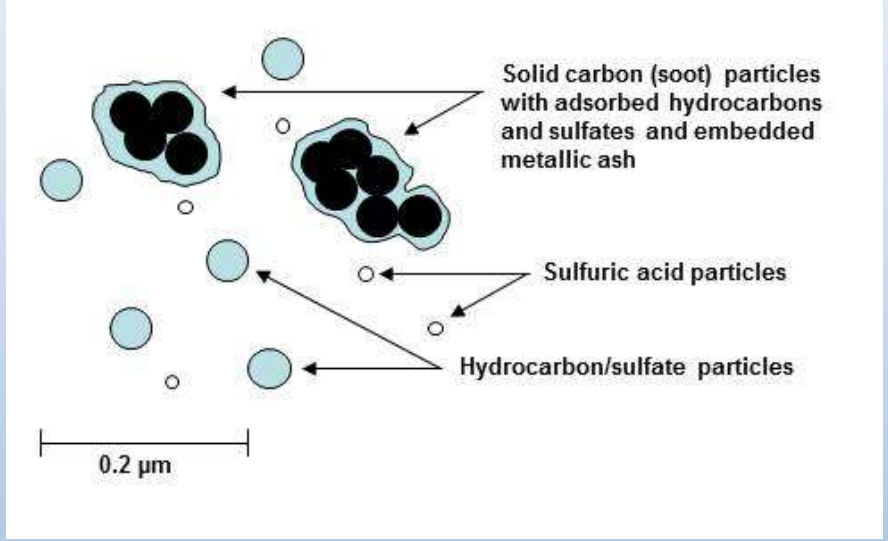
$PM_{2.5}$ – combustion
cardiovascular disease (CVD)

Diesel exhaust particles (DEP)
predominantly fine and ultrafine PM

# Diesel exhaust particles (DEP)

- Carbon core
- Adhered components: organic chemicals (**DEP-OC**) and metals

- Smallest particles most OC 50% of total mass
- Composition depends on temperature, fuel, engine type



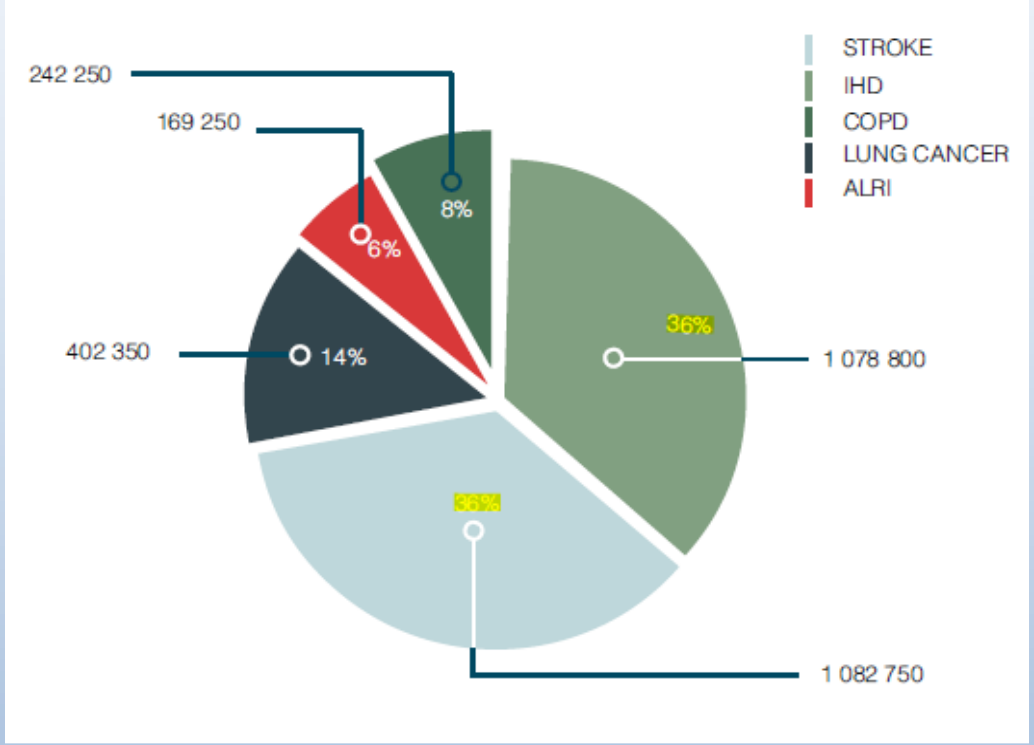

Øvrevik et al

# Deaths related to ambient air pollution
# Primarily caused by cardio vascular diseases (CVD)

Premature deaths attributed to ambient air pollution primarily caused by:

- Ischaemic heart disease (IHD)
- Stroke

Even low levels of $PM_{2.5}$ associated with CVD outcomes (<10 µg/m$^3$)

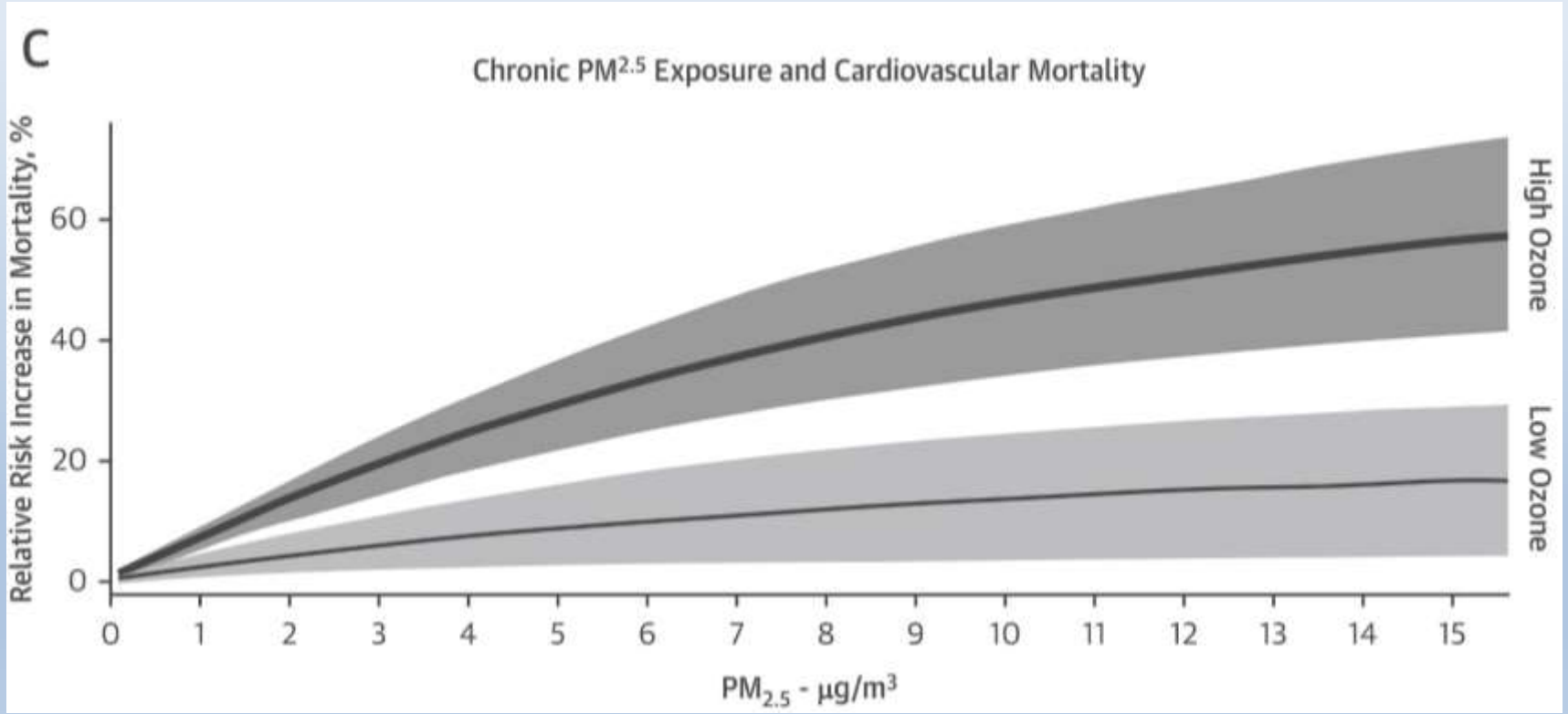



WHO 2014

Lung cancer, chronic obstructive pulmonary disease (COPD) and acute lower respiratory illness (ALRI) including asthma exacerbation

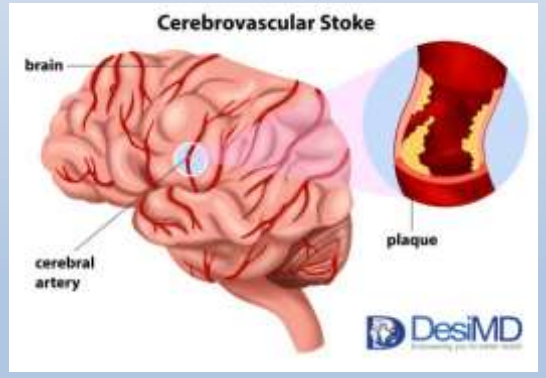
# Multiple exposures may interact

- Air pollution a complex mixture of gases and particles
- These toxicants may have additive or synergistic effects
- Ozone gas potentiates the effect of fine particulates substantially




*Rajagopalan et al 2018*

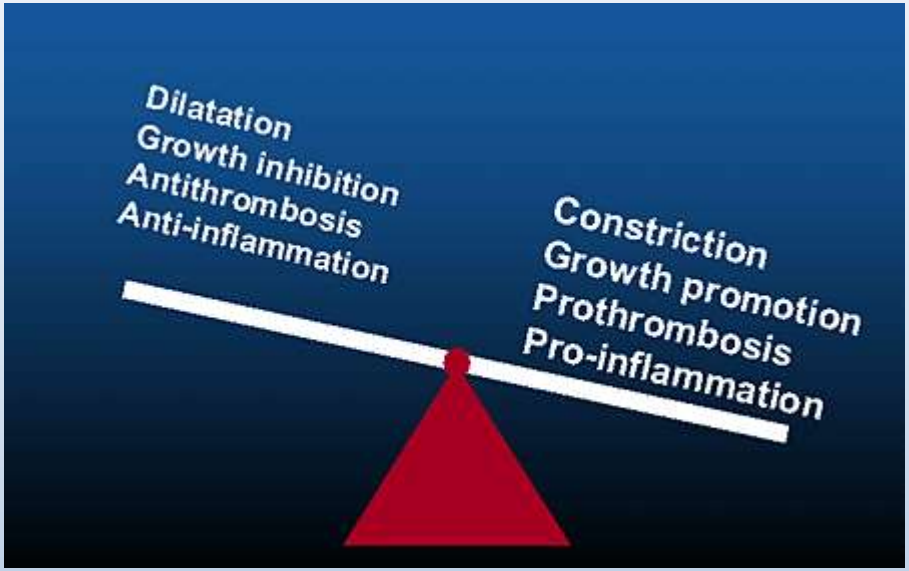
# Cardiovascular disease (CVD)

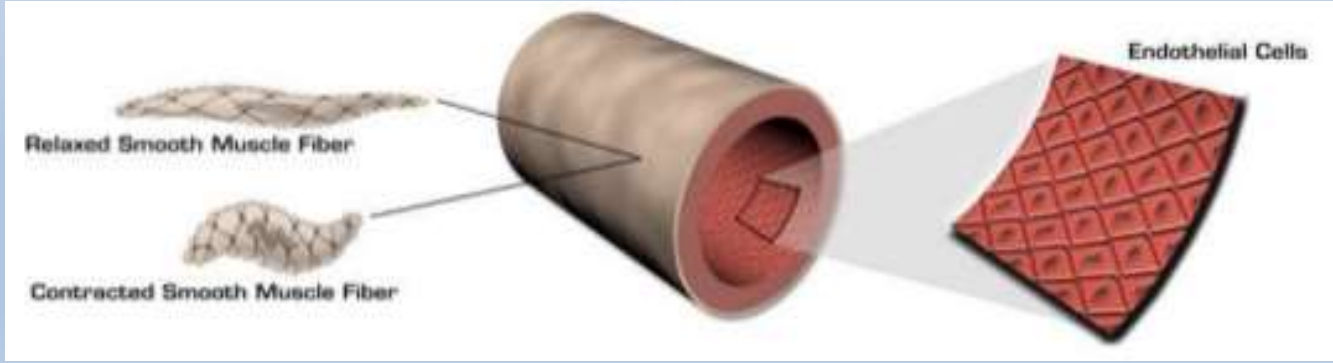



- Ischaemic heart disease (IHD) and stroke largely caused by vascular pathology
- Endothelial dysfunction – initial event
- Blood pressure increase
- Atherosclerosis – lipid plaques due to inflammation in vascular wall
- Systemic inflammation aggravate atherosclerosis inflammatory diseases (rheumatoid arthritis) increased risk of CVD
- Plaque destabilization and rupture – manifest disease

# Endotelial dysfunksjon

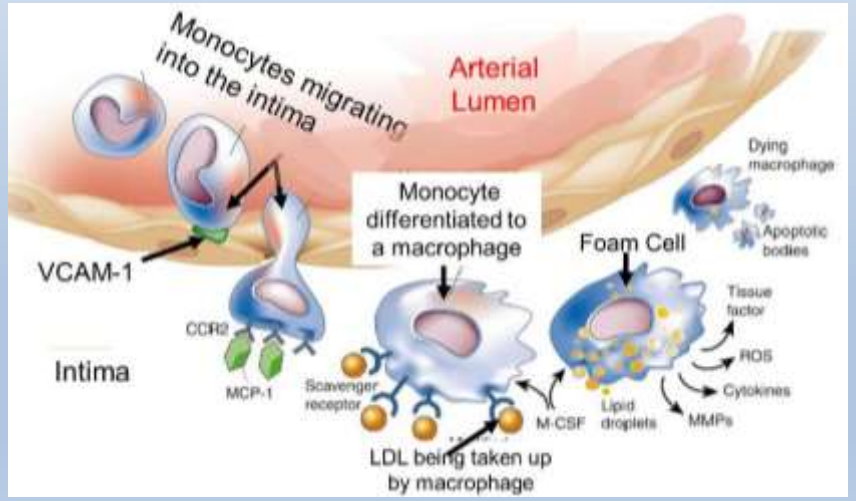
**Normal function** **Dysfunction**



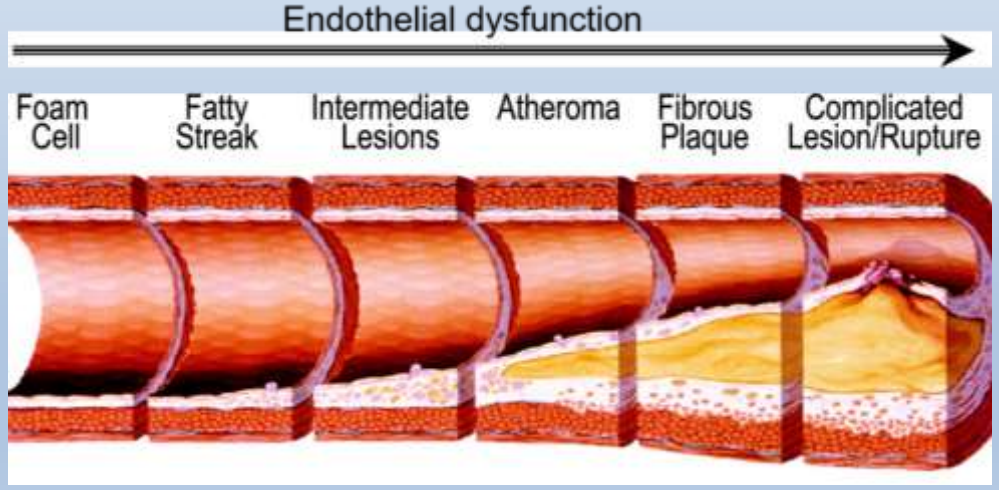



# Åreforkalkning (Aterosklerose)

- Betennelse i karveggen tiltrekker forsvarsceller (monocytter)
- Opphopning av disse cellene og fett i karveggen => Åreforkalkning
- Stabile plaque med bindevev => Angina pectoris
- Vedvarende betennelse => Plaque ruptur => Hjerteinfarkt og slag




*Modified from Libby 2002*




*Modified from Stary et al 1995*

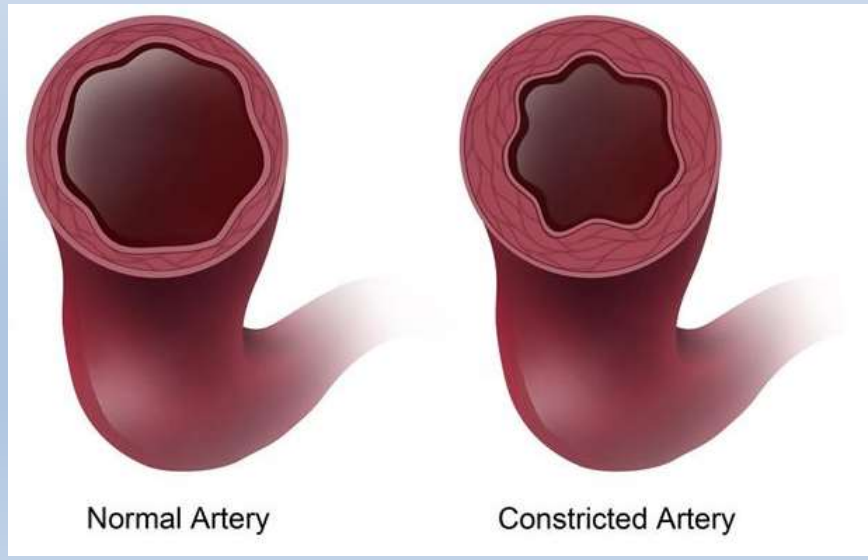
# Blood pressure

Blood pressure = cardiac output (CO) * systemic vascular resistance (SVR)

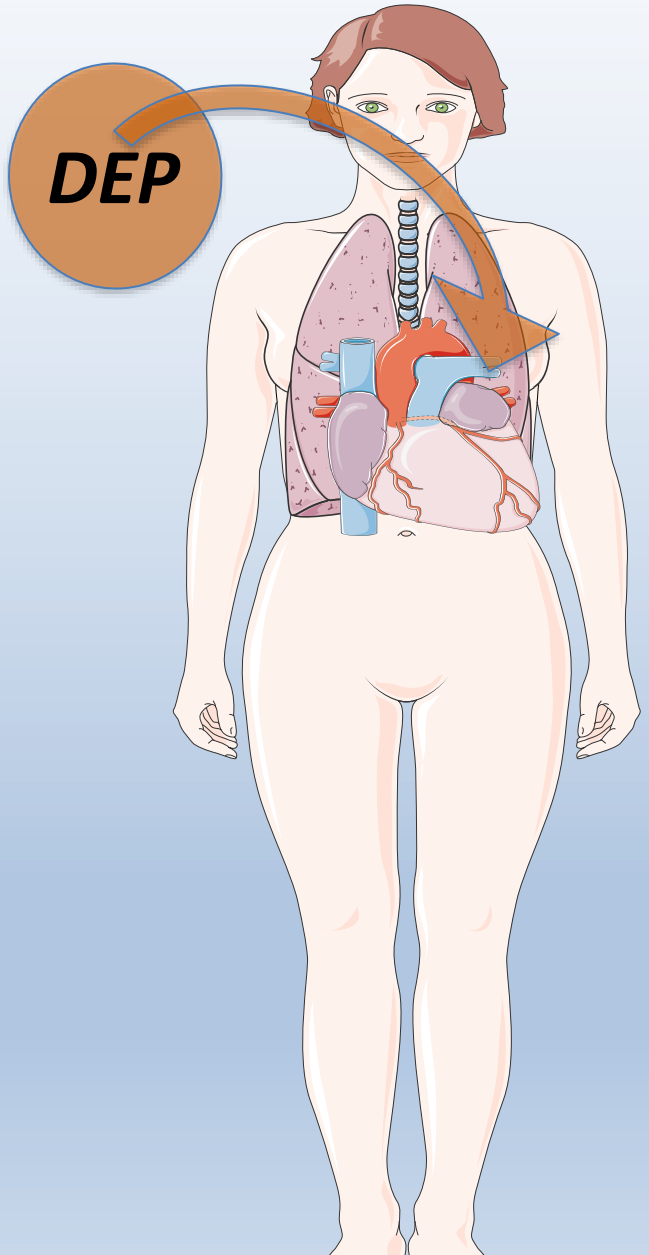
CO = heart rate * stroke volume

SVR = resistance of blood vessels

Endothelial dysfunction => Vasoconstriction => increased SVR



# How do inhaled DEP contribute to CVD?




1. Pulmonary inflammation systemic spill-over
2. Autonomic nervous system dysregulation

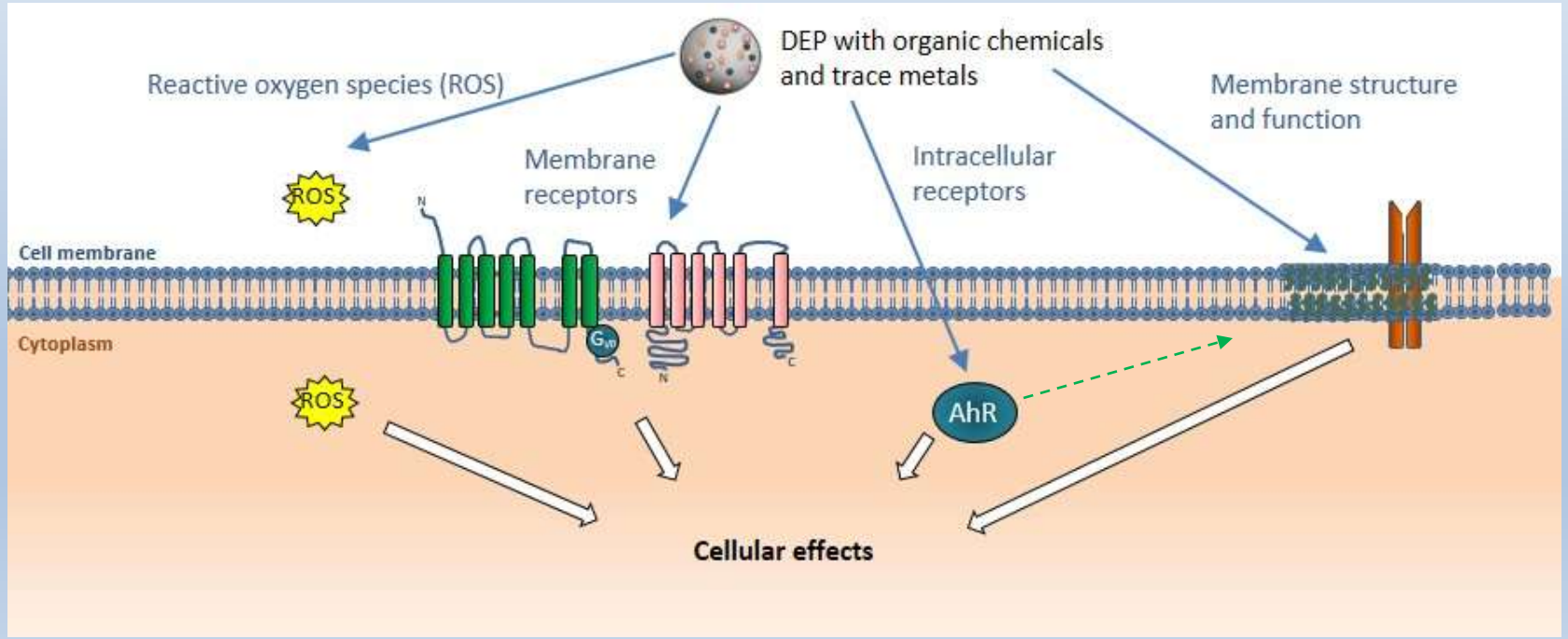
Indirect effects

3. Direct vascular effects of particles or adhered constituents

# Cellular effects of DEP

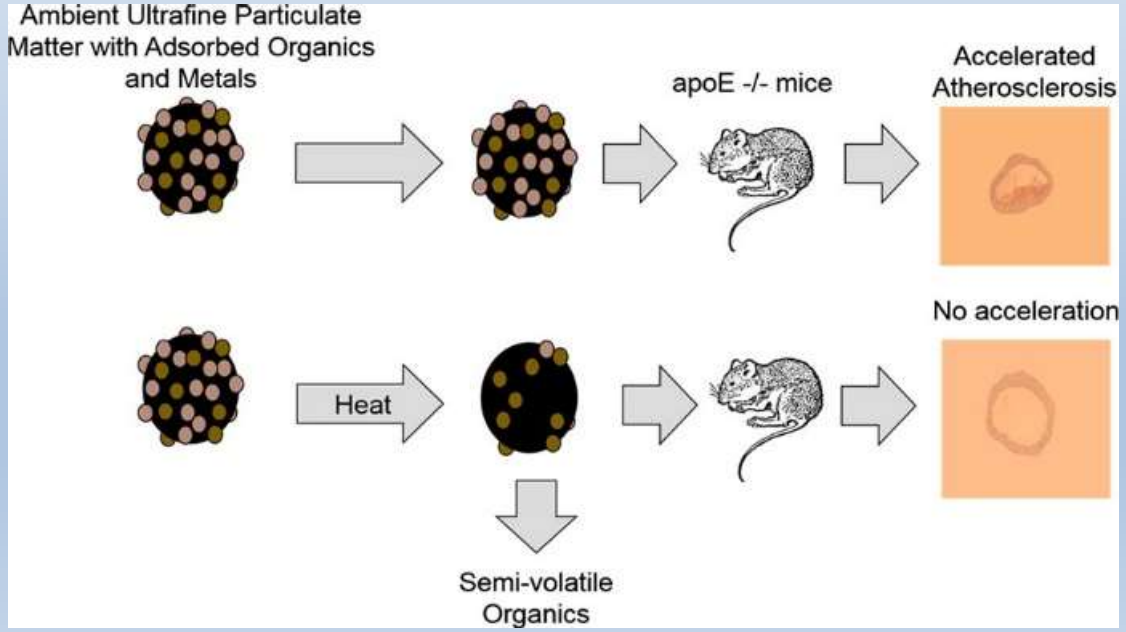
DEP and adhered components may trigger cellular effects via:

1. Reactive oxygen species (ROS)
2. Membrane receptors, transporters and channels
3. Intracellular receptors including aryl hydrocarbon receptor (AhR)
4. Altered membrane structure and function
5. Genotoxic effects – DNA damage



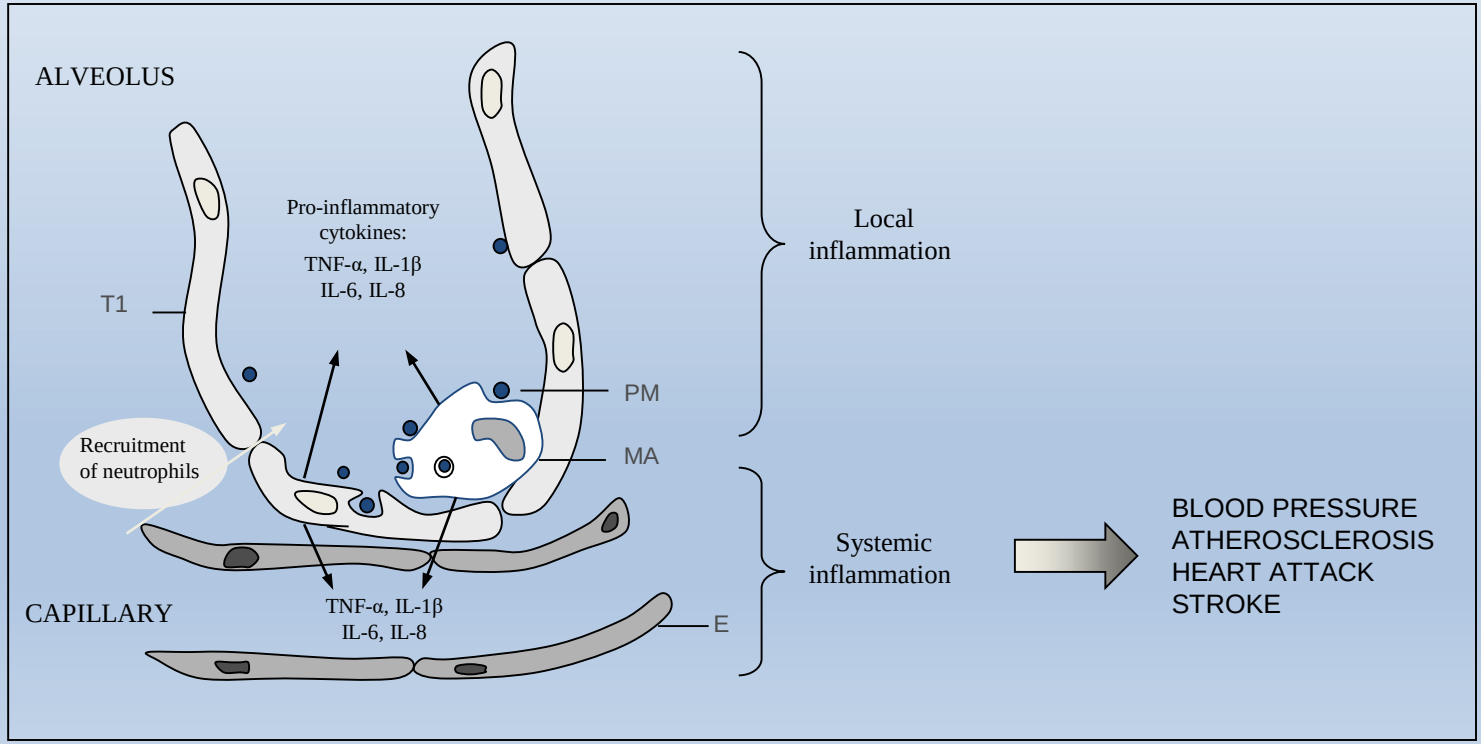
# Organic chemicals (OC) – central for vascular effects

- Keebaugh et al. exposed mice to concentrated ultrafine ambient particles (CAP) – accelerated atherosclerosis
- CAP thermally denuded of semi-volatile organic chemicals
- CAP without OC did not accelerate atherosclerosis development
- Bonvalot 2001: DEP-OC main drivers of epithelial inflammation
- We recently found that DEP-OC trigger endothelial cell inflammation



# 1. Pulmonary inflammation: systemic spill-over

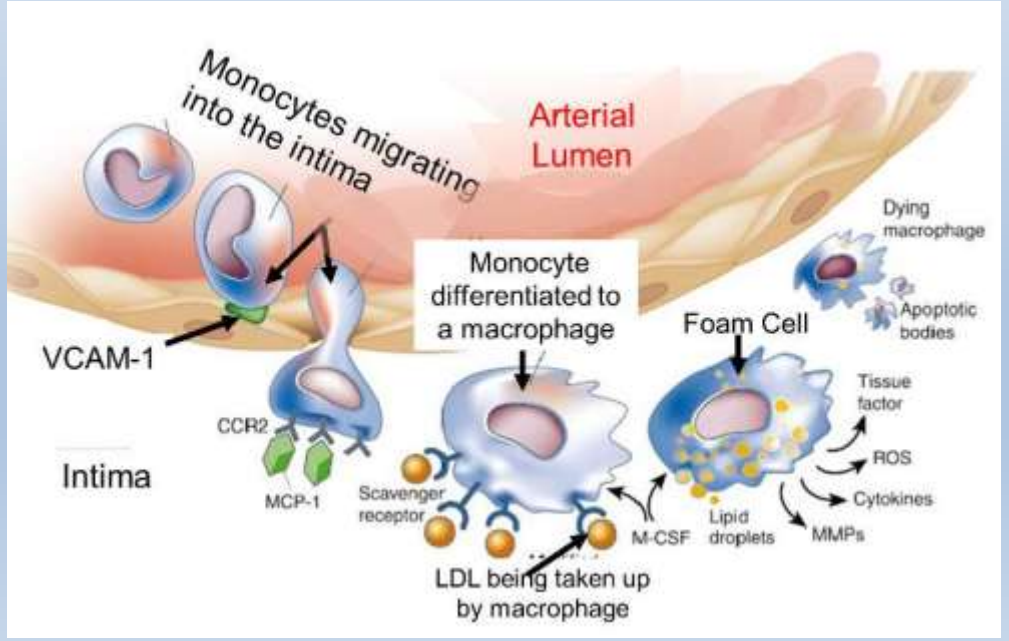
- Epithelial cells and macrophages (MA) exposed to DEP
- Oxidative stress and pro-inflammatory cytokines
- Recruitment of immune-cells (neutrophils)
- Cytokines “spill over” to circulation
- Systemic oxidative stress and inflammation promotes endothelial dysfunction – BP increase and atherosclerosis




*Bølling 2008*

# Systemic spill-over and atherosclerosis

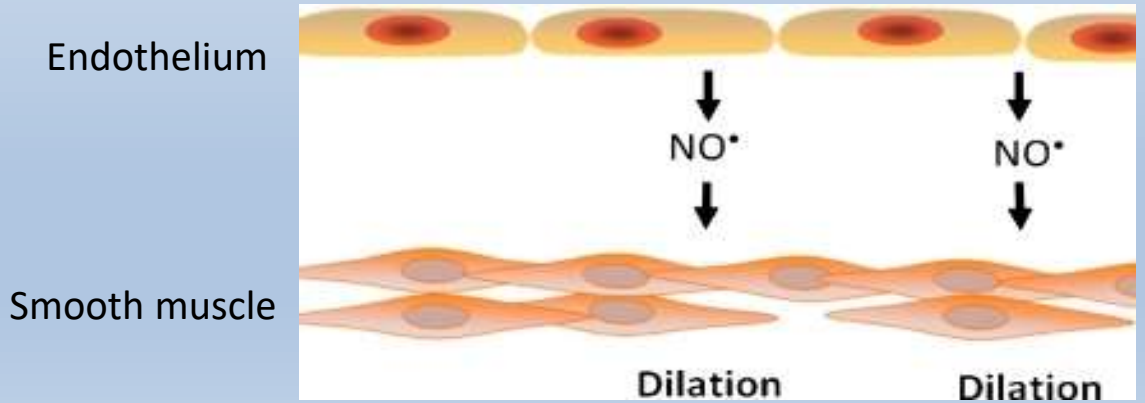
- Systemic inflammation and reactive oxygen species (ROS) trigger endothelial dysfunction – increased adhesion (VCAM1)
- Monocytes adhere to endothelium and enter vessel wall (intima)
- Takes up oxidized low density lipoprotein => Foam cells
- Vascular inflammation => atherosclerotic plaque build up, rupture




*Modified from Libby 2002*

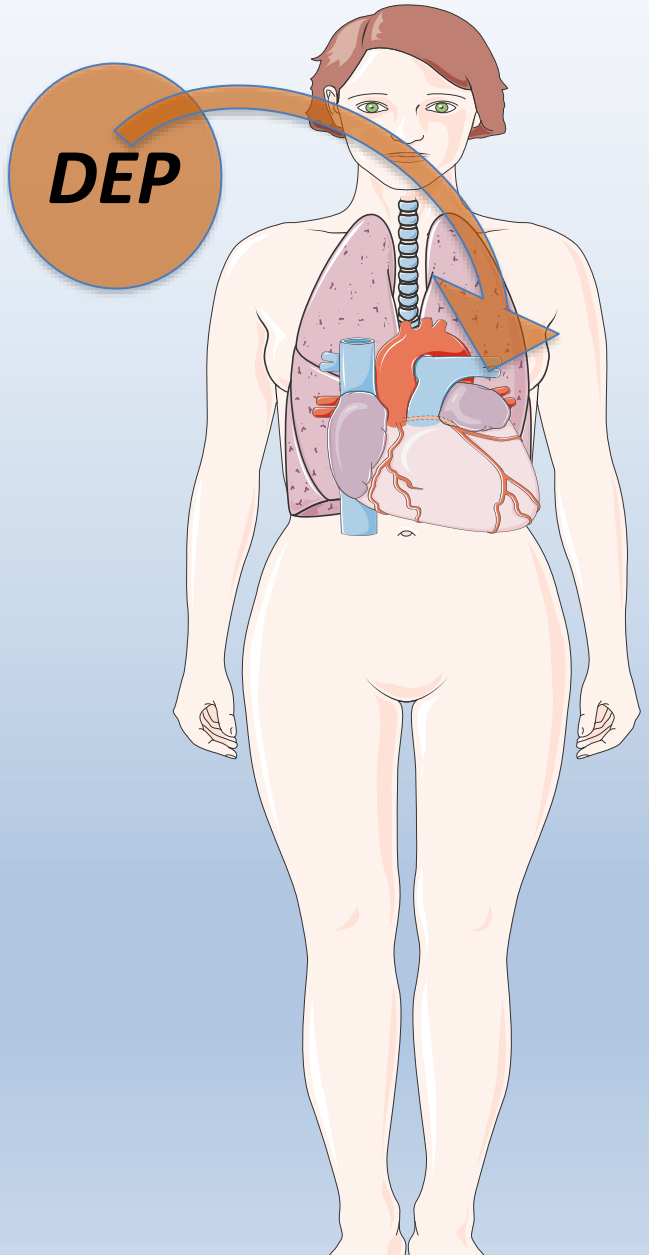
# Systemic spill-over and vasoconstriction

- Endothelial cells regulates vascular tone via nitric-oxide (NO)
- Inflammation and oxidative stress => endothelial dysfunction
- Reactive oxygen species (ROS) may disturb NO signalling
- Increased vascular resistance => elevated blood pressure




*Schelbert 2010*

# How do inhaled DEP contribute to CVD?




1. Pulmonary inflammation systemic spill-over:

   Systemic oxidative stress and inflammation trigger endothelial dysfunction

   Vasoconstriction => elevated BP

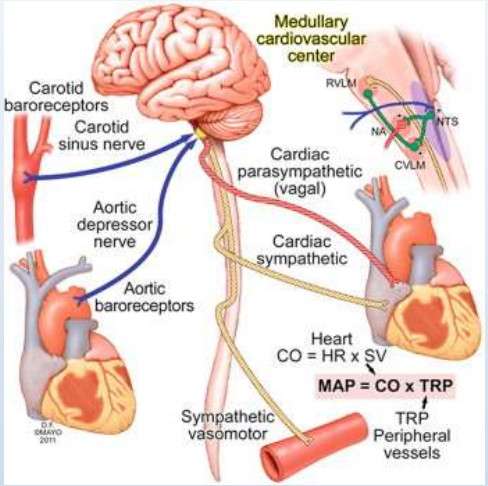
   Vascular inflammation and ROS

   Foam cell accumulation – atherosclerosis
   Plaque rupture and thrombosis

# 2. Autonomic nervous system dysregulation

- Autonomic nervous system (ANS) controls heart and vasculature via sympathetic and parasympathetic

- Changes in Heart rate variability (HRV) indicates sympathetic or parasympathetic dominance

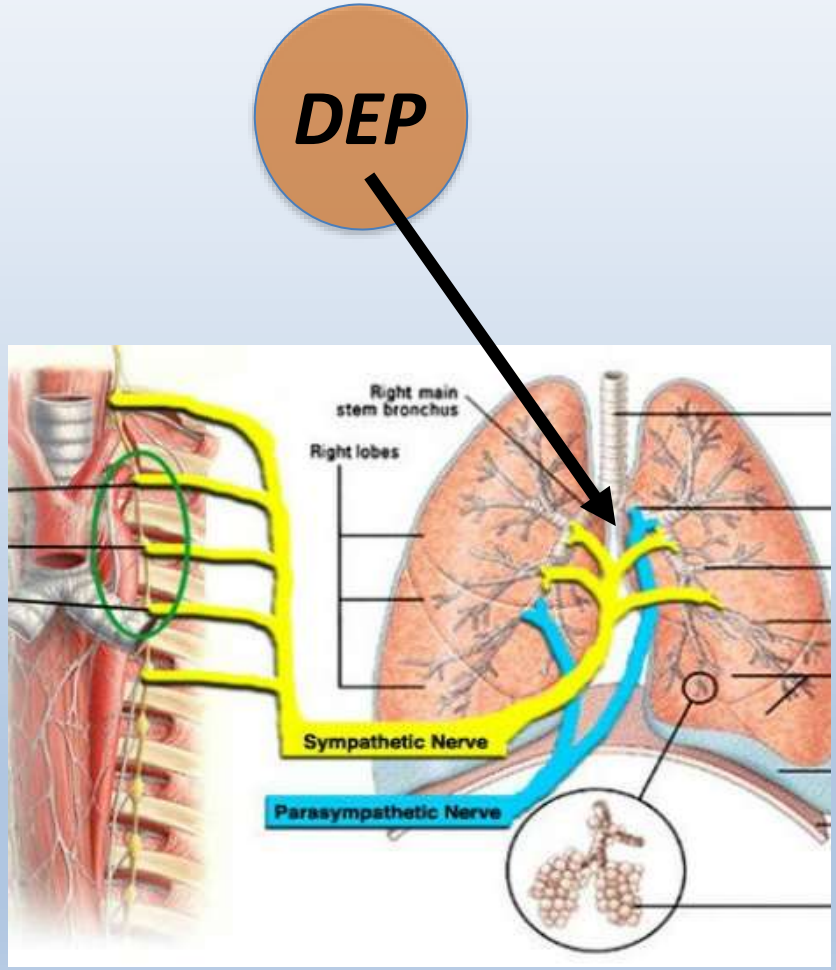



Fight or flight

Rest and digest

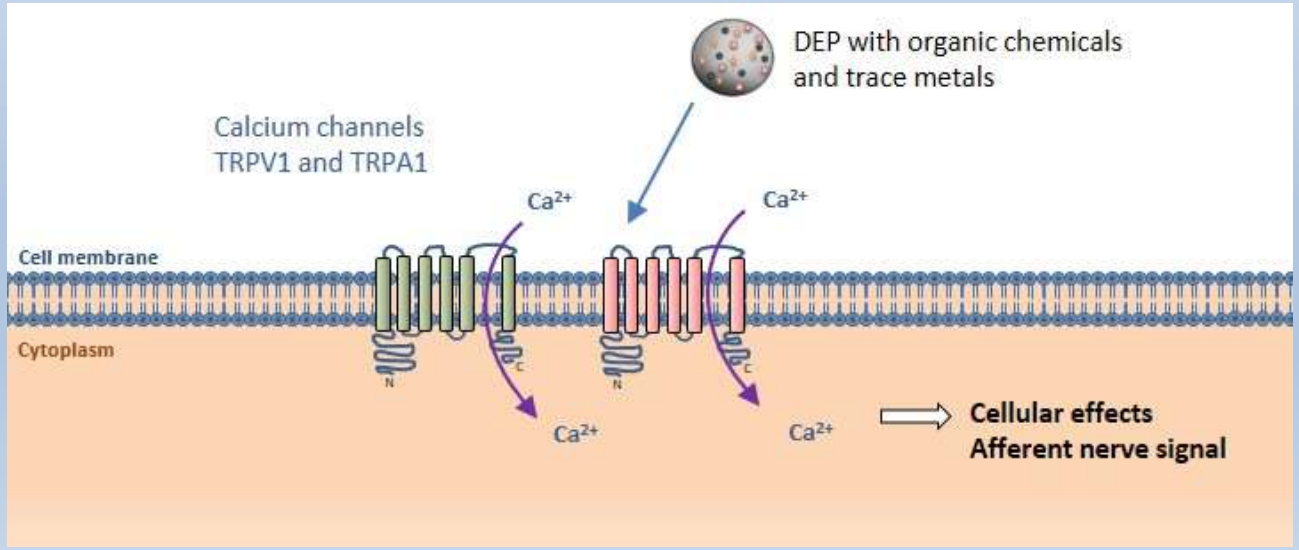
# Particles and nerves

- Inhalation of DEP has been found to decreased HRV
- PM and DEP exposure consistently linked to BP increase
- DEP deposited in lungs affect afferent nerve endings
- May affect autonomic nervous system and thus cardiovascular control



# Cellular effects – calcium channels

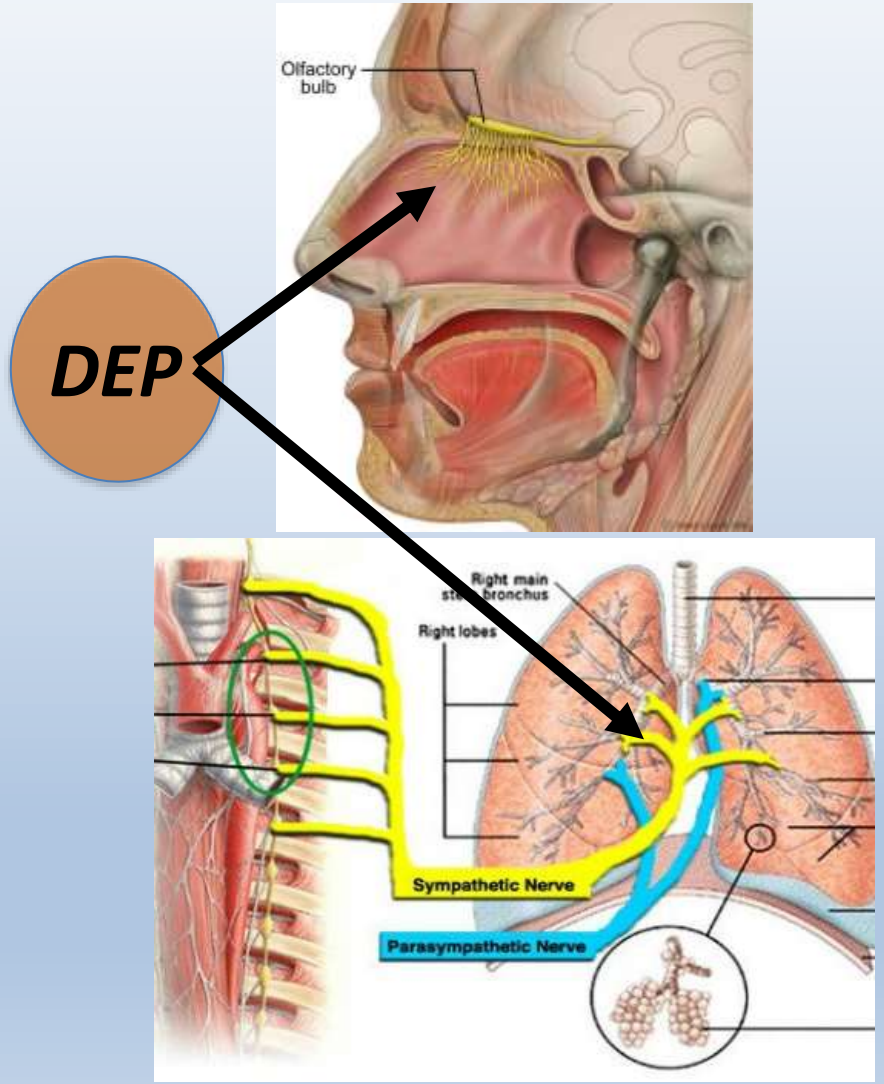
- DEP affects pain sensing C-fibers in airways => ANS dysfunction
- Calcium ($Ca^{2+}$) messenger molecule, low concentration in resting state
- DEP and adhered components interact with membrane bound calcium channels such as transient receptor potential (TRP)
- Increased intracellular calcium trigger afferent nerve signal.
  Calcium signalling implicated in epithelial and endothelial effects of DEP



# Particles and nerves

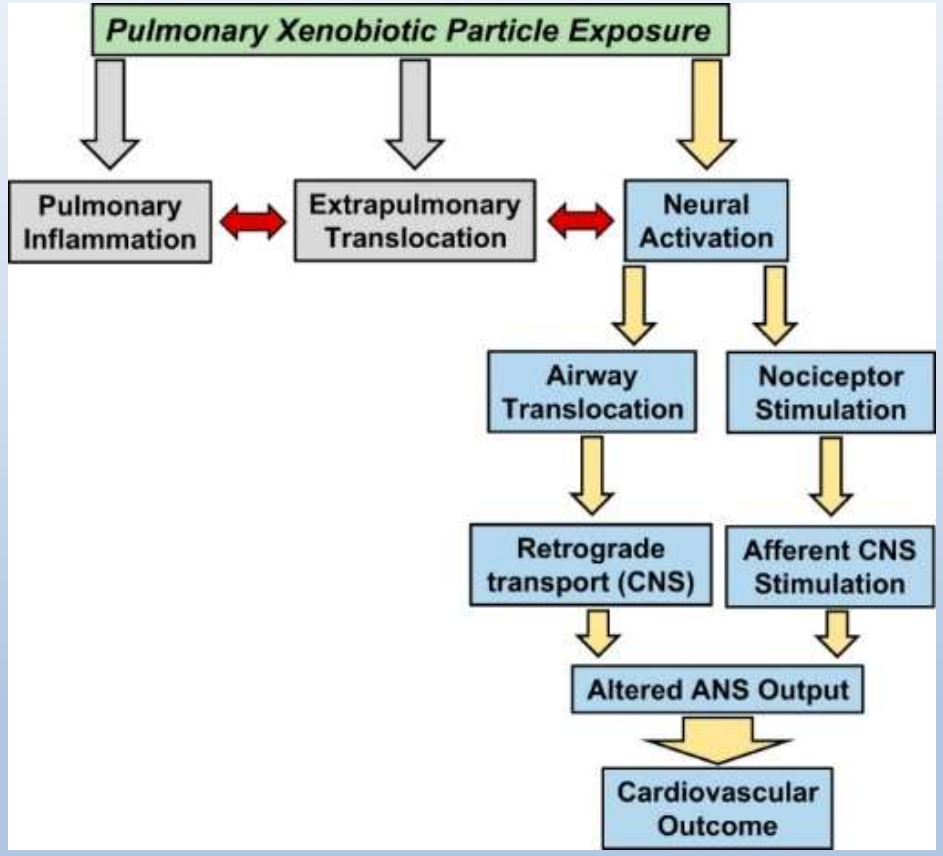
- UFP have been found to translocate to the central nervous system vi the olfactory bulb

- Has been suggested as a potential mechanism whereby DEP may dysregulate autonomic nervous system



# 2. Autonomic nervous system dysregulation

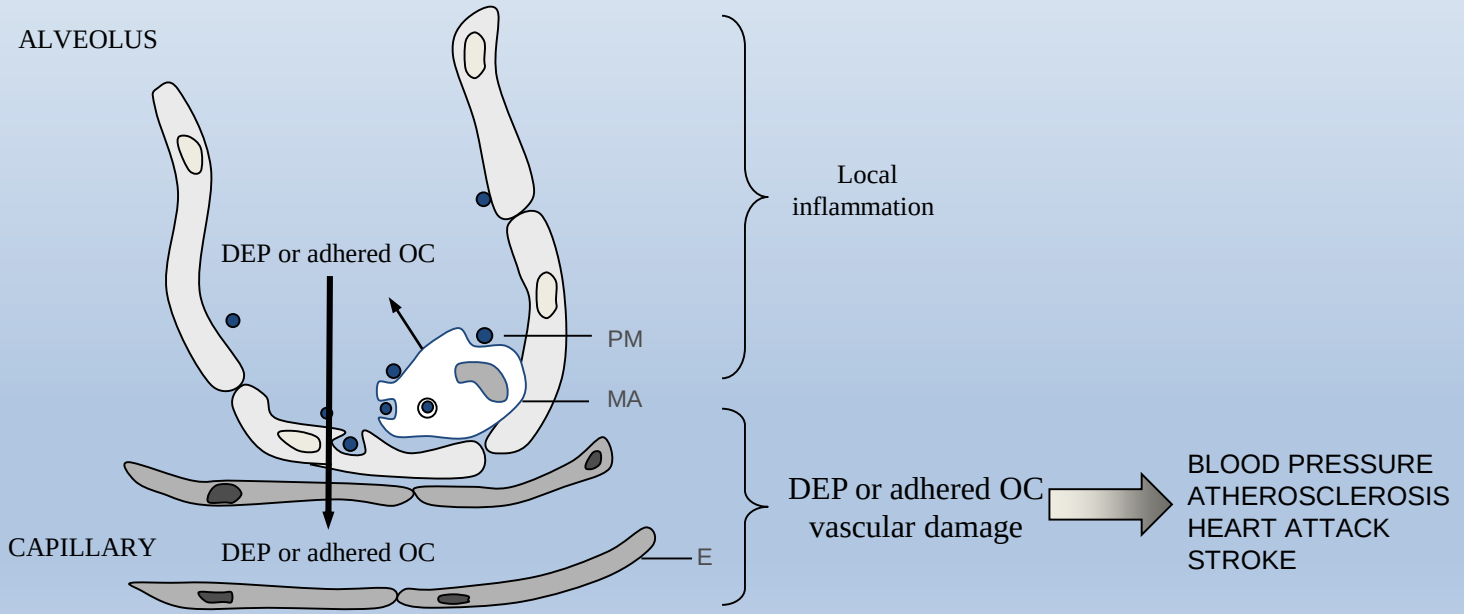
- DEP translocation via olfactory bulb or affects nerve endings via TRPs
- Autonomic nervous system dysregulation affects heart rate, volume and vascular resistance




*Stapelton 2015*

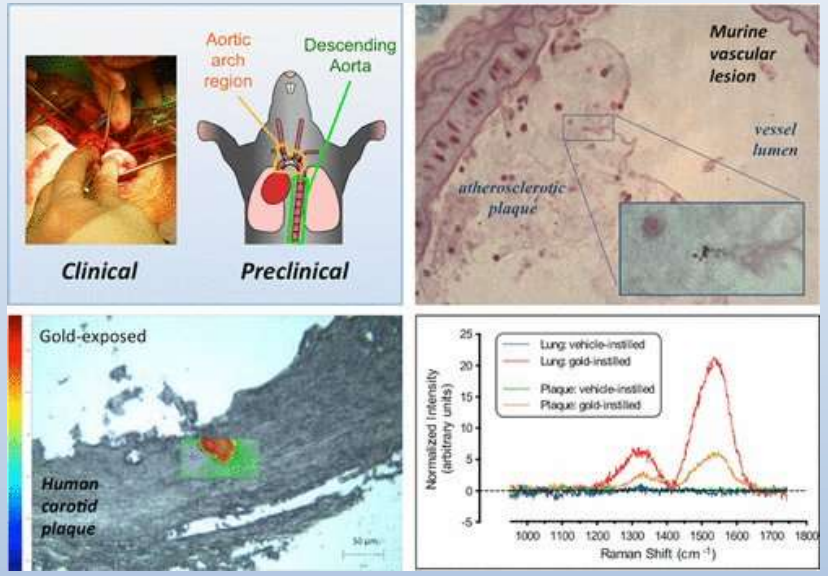
# 3. Direct vascular effects of particles or adhered constituents

- DEP or adhered constituents (organic chemicals (OC) and soluble metals) may penetrate airway epithelium and enter bloodstream
- May thus affect vasculature directly
- Endothelial dysfunction, vasoconstriction and atherosclerosis



# Direct vascular effects: translocation of ultrafine particles (UFPs)

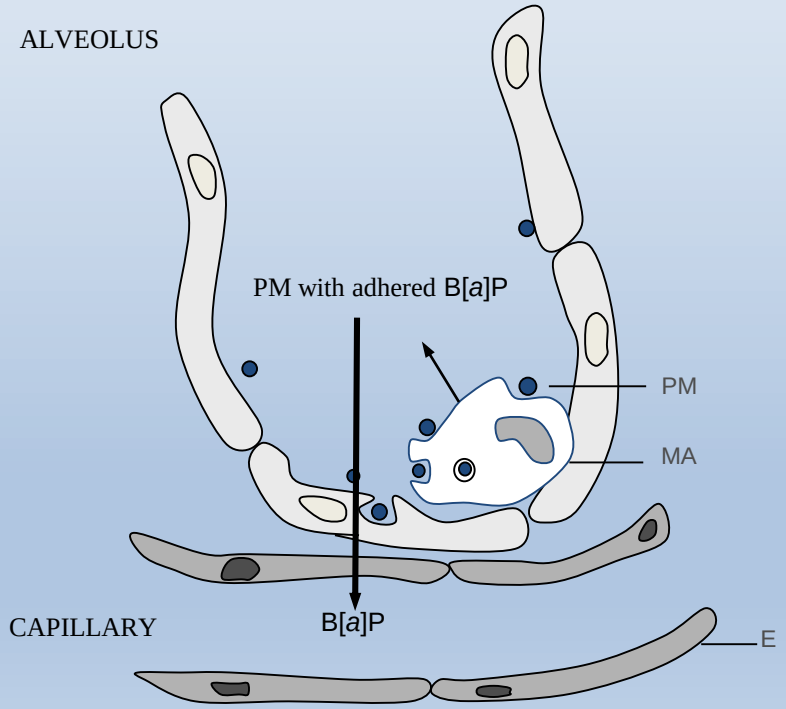
- Miller et al. exposed healthy humans to gold nano-particles; nano-particles in blood and urine 15 min, 24 h and 3 months post exposure
- Exposed mice; nano-particles accumulated in atherosclerotic plaques
- Patients at risk of stroke; gold nano-particles detected in carotid plaques surgically removed (endarterectomy)
- Amount of nano-particles that translocate is relatively small (0,02%)

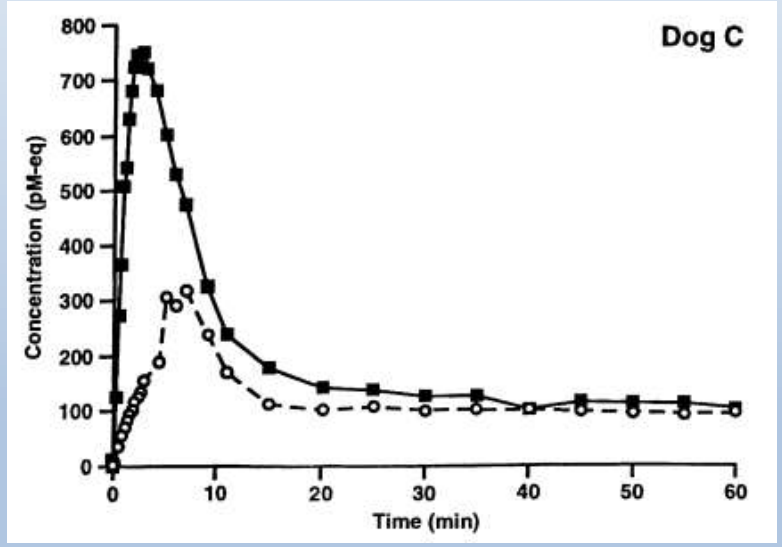



*Miller et al 2017*

# Direct vascular effects: DEP-OC

- Polycyclic aromatic hydrocarbons (PAH) such as benzo[*a*]pyrene (B[*a*]P), important group of DEP-OC
- Gerde et al. (2001) exposed dogs to particles with B[*a*]P
- 30 % of B[*a*]P entered blood within minutes



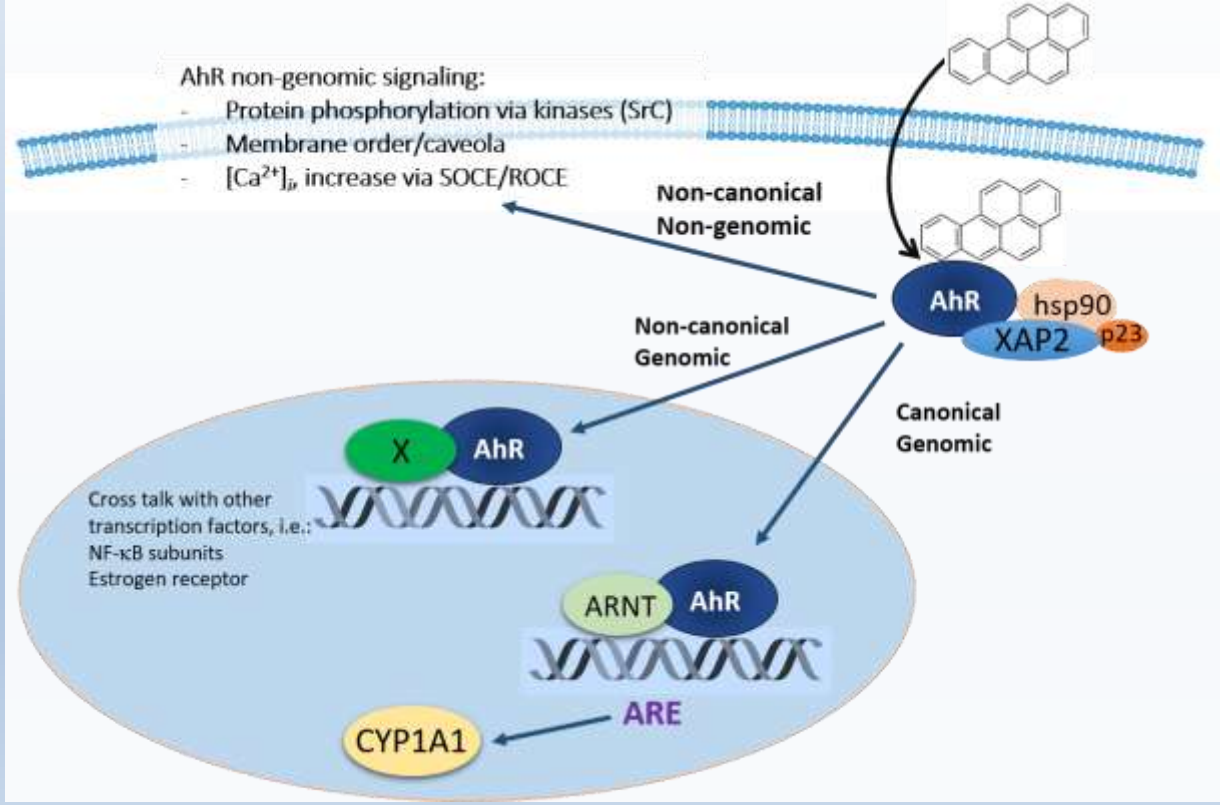




Aorta

Vena cava

# Aryl hydrocarbon receptor (AhR)

Intracellular receptor involved in many physiological functions
Important role in xenobiotic metabolism (getting rid of toxicants)
AhR may also mediate toxic effects of xenobiotics
DEP-OC such as polycyclic aromatic hydrocarbons (PAHs) and dioxins bind and activate AhR



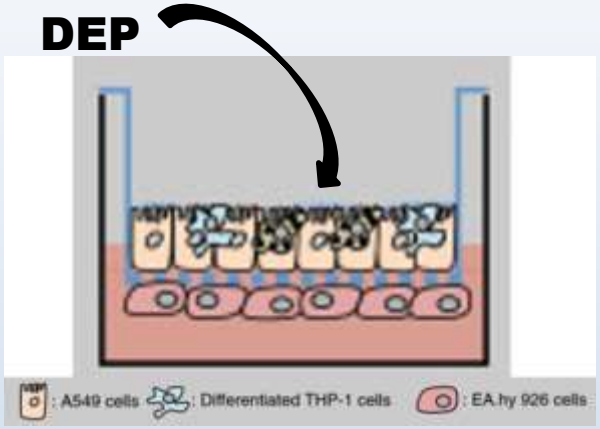
# Translocation and endothelial effects of DEP-OC

We exposed a 3D tri-culture mimicking alveolus to DEP with high OC content

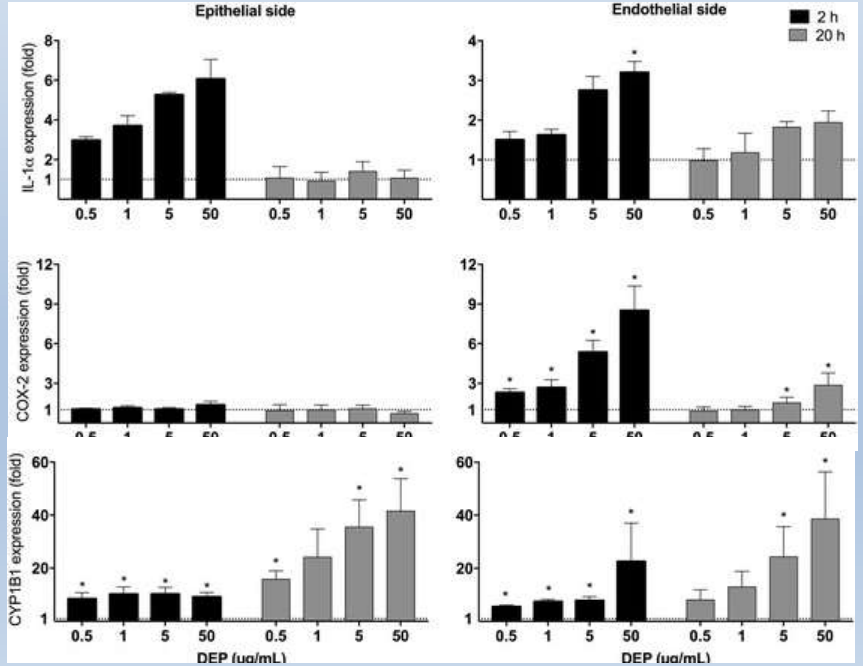
This induced inflammation-associated and AhR-regulated genes in endothelial cells

Clear effects on AhR-regulated genes indicates the presence of AhR-ligands in endothelial cells.

DEP-OC seemed to translocate through alveolar cells and affect endothelial cells directly

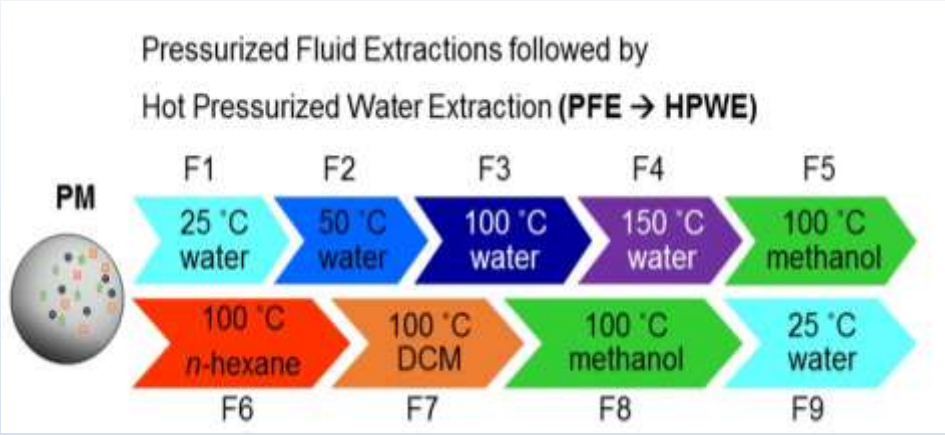


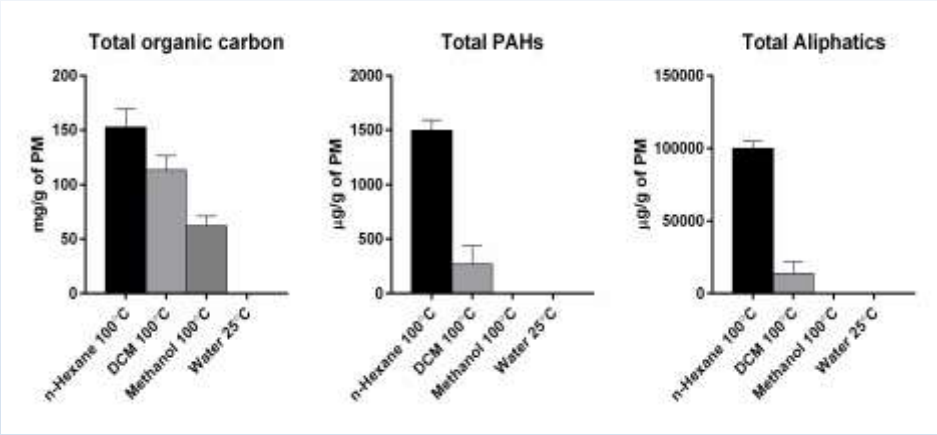

Modified from Klein et al 2013

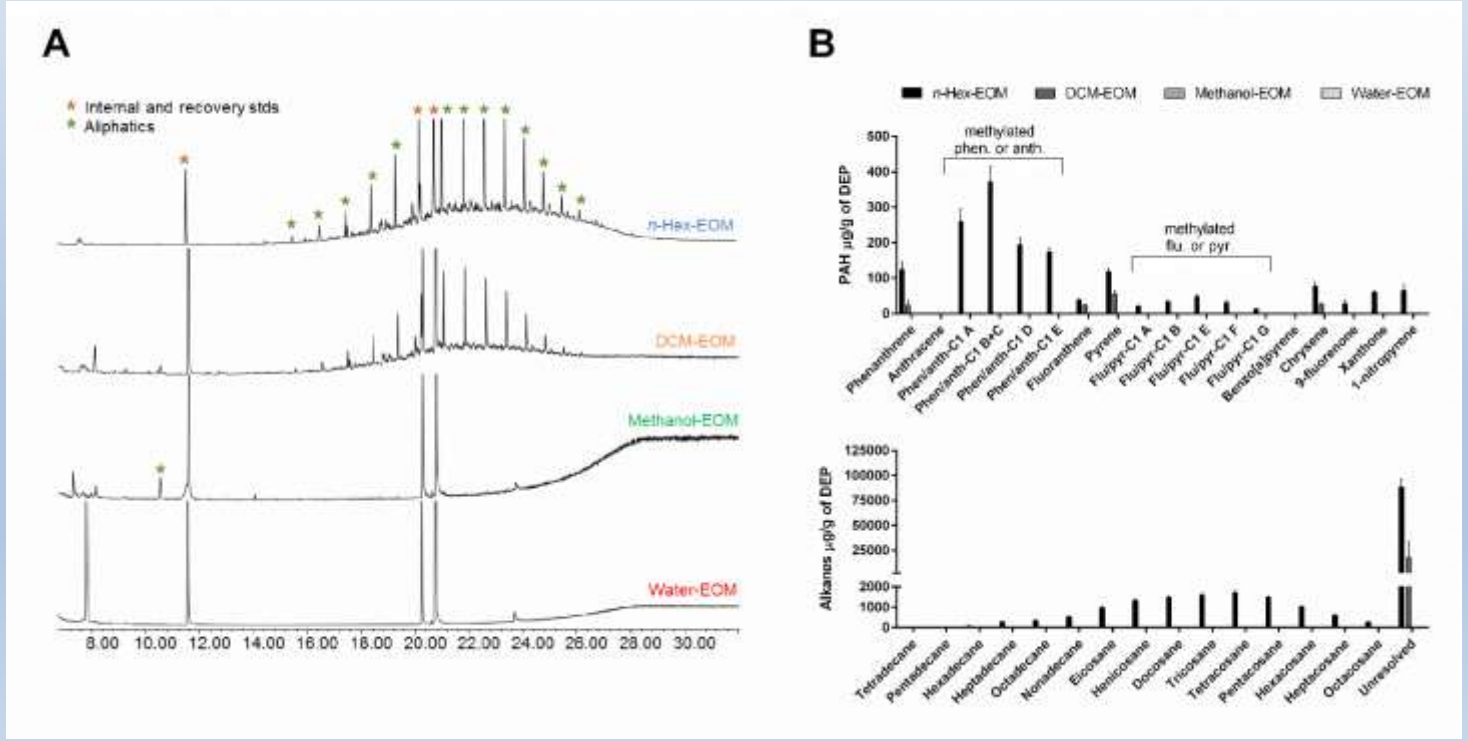



Brinchmann et al. 2018

# DEP-OC extracted and analysed in North Dakota USA



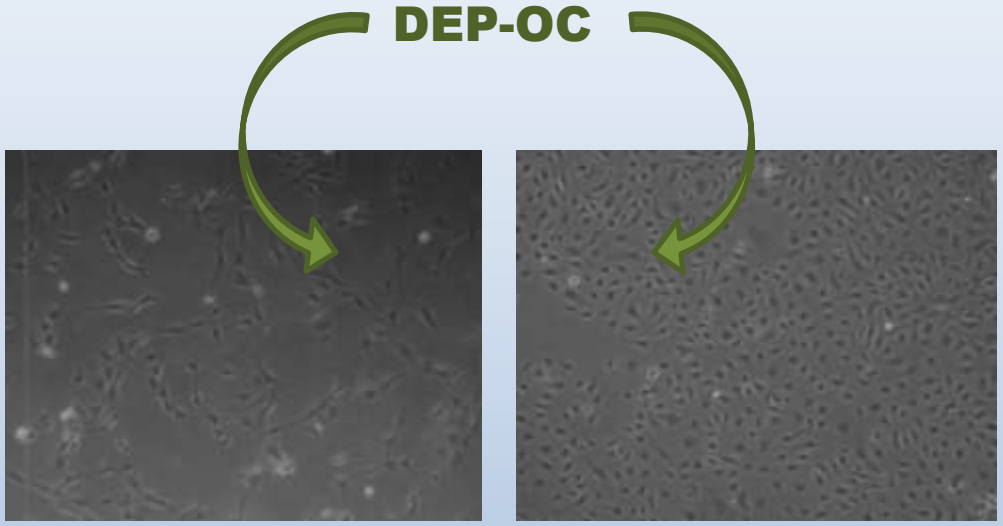
# Lipophilic DEP-OC induced inflammation-associated and AhR-regulated genes in endothelial cells

Endothelial cell line (HMEC-1) and primary human endothelial cells (PHEC) from 4 donors exposed to extracted DEP-OC

Lipophilic DEP-OC triggered pro-inflammatory responses and AhR regulated genes

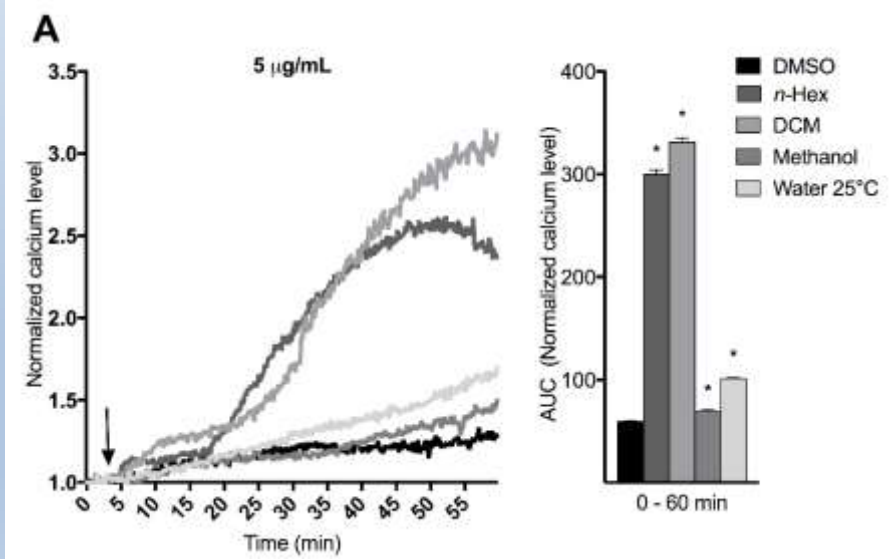
Effects in PHEC at low concentrations

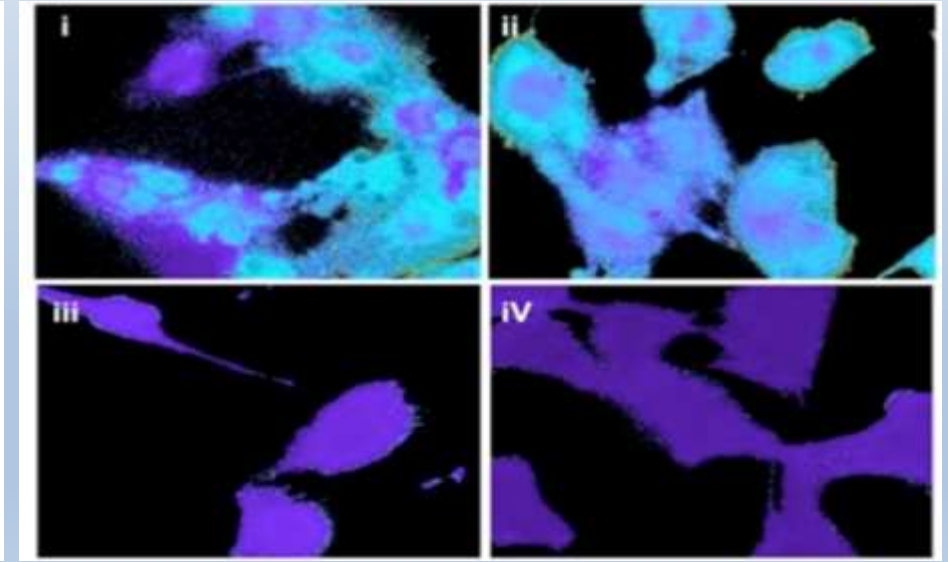
Inhibitors of AhR, PAR-2 as well as antioxidant treatment reduced some of the responses significantly



# Lipophilic DEP-OC increased intracellular calcium in endothelial cells

- Intracellular calcium: a central signalling molecule kept low in resting state
- Calcium increase represents cell activation
- Lipophilic DEP-OC increased intracellular calcium in HMEC-1
- Calcium signal inhibited by blocking AhR



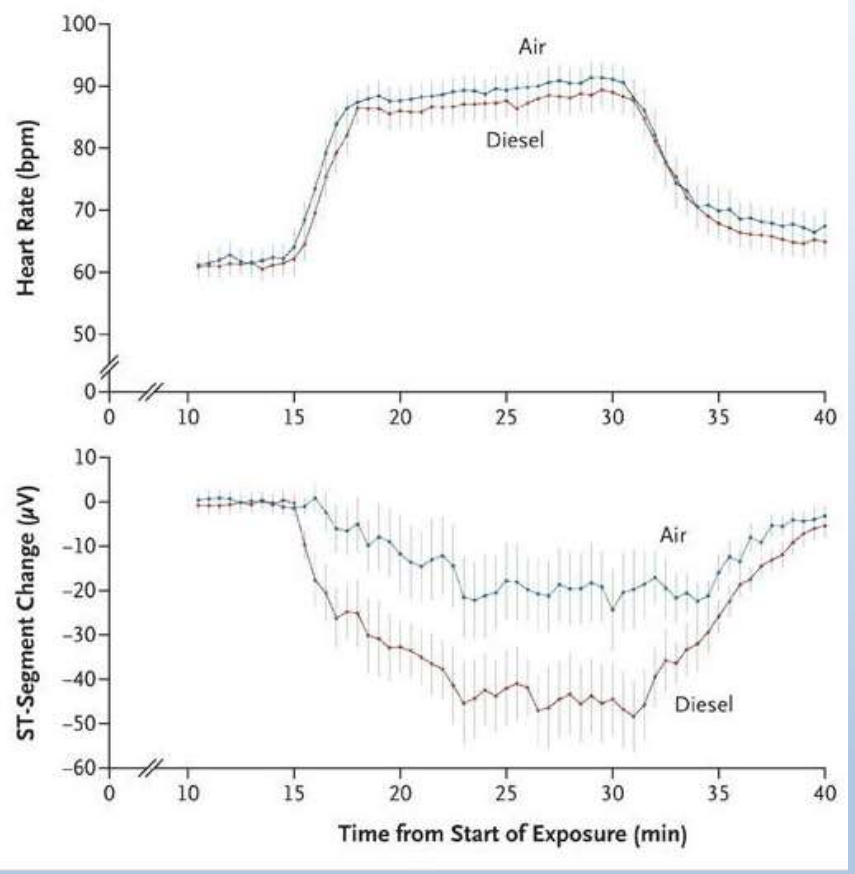



# Human exposure study, final example

- ST-segment depression indicates myocardial ischaemia

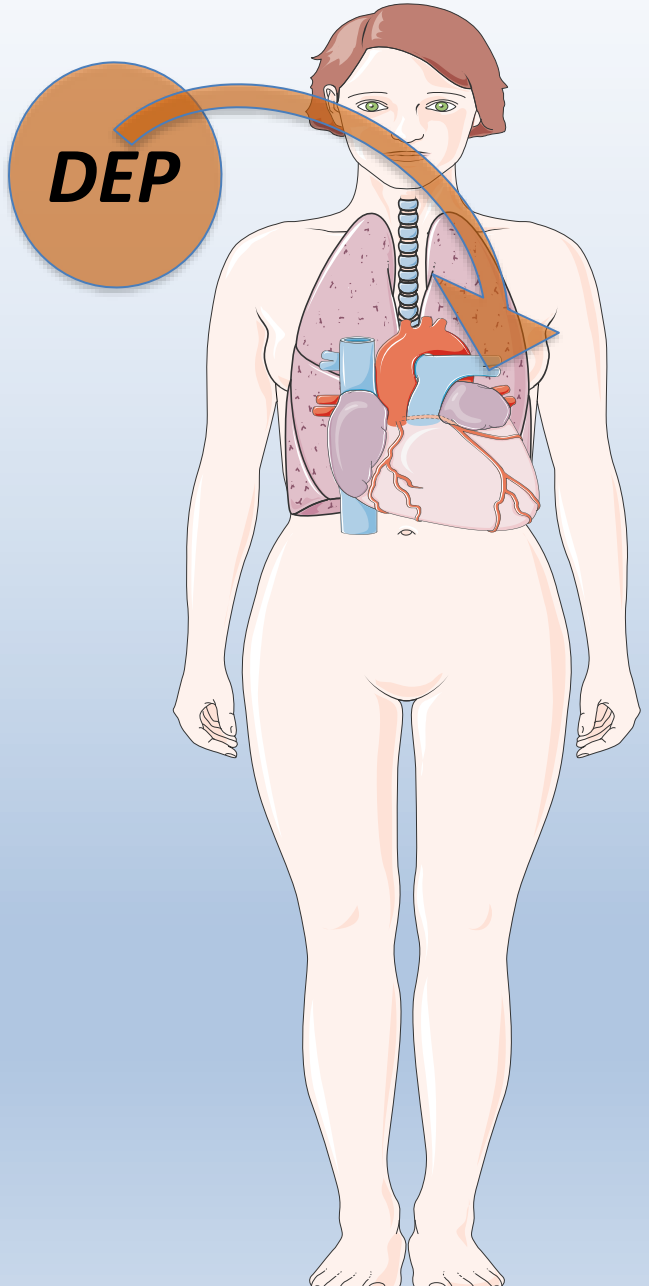
- 20 men with prior myocardial infarction
- Exposed 1 hour to diesel exhaust or filtered air
- 15 min physical exercise

- Diesel exhaust increased ST-segment depression




*Mills 2007*

# Summary




- DEP linked to cardiovascular disease
  Mechanisms intensly studied last decades

- DEP may trigger cellular effects via ROS, membrane receptors, intracellular receptors, plasma-membrane and DNA damage

- Mechanisms of vascular effects:

1. Pulmonary inflammation and oxidative stress: systemic spill-over
2. Autonomic nervous system dysregulation
3. Direct vascular effects of particles or particle-associated components

All three mechanisms may interact