National Research Centre
for the Working Environment

**Ulla Vogel**
Professor

# The use of Adverse Outcome Pathways (AOPs) in nanotoxicology (SmartNanoTox)

Ulla Vogel, professor

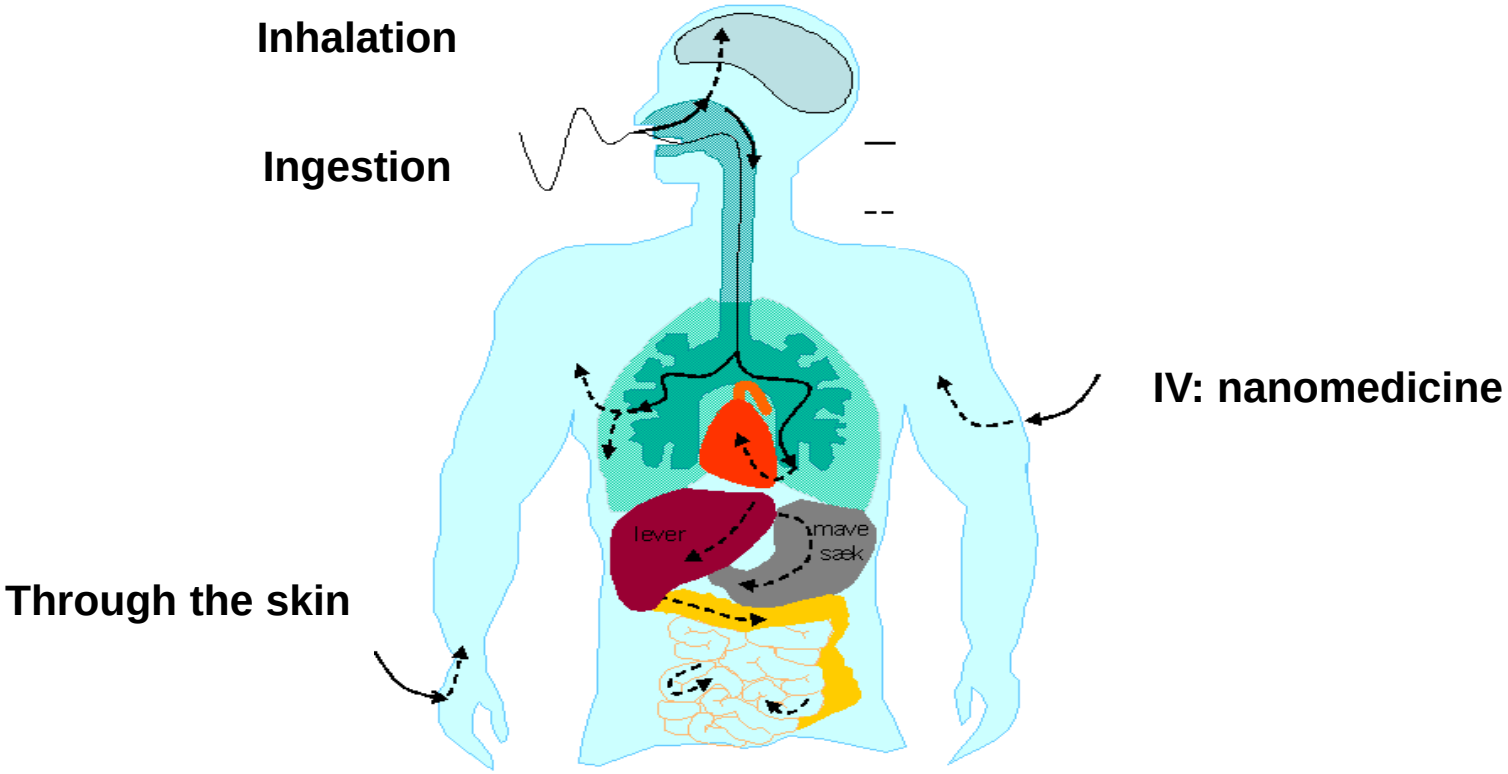
SmartNanoTox
Smart Tools for Gauging Nano Hazards

DTU Nanotech
Department of Micro- and Nanotechnology

# Nanosafety at the National Research Centre for the Working Environment

- Government research institute under the Ministry of Employment
- Nanosafety as strategic research area since 2005
- At present 35 persons in nanosafety research
- Advisors for the Danish Working Environment Authorities, EPA, EU, OECD, WHO
- Past and present partners in 20 EU projects on (nano)particle safety including NanoReg1 and 2

# SmartNanoTox has focus on inhalation of nanomaterials

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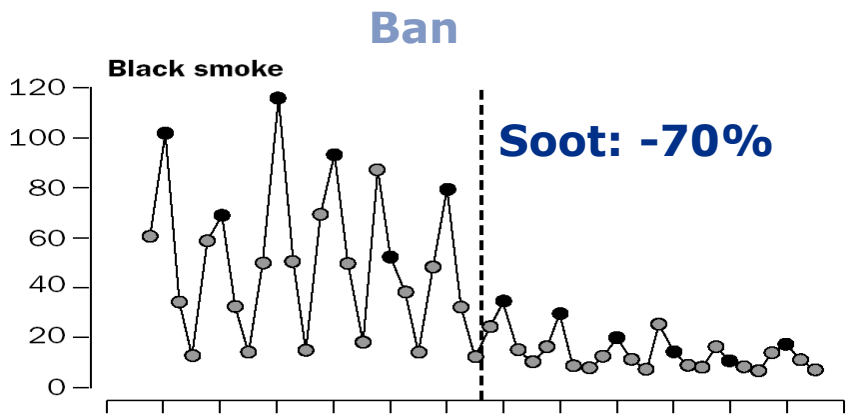
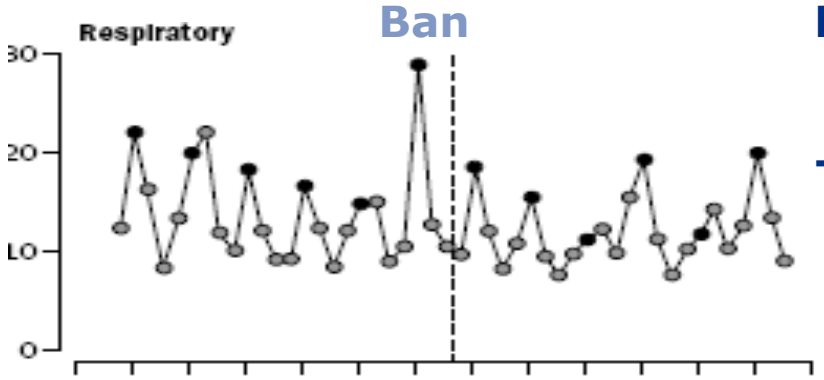
# Inhalation of particles affects health: Soot and death per 1000 person year before and after ban on coal in Dublin




**Soot: -70%**

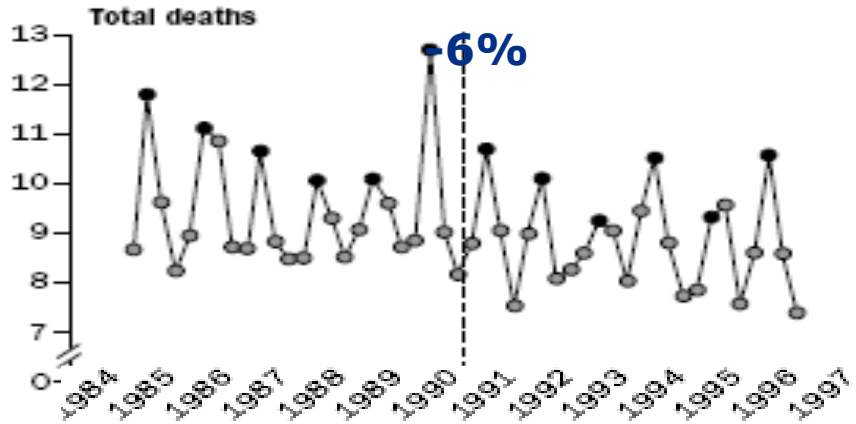


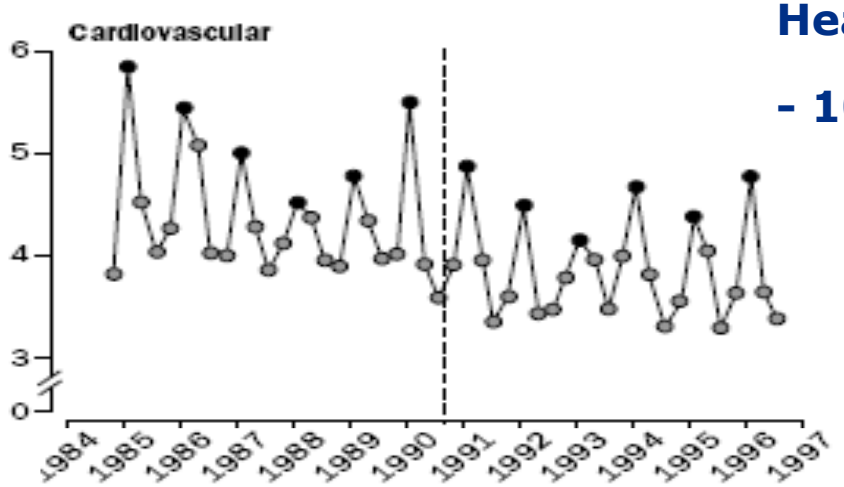

**Pulmonary related deaths:**

**-15%**

**All cause deaths:**

**-6%**




**Heart related deaths:**

**- 10%**

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Clancy et al. Lancet 360: 1210–14, 2002

# The vision

SmartNanoTox
AOPs &
assays for
testing of toxicity

Mechanism-based
understanding of
toxicological effect

Evidence-based risk assessment.
Prediction of toxicological effects on the basis of information on physico-chemcial properties

Grouping and ranking for regulation
Safe-by-design for innovation

Safe use of nanomaterials including high volume nanomaterials


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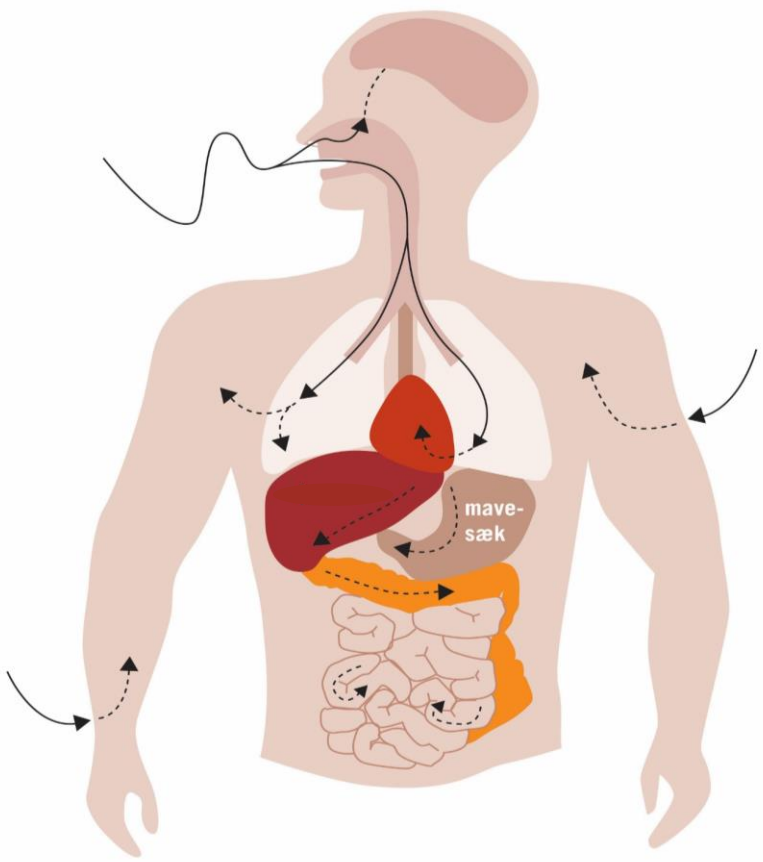
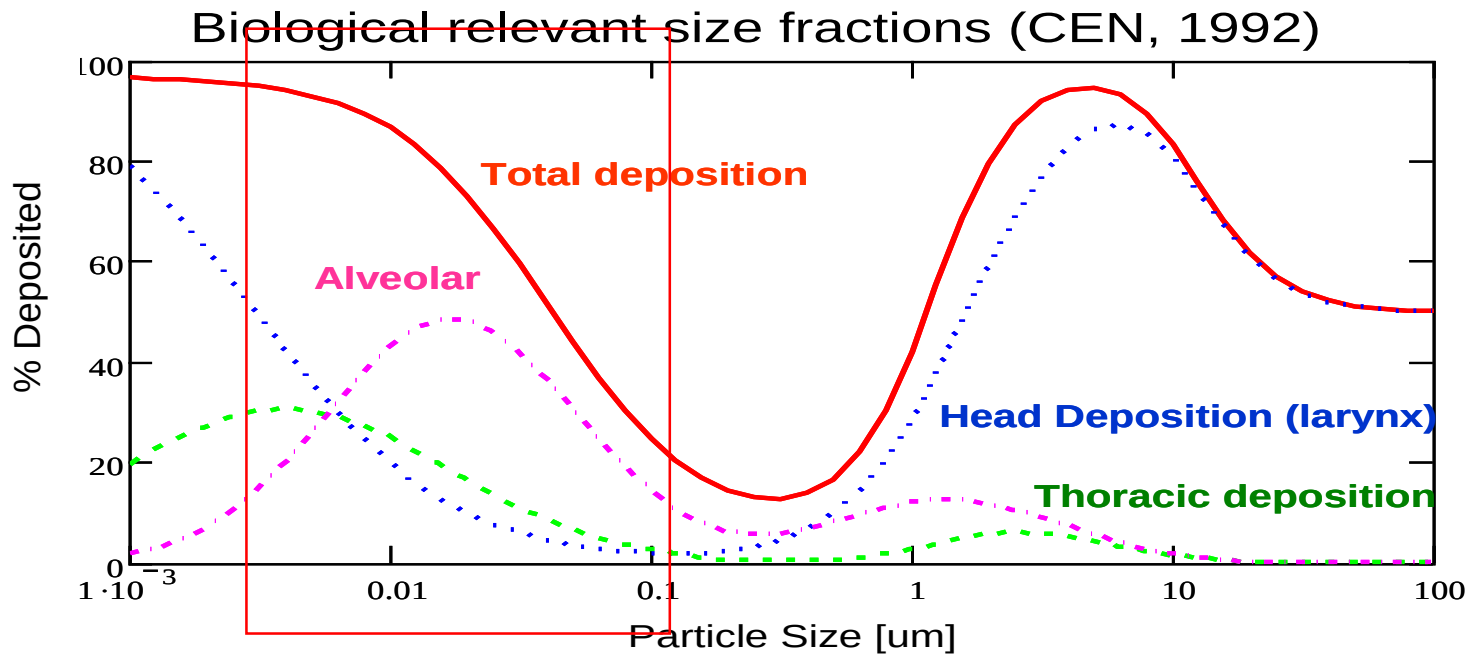
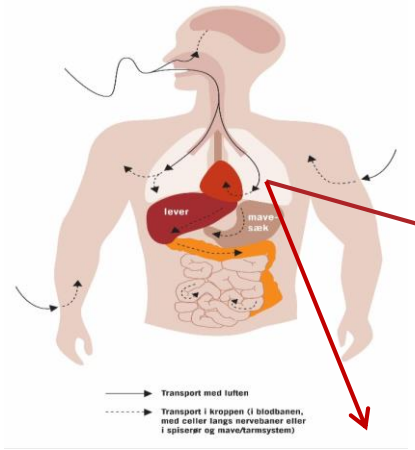
# Lung deposition is determined by particle size



# Low clearence of nanoparticles from the lung




Fine particles

Nanoparticles

Ultrafine particles

Airways

Particle deposition and removal by mucociliar clearance

Particle phagocytosis by macrophage

INFLAMMATION

release of mediators

ROS

Inflammatory cells and inflammatory mediators

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Marianne Dybdahl

# Inhaled $TiO_2$ nanoparticles in the lung are removed very slowly

Mice inhaled 40 mg/m$^3$ nanosized $TiO_2$ 1 hour daily for 11 days.

$TiO_2$ content in lung tissue was measured by ICP-MS.

| Exposure | Days after exposure | N | $TiO_2$ in lung (mg/kg) (mean ± sd) | Procent of deposited dose |
|---|---|---|---|---|
| $TiO_2$ | 5 | 3 | 63 ± 10 | 24% |
| Air | 5 | 3 | < 8 | |
| $TiO_2$ | 25 | 3 | 55 ± 30 | 21% |
| Air | 25 | 3 | < 1 | |

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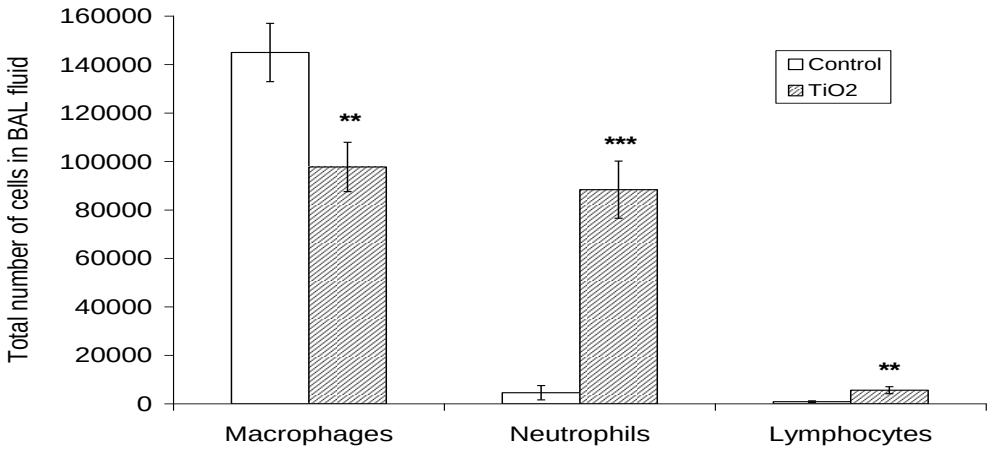
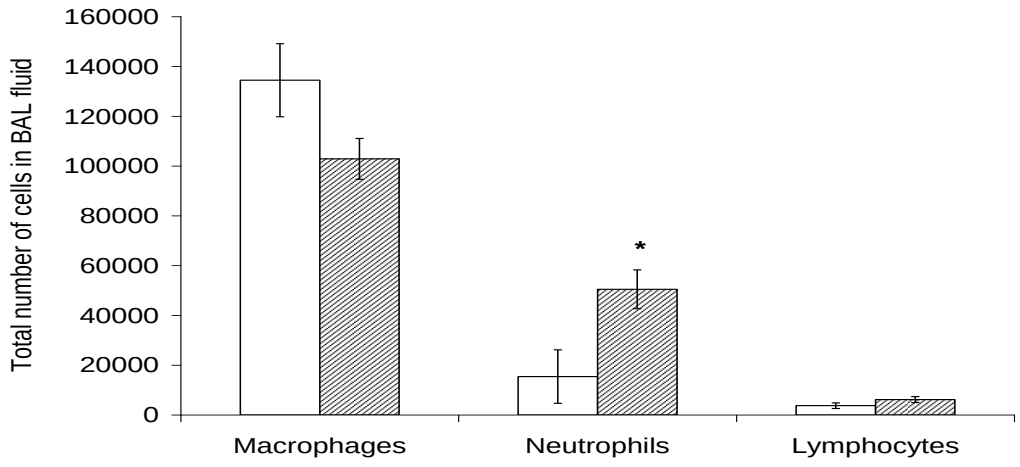
Hougaard et al, 2010, PF&T

# Inhalation of nano-$TiO_2$ results in long lasting inflammation

## After 5 days




## After 4 weeks




Types and numbers of cells in lung fluid

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Hougaard et al, 2010, PF&T

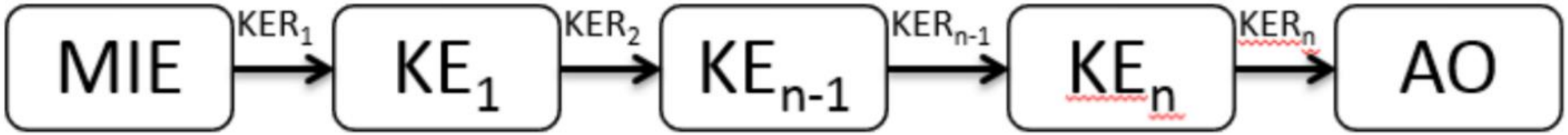
# Known health effects of (nano)particle inhalation exposure

- Inflammation (all insoluble nanoparticles)
- Cardiovascular disease (air pollution, welding fumes)
- Fibrosis (Quartz, carbon nanotubes)
- Lung cancer (carbon black, titanium dioxide, one carbon nanotube)
- Acute lung toxicity (Surface treatment spray products)

- SMARTNANOTOX DEVELOPS ADVERSE OUTCOME PATHWAYS (AOPs) FOR THESE HEALTH OUTCOMES

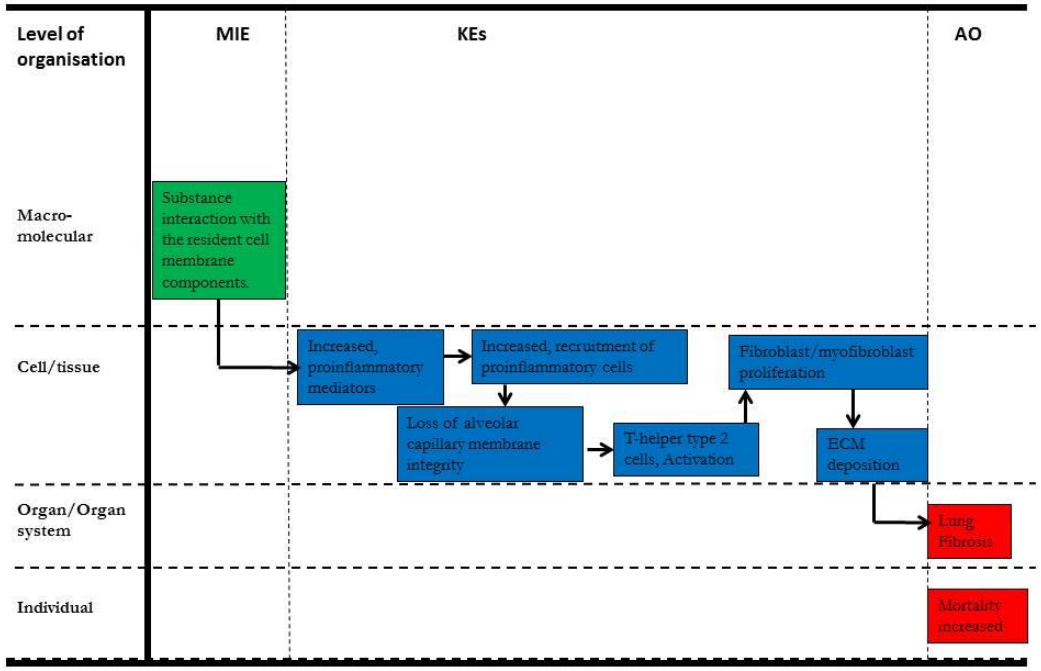
# SmartNanoTox will develop Adverse Outcome Pathways for nanomaterial-induced toxicity

- An **adverse outcome pathway** (**AOP**) is structured representation of biological events leading to adverse effects and is considered relevant to risk assessment
- The AOP links in a linear way existing knowledge along one or more series of causally connected **key events** (**KE**) between two points — a **molecular initiating event** (**MIE**) and an **adverse outcome** (**AO**) that occur at a level of biological organization relevant to risk assessment.[2] The linkage between the events is described by **key event relationships** (**KER**) that describe the causal relationships between the key events.




https://aopwiki.org/

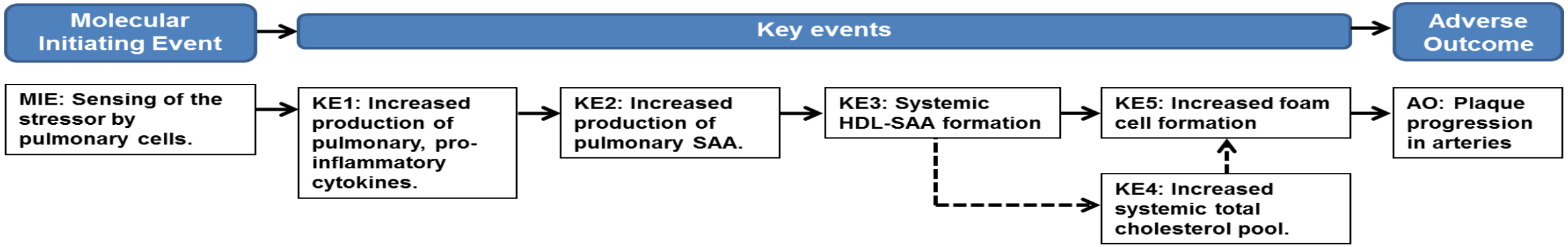
# AOP 173: Increased substance interaction with the resident cell membrane components leading to lung fibrosis




https://aopwiki.org/aops/173

Sabina Halappanavar

# AOP 237: Secretion of inflammatory cytokines after cellular sensing of the stressor leading to plaque progression





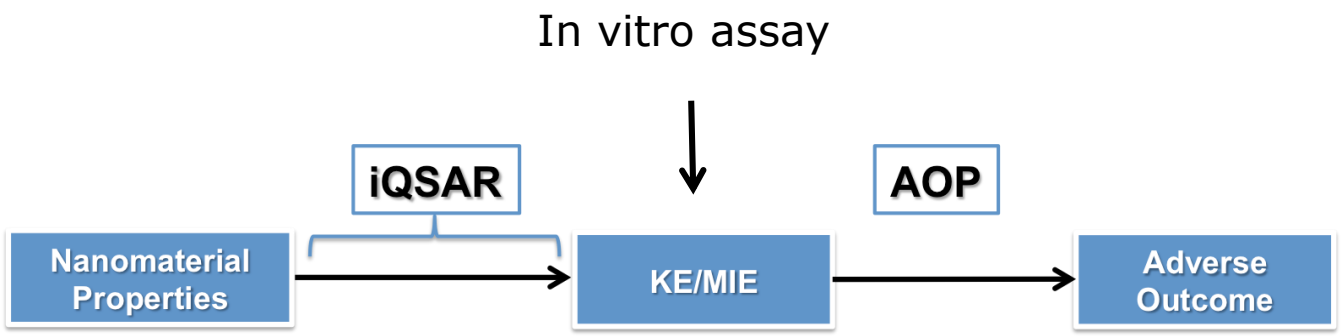

https://aopwiki.org/aops/237

Sarah Søs Poulsen

# SmartNanoTox ambitions

- Submit 5 AOPs to OECD AOP sponsorship program
- AOP 173 has been approved
- AOP 237 has been submitted
- 3 more are under development in SmartNanoTox
- Next step:

PATROLS:
Focus on in vitro assays to detect KEs



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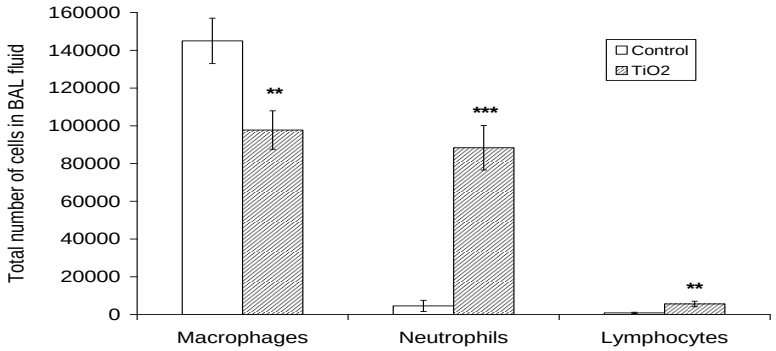
# An Example:
# AOP for ENM-induced risk of developing atherosclerotic plaques

# Inhalation of $TiO_2$ NP induced inflammation and acute phase response in mice. Acute phase genes were the most differentially expressed genes in lung tissue



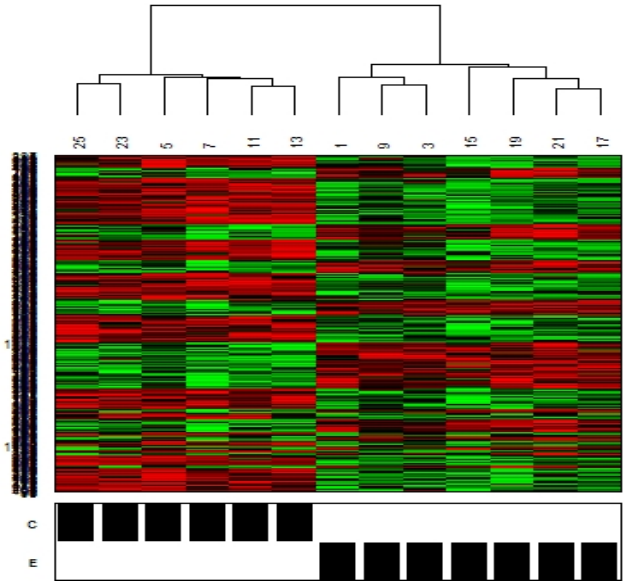




**TABLE II. List of all Acute Phase Response Genes Showing Fold Changes Higher Than 1.2 in exposed mice**

| Acute phase reactants | *P* value | Fold change[a] |
|---|---|---|
| **Serum amyloid A1** | **0.00** | **2.24** |
| **Serum amyloid A3** | **0.00** | **4.71** |
| **Complement protein C3** | **0.00** | **1.37** |
| **Complement component 1, s (C1s)** | **0.00** | **1.28** |
| **Complement component 3a receptor 1 (C3ar1)** | **0.00** | **1.15** |
| **Complement component 1, q beta polypeptide (C1qb)** | **0.00** | **1.30** |
| **Complement component 1, r subcomponent (C1r)** | **0.00** | **1.31** |
| **Complement component C1RB (C1rb)** | **0.00** | **1.21** |
| Fibrinogen | 0.01 | 2.05 |
| Coagulation factor II (F2) | 0.01 | 1.72 |
| Mannose binding protein | 0.02 | 1.70 |
| Albumin | 0.01 | 1.79 |
| apoA1 | 0.01 | 1.51 |
| apoAII | 0.03 | 1.61 |
| alpha2-HS glycoprotein | 0.00 | 1.85 |
| S100A8 (calgranulin A) | 0.01 | −1.85 |
| Serpina3n | 0.00 | 1.37 |

Gene names in bold indicate FDR adjusted *P* value > 0.05.
[a]Average fold change compared with matched controls.

Halappanavar et al. EMM 2011

# The acute phase response: A risk factor for cardiovascular disease

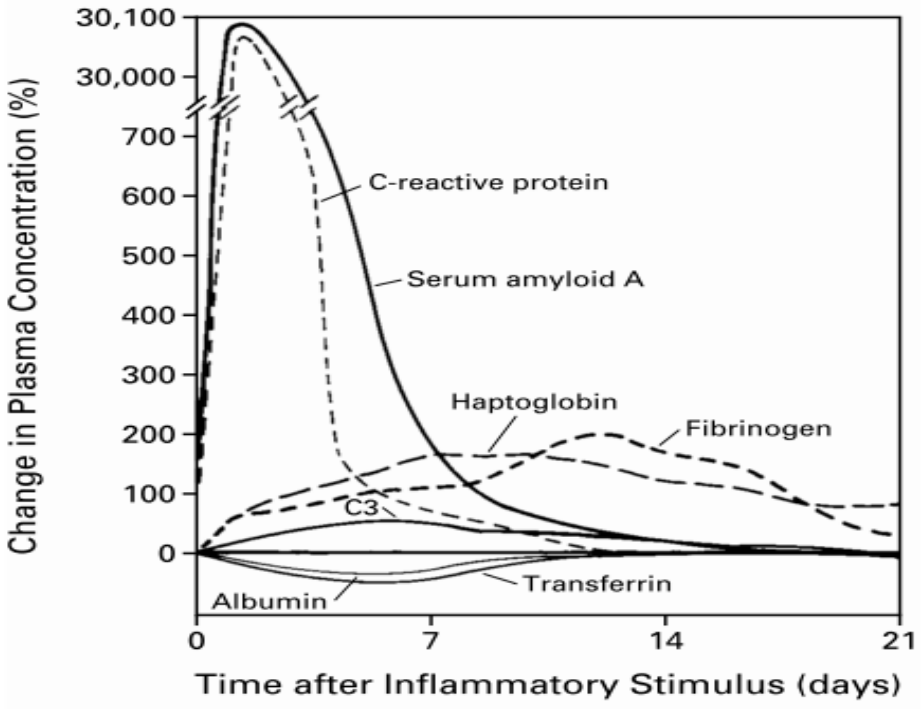



**Figure 1.** Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus.
Modified from Gitlin and Colten[5] with the permission of the publisher.

- The acute phase response is the systemic response to acute and chronic inflammatory states caused by fx bacterial infection, trauma and infarction.

- Conditions that induce acute phase response are associated with risk of cardiovascular disease, including asthma and air pollution exposure.



Gabay and Kushner 1999, NEJM

# Acute phase proteins CRP & SAA are associated with risk of CVD in prospective epidemiological studies

Nurses' Health Study : 120.000 participants

**TABLE 3.** RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

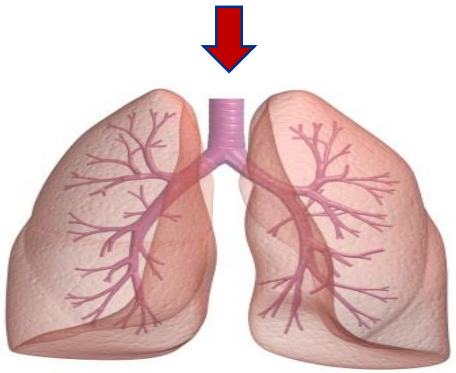
| VARIABLE | QUARTILE OF PLASMA LEVEL | | | | P VALUE FOR TREND |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| High-sensitivity C-reactive protein | | | | | |
| Median — mg/dl | 0.06 | 0.19 | 0.38 | 0.85 | |
| Relative risk (95% CI) | 1.0 | 2.1 (1.0–4.5) | 2.1 (1.0–4.4) | 4.4 (2.2–8.9) | <0.001 |
| Serum amyloid A | | | | | |
| Median — mg/dl | 0.25 | 0.43 | 0.62 | 1.17 | |
| Relative risk (95% CI) | 1.0 | 1.8 (0.9–3.6) | 1.9 (0.9–3.8) | 3.0 (1.5–6.0) | 0.002 |

Ridker *et al.* 2000, NEJM

# Proposed mechanism of action

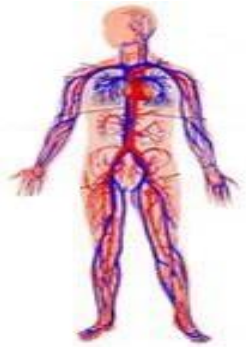
Inhaled particles promote atherosclerosis via acute phase response

Inhalation of (nano)particles

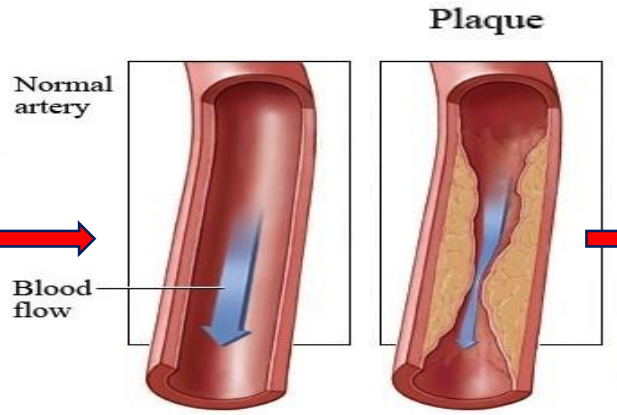


Inflammatory & acute phase response

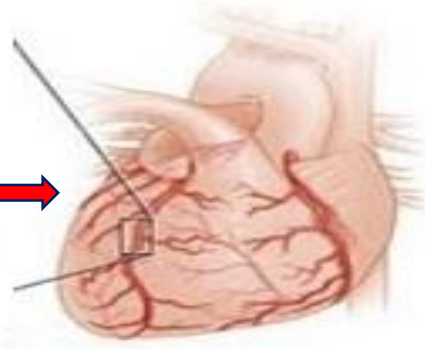
Systemic circulation of acute phase proteins



Atherosclerosis




Cardiovascular disease



New knowledge

Established fact

# Time- and dose-dependent pulmonary acute phase response in mice

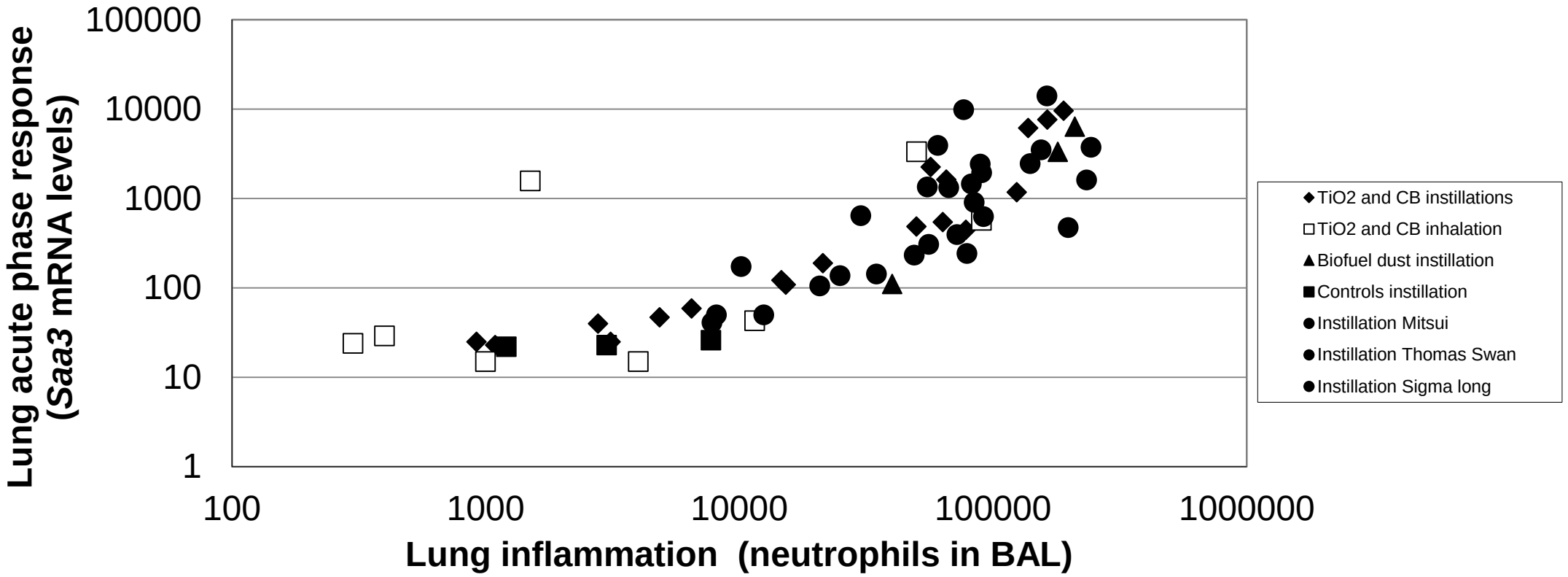
**TABLE 1** | Differential Expression of Murine Acute Phase Genes and *Saa3* Expression Levels after Exposure to Different Nanomaterials and at Different Time Points

| Post Exposure Day | 1 | | | 3 | | | 28 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dose/Animal | 18 µg | 54 µg | 162 µg | 18 µg | 54 µg | 162 µg | 18 µg | 54 µg | 162 µg | Ref |
| $TiO_2$ nanoparticles | → | | | | | | | | | |
| N acute phase genes[1] | 0 | 5 | 10 | 3 | 1 | 3 | 1 | 2 | 3 | 28 |
| Fold increase of *Saa3* mRNA[2] | 1.8 | 87 | 368 | 1.1 | 2.6 | 19 | 1 | 1.8 | 5.5 | 11 |
| Carbon Black nanoparticles | → | | | | | | | | | |
| N acute phase genes[1] | 0 | 7 | 10 | 0 | 0 | 4 | 0 | 0 | 2 | 42 |
| Fold increase of *Saa3* mRNA[2] | 63 | 237 | 294 | 8.3 | 24 | 51 | 1.1 | 5 | 22 | 11 |
| Multiwalled Carbon nanotubes | → | | | | | | | | | |
| N acute phase genes[1] | 5 | 5 | 10 | ND | ND | ND | ND | 1 | ND | 35 |
| Fold increase of *Saa3* mRNA[2] | 52 | 151 | 95 | 39 | 152 | 612 | 7.9 | 29 | 88 | 11 |

Saber *et al.* 2014

WIREs Nanomed nanobiotech

# Close correlation between *pulmonary acute phase response* and pulmonary inflammation across particles, doses, time points

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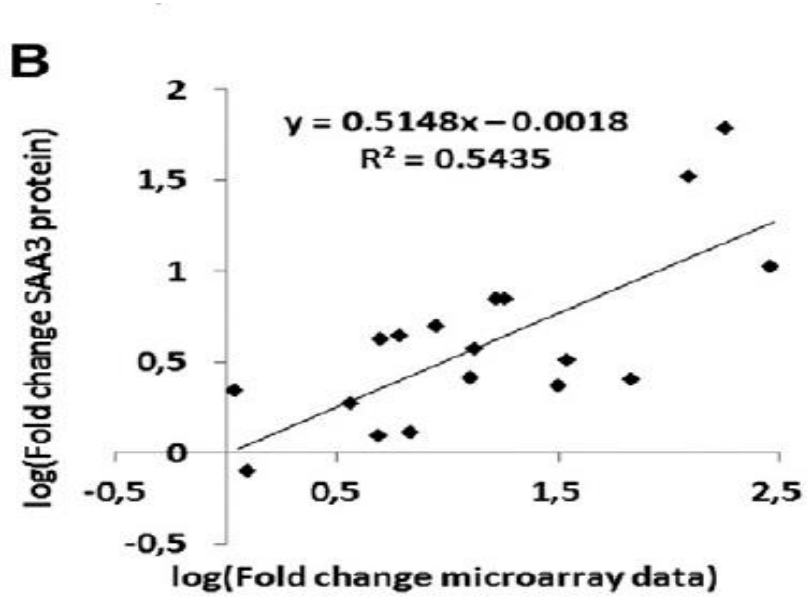
Saber AT et al, Plos One, 2013

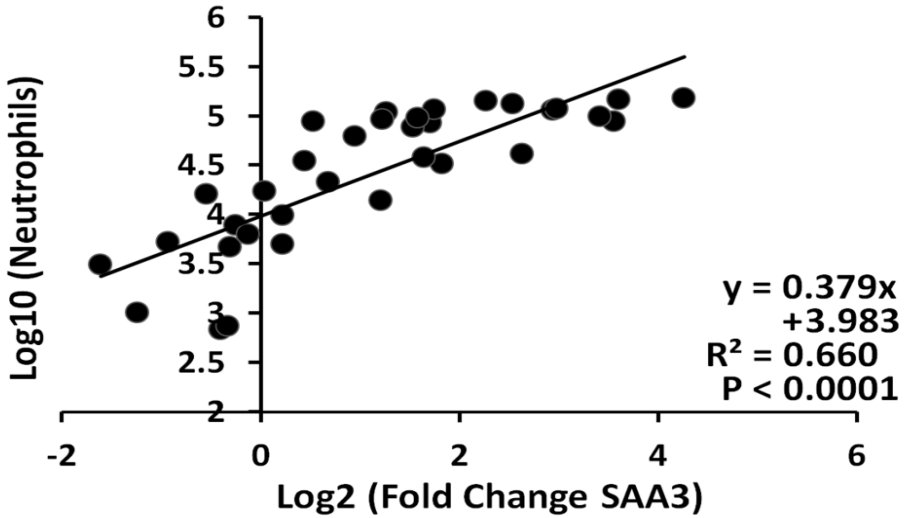
# Plasma levels of acute phase protein SAA3 correlates with lung responses

*Saa3* mRNA in lung correlates with plasma SAA3




Poulsen *et al* 2015, TAAP

Plasma SAA3 levels and neutrophil influx




Poulsen *et al.* 2017, Plos One

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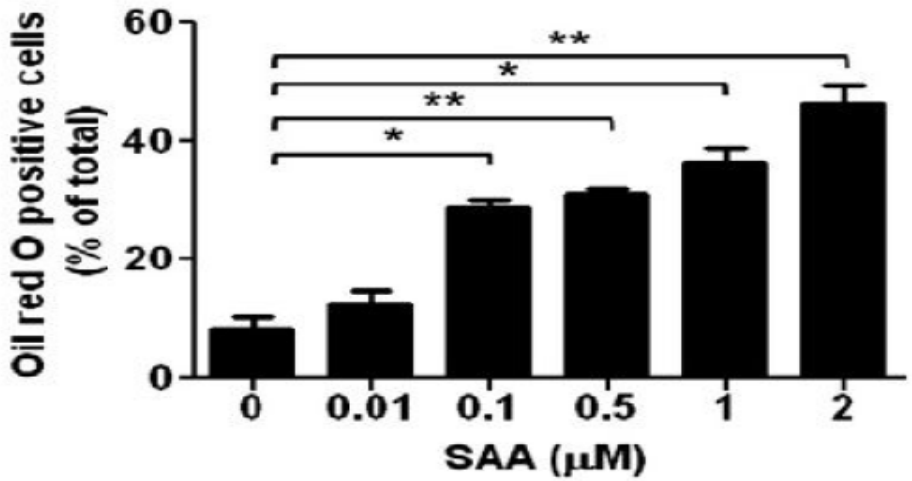
# SAA: an acute phase protein that directly promotes formation of foam cells

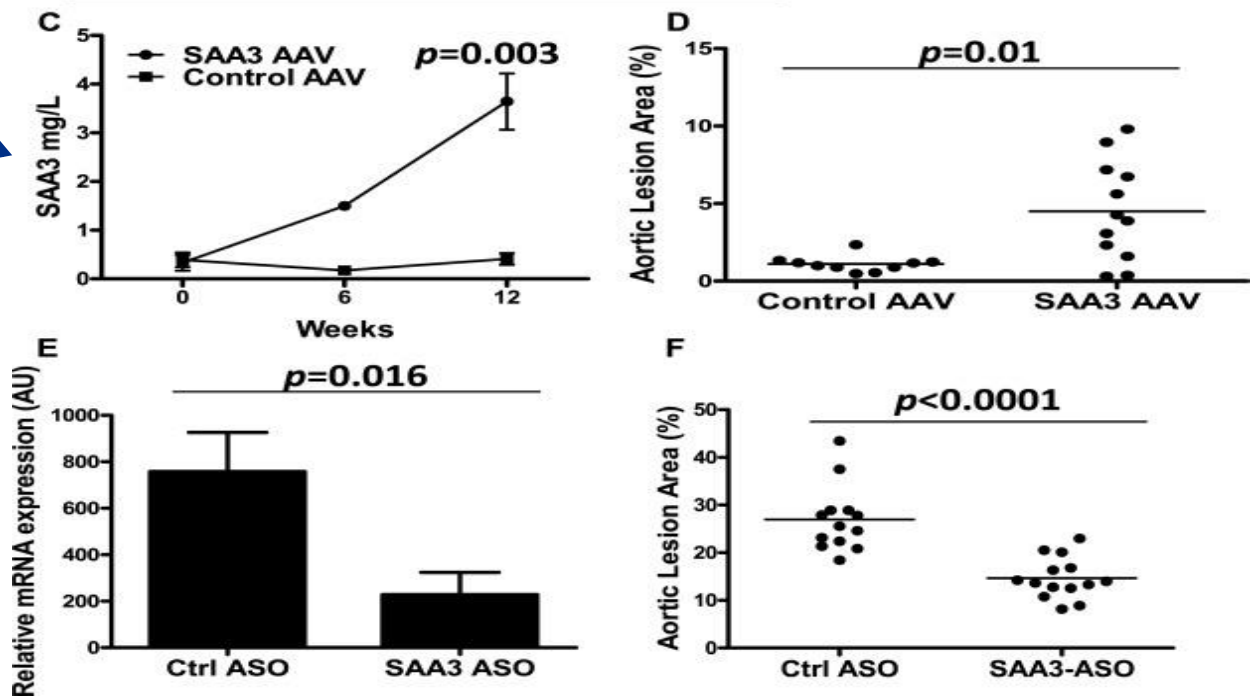
- SAA can replace ApoA-1 as the major HDL protein.
- This inhibits HDLs role in reverse cholesterol transport.
- SAA induces foam cell formation in macrophages [1].




[1] Lee et al, 2013, BBRC

# Acute phase protein SAA is causally implicated in plaque progression

- Mice have 3 inducible SAA isogenes (*Saa1, Saa2, Saa3*)
- Over-expression of SAA3 increases plaque progression (Thompson 2018)
- Inactivation (KO) of all SAA isogenes results in reduced plaque progression (Thompson 2018)

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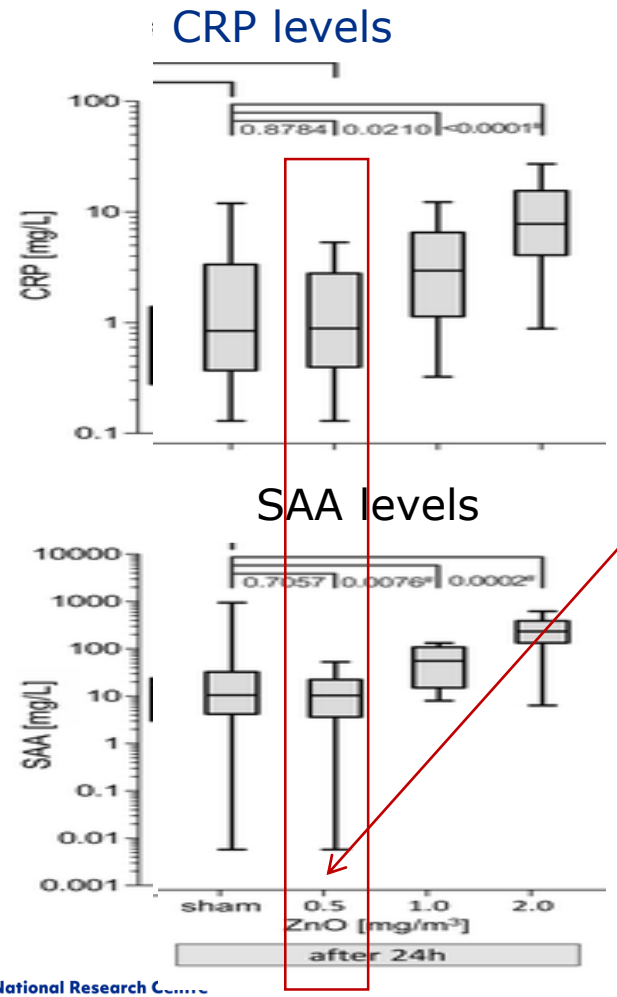
# Any Human relevance ?: Yes; inhalation of ZnO induces acute phase response in human volunteers




- No effect level:
- 0.5 mg/m$^3$

Study set up:

- 16 volunteers
- Exposed to 0, 0.5, 1 or 2 mg/m$^3$ ZnO particles for 4 h
- OEL: 5 mg/m$^3$ for 8 h
- Acute phase response proteins CRP and SAA

Acute phase response was induced after ZnO inhalation at concentrations well below incurrent OEL

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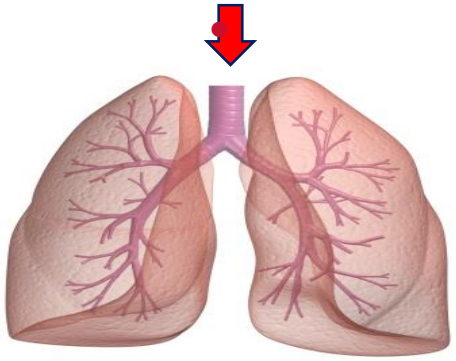
Adapted from Monsé et al Part Fibre Toxicol. 2018 15(1):8

# Summary: The Adverse Outcome Pathway for particle-induced cardiovascular disease

Inhaled particles promote atherosclerosis via acute phase response

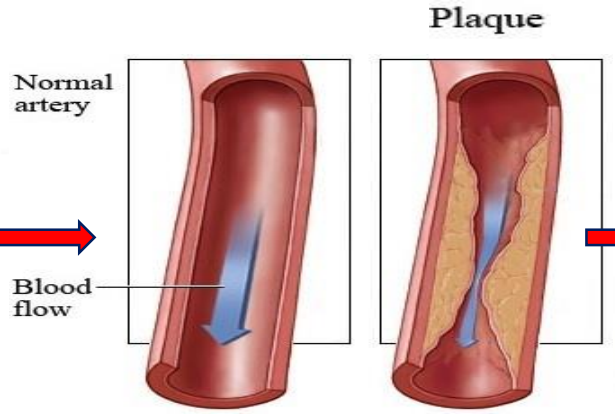
Inhalation of (nano)particles



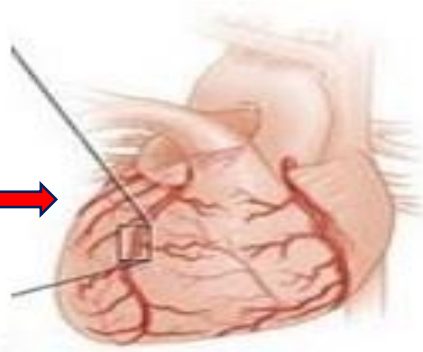
Inflammatory & acute phase response

Systemic circulation of acute phase proteins

Atherosclerosis




Cardiovascular disease



New knowledge

Established fact

# Acknowlegdements and funding

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Health Canada Santé Canada


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- European Union's Horizon 2020 research and innovation programme under grant agreement No 686239 (caLIBRAte)
- European Union's Horizon 2020 research and innovation programme under grant agreement No 760813 (PATROLS)


SmartNanoTox
Smart Tools for Gauging Nano Hazards

caLIBRAte
nano risk governance

PATROLS
Advanced Tools for NanoSafety Testing