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Nanosafe 2018

Inhalation of (nano)particles, acute phase response and cardiovascular disease

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DTU Nanotech
Department of Micro- and Nanotechnology

Nanosafety at the National Research Centre for the Working Environment

- Government research institute under the Danish Ministry of Employment
- Nanosafety as strategic research area since 2005
- Past and present partners in more than 20 EU projects on nanosafety
- Danish Centre for Nanosafety 2012-2019

The vision

Research

Mechanism-based understanding of toxicological effect

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

Grouping and ranking for regulation
Safe-by-design

Safe use of nanomaterials including high volume chemicals

Safe-by-design:

Lung cancer
Fibre-paradigm

Mineralwool

Painters syndrome
Organic solvents

Water-based paint
MAL kodes

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Cardiovascular disease constitutes a major fraction of preventable air pollution-induced morbidity

EXAMPLE:

- Heating with coal in private households was banned in Dublin, Ireland in 1991:
- Black smoke levels in ambient air were reduced by 0.036 mg/m^3

- Mortality rates were reduced by 75 per 100 000 person-years
- 77% cardiovascular (!)

- Effects were adjusted for death rates in the rest of Ireland

- Morbidity was not assessed

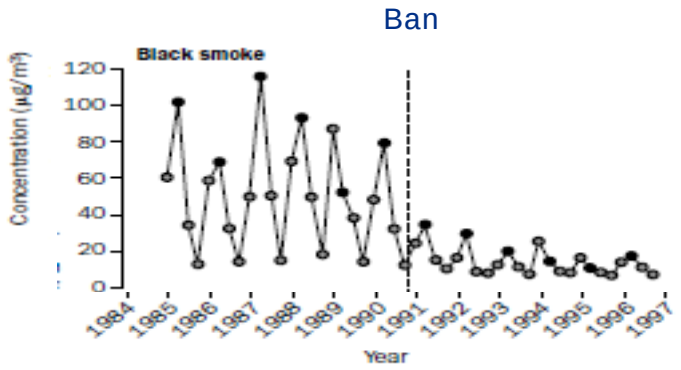
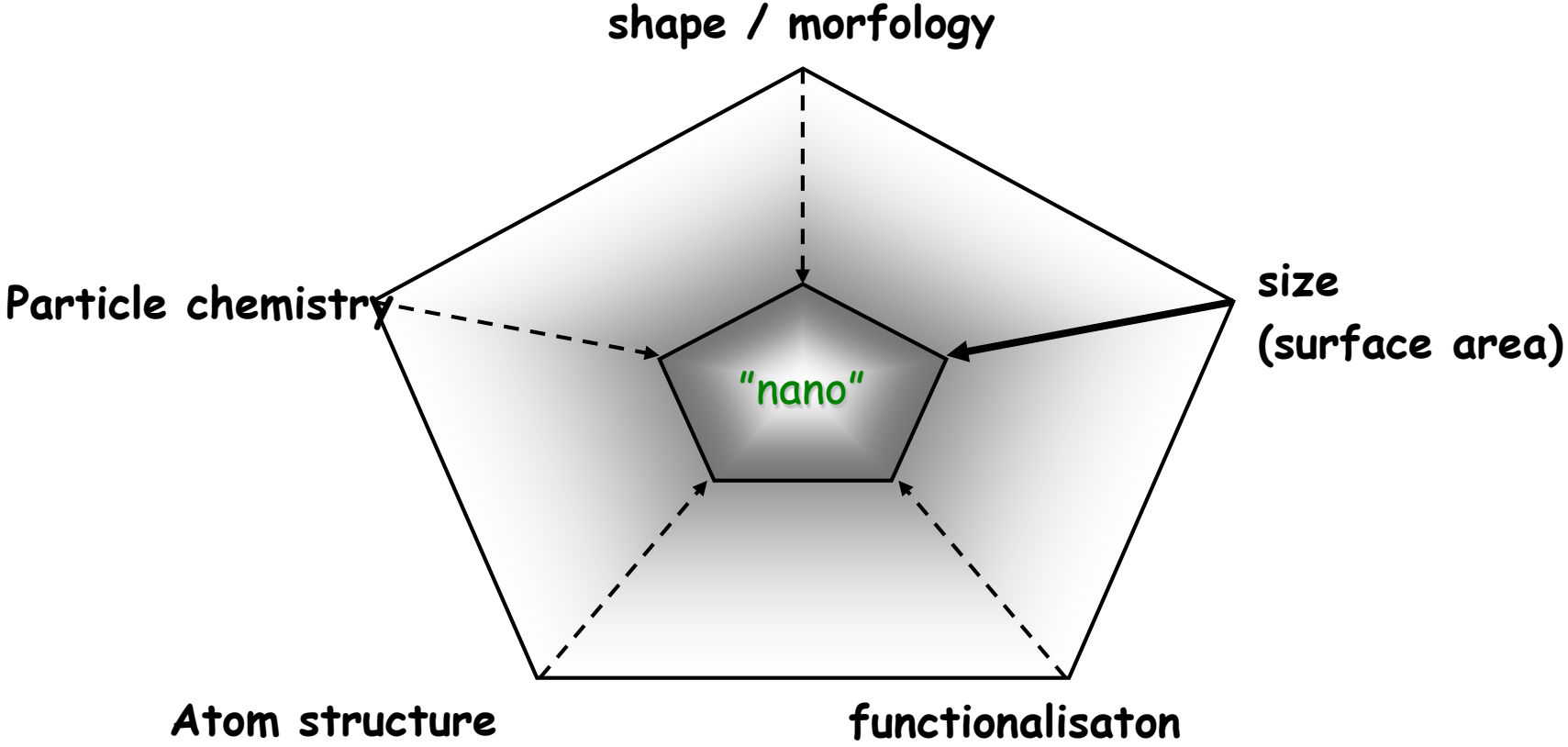


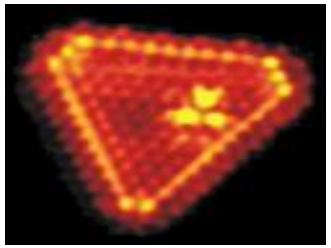
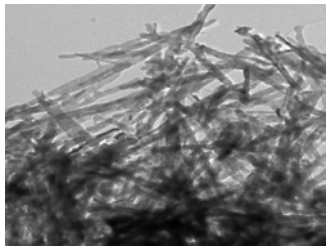
Table 2: **Age-standardised mortality rates for Dublin County Borough before (1984–90) and after (1990–96) ban of sale of coal, by season**

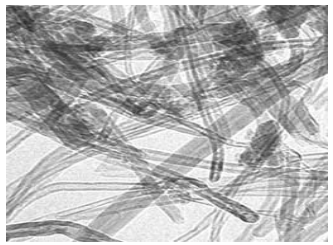
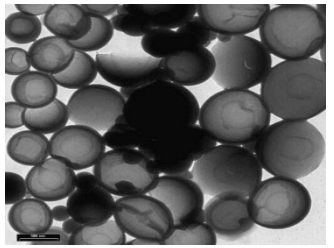
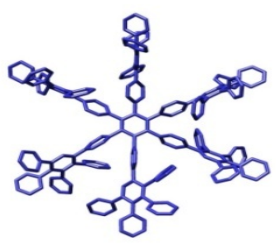
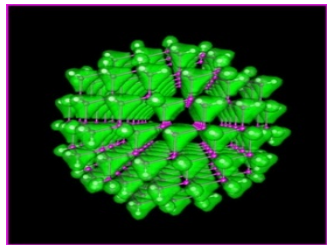
	1984–90	1990–96	Change	p
Deaths per 1000 person-years				
Non-trauma				
Autumn	8·73	8·54	–0·19	<0·0001
Winter	11·03	9·88	–1·15	<0·0001
Spring	9·49	8·66	–0·83	<0·0001
Summer	8·40	7·56	–0·85	<0·0001
Total	9·41	8·65	–0·75	<0·0001
Cardiovascular				
Autumn	4·01	3·67	–0·34	<0·0001
Winter	5·18	4·47	–0·71	<0·0001
Spring	4·41	3·71	–0·69	<0·0001
Summer	3·89	3·29	–0·59	<0·0001
Total	4·37	3·78	–0·58	<0·0001

(Adapted from Clancy et al, Lancet, 2002)

Many different nanomaterials



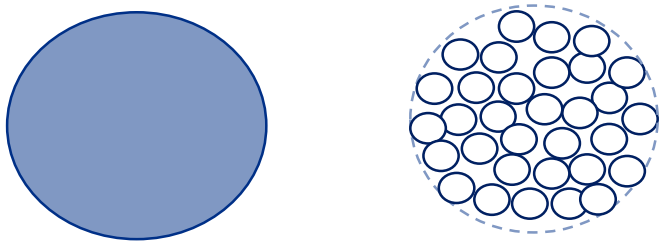
Inhalation of particles and risk of cardiovascular disease

- Epidemiological studies show associations between air pollution and risk of cardiovascular disease
- Several different mechanisms have been proposed related to
  - Inflammation
  - Inflammation-induced hepatic acute phase response
  - Direct effect of translocated particles
  - Vascular function

The Known: Nanomaterial-induced pulmonary inflammation

- Inhalation of inert and insoluble nanoparticles induces pulmonary inflammation in terms of neutrophil influx and increased pulmonary transcription and expression of cytokines
- Inflammation (neutrophil influx) correlates with the total surface area of deposited particles

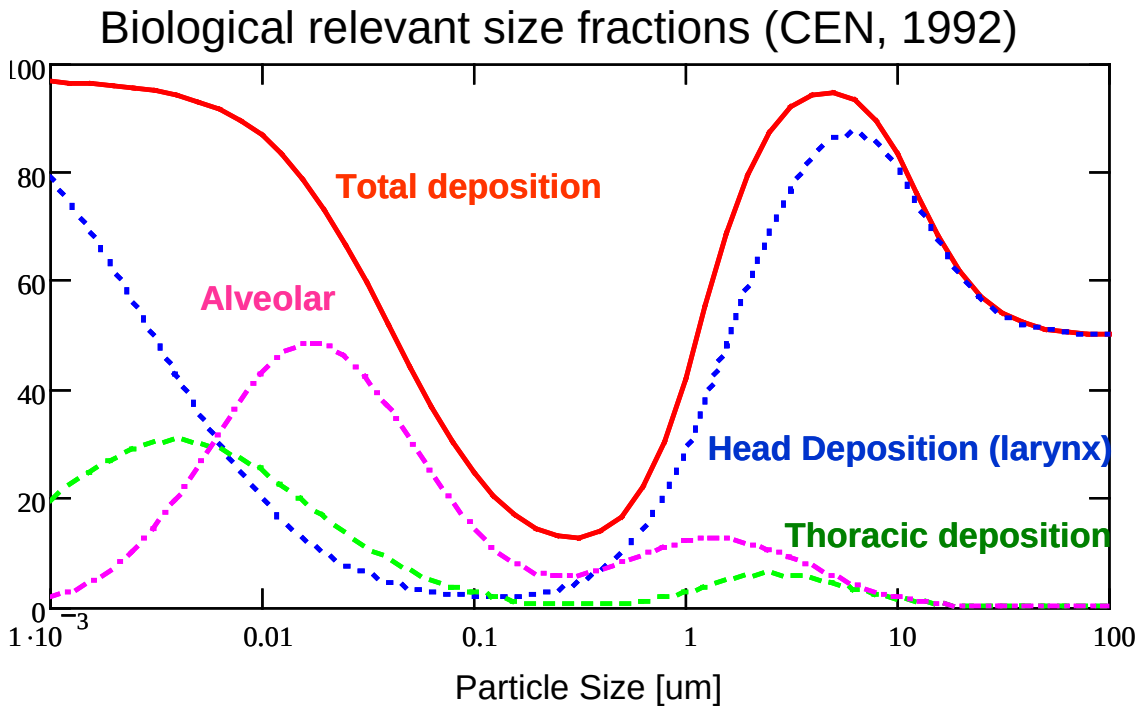
The Nano Issue: More particles & larger total surface area per mass unit, and more alveolar deposition after inhalation



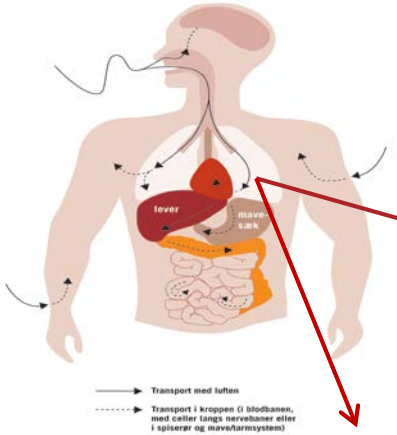
Smaller particles:

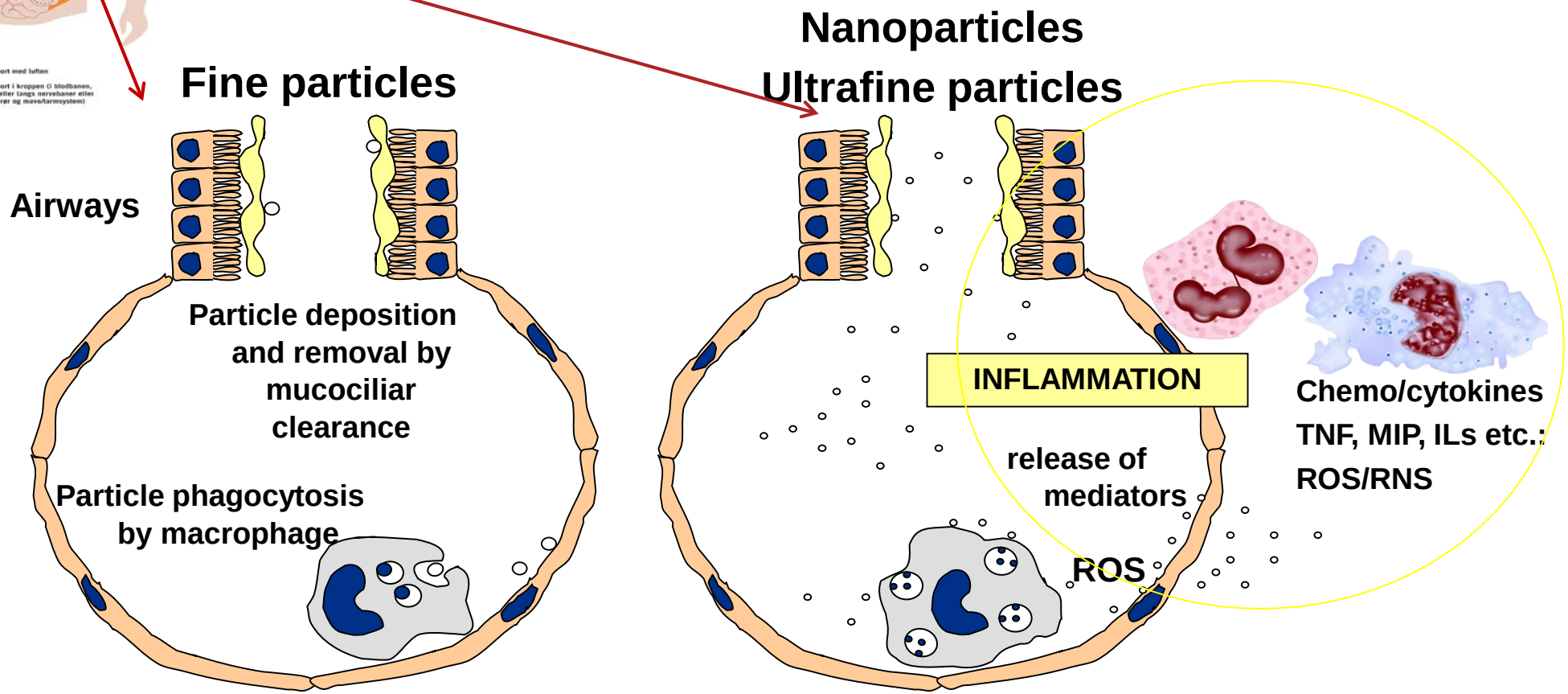
More particles pr mass unit

Larger surface area pr mass unit



Inhalation of nanoparticles: particle accumulation and inflammation





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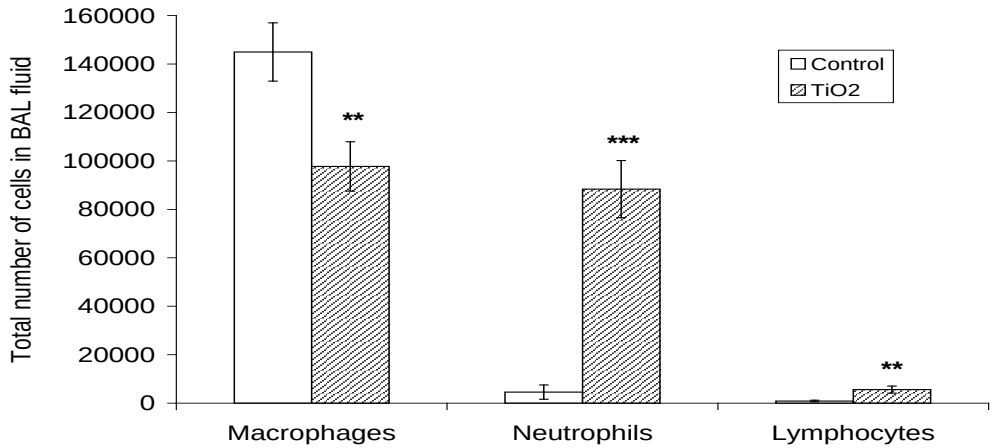
Inhalation of nano-TiO_2 induces pulmonary inflammation in mice

Mice inhaled 40 mg/m^3 nanosized TiO_2 1 hour daily for 11 days.
Types and numbers of cells in lung fluid:

Cell composition in bronchoalveolar lavage fluid 5 days post-exposure



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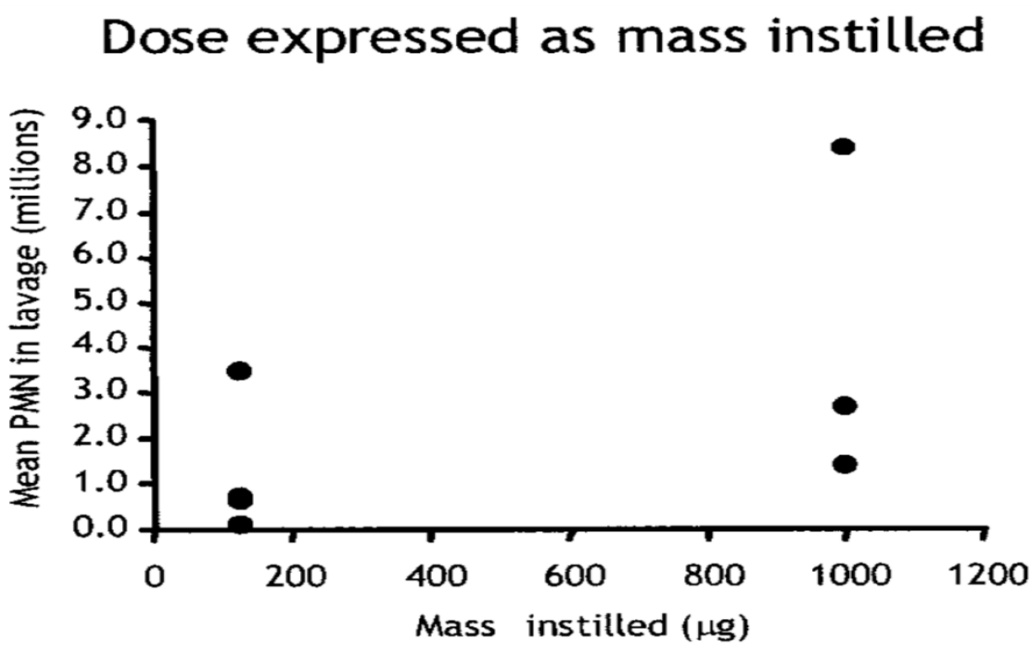
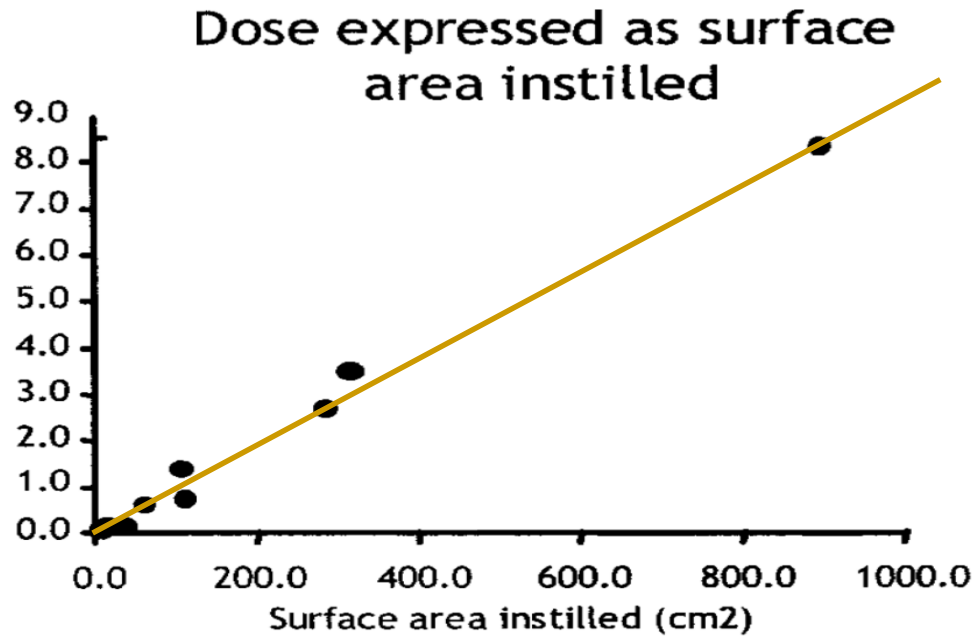
Hougaard et al, PT&T 2010

Deposited surface area is a predictor of pulmonary inflammation





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Donaldson et al., 2002

Global gene expression in lung tissue (day 5): Acute phase reponse !

The most differentially regulated genes

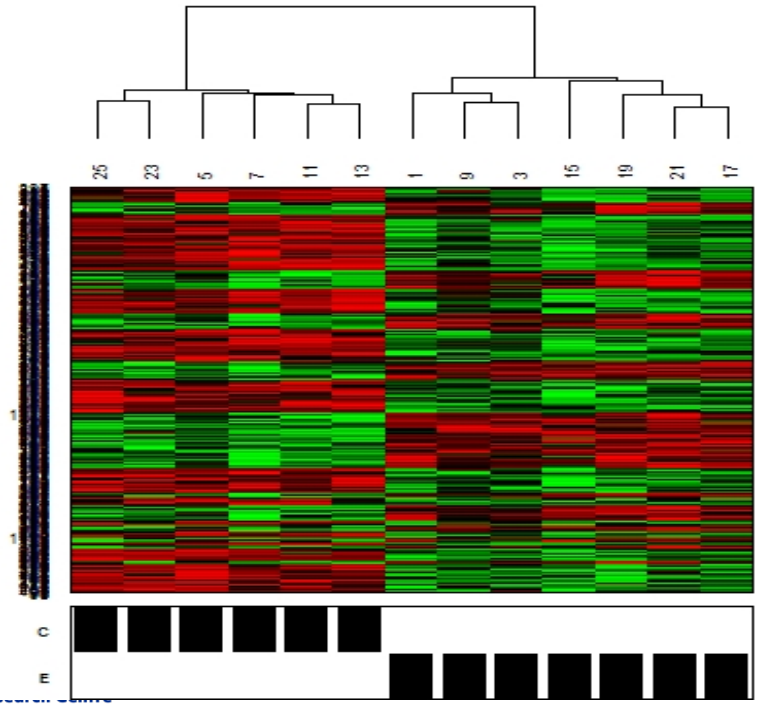


TABLE II. List of all Acute Phase Response Genes Showing Fold Changes Higher Than 1.2 in exposed mice

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

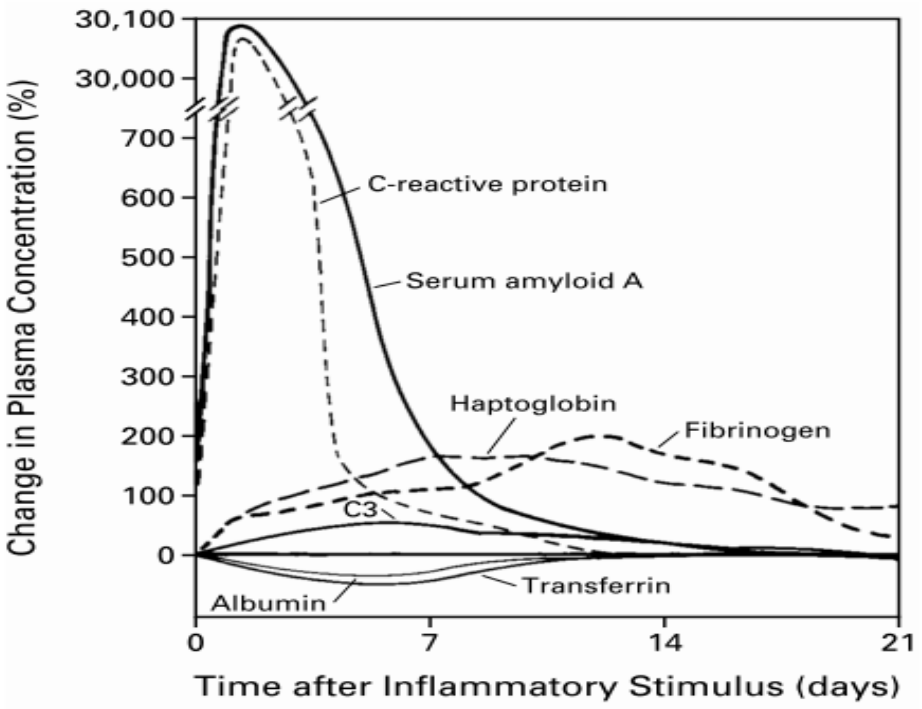


Figure 1. Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus.
Modified from Gitlin and Colten[5] with the permission of the publisher.

- 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.*

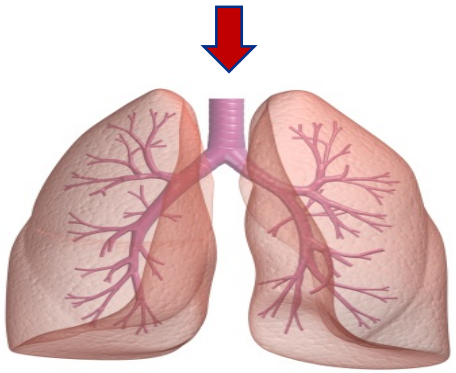
VARIABLE	QUARTILE OF PLASMA LEVEL				P VALUE FOR TREND
	1	2	3	4	
High-sensitivity C-reactive protein					
Median — mg/dl	0.06	0.19	0.38	0.85	
Relative risk (95% CI)	1.0	2.1 (1.0–4.5)	2.1 (1.0–4.4)	4.4 (2.2–8.9)	<0.001
Serum amyloid A					
Median — mg/dl	0.25	0.43	0.62	1.17	
Relative risk (95% CI)	1.0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

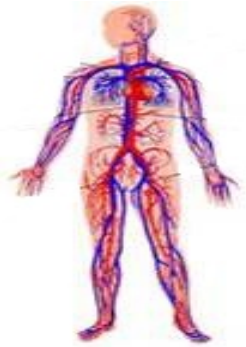
Inhaled particles promote atherosclerosis via acute phase response

Inhalation of (nano)particles

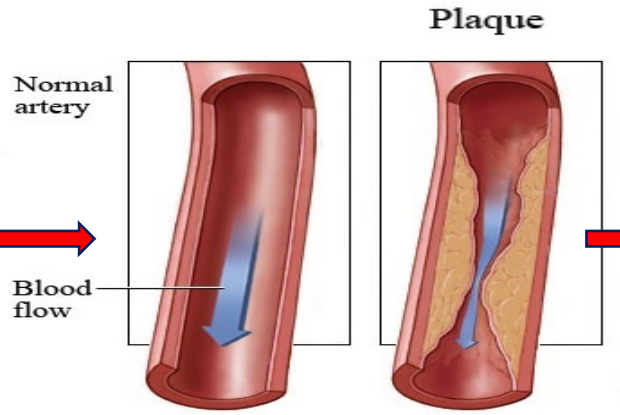


Inflammatory & acute phase response

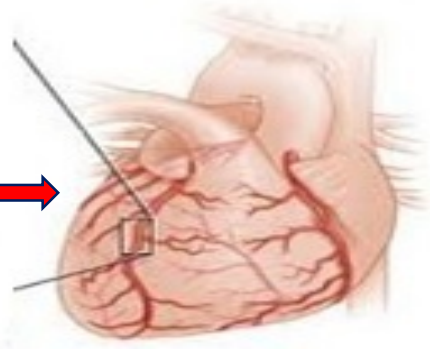
Systemic circulation of acute phase proteins



Atherosclerosis



Cardiovascular disease



New knowledge

Established fact

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

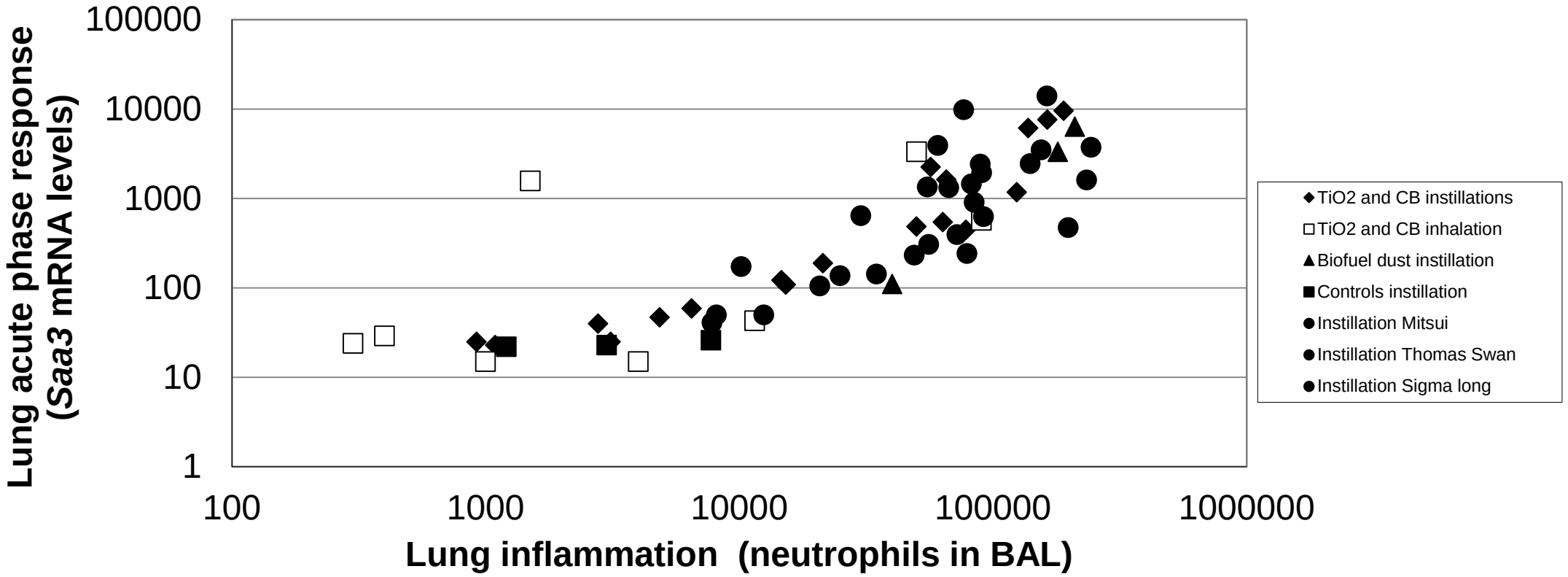
TABLE 1 | Differential Expression of Murine Acute Phase Genes and *Saa3* Expression Levels after Exposure to Different Nanomaterials and at Different Time Points

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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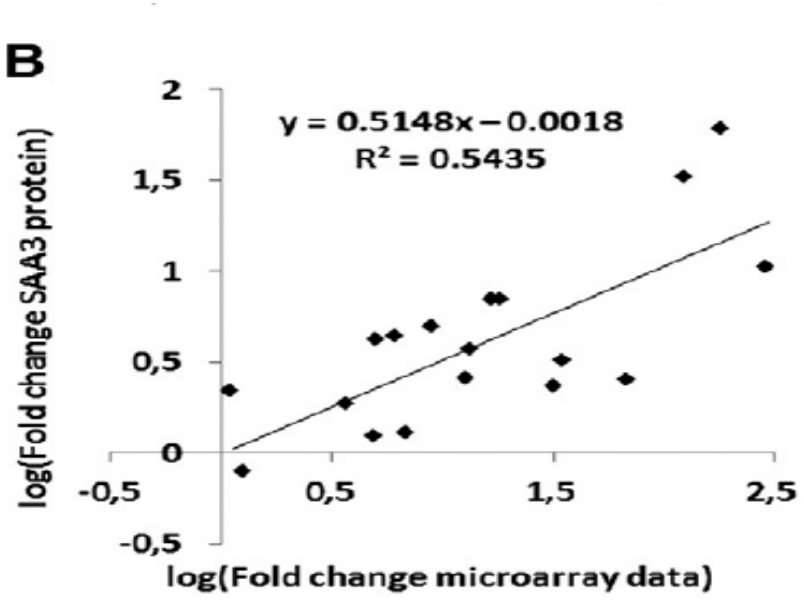
Saber AT et al, Plos One, 2013

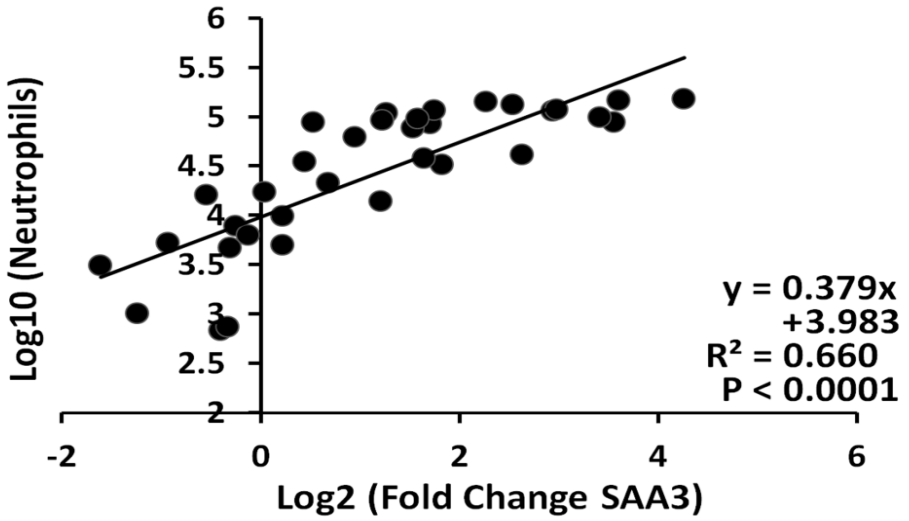
Plasma levels of acute phase protein SAA3 correlates with lung responses

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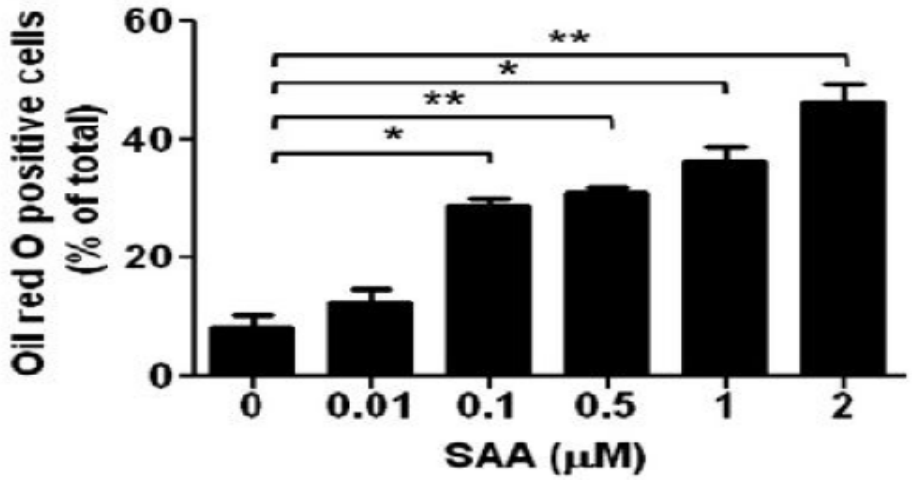
SAA: an acute phase protein that directly promotes formation of foam cells

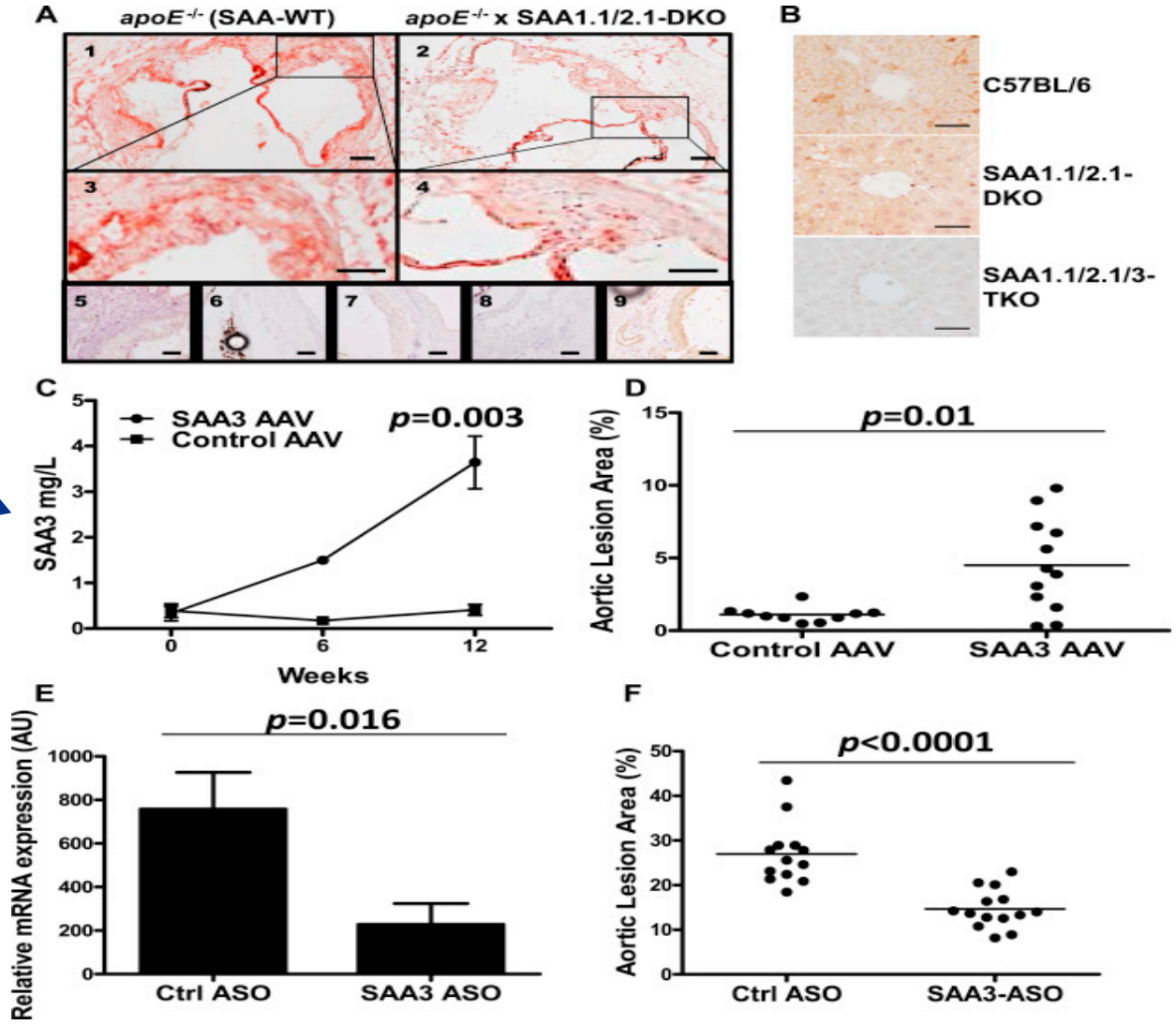
- SAA can replace ApoA-1 as the major HDL protein.
- This inhibits HDLs role in reverse cholesterol transport.
- SAA induces foam cell formation in macrophages [1].



[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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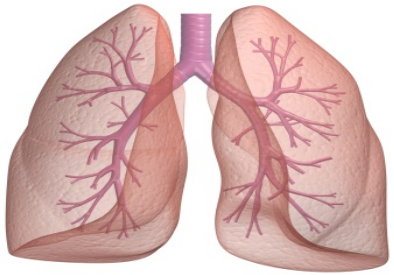
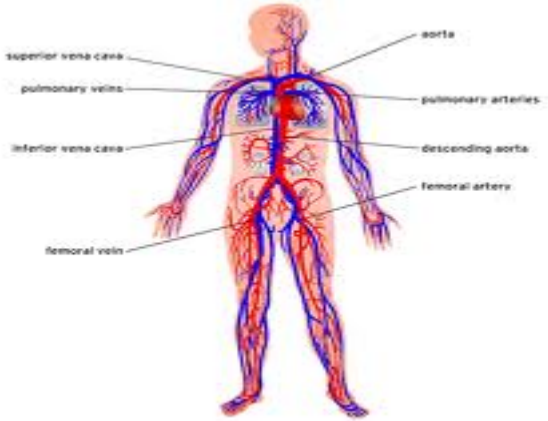
Particle-induce atherosclerosis

Particles

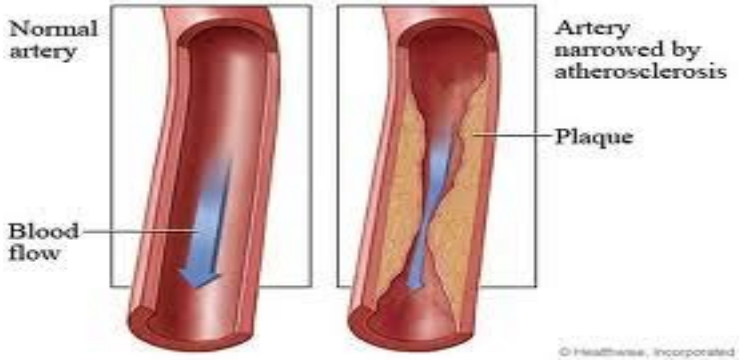


Inflammatory & acute phase response

Systemic circulation of acute phase proteins



Atherosclerosis



Coronary heart disease

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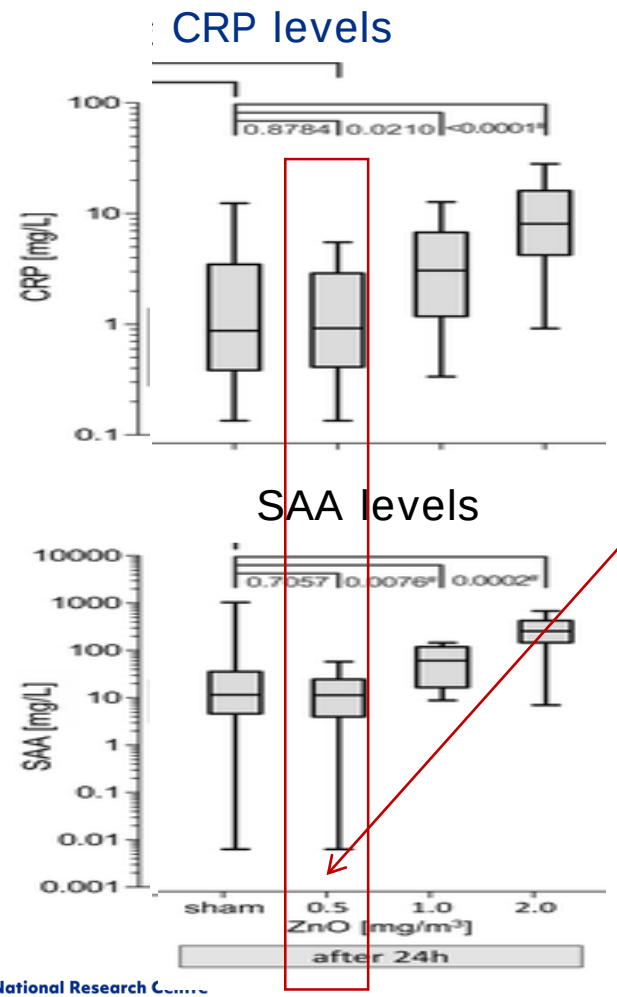
Any Human relevance ?: Yes; inhalation of ZnO induces acute phase response in human volunteers



- No effect level:
- 0.5 mg/m³

Study set up:

- 16 volunteers
- Exposed to 0, 0.5, 1 or 2 mg/m³ ZnO particles for 4 h
- OEL: 5 mg/m³ 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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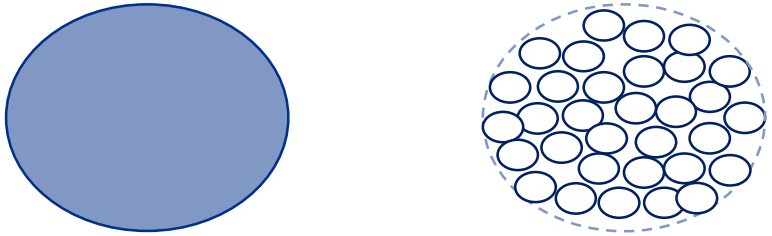
Adapted from Monsé et al Part Fibre Toxicol. 2018 15(1):8

And more..

- Correlation between exposure to organic dust and serum levels of acute phase proteins SAA and CRP among 33 greenhouse workers (Madsen et al, 2016, Environmental Health)
- Correlation between exposure to respirable dust and serum levels of SAA among 101 welders (Li et al, 2015, Plos One)
- Correlation between occupational exposure to paper mill dust and SAA and CRP levels (Westberg, Int Arch Occup Environ Health. 2016)
- Exposure to welding fumes with ZnO and/or CuO increase CRP and SAA levels in human volunteers (Baumann R et al, J Expo Sci Environ Epidemiol, 2018)
- Exposure to fumes from military small arms increased CRP levels in human volunteers (Sikkeland et al, Am J Respir Crit Care Med. 2017)

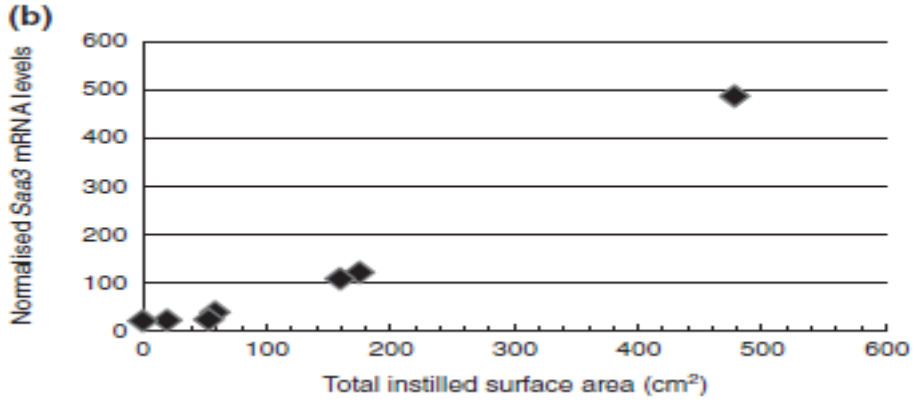
Risk assessment of nanoparticles



10 times smaller particles:

1.000 more particles pr mass unit

10 times larger surface area pr mass unit



Correlation between deposited surface area and pulmonary acute phase response

Summary

- Acute phase response is causally related to atherosclerosis and cardiovascular disease
- Airway exposure to nanoparticles and nanomaterials induces a time- and dose dependent acute phase response in mice
- The acute phase response correlates with markers of lung inflammation and deposited surface area
- Cardiovascular disease is an important particle-related occupational disease

Thank you for your attention!

Sabina Halappanavar
Health Canada

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