National Research Centre for the Working Environment

Ulla Vogel
Professor

Nanoparticle-relevant AOPs

Sarah S. Poulsen, Anne T. Saber, Nicklas R.Jacobsen, Jorid Sørli, Pernille Danielsen, Sabina Halappanavar and Ulla Vogel

DTU

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. Danish Centre for nanosafety 2012-2019.
- At present 35 persons involved in research in chemical working environment including nanosafety
- Advisors for the Danish Working Environment Authorities, EPA, EU, OECD, WHO
- Past and present partners in 27 EU projects on (nano)particle safety

AOPs are useful in occupational health

- Can be used to identify relevant AOs based on MIE or KE
- Identify and provide evidence for causal relationships between agent and disease for classification and labelling (regulation)
- Understanding of the underlying mechanism of action guides preventive measures in the working environment
- Understanding which physico-chemical properties that drive the toxic response guide safe-by-design approaches in innovation of new nanomaterials

ORIGINAL ARTICLE

Respiratory disease mortality among US coal miners; results after 37 years of follow-up

Judith M
Michael

Real-life exposures to nanoparticles are complex and cause several diseases

Region									
Eastern Pennsylvania	181	365.29	11	0.61	26	0.79	77.0	97.7	2.6
Eastern Appalachia	65	86.91	48	1.26	77	1.01	82.4	77.4	2.8
Western Appalachia	101	38.21	157	1.05	334	1.17†	82.3	82.3	3.8
Midwest	14	17.38	53	1.44†	101	1.47†	86.7	72.8	2.7
West	42	53.74	40	1.10	30	0.49†	81.7	77.5	2.5
Race									
White	389	67.44	293	1.09	542	1.09†	82.5	80.4	3.3
Black	14	129.84	16	1.60	26	0.88	78.1	90.4	3.8
Baseline radiograph									
Category 0	237	48.50	250	1.06	510	1.13†	83.0	76.4	3.2
Category 1	56	92.67	29	1.15	39	0.89	78.9	99.3	3.6
Category 2	65	192.82	24	1.85†	14	0.62	75.7	113.0	3.6
Category 3	45	409.78	6	1.39	5	0.61	82.5	122.0	3.6
Calendar year of death									
1970–1989	170	63.25	85	0.86	233	0.95	83.60	n/a	
1990–1999	143	106.20	118	1.19	208	1.31†	81.90		
2000–2007	90	127.59	106	1.26†	127	1.23†	80.90		

*SMRs from pneumoconiosis are artificially high as there is no valid comparison group in the general population. We included them to show comparisons within levels of covariates and for consistency with previously published studies of this cohort. We do not include statistical testing given the lack of a valid comparison group.
†Statistically significant at p<0.05

ORIGINAL ARTICLE

Respiratory disease mortality among US coal miners; results after 37 years of follow-up

Judith M Graber,[1,2] Leslie T Stayner,[1] Robert A Cohen,[3] Lorraine M Conroy,[3] Michael D Attfield[4]

- 8827 miners
- 37 years of follow-up (1969/71-2007)
- 67% dead (cause of death known)

Table 2 Overall and stratified SMRs for selected underlying causes of death and percentage of ever smokers, mean cumulative coal mine dust and respirable silica exposure category by coal-rank region, radiographic status at enrolment, race and calendar year of death

	Pneumoconiosis*		COPD		Lung cancer		Ever smoked at enrolment	Mean cumulative exposure*	
Category	Obs.	SMR	Obs.	SMR	Obs.	SMR	Per cent	Coal mine dust mg/m^3-years	Respirable silica
Total	403	79.70	309	1.11	568	1.08	79.6	81.0	3.2
Region									
Eastern Pennsylvania	181	365.29	11	0.61	26	0.79	77.0	97.7	2.6
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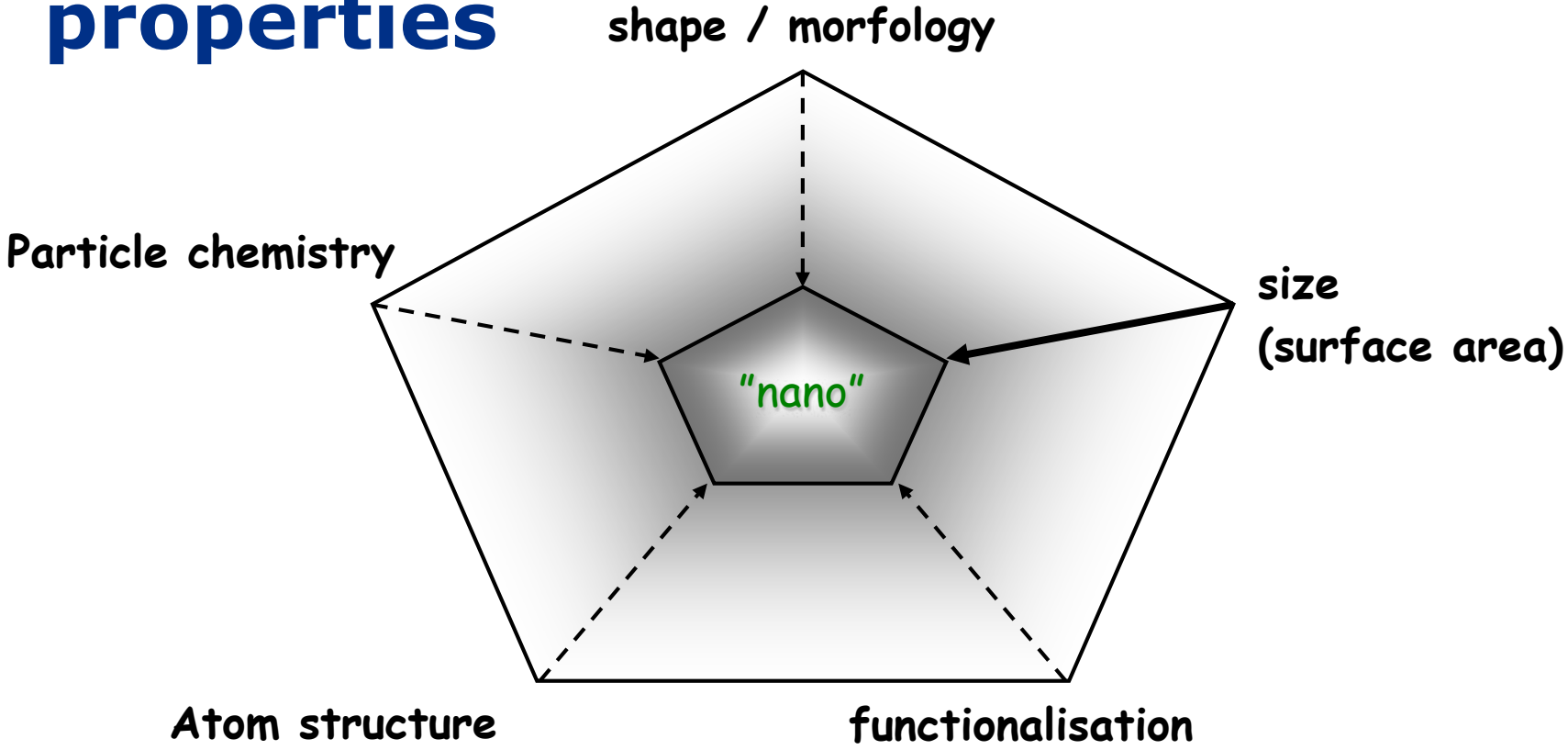
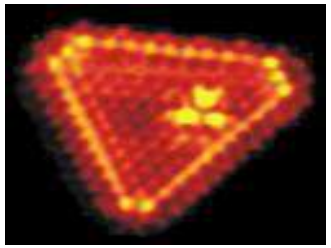
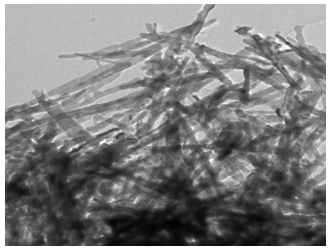
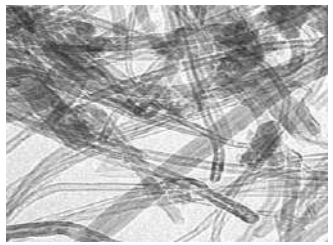
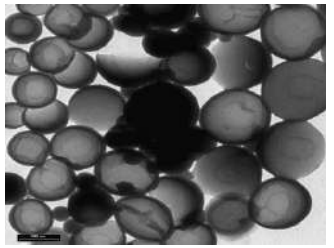
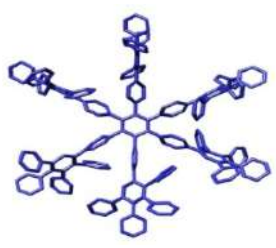
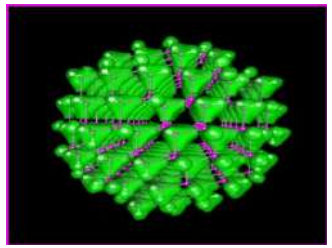
Graber JM, *et al. Occup Environ Med* 2014;**71**:30–39. doi:10.1136/oemed-2013-101597

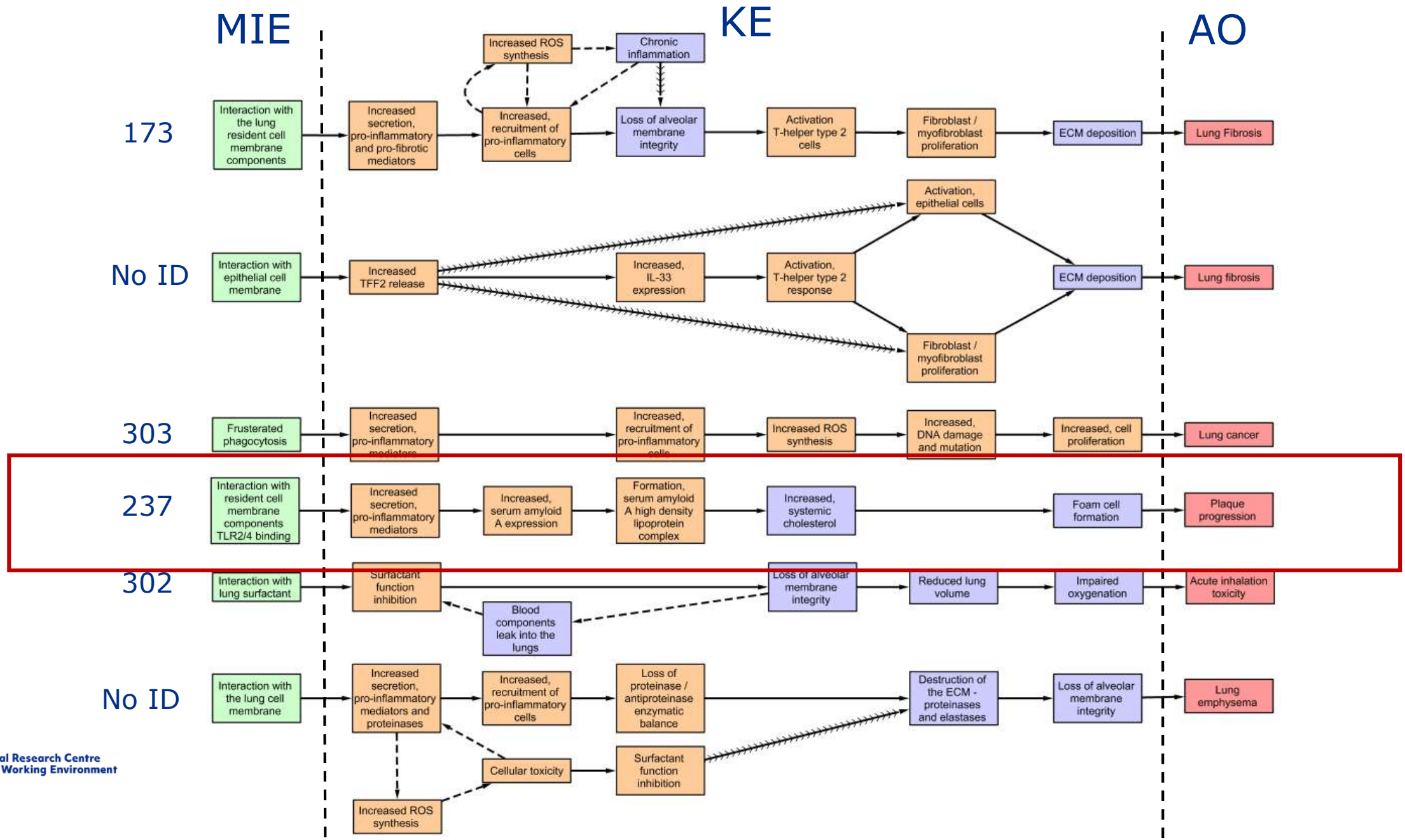
The second challenge: Many different nanomaterials with varying physico-chemical properties



Nano-relevant AOPs developed in the EU H2020 project SmartNanoTox



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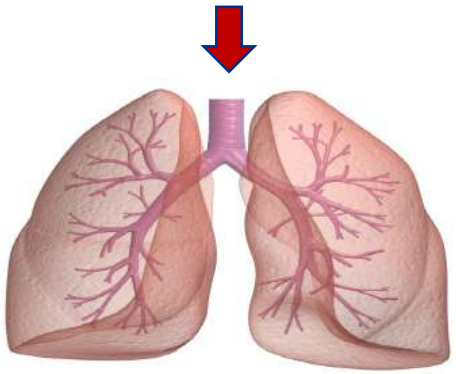
Proposed mechanism of action of particle-induced cardiovascular disease

Inhaled particles promote atherosclerosis via acute phase response

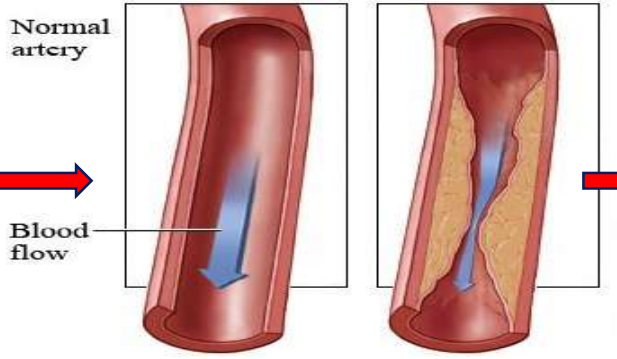
Inhalation of (nano)particles



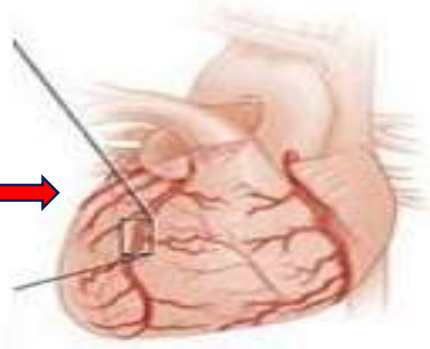
Inflammatory & acute phase response

Systemic circulation of acute phase proteins

Atherosclerosis



Cardiovascular disease



New knowledge

Established fact

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

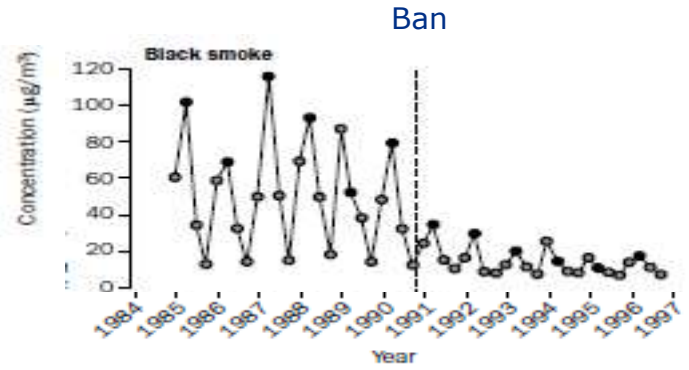


Table 2: **Age-standardised mortality rates for Dublin County Borough before (1984–90) and after (1990–96) ban of sale of coal, by season**

	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)

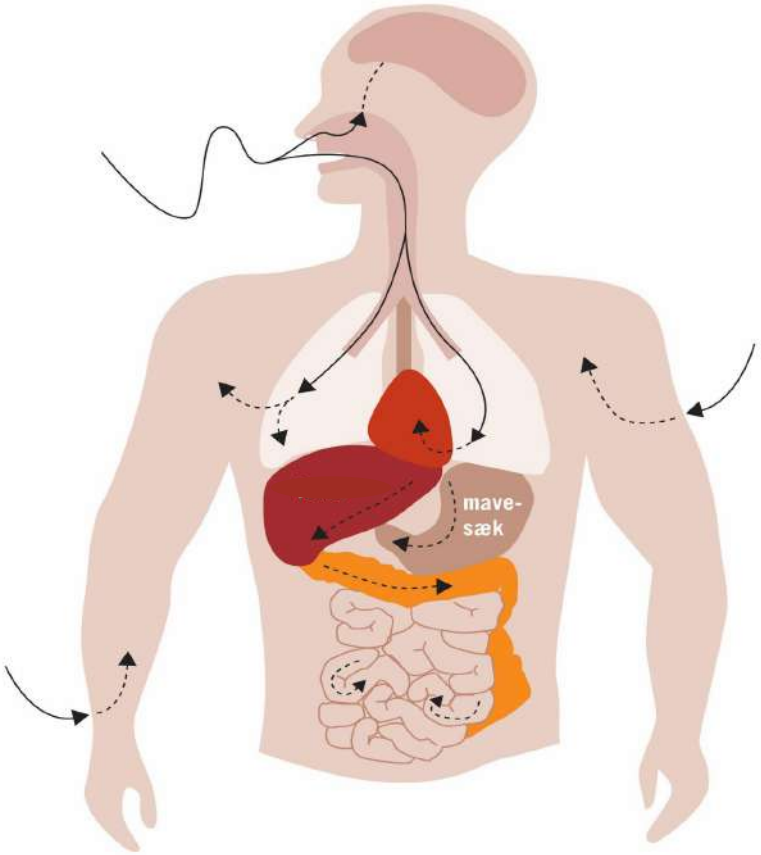
Inhalation of particles and risk of cardiovascular disease

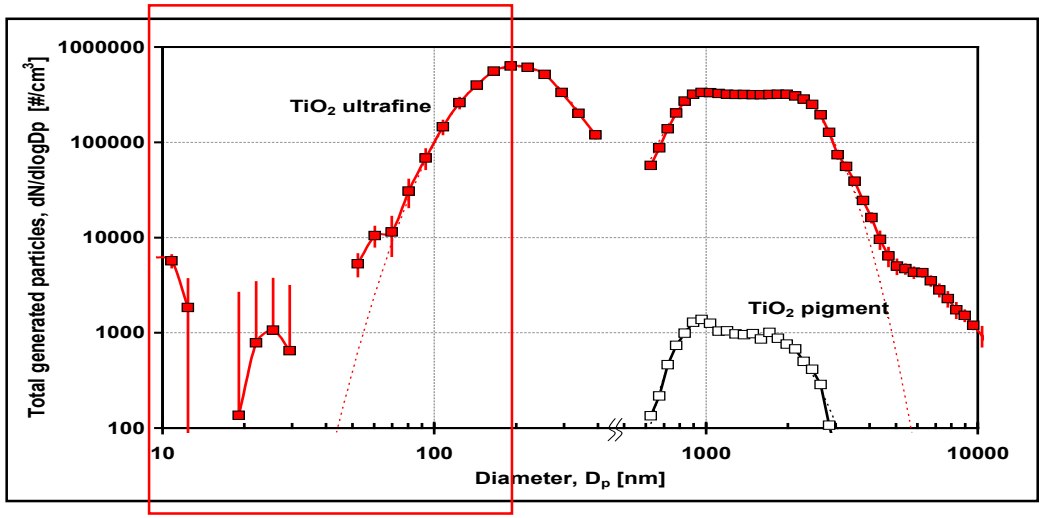
- Epidemiological studies show associations between air pollution and risk of cardiovascular disease

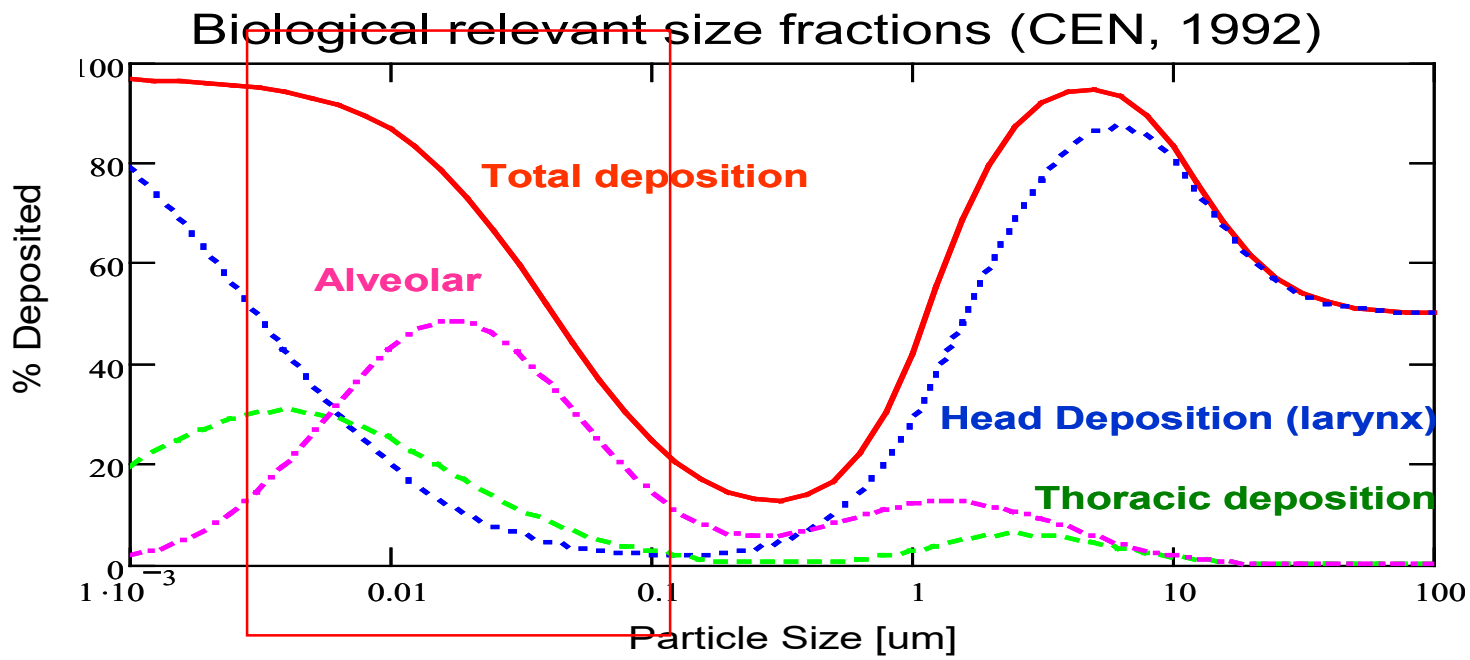
- Several different mechanisms have been proposed related to
  - Inflammation
  - Inflammation-induced hepatic acute phase response
  - Direct effect of translocated particles
  - Vascular function

Aerodynamic size in air predicts pulmonary deposition during inhalation exposure

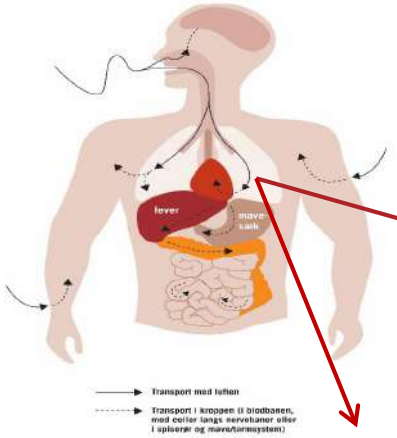
Nanomaterials aggregate in air







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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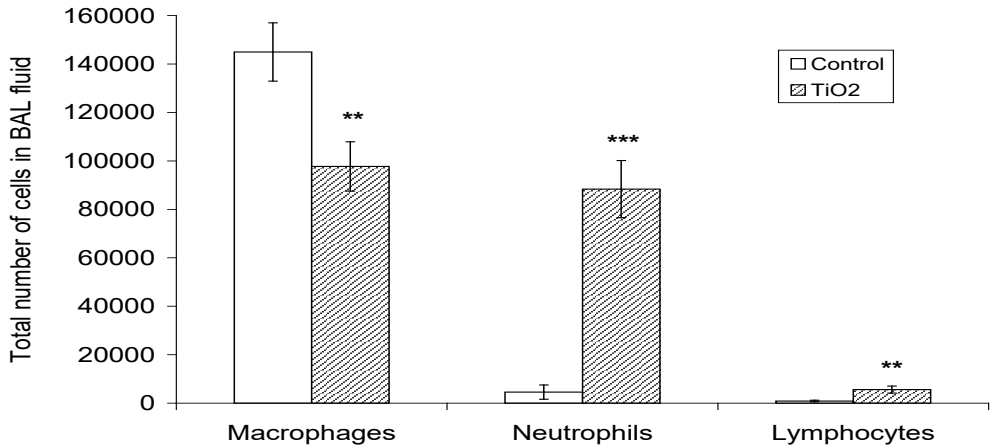
Marianne Dybdahl

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.
Current Danihs Occupational Exposure Limit: 10 mg/m^3 for 8 hours.
Types and numbers of cells in lung fluid:

Cell composition in bronchoalveolar lavage fluid 5 days post-exposure



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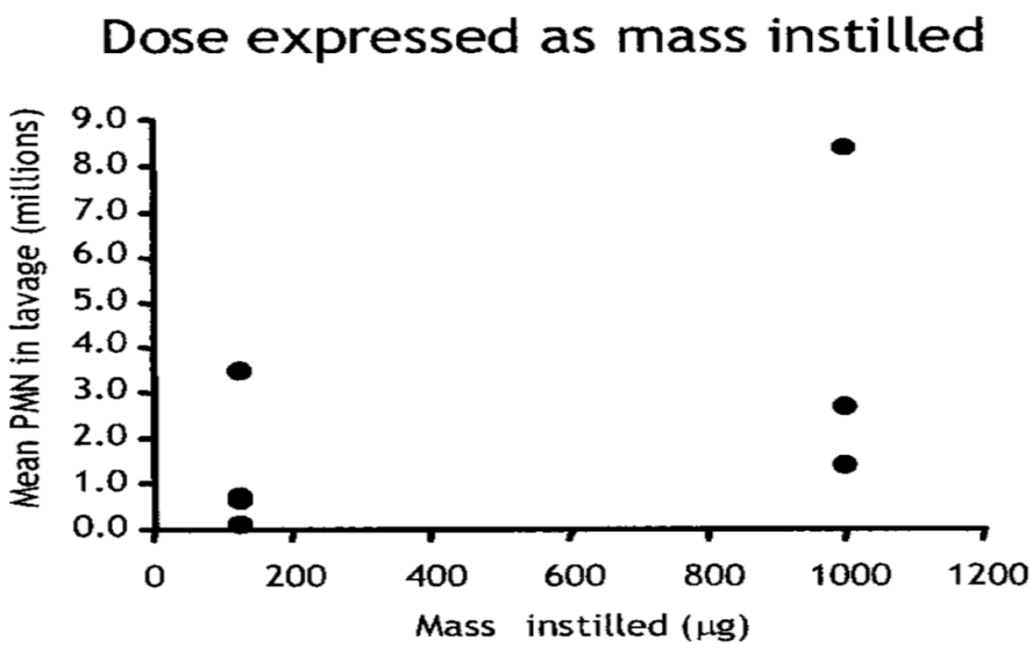
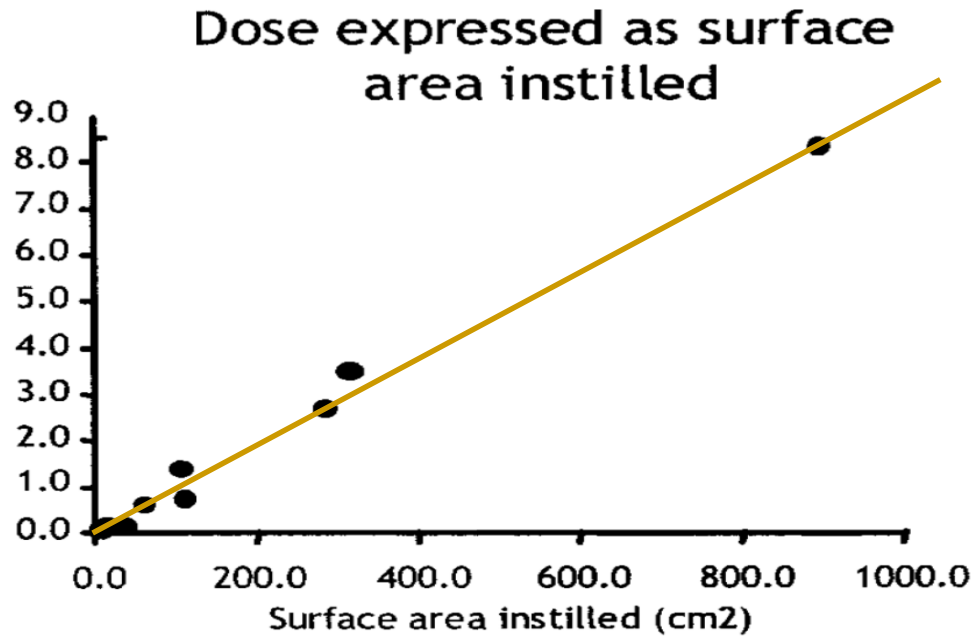
Hougaard et al, PT&T 2010

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

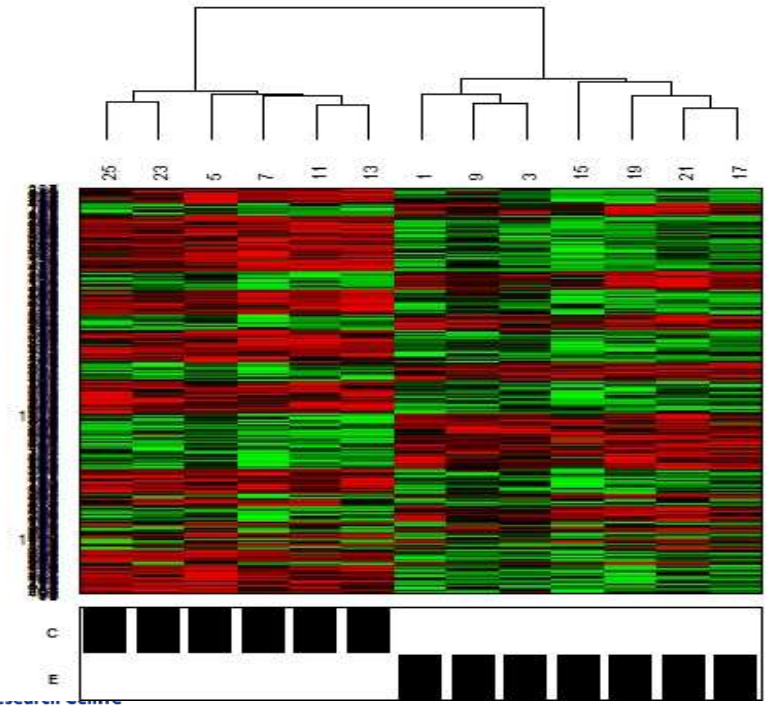

TABLE II. List of all Acute Phase Response Genes Showing Fold Changes Higher Than 1.2 in exposed mice

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

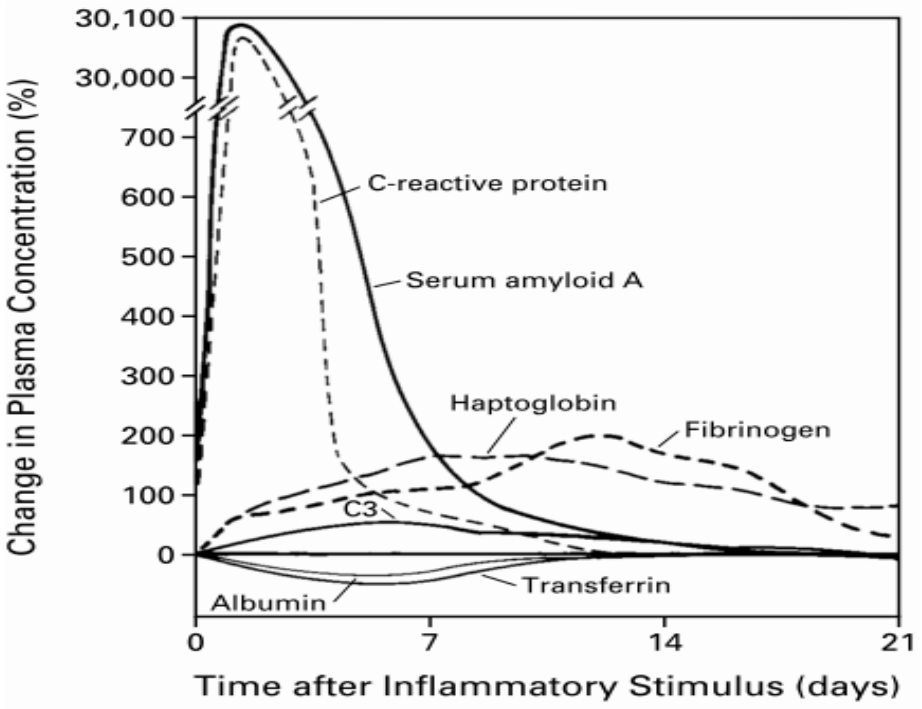

Figure 1. Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus.
Modified from Gitlin and Colten[5] with the permission of the publisher.

- 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, HIV infection, antibiotic treatments 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

Time- and dose-dependent pulmonary acute phase response following airway exposure of mice to different nanomaterials

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

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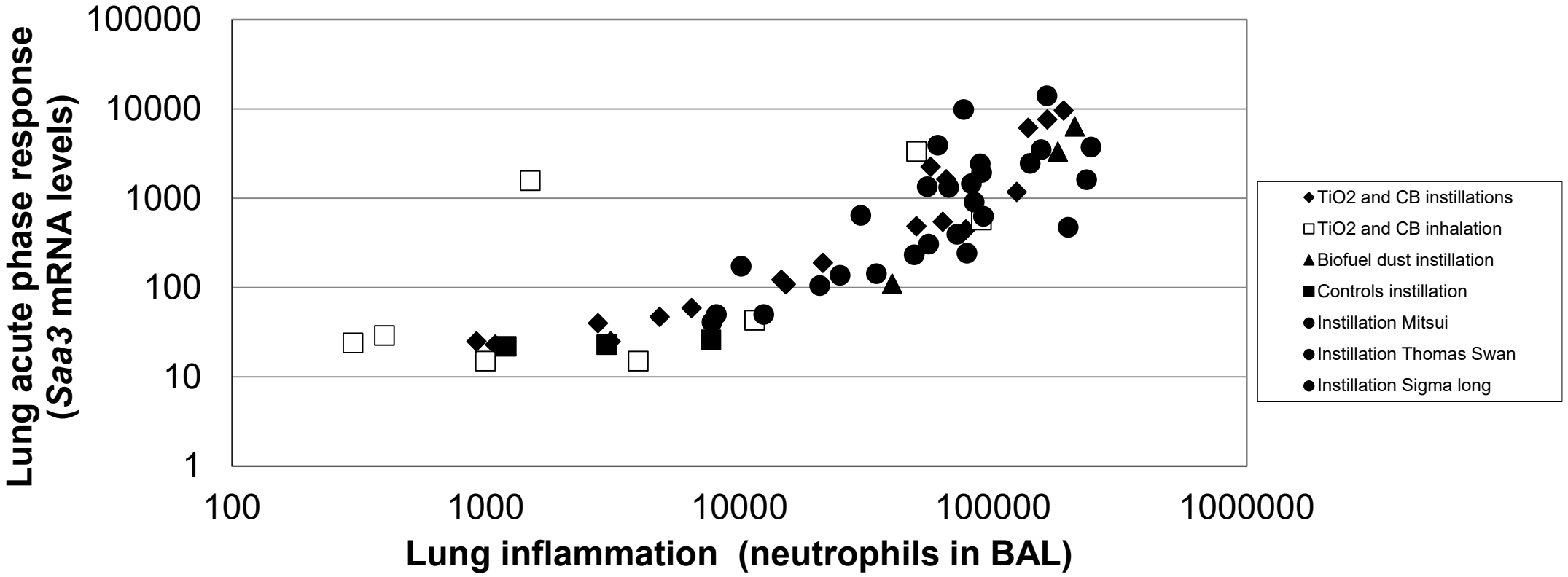
Close correlation between *pulmonary acute phase response* and pulmonary inflammation across particles, doses, time points



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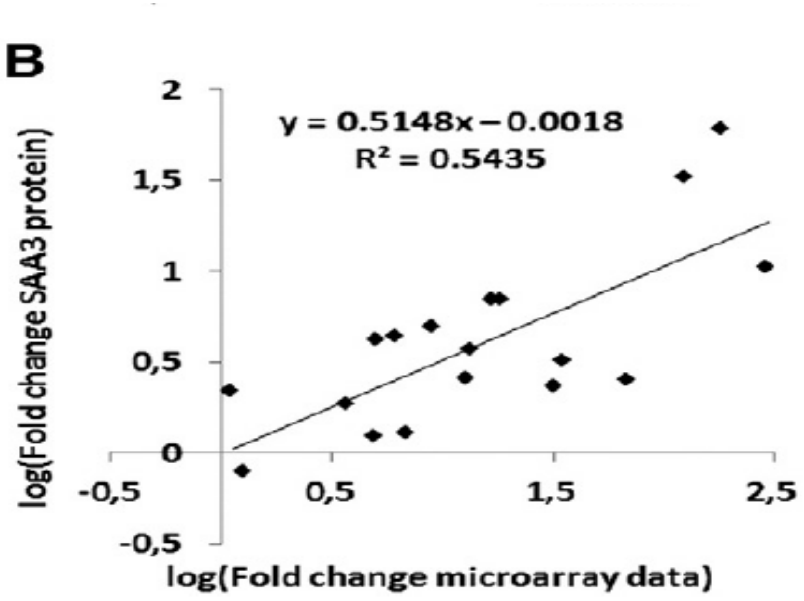
Saber AT et al, Plos One, 2013

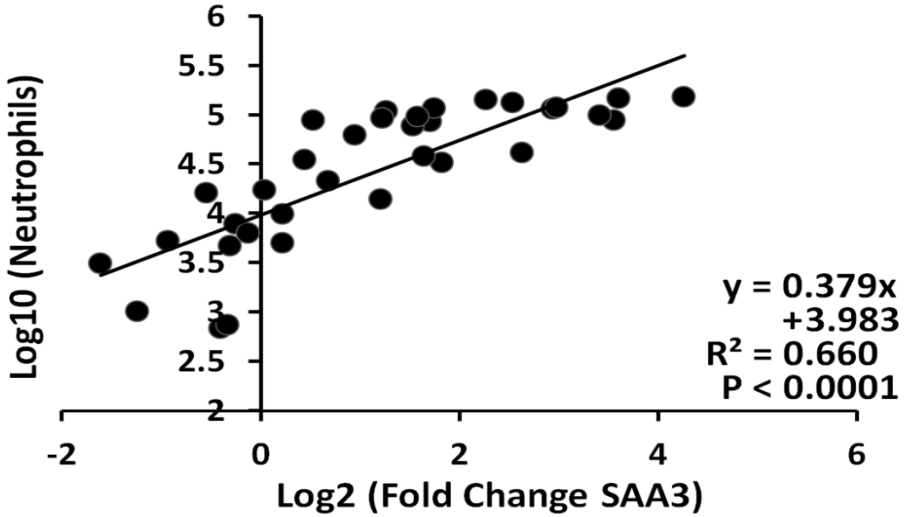
Plasma levels of acute phase protein SAA3 correlates with lung responses

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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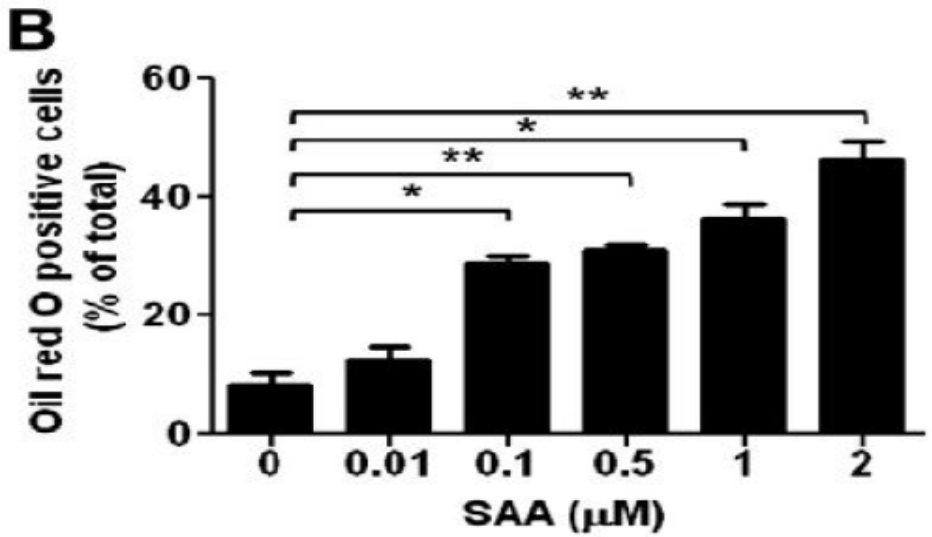
SAA: an acute phase protein that directly promotes foam cell formation and atherosclerosis

- 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 (Lee, 2013).

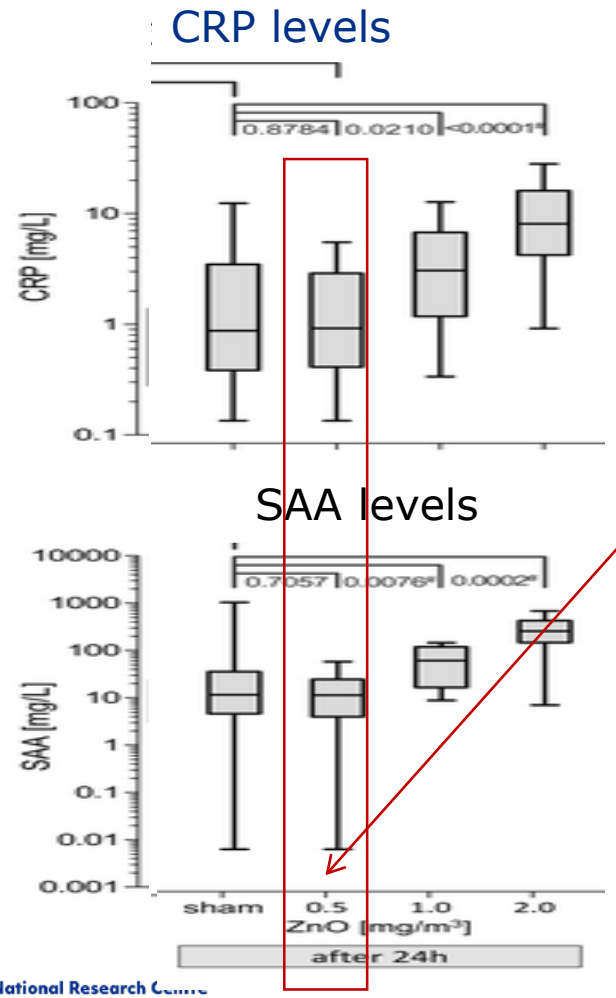
- Mice have 3 inducible SAA isogenes (*Saa1, Saa2, Saa3*)
- Over-expression of SAA3 or SAA1 increases plaque progression (Thompson 2018, Deng, 2011)
- Inactivation (KO) of all SAA isogenes results in reduced plaque progression (Thompson 2018)

Dose-dependent foam cell formation



[1] Lee et al, 2013, BBRC

Human relevance: 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

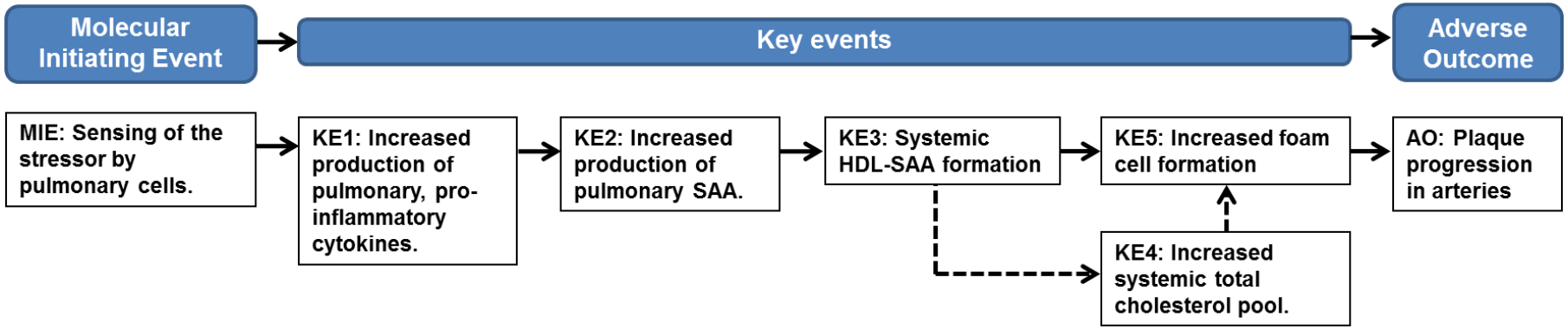
And more..

- 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)

AOP

- Upregulation of *Il6, Il1β* and *TNFa* leading to transcription of Saa genes
- SAA synthesis. Translocation to the blood. Replacement of Apo-A1 as the major HDL-associated protein.
- Impaired reverse cholesterol transport → Larger cholesterol pool.
- HDL-SAA and SAA stimulate the transformation of macrophages into foam cells.
- Foam cells are primary components of atherosclerotic plaques.

AOP 237



- The AOP was accepted into the OECD AOP program in June 2017

New health-based occupational exposure limit for Zinc oxide

- The Danish Working Environment Authority has asked NFA to provide the scientific basis for a new health-based occupational exposure limit for ZnO
- NFA will submit the OEL proposal in 2020

Thank you for your attention!

Sabina Halappanavar
Health Canada

Health Canada Santé Canada

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National Research Centre for the Working Environment

SmartNanoTox
Smart Tools for Gauging Nano Hazards

PATROLS
Advanced Tools for NanoSafety Testing