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European Aerosol Conference

Health effects of inhaled nanomaterials

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DTU

Nanosafety at the National Research Centre for the Working Environment, Copenhagen, Denmark

- Government research institute under the Ministry of Employment
- Nanosafety as strategic research area since 2005
- At present 35-40 persons in nanosafety research
- Advisors for the Danish Working Environment Authorities, EPA, EU, OECD, WHO
- Past and present partners in 25 EU projects on (nano)particle safety

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Health effects of inhaled nanomaterials – the short version:

Are nanoparticles more hazardous by inhalation compared to larger particles with the same chemical composition ?

Yes! (by mass)

Is there occupational and/or environmental exposure to nanomaterials?

Yes

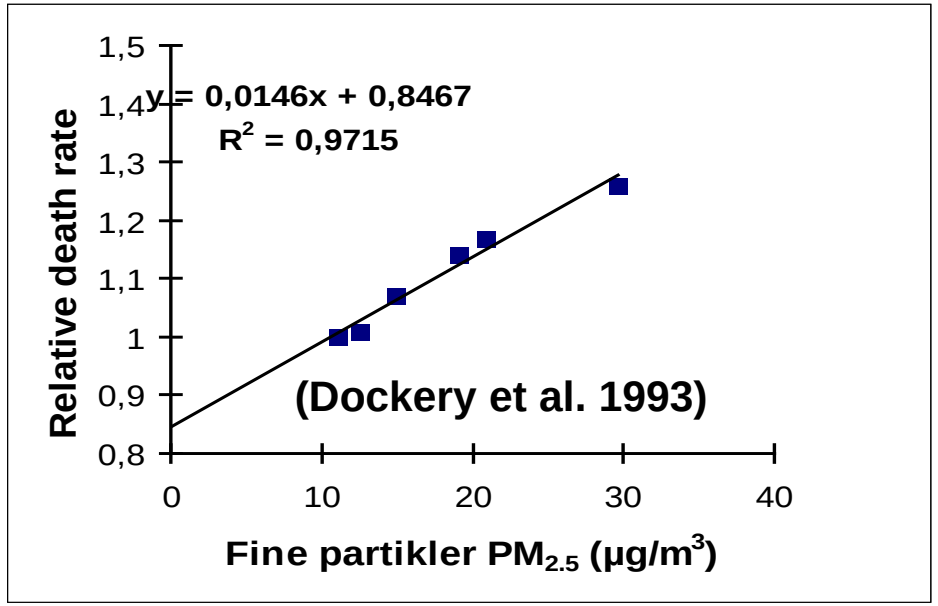
Are exposure levels high enough to pose a potential health risk ?

Yes

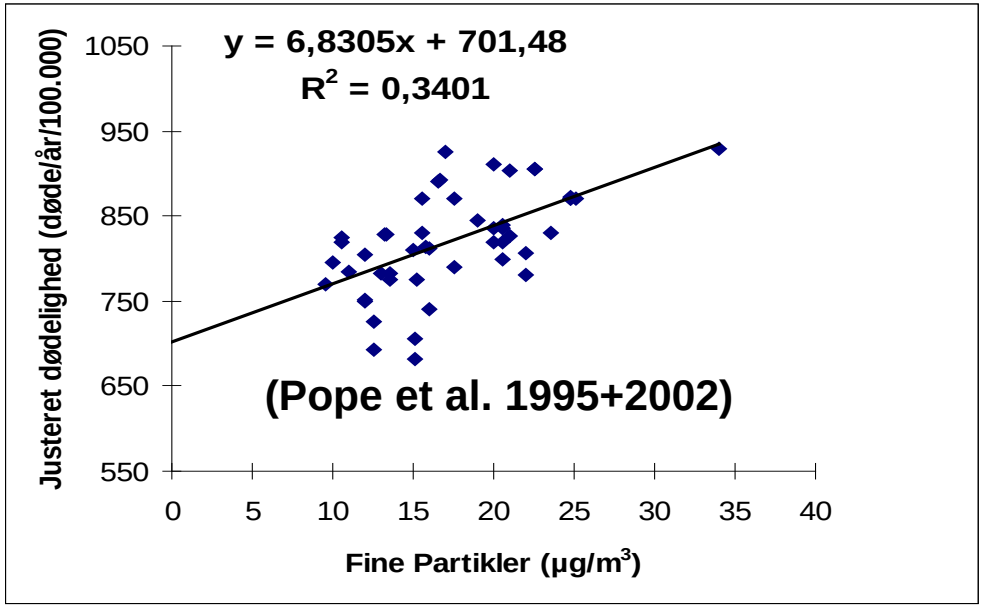
✓ Need for regulation of (occupational) exposure

Concern about nanomaterials: Association between particulate air pollution and mortality

6 cities with 8000 people



151 urban areas with 500.000 people



Direct correlation between mortality and particle concentration ($PM_{2.5}$):

7 deaths/100 000 persons/year/ ug/m^3 PM 2.5

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RISK = Exposure x Hazard

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Keld A Jensen

The vision for safe use of nanomaterials

Research → **Mechanism-based understanding of toxicological effect** → Evidence-based risk assessment.
Prediction of the toxicological effects on the basis of physico-chemical properties

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

Evidence-based risk assessment. → Safe use of engineered and process-generated nanomaterials

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Safe-by-design:

Asbestos

Lung cancer
Fibre-paradigm

Mineralwool

Paint based on organic solvents

Painters syndrome
Organic solvents

Water-based paint
MAL kodes

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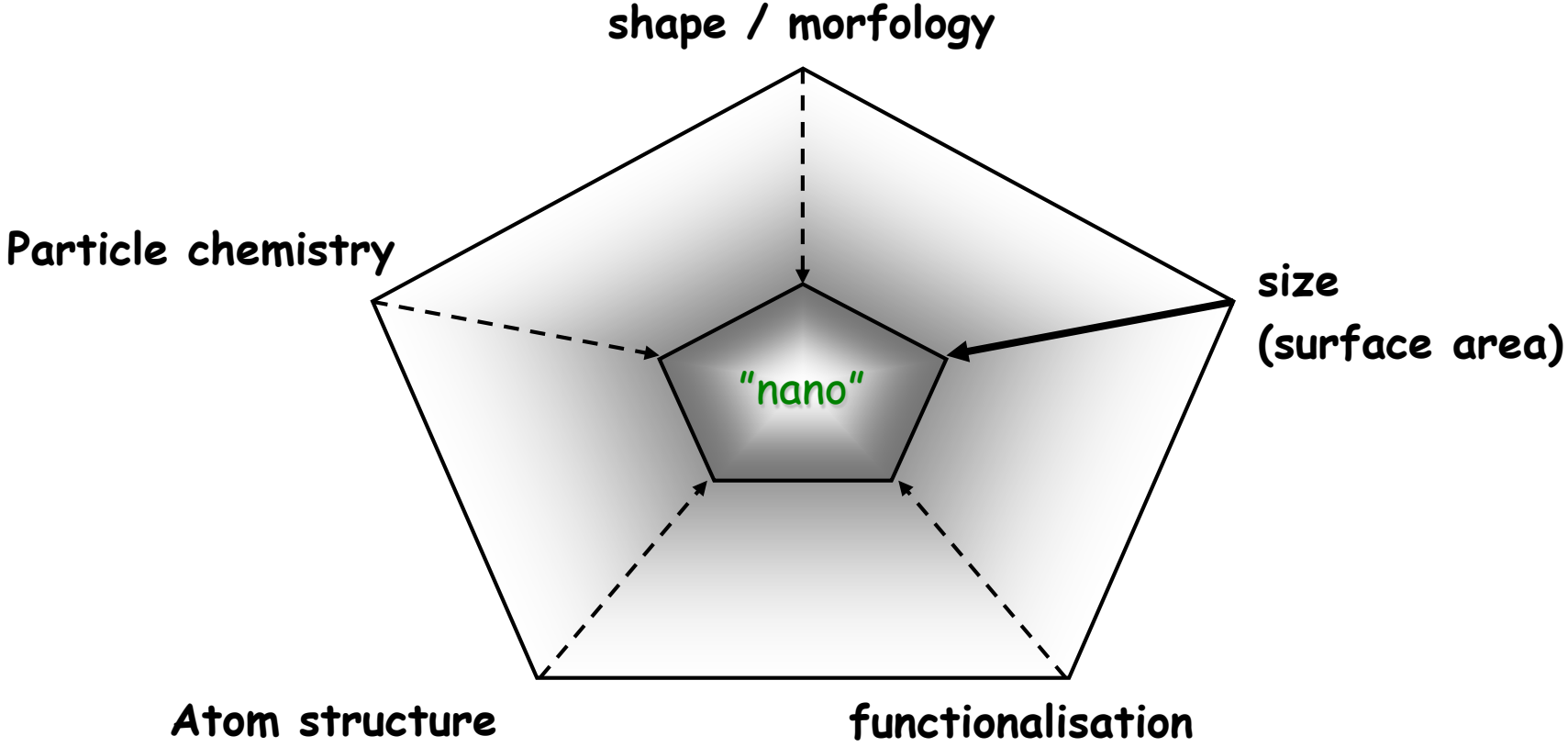
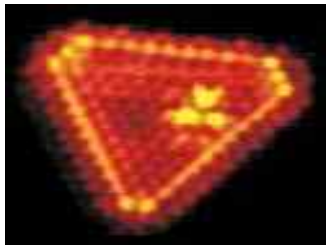
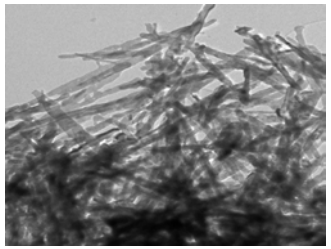
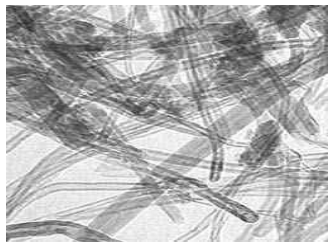
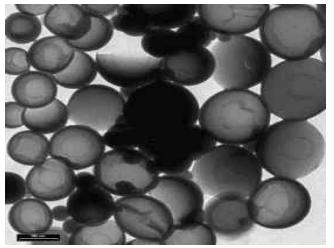
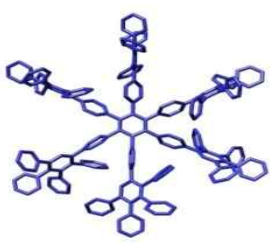
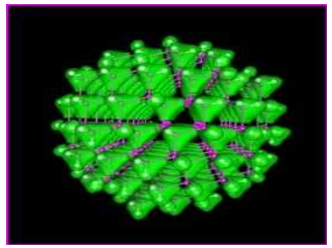
The challenge: Many different nanomaterials with varying physico-chemical properties



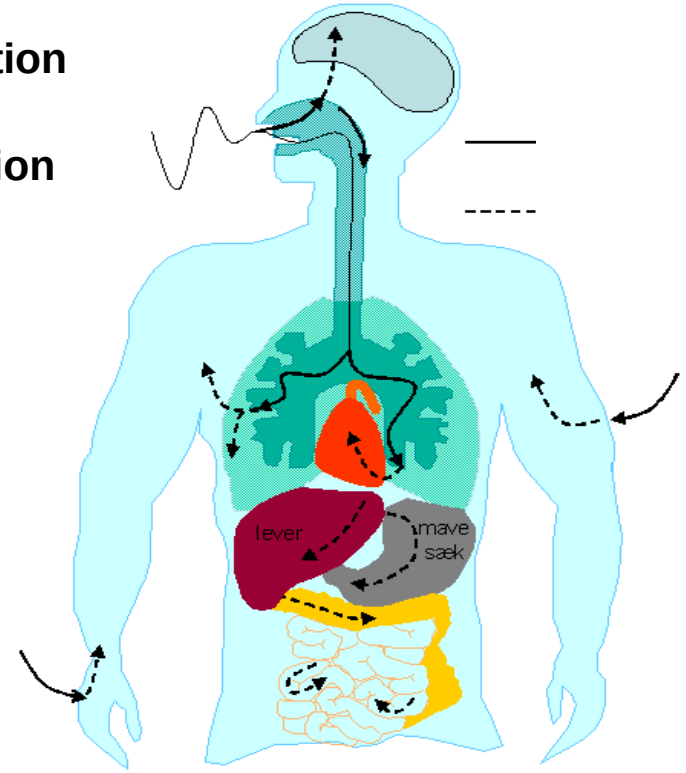





Inhation is the most relevant exposure route for nanomaterials for consumers and workers

Inhalation

Ingestion

Through the skin



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Key questions

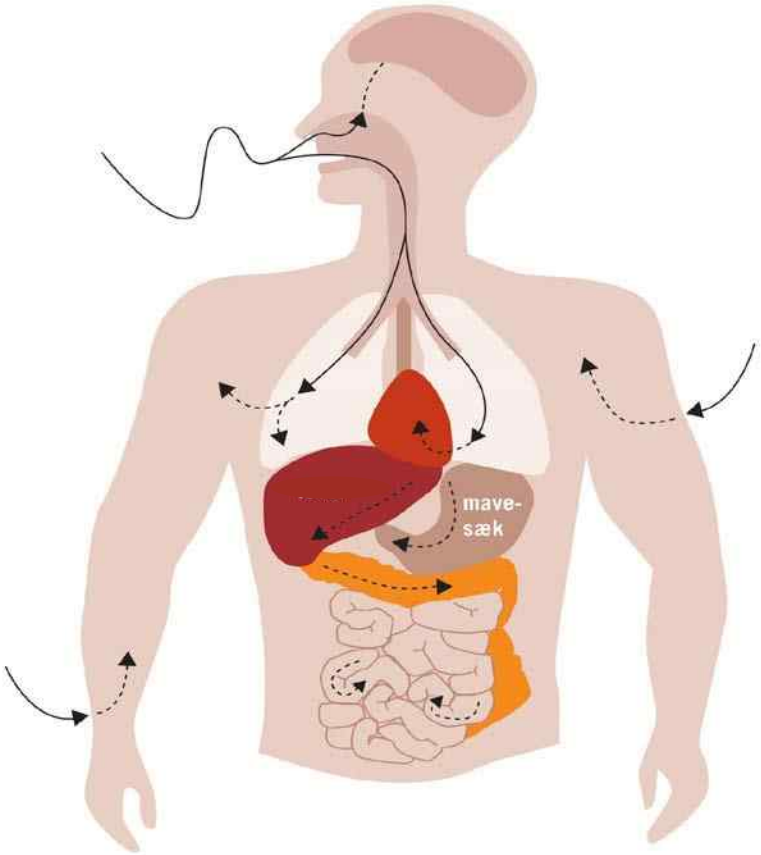
- What is going on ? (the biological mechanisms of action)
- Which physico-chemical properties drive the toxic responses ?

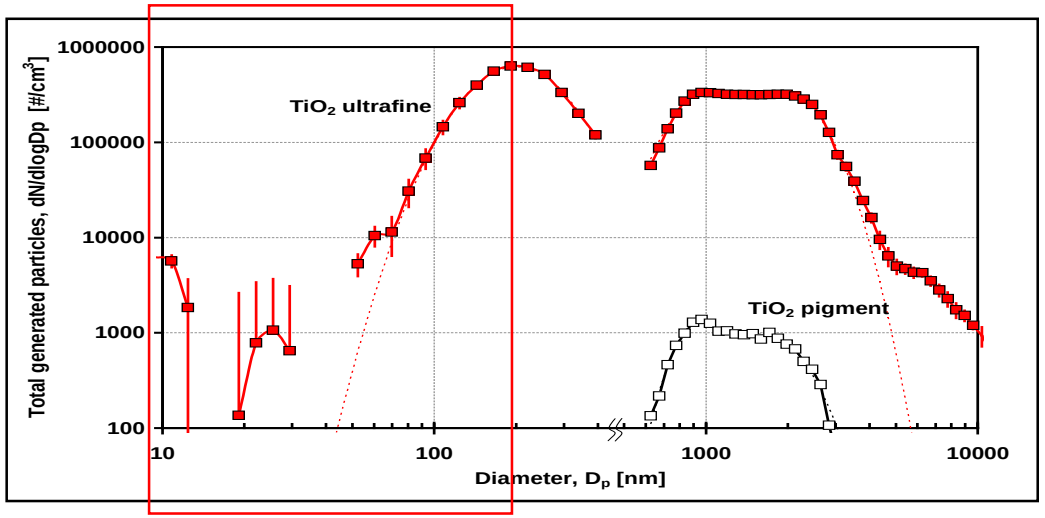
Focus on:

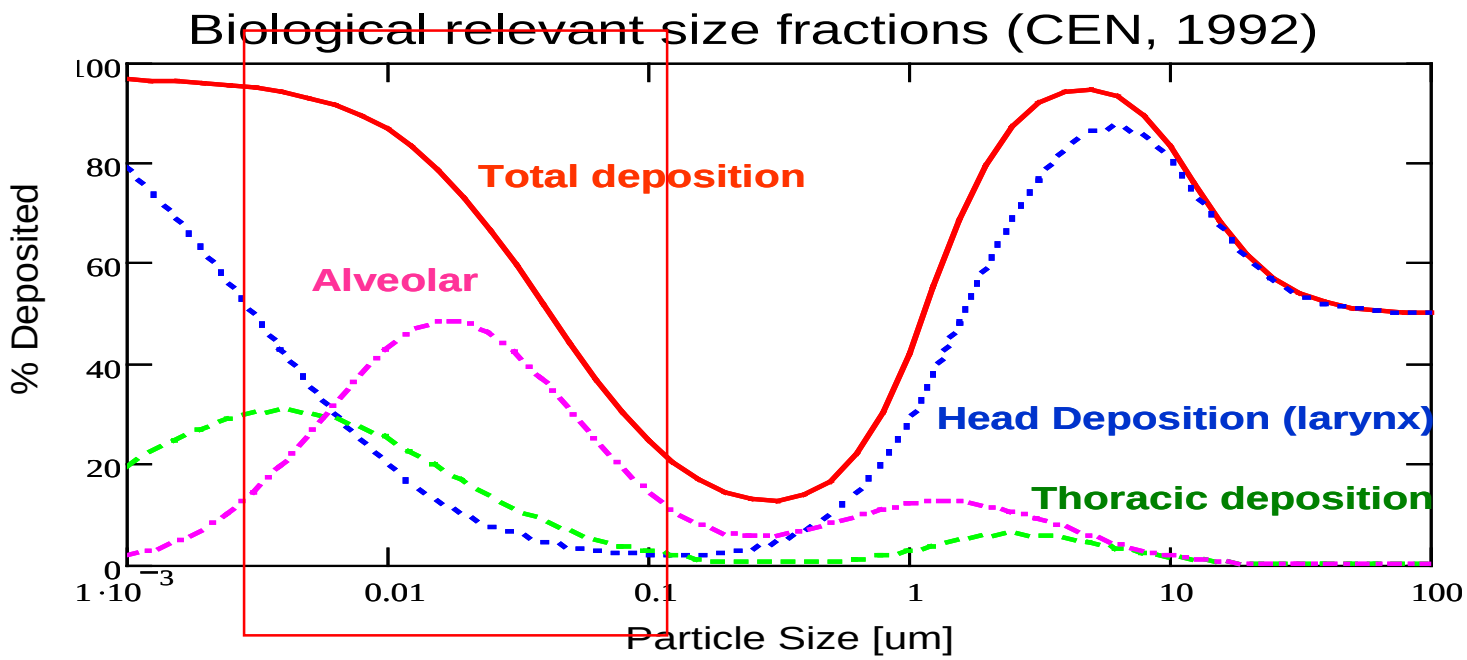
- Cardiovascular disease
- Cancer
- Risk assessment

Aerodynamic size in air is the important predictor of pulmonary deposition during inhalation exposure

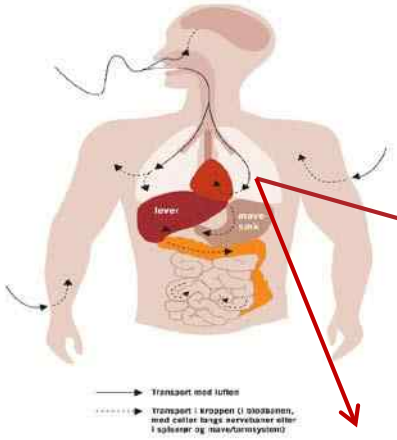
Nanomaterials aggregate in air







Low clearence of nanoparticles from the lung



Nanoparticles
Ultrafine particles

Fine 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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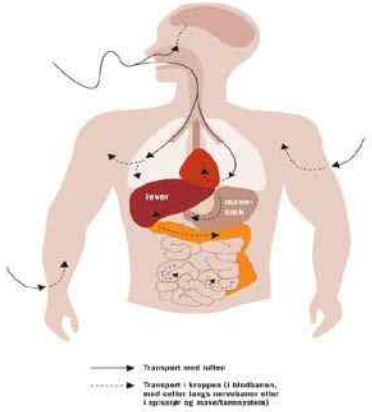
Marianne Dybdahl



Even lower clearance of High Aspect Ratio nanomaterials

Fine particles

Airways

Particle deposition and removal by mucociliar clearance

Particle phagocytosis by macrophage

Nanoparticles

Ultrafine particles

INFLAMMATION

release of mediators

ROS

Carbon nanotubes

INFLAMMATION

release of inflamatory mediators

ROS

Frustrated fagocytosis

Very low clearence

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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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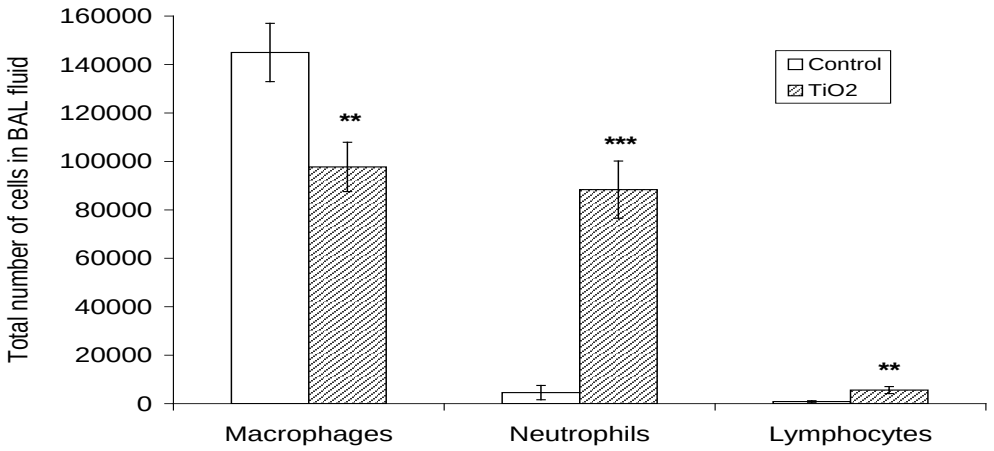
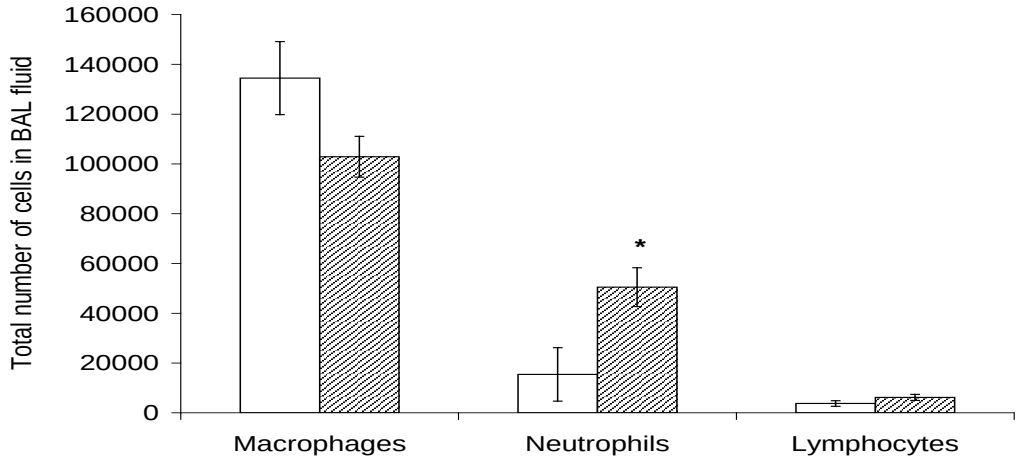
Hougaard et al, PF&T, 2010

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

Global gene expression in lung tissue af inhalation of TiO_2 NPs (day 5): acute phase reponse !

The most differentially regulated genes

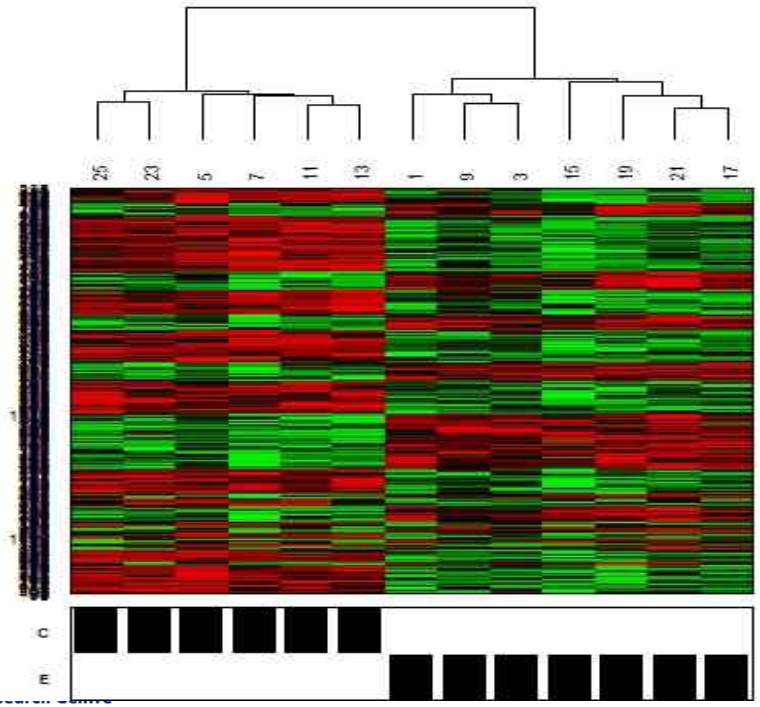


TABLE II. List of all Acute Phase Response Genes Showing Fold Changes Higher Than 1.2 in exposed mice

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

Acute phase response is causally linked to cardiovascular disease

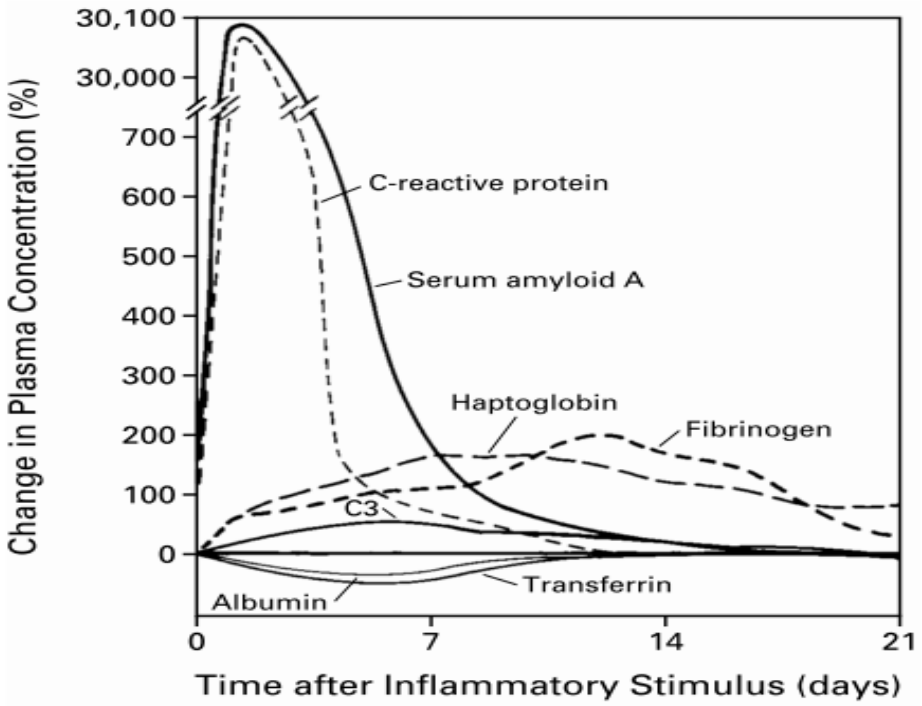


Figure 1. Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus.

Modified from Gitlin and Colten[5] with the permission of the publisher.

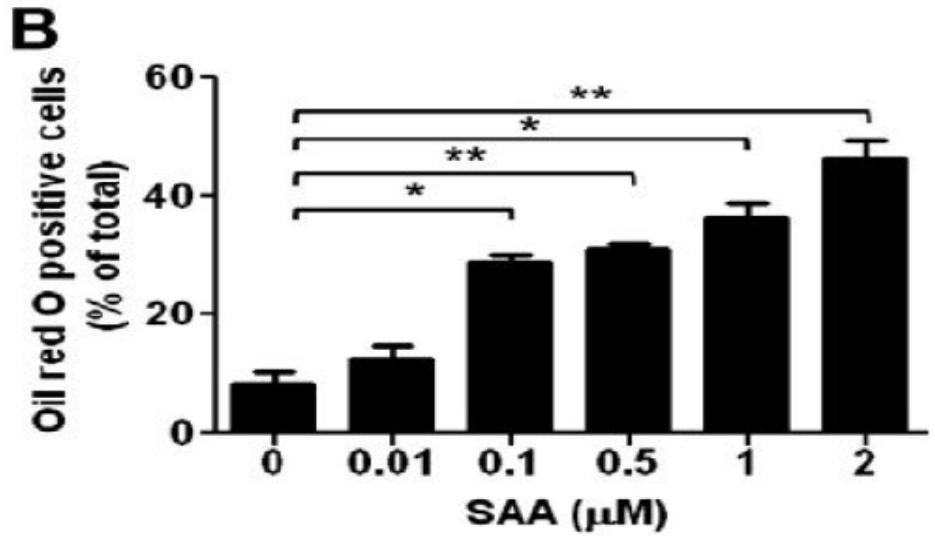
- The acute phase response is the systemic response to acute and chronic inflammatory states caused by fx bacterial infection, trauma and infarction
- Conditions that entail acute phase response are associated with risk of cardiovascular disease including HIV infection, asthma, air pollution exposure
- Acute phase protein Serum Amyloid A is directly implicated in atheroscleorosis (Thompson et al, 2018)

Gabay and Kushner, 1999

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

- Mice have 3 inducible SAA isogenes (*Saa1, Saa2, Saa3*)
- SAA induces foam cell formation in macrophages (Lee, 2013).
- Over-expression of SAA3 or SAA1 increases plaque progression (Thompson 2018,)
- Inactivation (KO) of all SAA isogenes results in reduced plaque progression (Thompson 2018)

Dose-dependent foam cell formation



[1] Lee et al, 2013, BBRC

Acute phase proteins CRP & SAA are associated with risk of cardiovascular disease 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

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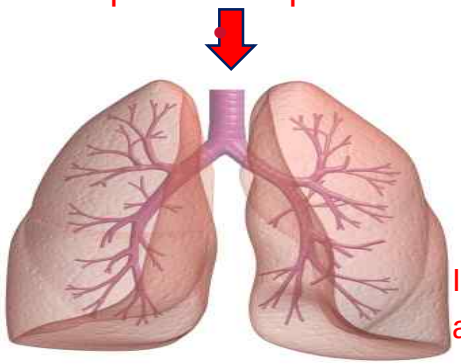
Ridker et al, NEJM, 2000

Proposed mechanism-of-action for (nano)particle-induced atherosclerosis

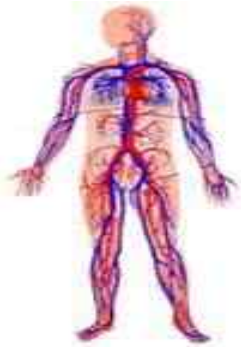
Inhaled particles promote atherosclerosis via acute phase response

Inhalation of particles below occupational exposure limits

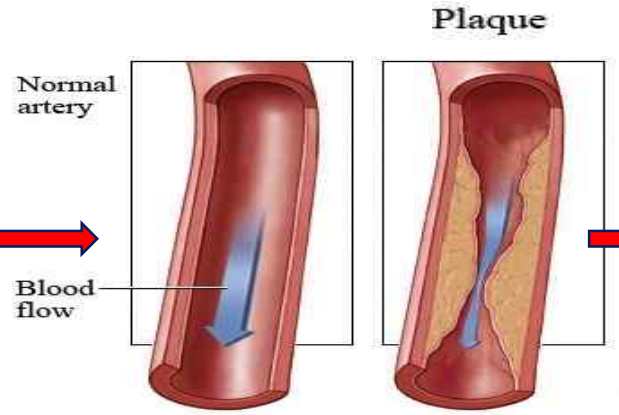


Inflammatory & acute phase response

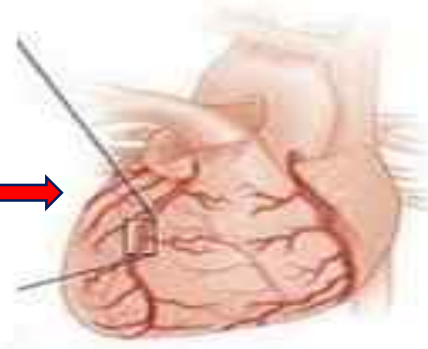
Systemic circulation of acute phase proteins



Atherosclerosis



Cardiovascular diseases



New knowledge

Established fact

Dose dependent induction of pulmonary acute phase response by different nanomaterials including CNTs

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 a										
Fold										
Carbo										
N a										
Fold										
Multi										
N a										
Fold increase of Saa3 mRNA[b]	52	151	95	39	152	612	7.9	29	88	

All assessed nanomaterials induce dose-dependent acute phase response in mice

Humans and mice induce acute phase response

Rats have a fundamentally different acute phase reponse

ND, not determined.

[a]The number of differentially expressed acute phase genes as observed in DNA microarray. Murine acute phase genes were identified through the Gene category on NCBI. The following genes were included: *ACADM, ARSG, ANGPTL4, APEX1, CD163, CREB3L1, CRP, CSF3, EPO, ESRRA, F2, F8, FGF21, FN2, HAMP, HNF4A, HP, ICIRN, IL22, IL6, IL6ST, INS2, ITIH4, LBP, LCN2, MRC1PEA3, NR1H4, NR3A2, ORM1, ORM2, PRLR, REG1A, REG3B, REG3G, RELA, SEPP1, SERPINA1B, SERPINA3N, SERPINF2, SIGIRR, STM2, SMAD3, STAT1, STAT5B, SULT2A1, SAA1, SAA2, SAA3, SAA4, SAA1.3, TSC2,* and *VIMP*. Genes with a fold change ± 1.5 and FDR *P*-values ≤ 0.05 were considered as differentially expressed.

[b]Fold increase of mRNA *Saa3* was determined using qRT-PCR. *Saa3* levels were normalized to 18S levels.

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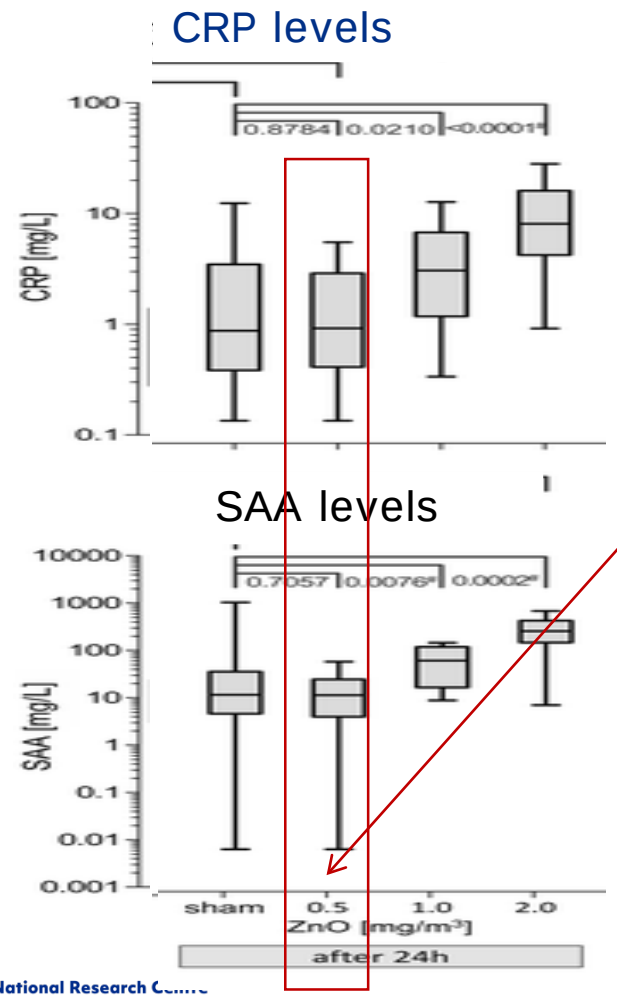
Saber et al 2014 WIREs Nanomed nanobiotech

Human relevance: Inhalation of ZnO induces dose-dependent 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 induced at ZnO concentrations well below current OEL

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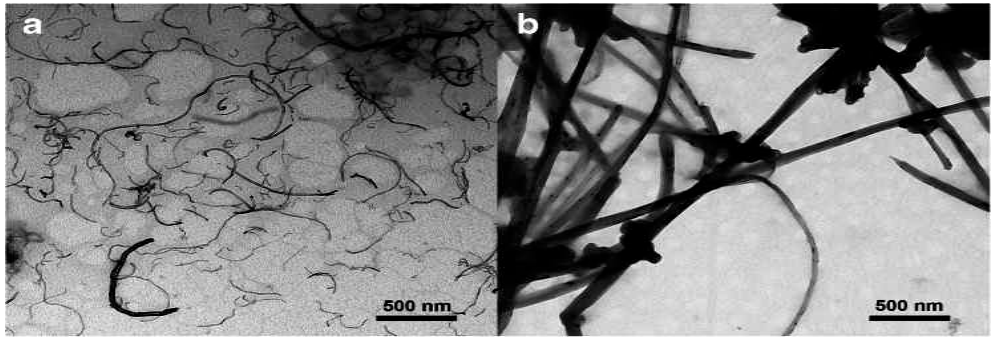
Adapted from Monsé et al Part Fibre Toxicol. 2018 15(1):8

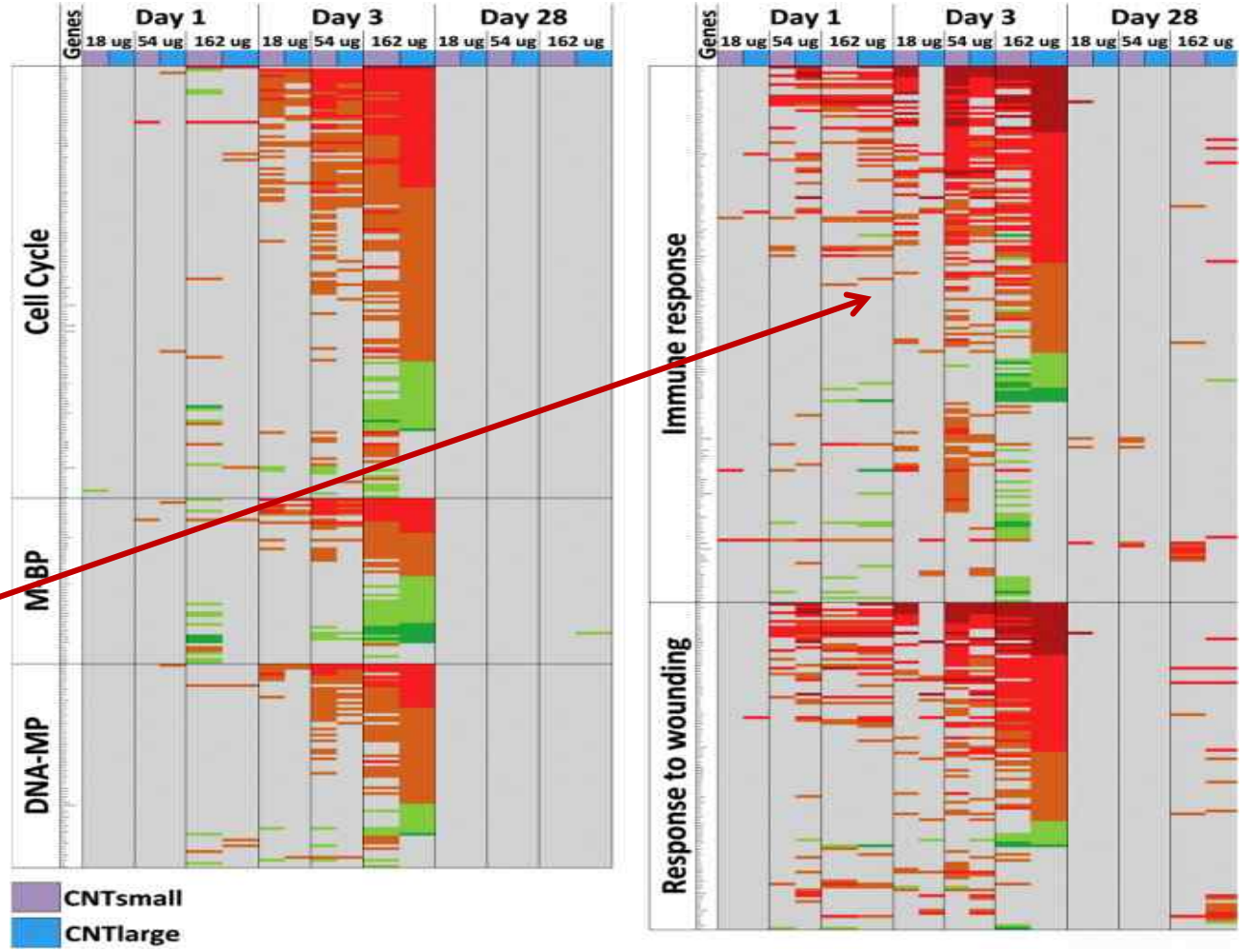
Lung response following pulmonary exposure to two very different CNTs: acute phase response



Small and thin MWCNT

Thick and long MWCNT

Strongest transcriptional signal:
acute phase response



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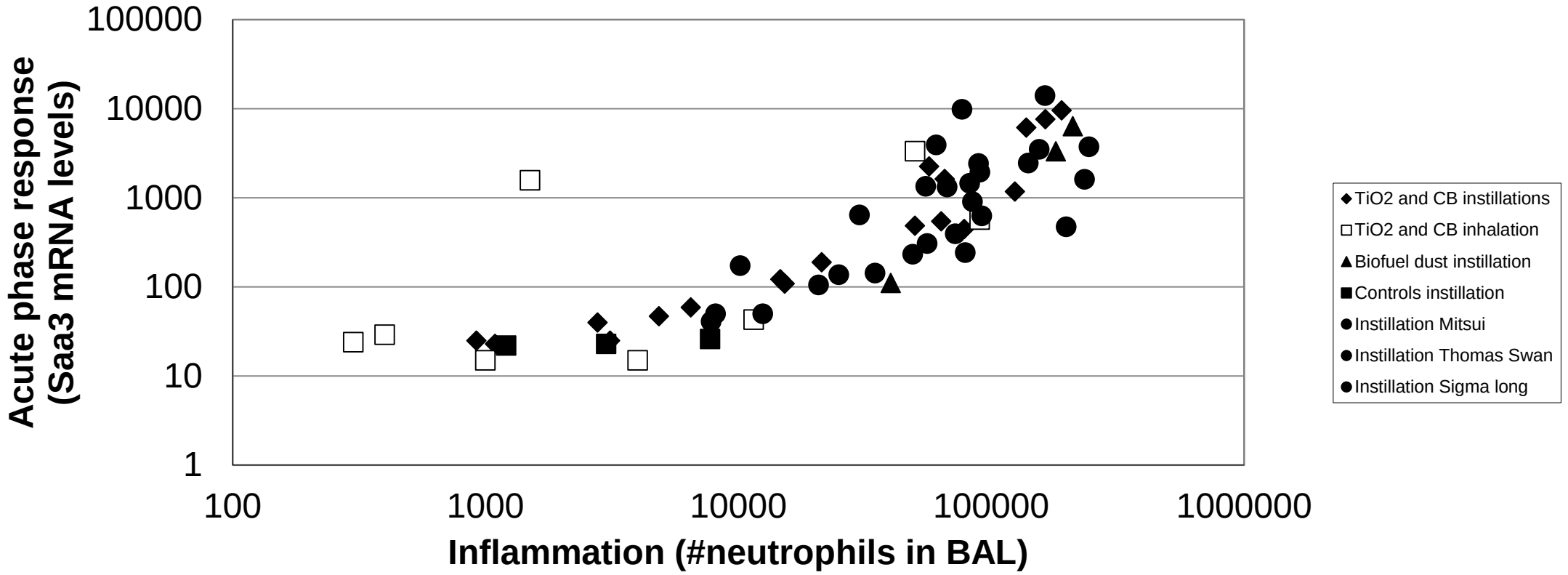
SS Poulsen et al TAAP 2015

Property-response comparison. Change in the expression of genes in five highly regulated selected GO biological processes relative to control mice. The genes are organized by the size of changes in expression after exposure to CNTLarge at the 162 µg dose.

Close correlation between acute phase response and inflammation across doses, time points, material types, and exposure type



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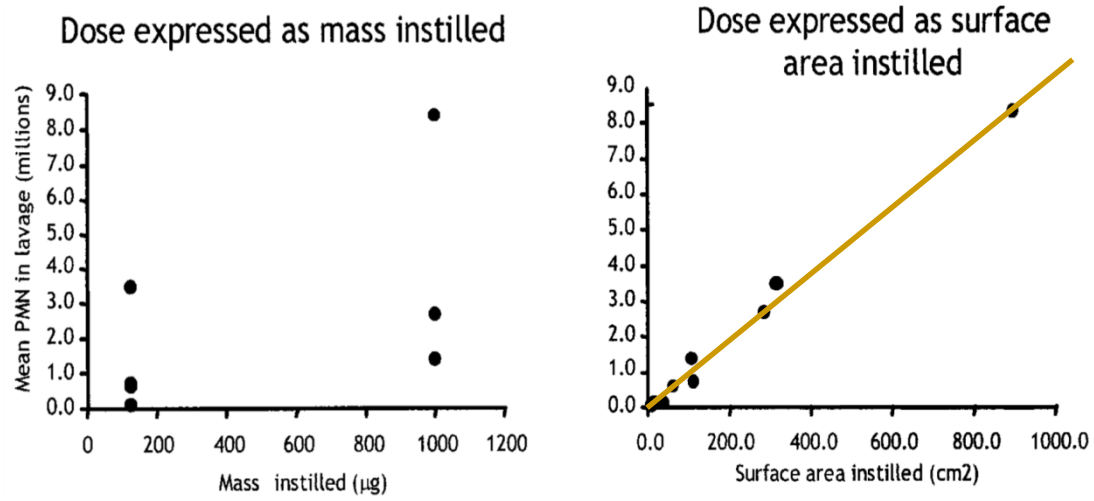
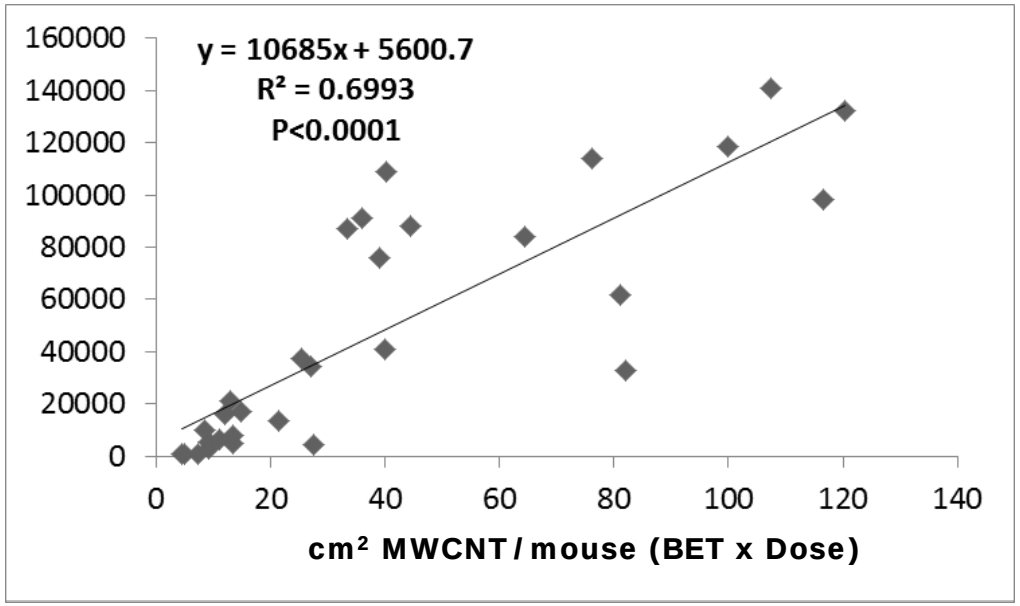
Saber AT et al, Plos One, 2013

Surface area is a predictor of pulmonary inflammation (& acute phase response)

Deposited surface area of particles predicts pulmonary inflammation



Deposited surface area of CNTs predicts inflammation



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Donaldson et al., 2002

SS Poulsen et al, 2016 Nanotoxicology

A number of nanomaterials are classified as carcinogenic or possibly carcinogenic by IARC

- Diesel engine exhaust (1)
- Gasoline engine exhaust (2B)
- Welding fumes (1)
- Carbon black (2B)
- Titanium dioxide nanoparticles (2B)
- Mitsui-7 carbon nanotubes (2B)

Proposed key characteristics of carcinogens

Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

Release of toxic substances; fx PAH, metals

Surface-dependent ROS generation

- Deposited total surface area
- Shape (HARN)

Smith MT EHP, 2016, PMID: 26600562

Mutagenicity of carbon nanoparticles: Surface mediated generation of Reactive Oxygen Species

Carbon black (black pigment)

- Carbon black is classified as possibly carcinogenic (2B) by IARC
- Carbon black is mutagenic in vivo and in vitro
- Carbon Black generates reactive oxygen species in vivo and in vitro
- The mutation spectrum is consistent with being caused by ROS

SOME CNTS MAY FIT ‘THE FIBRE PARADIGM’ – the reason for asbestos-induced lung cancer and methotheliomas

Rule 1 – the fibre is thin enough to be inhaled (D<5 µm)

Rule 2 – the fibre should be so long that the macrophages cannot engulf the fibre (L>5-15 µm)

Rule 3 – the fibre should be insoluble in the lung

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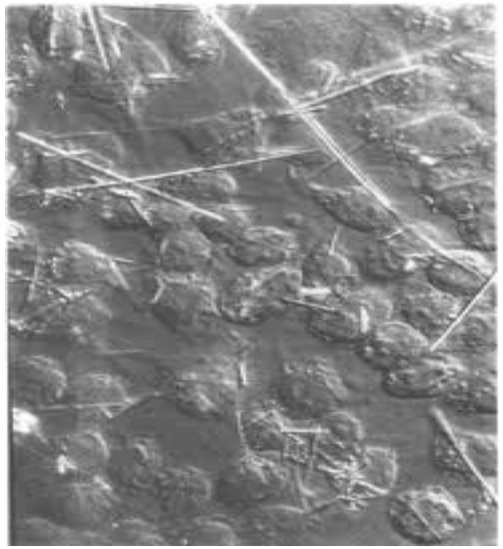


Sub-chronic inhalation study of carbon nanotubes

- 4 published sub-chronic (13 weeks) inhalation studies in the open litterature
- Pauluhn, Tox Sci 2010:
- Rats were exposed on 6 h/day, 5 days per week for 13 consecutive weeks to 0, 0.1, 0.4, 1.5, and 6 mg/m^3.
- Biomarkers assessed 4, 13 and 26 weeks after exposure
- Baytubes, MWCNT
- A number of biomarkers of lung inflammation and toxicity

- Pauluhn 2010

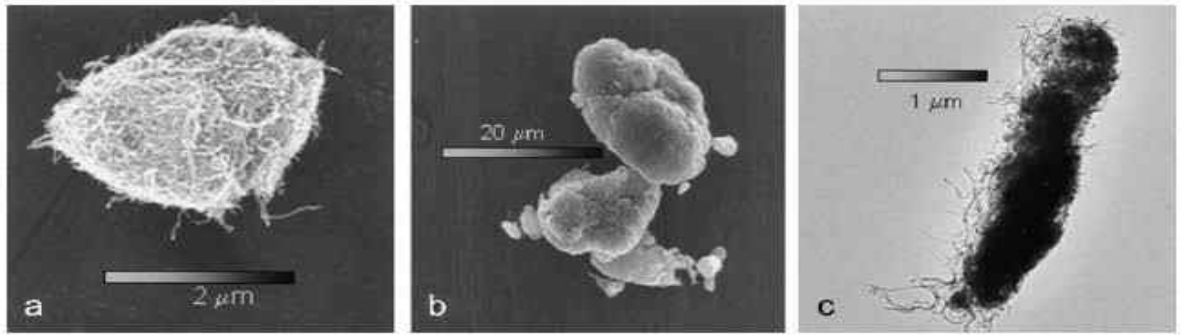
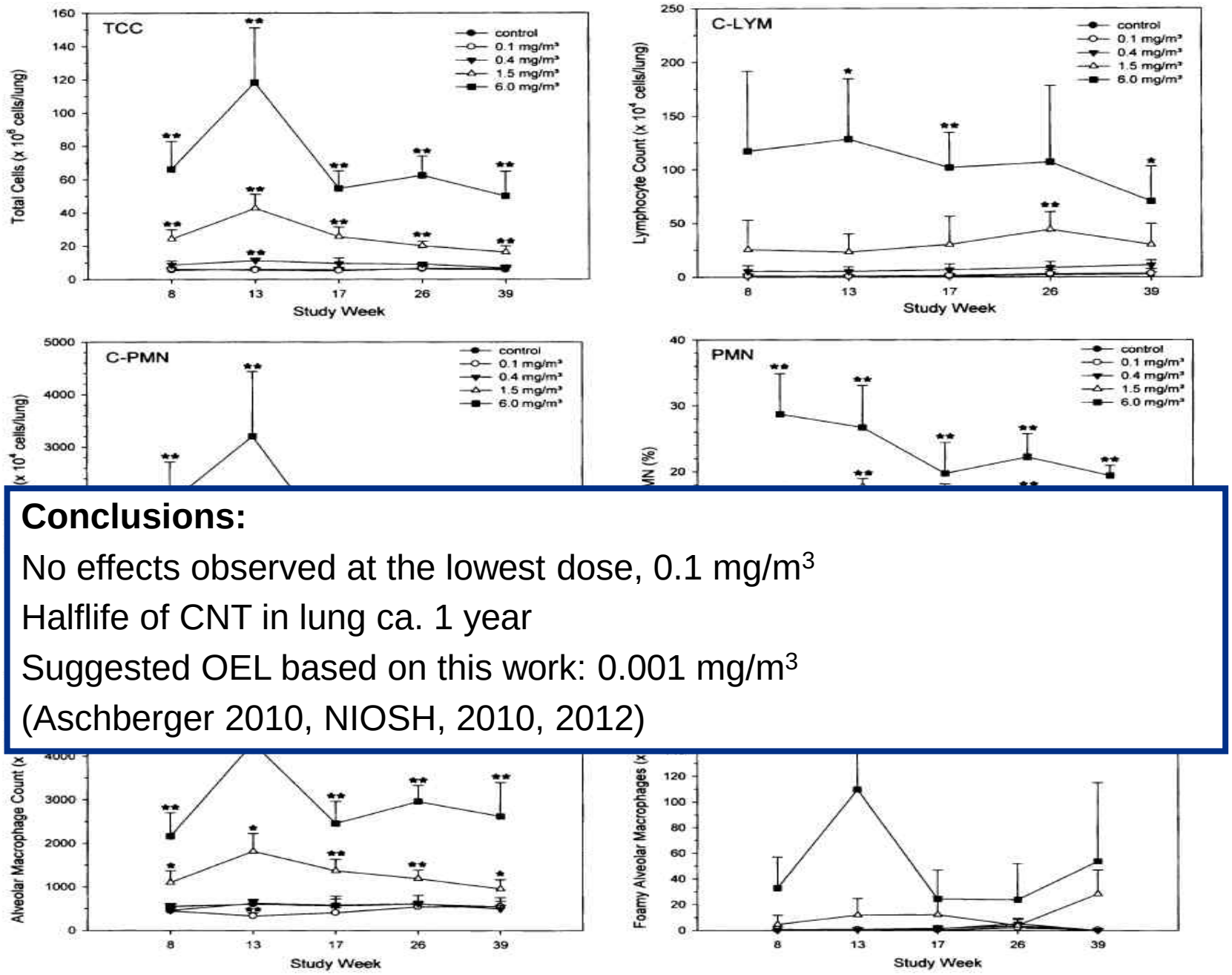


FIG. 2. Image analysis by SEM of Baytubes dispersed into inhalation chambers. (a) Morphology of MWCNT agglomerate collected on an impaction plate of a critical orifice cascade impactor, (b) MWCNT agglomerates collected on the cascade impactor stage 8–16 μm, and (c) TEM image of Baytubes collected directly into TEM grids.



Conclusions:

No effects observed at the lowest dose, 0.1 mg/m^3

Halflife of CNT in lung ca. 1 year

Suggested OEL based on this work: 0.001 mg/m^3

(Aschberger 2010, NIOSH, 2010, 2012)

FIG. 8. Comparison of cellular inflammatory end points in BAL from rats exposed for 8 weeks (interim sacrifice) and 13 weeks (6 h/day, 5 days/week). Data points represent the mean ± SD of six male rats examined on study weeks 13 (postexposure day 1), 17, 26, and 39. Asterisks denote statistical significance to the time-matched control (*$p < 0.05$, **$p < 0.01$).

Dose-dependent CNT-induced cancer

A short and thin MWCNT did not cause cancer

- Dose-dependent induction of mesothelioma after IP injection (Takagi et al, 2012)
- One specific thick and long MWCNT (MWNT-7) is classified as possibly carcinogenic (2B) by IARC (WHO's cancer research institute)
- Other thick and long MWCNTs also induce adenomas
- A short and thin MWCNT did not induce cancer after IP injection (20 mg/animal) in a two year cancer study

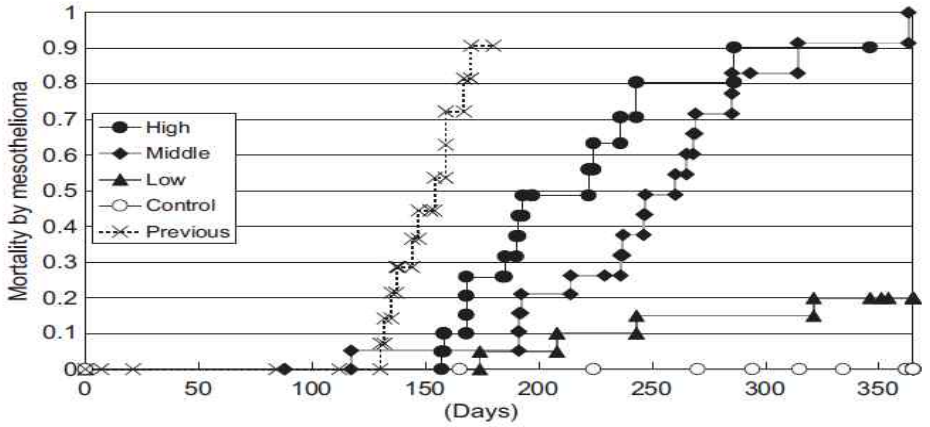
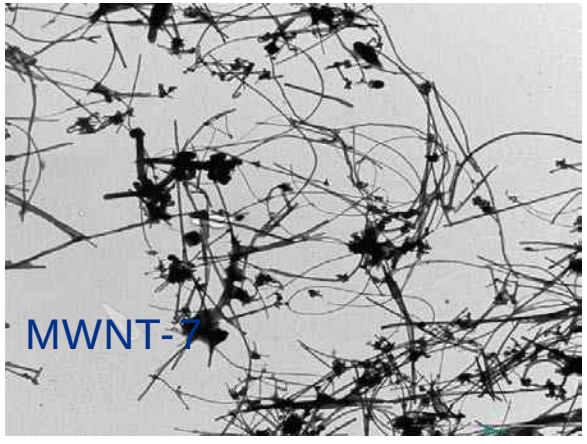


Fig. 1. Dose-dependent induction of mesotheliomas by micrometer-sized multi-wall carbon nanotubes (μm-MWCNT). Mice with lethal mesotheliomas are plotted using the Kaplan–Meier method. High: 300 μg/mouse, corresponding to 1×10^8 fibers/mouse; middle: 30 μg/mouse, corresponding to 1×10^7 fibers/mouse; low: 3 μg/mouse, corresponding to 1×10^6 fibers/mouse); previous: data from a previous study (i.e. 3 mg/mouse, corresponding to 1×10^9 fibers/mouse). No mesothelioma was observed in the vehicle control group.

Takagi, et al, 2012



MWNT-7

Muller et al, 2009

Dose dependent MWCNT-induced lung cancer following inhalation exposure in rats

2 year inhalation study in rats

MWNT-7 MWCNT

- Male and female F344/DuCrlCrlj

(N=50/group)

- 0, 0.02, 0.2, 2 mg/m^3
- 6h/day, 5 days/week for 104 weeks
- Aerosolised as single fibers
- Very low levels of contaminations

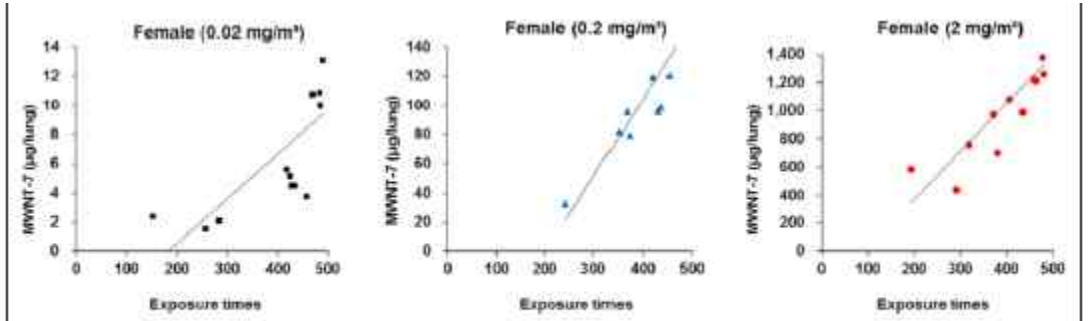


Fig. 5 Results of lung burden analysis. MWNT-7 fibers in the lung were measured for rats that died or were sacrificed in a moribund condition before the end of the experiment. The lung burden increased with concentration and time of exposure.

Results:

- 2 year inhalation study:
- 6 h/day, 5 days/week
- Lung cancer at 2 (M, F) and 0.2 mg/m^3 (M)
- Biopersistence: Accumulation of MWCNTs in lung throughout the exposure period
- Scaling to a risk acceptance level of 1:1000 (instead of 22%), and 40 years exposure (instead of 2), the acceptable air concentration would be 0.000 045 mg/m^3 = 45 ng/m^3

Kasai et al, PF&T, 2016, PMID: 27737701

Animal models of particle-induced lung cancer

- Only inhalation studies are (normally) used for risk assessment
- 2 year chronic inhalation studies in rats (no cancer in mice and hamsters)
- We want to estimate human lung cancer risk (0.1% - 0.001%) during 40 years of exposure based on groups of 50-100 rats exposed for 2 years (the detection limit is ca. 5% cancer), so the air concentrations should at least be 50 (5%/0.1%) x 20 (40 years/2 years) = 1000 fold higher in the animal studies
- Concern has been raised that impaired clearence (overload) will lead to over-estimation of cancer risk

Inhalation and instillation of two different CNTs gives the same dose response relationship for inflammation in rats

Toxicology and Applied Pharmacology 375 (2019) 17–31

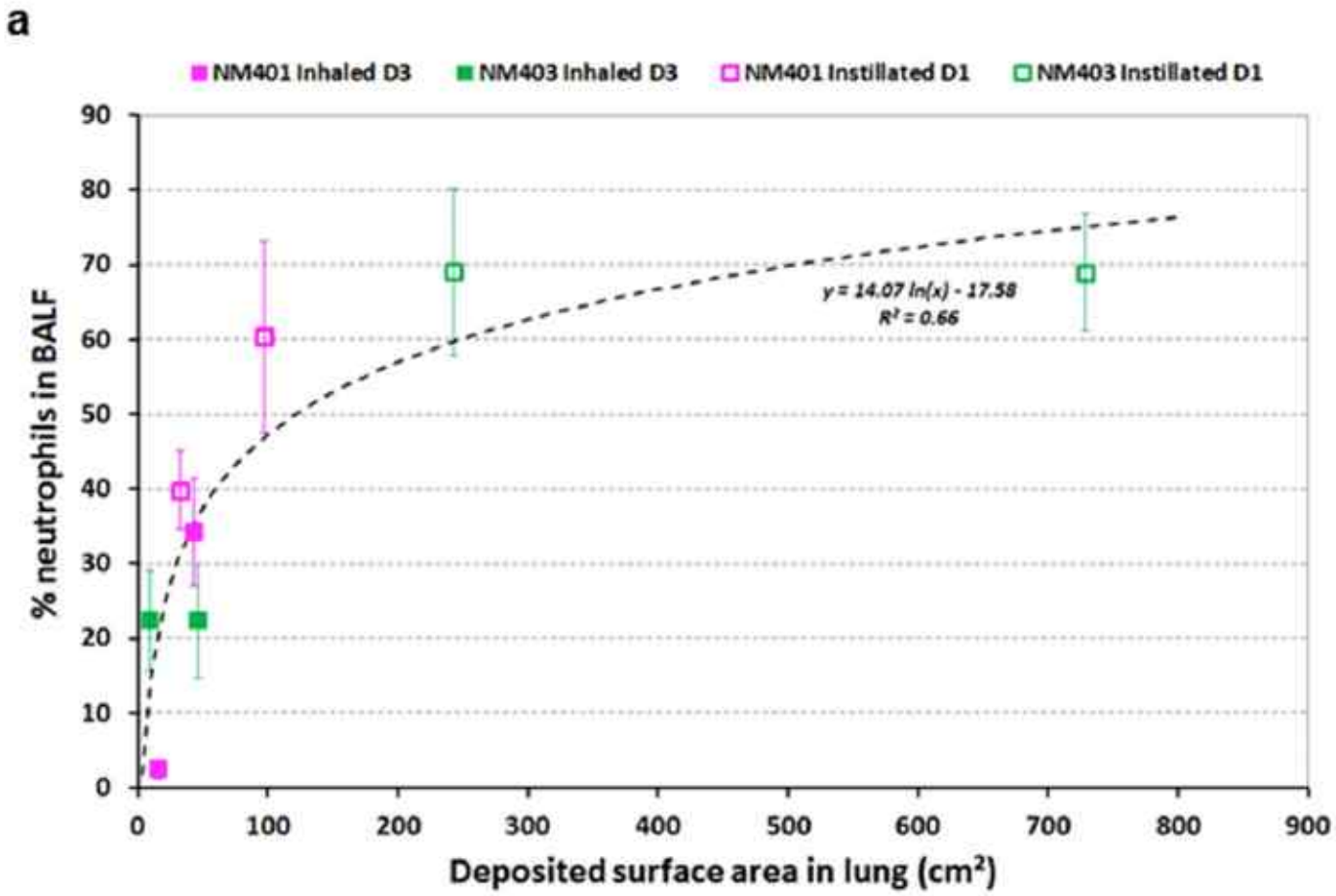


Fig. 7. Correlation between the percentage of BALF neutrophils and the pulmonary MWCNT-deposited surface area. (a) Percent neutrophils in BAL fluid on day 1 (IT) or 3 (inhalation) as function of pulmonary deposited surface area (estimated in the lung at the end of exposure) of rats exposed to NM-401 and NM-403 by inhalation (full square) or IT (open square). (b) Percentage of neutrophils in BAL fluid on day 28 (IT) or 30 (inhalation) as a function of pulmonary deposited surface area (estimated in the lung at the end of exposure) of rats exposed to NM-401 and NM-403 by inhalation (full square) or IT (open square). The logarithmic curves "models" were established from the set of individual data.

Gaté et al, TAAP, 2019

Nanosize matters: increased carcinogenic potency in chronic inhalation studies of TiO_2

	Air concentration	Total number of lung tumors	
Fine, rutile TiO_2	250 mg/m^3	39/151 rats= 26%	Lee et al (1985)
Nano TiO_2 (P25)	10 mg/m^3	32/100 rats= 32%	Heinrich et al (1995)

Fine, rutile TiO_2 did not cause cancer in 2-year inhalation studies at 5, 10 or 50 mg/m^3 (Muhle et al 1991, Lee et al , 1985)

Concern: the overload hypothesis

- Rats develop lung cancer after particle inhalation (2 years)
- Mice and hamsters do not
- Dose-dependent differences in particle retention
- Overload is seen for CB Printex90 at 50 mg/m^3 (13 weeks)
- It is argued that rats therefore overestimate human cancer risk

Nano-sized carbon black particles (P90) at different exposure levels

TABLE 4
Particle Retention Half Times for Rats, Mice, and Hamsters following 13 Weeks of Exposure to Carbon Black

	Rats	Mice	Hamsters
HSCb, 1 mg/m^3	64[a]	133	42
HSCb, 7 mg/m^3	115	343	53
HSCb, 50 mg/m^3	*No significant clearance*	322	309

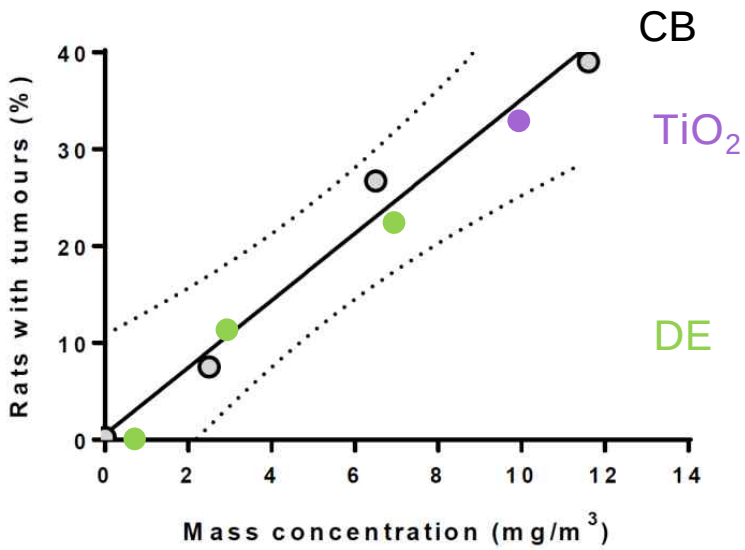
[a]Times are reported in days and were calculated from the retention curves using a two-parameter monoexponential decay function. For the mice, the half times were estimated from the retention curves in Figure 4b.

Elder et al, 2005, Tox Sci, 88(2) 614

Similar carcinogenic potency of three insoluble NMs (DEP, TiO_2 and CB) after chronic inhalation in rats at air concentrations that did not induce overload

Two year inhalation study in female (and male) rats exposed to diesel exhaust (DE), nano-TiO_2 (P25) and nano-CB (Printex90) (**Heinrich et al., 1995**).

Cancer incidence in female rats



Lung cancer incidence in rats exposed to diesel exhaust particles (DEP), carbon black (CB) and titanium dioxide (TiO_2) after 30 months (24 months of exposure followed by 6 months in clean air) (Heinrich et al. 1995).

	Average particle exposure (mg/m³)					
	Clean air	DE			CB	TiO_2
Exposure concentration	0	0.8	2.5	7.0	11.6	10
Number of rats with tumours						
with benign tumours	1/217	0/198	11/200	22/100	39/100	32/100
without benign tumours			4/200	9/100	28/100	19/100

National Research Centre for the Working Environment

Clearence half-times for CB (Printex90), TiO_2 (P25) and diesel exhaust particles in the two year inhalation study

TABLE 9. Half-Times of Pulmonary Tracer Clearance (^{59}Fe)

	Half-time of alveolar clearance (days)			
Exposure	3 mo	12 mo	18 mo	18 mo Recovery
Control	61	72	96	93
Diesel soot, 0.8 mg/m^3	94[a]	121[a]	221[a]	n.d.[b]
Diesel soot, 2.5 mg/m^3	119[a]	254[a]	272[a]	n.d.[b]
Diesel soot, 7.0 mg/m^3	330[a]	541[a]	687[a]	1068[a]
Carbon black	244[a]	368[a]	363[a]	591[a]
TiO_2	208[a]	403[a]	357[a]	368[a]

[a]Significant at $p < .01$ (Dunnett's test).
[b]n.d., Not determined.

Heinrich et al, 1995

Comparison between rat inhalation and human epidemiology: Cumulative dose-response relationship between diesel exhaust exposure and lung cancer risk in three epidemiologial studies

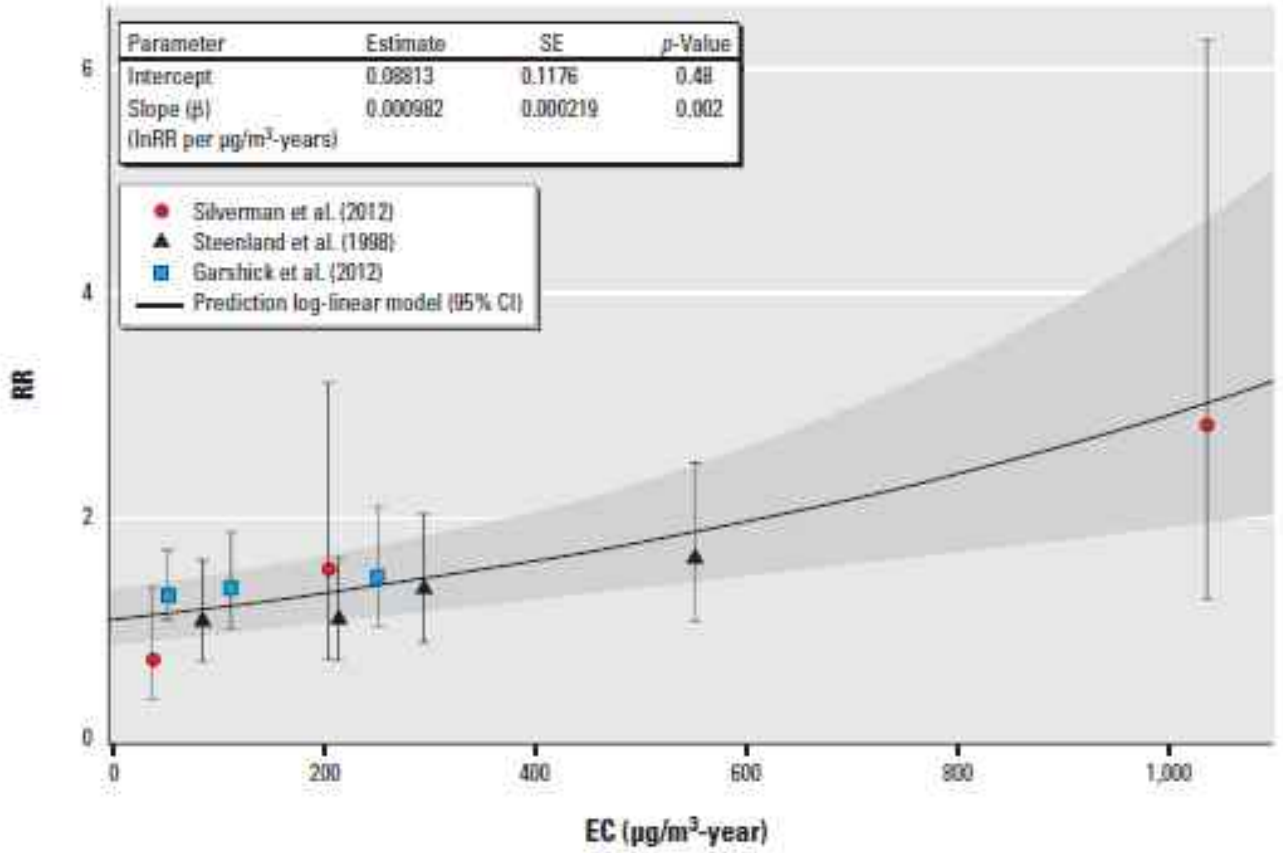


Figure 1. Predicted exposure–response curve based on a log-linear regression model using RR estimates from three cohort studies of DEE and lung cancer mortality. Individual RR estimates [based on HRs reported by Garshick et al. (2012) or ORs reported by Silverman et al. (2012) and Steenland et al. (1998)] are plotted with their 95% CI bounds indicated by the whiskers. The shaded area indicates the 95% CI estimated based on the log-linear model. The insert presents the estimates of the intercept and beta slope factor, the SE of these estimates, and the associated *p*-values.

National Research Centre for the Working Environment

Vermeulen et al, 2014, EHP

Risk estimate for DEP based on epidemiological evidence

Table 1. Exposure–response estimates (lnRR for a 1-μg/m^3 increase in EC) from individual studies and the primary combined estimate based on a log-linear model.

Model[a]	Intercept	β (95%CI)
All studies combined	0.088	0.00098 (0.00055, 0.00141)
Silverman et al. (2012) only	–0.18	0.0012 (0.00053, 0.00187)
Steenland et al. (1998) only	–0.032	0.00096 (0.00033, 0.00159)
Garshick et al. (2012) only	0.24	0.00061 (–0.00088, 0.00210)

[a]Log-linear risk model (lnRR = intercept + β × exposure). Exposure defined as EC in μg/m^3-years.

Table 2. Excess lifetime risk per 10,000 for several exposure levels and settings, United States in 2009.

Exposure setting	Average EC exposure (μg/m^3)	Excess lifetime risk through age 80 years (per 10,000)
Worker exposed, age 20–65 years	25	689
Worker exposed, age 20–65 years	10	200
Worker exposed, age 20–65 years	1	17
General public, age 5–80 years	0.8	21

Based on linear risk function, lnRR = 0.00098 × exposure, assuming a 5-year lag, using age-specific (5-year categories) all cause and lung cancer mortality rates from the United States in 2009 as referent.

The EU OEL for DEP is 50 ug/m^3

Vermeulen et al, EHP

Dutch Committee on Occupational Safety (DECOS):

- The DECOS estimates that the exposure concentrations of respirable elemental carbon (REC) in the air, which serve as parameter for exposure to diesel engine exhaust powered by petroleum-diesel fuels, and which corresponds to:
- 4 extra death cases of lung cancer per 100,000 (target risk level), for 40 years of occupational exposure, equals to 0.011 µg REC/m^3,
- 4 extra death cases of lung cancer per 1,000 (prohibition risk level), for 40 years of occupational exposure, equals to 1.03 µg REC/m^3.
- The exposure levels are 8-hour time-weighted average concentrations.

https://www.healthcouncil.nl/documents/advisory-reports/2019/03/13/diesel-engine-exhaust.

Can chronic inhalation studies in rats be used for risk assessment in humans ? YES, I think so

Diesel exhaust as a case:

Diesel exhaust (DE) cause cancer, filtered DE does not.

In chronic (2 year) inhalation studies in rats,

- 2.5 mg/m^3 DE induced cancer in 5.5% of the exposed rats
- 40 years instead of 2
- 20:10 000 instead of 5.5%:
- 4.5 ug/m^3 will induce 20 excess lung cancer cases
- Epidemiological meta-analysis: 1 ug/m^3 induce 17 lung cancers:10 000 exposed
- the chronic inhalation in rats does not over-estimate lung cancer risk for DE

It works: Reduction of particle exposure reduces mortality

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
- Assuming 45 year work life and that we work 20% of the time, reducing occupational exposure with 1 ug/m^3 should save 19 per 100.000 = 2/10 000

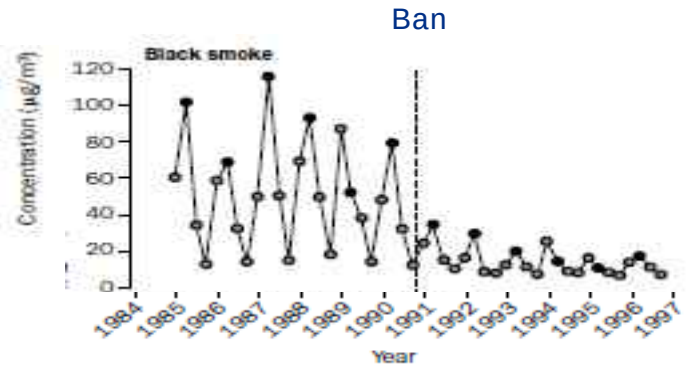


Table 2: **Age-standardised mortality rates for Dublin County Borough before (1984–90) and after (1990–96) ban of sale of coal, by season**

	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)

Take home: Physico-chemical predictors of pulmonary toxicity for nanomaterials

- **Aerosolised size** will determine pulmonary depostion rate and distribution
- **Specific surface area** of retained dose predicts inflammation and acute phase response for **insoluble particles**
- **Shape** and **size** are strong determinants of clearence rates (and hence deposited dose)
- **High aspect ratio** may predict cancer risk
- **Surface-mediated generation of radicals/ROS** may reslut in genotoxicity
- **Dissolution/solubility** of low toxicity chemicals may lower toxicity
- Dissolution/release of toxic metals/chemicals may enhance toxicity

- Comparison of risk assessment of diesel exhaust suggests that chronic inhalation studies in rats do not overestimate human risk and can be used for risk assessment of nanomaterials

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SmartNanoTox
Smart Tools for Gauging Nano Hazards

caLIBRAte
nano risk governance

Health Canada
Santé Canada

Example of insoluble particles: Association between air pollution levels and CRP

Association between PM2.5 and CRP levels in 30 000 Taiwanese:
0.014 mg/L CRP pr 5 ug/m^3 increment in PM 2.5

Table 2. Associations of CRP with long-term exposure to $PM_{2.5}$ in baseline analysis among Taiwanese adults

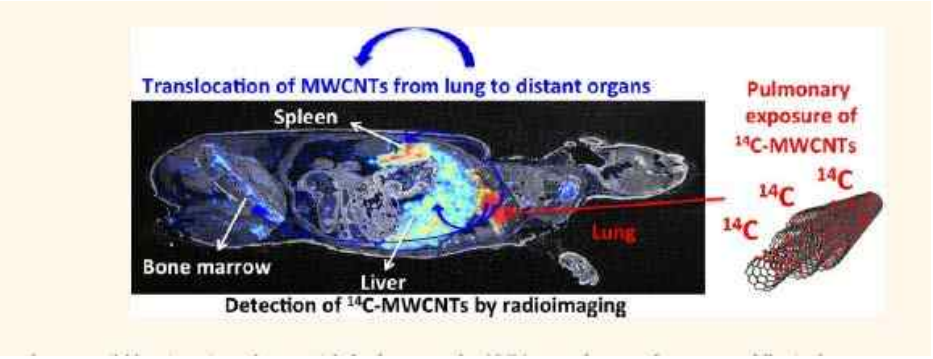
	Crude model			Model 1[a]			Model 2[a]		
	Mean (SE)[b]	% difference (95% CI)	*P*	Mean (SE)[b]	% difference (95% CI)	*P*	Mean (SE)[b]	% difference (95% CI)	*P*
Men ($N = 17761$)									
1st quartile	0.96 (1.01)	Ref	Ref	0.92 (1.03)	Ref	Ref	0.98 (1.05)	Ref	Ref
2nd quartile	0.96 (1.01)	0.34 (−1.36, 2.05)	0.69	0.95 (1.03)	1.18 (−0.01, 2.89)	0.18	1.01 (1.05)	1.49 (−0.01, 3.05)	0.06
3rd quartile	0.98 (1.01)	0.93 (−0.77, 2.62)	0.28	0.97 (1.03)	1.94 (0.23, 3.65)	0.03	1.03 (1.05)	2.19 (0.63, 3.76)	0.006
4th quartile	1.07 (1.01)	4.78 (3.09, 6.48)	< 0.001	1.03 (1.03)	4.71 (3.01, 6.41)	< 0.001	1.08 (1.05)	4.27 (2.72, 5.82)	< 0.001
trend test[c]	-	-	< 0.001	-	-	< 0.001	-	-	< 0.001
every 5 $\mu g/m^3$ increment[d]	-	1.42 (0.96, 1.87)	< 0.001	-	1.35 (0.89, 1.81)	< 0.001	-	1.21 (0.79, 1.63)	< 0.001
Women ($N = 12273$)									
1st quartile	0.76 (1.02)	Ref	Ref	0.70 (1.06)	Ref	Ref	0.85 (1.07)	Ref	Ref
2nd quartile	0.68 (1.02)	−4.79 (−0.01, -2.42)	< 0.001	0.67 (1.06)	−1.52 (−0.01, 0.78)	0.19	0.84 (1.07)	−0.82 (−0.01, 1.23)	0.43
3rd quartile	0.70 (1.02)	−3.54 (−5.91, -1.16)	0.004	0.70 (1.06)	0.37 (−1.95, 2.70)	0.75	0.88 (1.07)	1.44 (−0.63, 3.51)	0.17
4th quartile	0.82 (1.02)	3.30 (0.92, 5.68)	0.006	0.79 (1.06)	5.19 (2.88, 7.5)	< 0.001	0.95 (1.07)	4.89 (2.84, 6.95)	< 0.001
trend test[c]	-	-	< 0.001	-	-	< 0.001	-	-	< 0.001
Every 5 $\mu g/m^3$ increment[d]	-	1.27 (0.66, 1.89)	< 0.001	-	1.64 (1.04, 2.24)	< 0.001	-	1.62 (1.09, 2.15)	< 0.001

Zhang et al, Int J Epidemiol. 2017

Biodistribution: Inhaled nanomaterials including CNTs translocate and reach distal organs

CNT translocation was studied with ^{14}C-skeleton labelled MWCNTs:

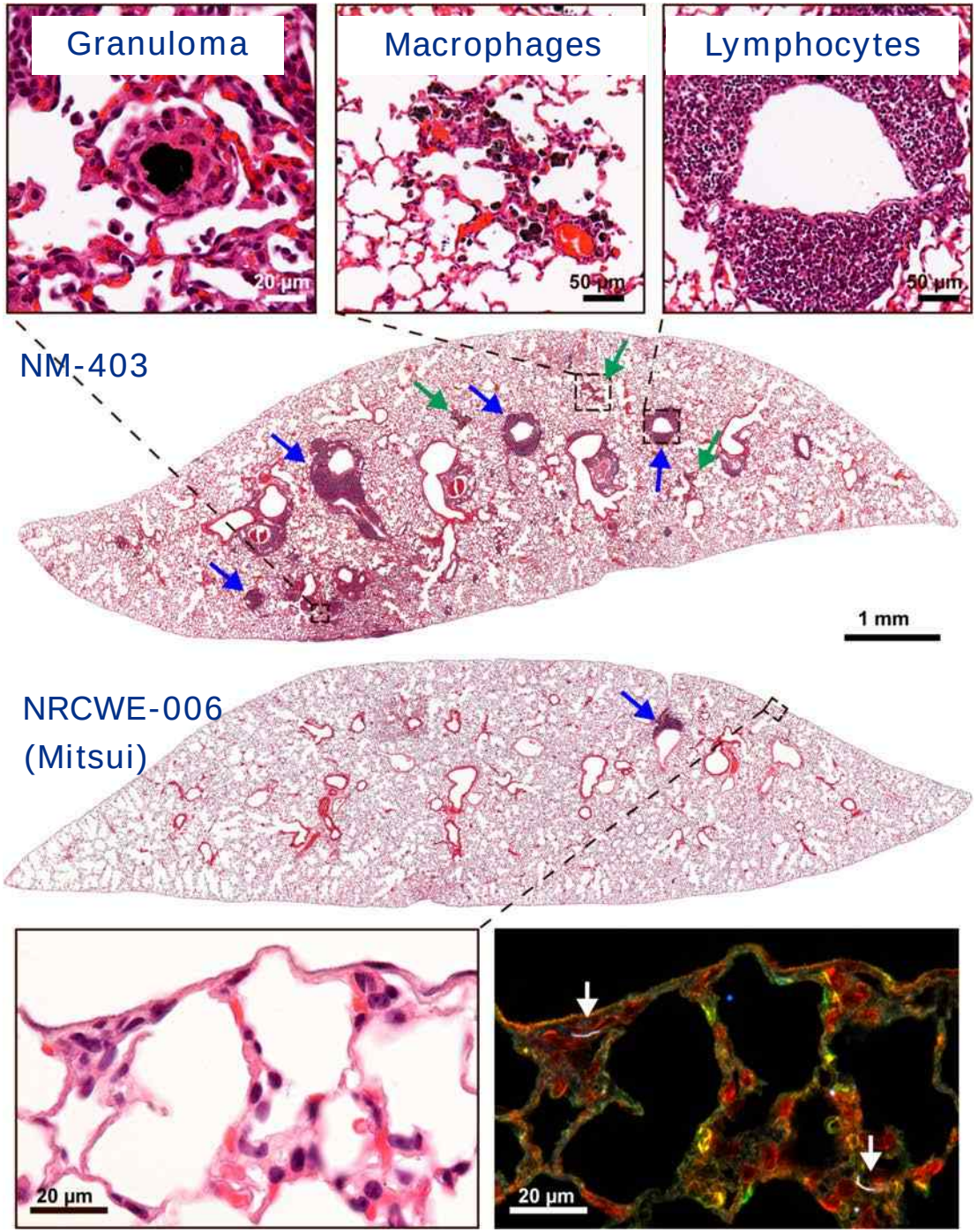
- Mice were exposed to 20 ug radioactively labelled MWCNT and followed up to 1 year
- 1% of total dose was found in liver and spleen 1 year post-exposure
- No uptake detected after oral exposure



Czarny, ACS nano 2014

^{14}C labelled Few-Layered Graphene (G):

- Biopersistence: Pulmonary dosed G, GO and rGO is found in lung 1-3 mo post-exposure (PMID: 26864058, PMID: 28570647)
- Translocation of G to liver (1%) and spleen (0.2 %) after 1 mo (PMID: 26864058)
- No uptake from oral dosing (PMID: 26864058)



Phys-chem-related difference in MWCNT distribution in lung 1 year after IT of 54µg

Thin, short MWCNT (negative in cancer study)

(D~11-26nm, L~1µm)

- Black aggregates in macrophages and granuloma
- Chronic low-level inflammation for 7/11 MWCNT

Mitsui-7 and NM-401 (lung cancer by inhalation)

(D~70nm, L~5um)

- Single fibers in interstitium
- Tissue reaction ~ vehicle controls

- Knudsen et al, BCPT, 2018

Epidemiological data are not always available and is only feasible for relatively potent carcinogens

Relative risk of lung cancer for carcinogens that cause 1%, 0.1% or 0.01% excess lung cancer risk in a population with the current Danish lung cancer incidence

	Men	Women
Life time lung cancer risk (0-74 years) 2011-15 in Denmark	4.9%	4.5%
Excess lung cancer risk level	RR	RR
1:100	RR= (4.9+1)/ 4.9= 1.20	RR= (4.5+1)/4.5= 1.22
2:1000	RR= (49+2)/49= 1.041	RR= (45+2)/45=1.044
1:1 000	RR= (49+1)/49= 1.02	RR= (45+1)/45=1.02
1:10 000	RR= (490+1)/490= 1.002	RR= (450+1)/450= 1.002
1:100 000	RR= (4900+1)/4900= 1.000 2	RR= (4500+1)/4500= 1.000 2

Power calculation:

Detection of 1% excess cancer incidence with 5% lung cancer incidence: 8 000 participants (with 80% chance of detecting the effect at 5% significance level).

Detection of 0.1% excess lung cancers (1 per 1 000, which is the acceptance level in the US), corresponds to a RR of 1.02, which requires group sizes of 750 000 persons if the background cancer incidence is 5%.

The preferred evidence for regulation

- Identification of critical effects (cancer, cardiovascular disease, reprotoxicity, astma, COPD ect)
- Human evidence from epidemiological studies with dose-response relationship
- Chronic inhalation studies in rodents (rats)
- Sub-chronic inhalation studies

- Mechanism-of-action:
- Threshold/non-threshold ?