ATS BRESCIA - ATS BRIANZA - ATS INSUBRIA - ATS MILANO - ATS MONTAGNA - ATS VAL PADANA
Seminario per le Imprese
Lo sviluppo di nanotecnologie e la valutazione e gestione del rischio:
aspetti salienti di un binomio possibile

Palazzo Lombardia, Sala Biagi, 10 Ottobre 2018

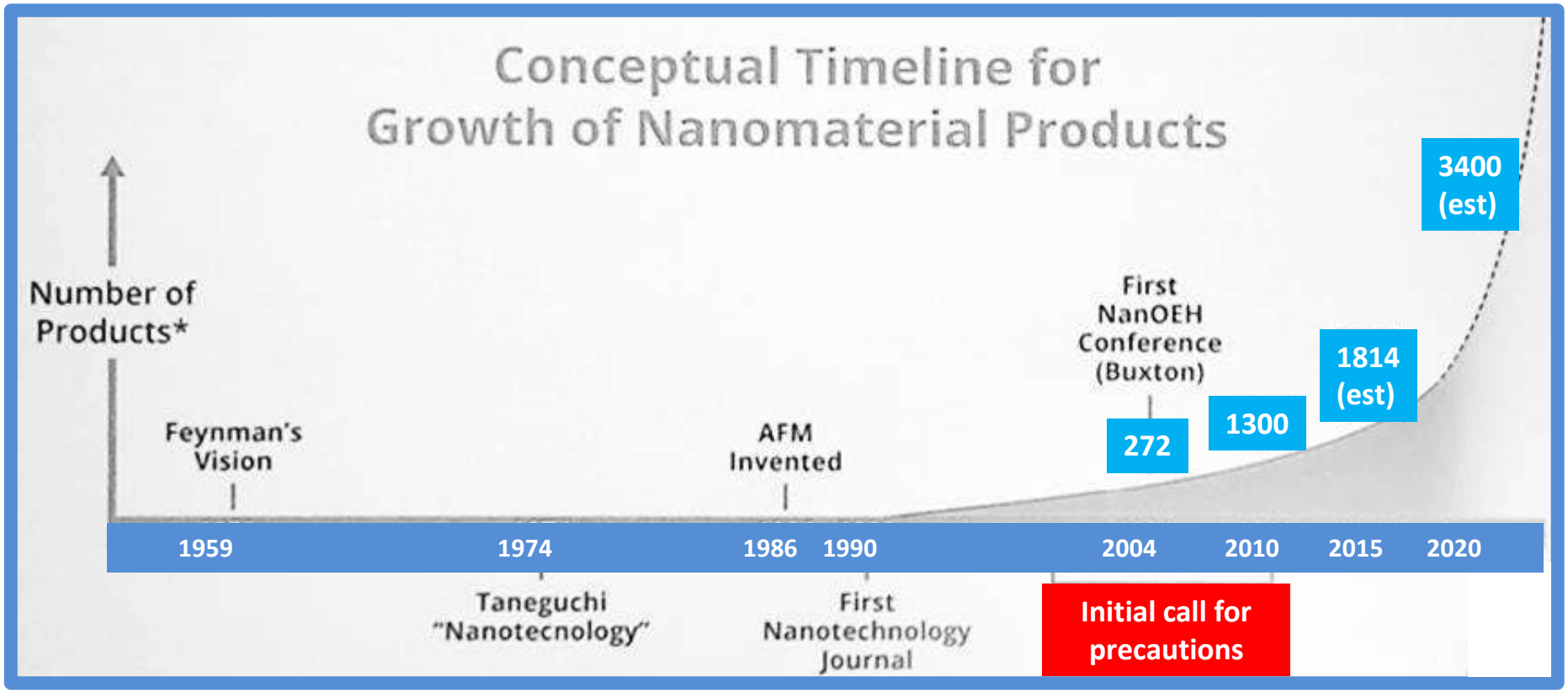
Sistema Socio Sanitario
Regione Lombardia

NANOMATERIALI ED EFFETTI TOSSICOLOGICI NOTI

Prof. Giovanni DE VITO

1998-2018 20 UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA VENTESIMO ANNIVERSARIO
UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA
DIPARTIMENTO DI MEDICINA E CHIRURGIA
SCHOOL OF MEDICINE AND SURGERY

UOC Medicina del Lavoro
Sistema Socio Sanitario
Regione Lombardia
ASST Lecco


Conceptual Timeline for
Growth of Nanomaterial Products
Number of Products*
Feynman's Vision
AFM Invented
First NanOEH Conference (Buxton)
272
1300
1814 (est)
3400 (est)
1959
1974
1986
1990
2004
2010
2015
2020
Taneguchi "Nanotecnology"
First Nanotechnology Journal
Initial call for precautions

Fattori che influenzano la scoperta degli effetti dei nanomateriali sulla salute dei lavoratori

- ENM (Engineered Nano Materials)
  - Gruppo numeroso ed eterogeneo
  - Molteplici meccanismi tossicologici
  - Molti fattori influenzano la tossicità
  - Devo essere analizzarli per tipologia
- Siamo all'inizio della storia commerciale
  - Pochi lavoratori
  - Decentralizzati
  - Esposizioni relativamente basse
  - Latenza degli effetti cronici
  - Manca l'evidenza su quali siano gli indicatori precoci

Summary of Epidemiological and Animal Data for ENMs by commercial volume

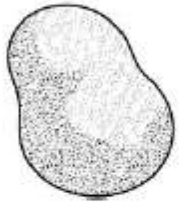
Nanomaterial	Commercial Tonnage (Tons)	Epidemiologic findings pathologic effects in workers	Potential biomarkers of adverse effects in epidemiological studies of workers	Adverse effects in animals
Carbon black	9.600.000			
Synthetic amorphous silica	1.500.000			
Aluminium oxide	200.000			
Bariun titanate	15.000			
Titanium dioxide	10.000			
Cerium dioxide	10.000			
Zinc oxide	8.000			
Carbon nanotubes/ nanofibers	100 - 3000			
Silver nanoparticles	20			

Based on the WHO report 2017

ISO/TS 27687:2008 (en)

Nanotechnologies -Terminology and definitions for **nano-objects** (**NOAA**) - Nanoparticle, nanofibre and nanoplate

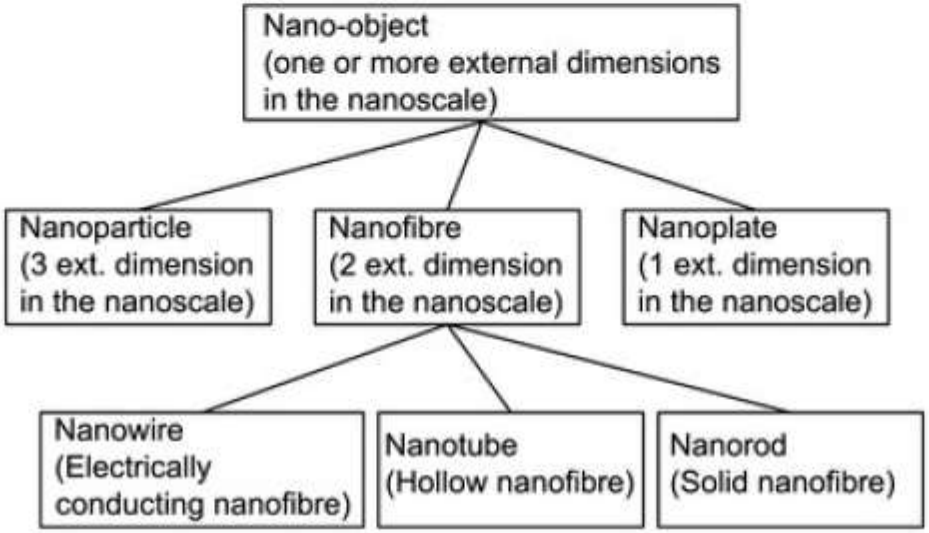
*This standard has been replaced by **ISO/TS 80004-2:2015***



a) nanoparticle

b) nanofibre

c) nanoplate



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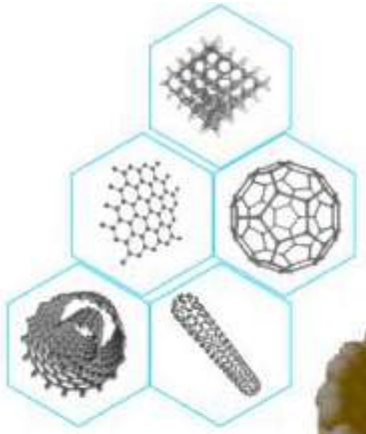
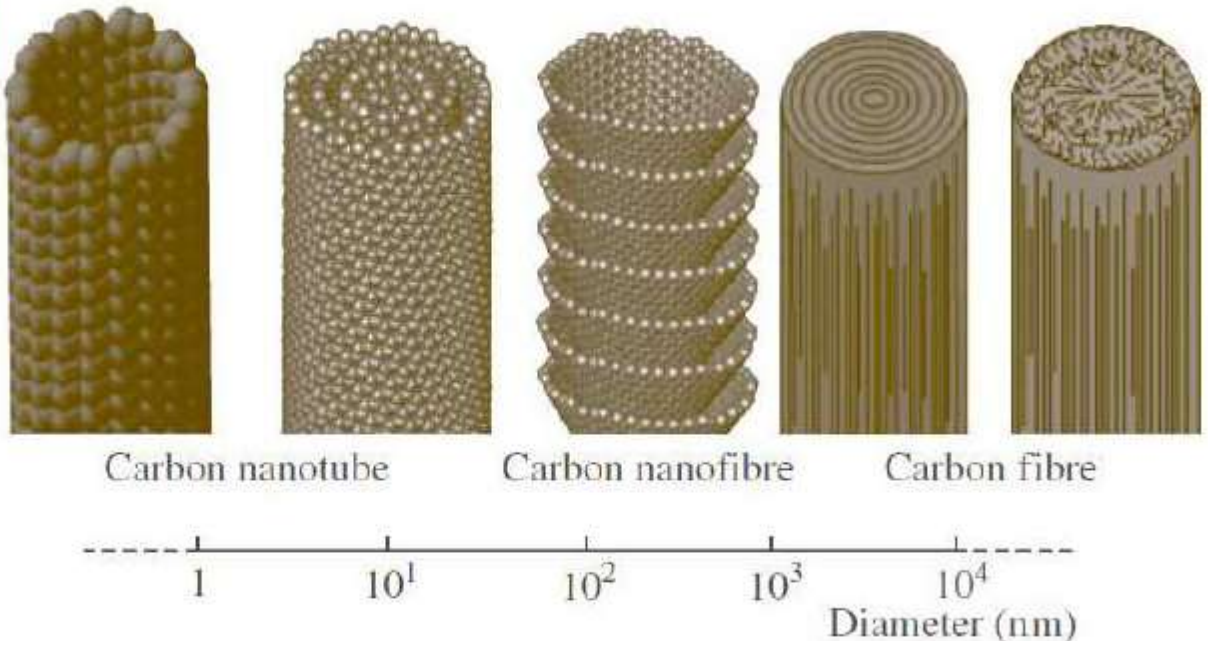
Based on the WHO report 2017

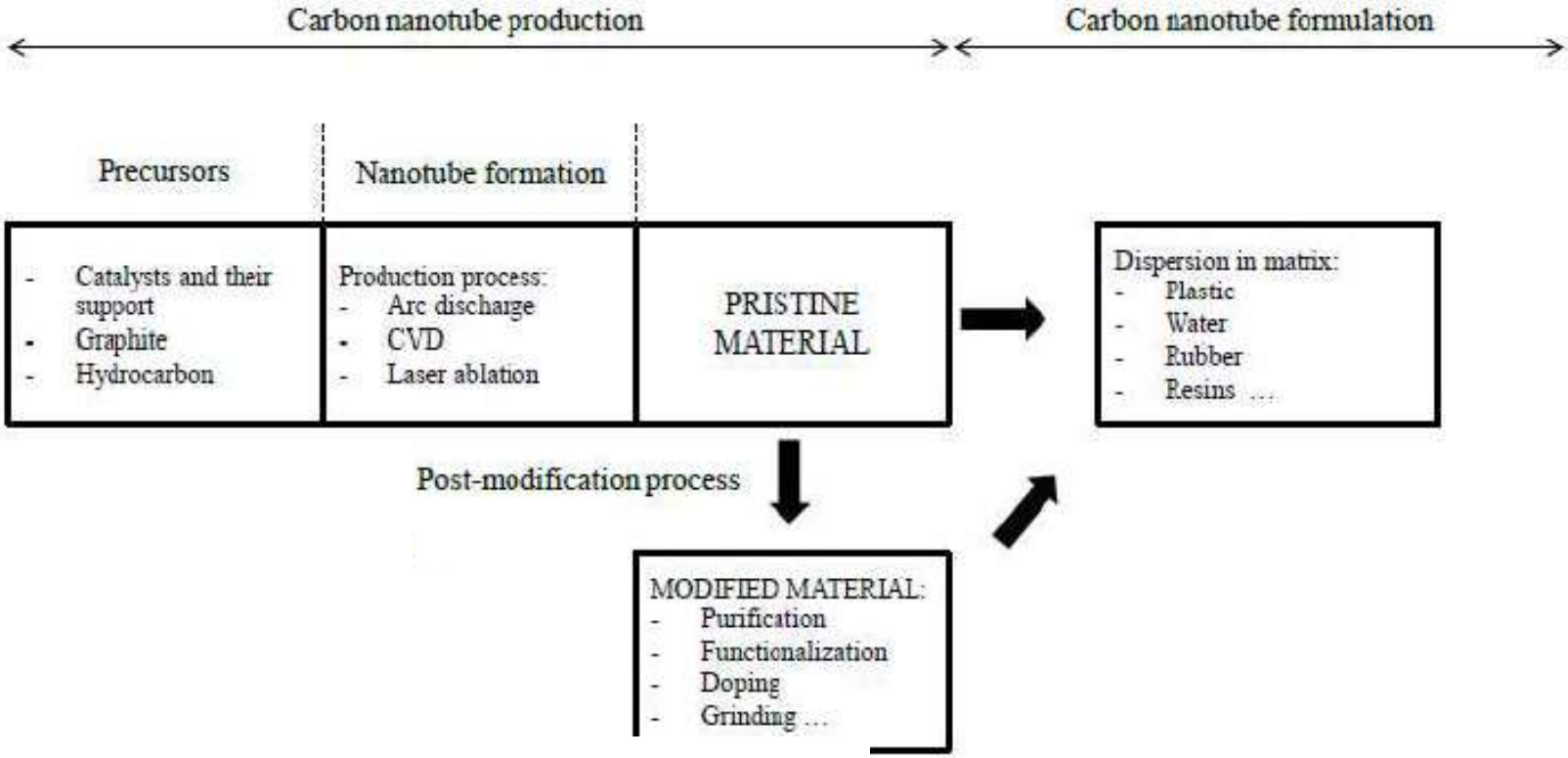


Confronto schematico fra i vari tipi di materiali fibrosi a base di carbonio (*scala logaritmica*)



IARC MONOGRAPHS – 111

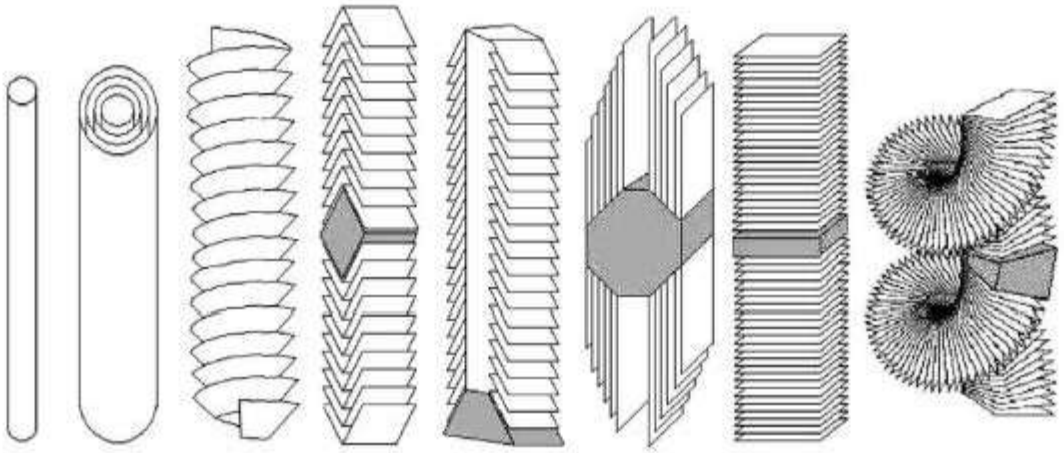
Processo produttivo dei nanotubi di carbonio



In situ HRTEM images and depictions of initial stages of CNT nucleation.
from: S. Hofmann et al. Nano Lett., 2007, 7, 602–608. Copyright 2007 American Chemical Society.

Eterogeneità strutturale dei nanotubi e delle nanofibre di carbonio

Guseva Canu
et al., 2016



I CNTs non sono materiali «puri»

Unpurified nanotube sample

Nanotubes

Single-walled

1-3 nm diameter
Often form bundles

Multi-walled

10-100 nm diameter

Widely varying lengths, typically tens of microns

Residual material

Metals

Typically Co, Fe, Ni, Mo

Nanoparticulate Carbon-encapsulated Oxides

Organics

Amorphous carbon

Microstructured carbon (carbon blacks, onions, fibres etc.)

Support

Typically fine alumina, magnesium oxide or silica.

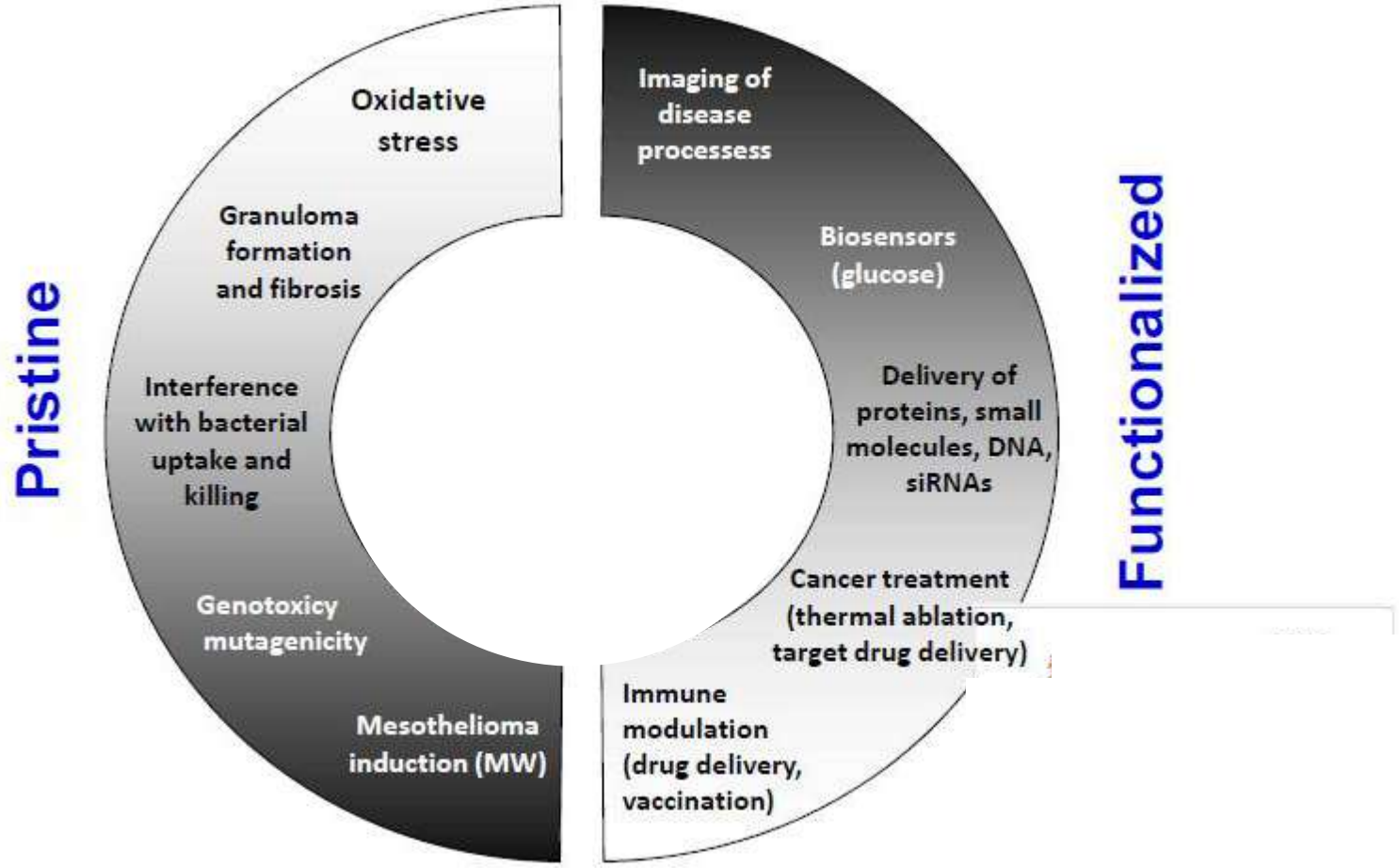
Le peculiari ed innovative proprietà chimico-fisiche ne fanno uno dei materiali più promettenti per una serie di applicazioni altamente tecnologiche

CNT - Properties

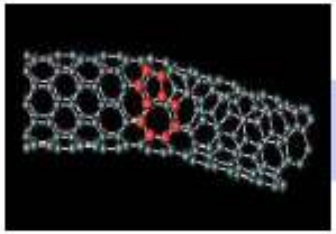
New Industry/ Product Opportunities for Carbon Nanotubes

- Conduttività elettrica
- Resistenza meccanica
- Conduttività termica

CNTs are ENM with Janus-like properties

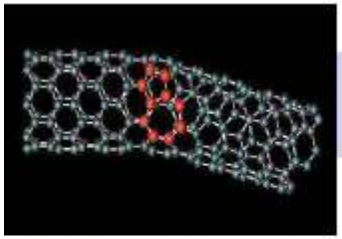


From: *Shvedova et al., 2009 - modified*



Evidenze tossicologiche sui CNT

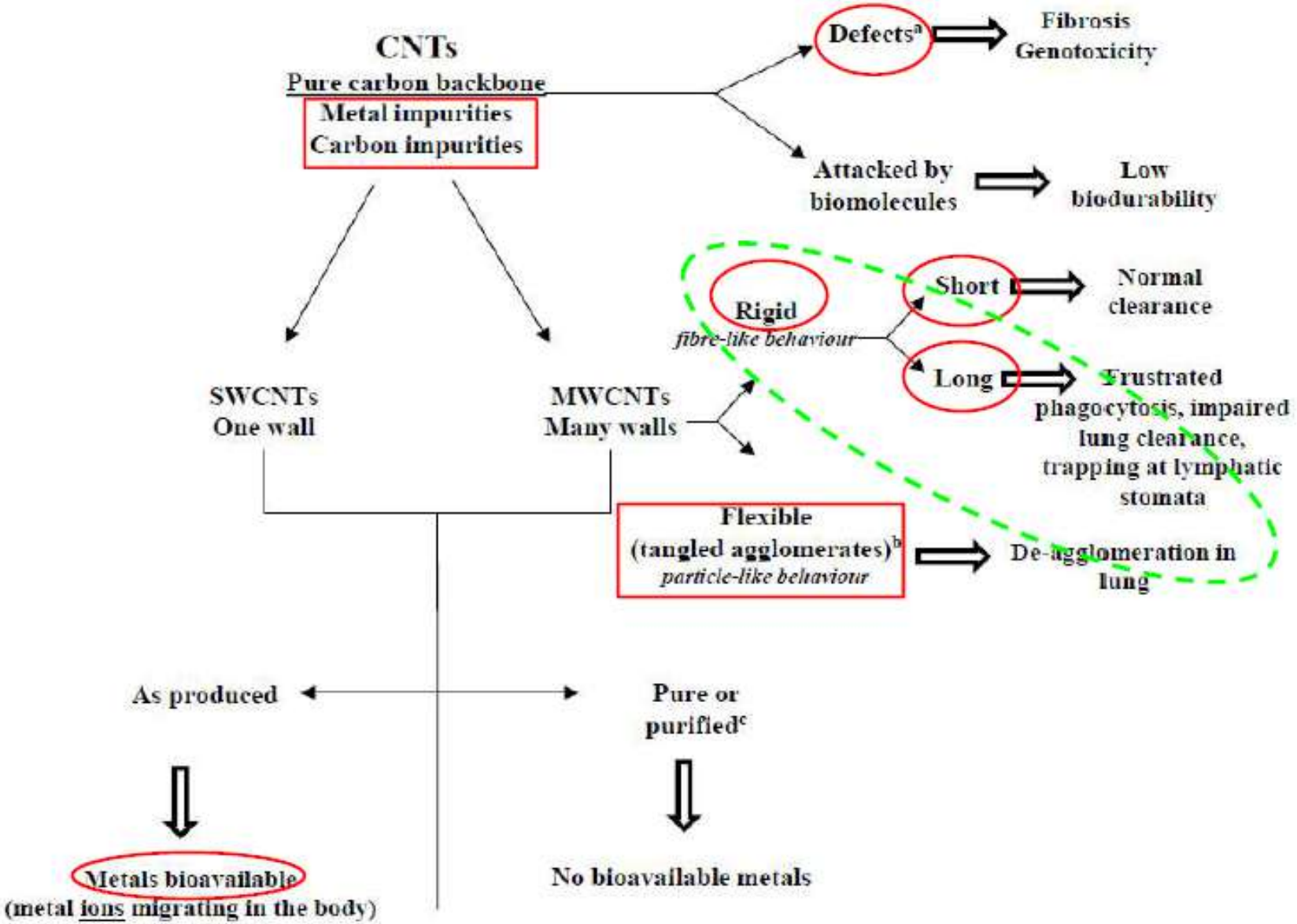
Deposit in the alveoli - *if single fibre*	*Shvedova et al., 2005* *Mercer et al., 2007*
Evade phagocytosis and enter alveolar walls	*Mercer et al., 2007*
Produce low and transient inflammatory changes	*Warheit et al., 2004*
Cause epithelial hypertrophy/hyperplasia	*Muller et. al., 2004* *Shvedova et al., 2005*
Produce lung fibrosis - >> equal mass of quartz or carbon black	*Shvedova et al., 2005* *Mercer et al., 2007*
Form non dose-dependent granulomas at deposition sites	*Warheit et al., 2004* *Shvedova et al., 2005* *Poland et al., 2008*
Induce plaques on vessel walls	*Li et al, 2007*
Affect the permeability of lung epitelial barrier depending on lenght and shape	*Rotoli et al., 2008*
Enter cell nuclei - + *if short*	*Porter et al., 2007*
Affect macrophagic clearance	*Shvedova et al., 2007*



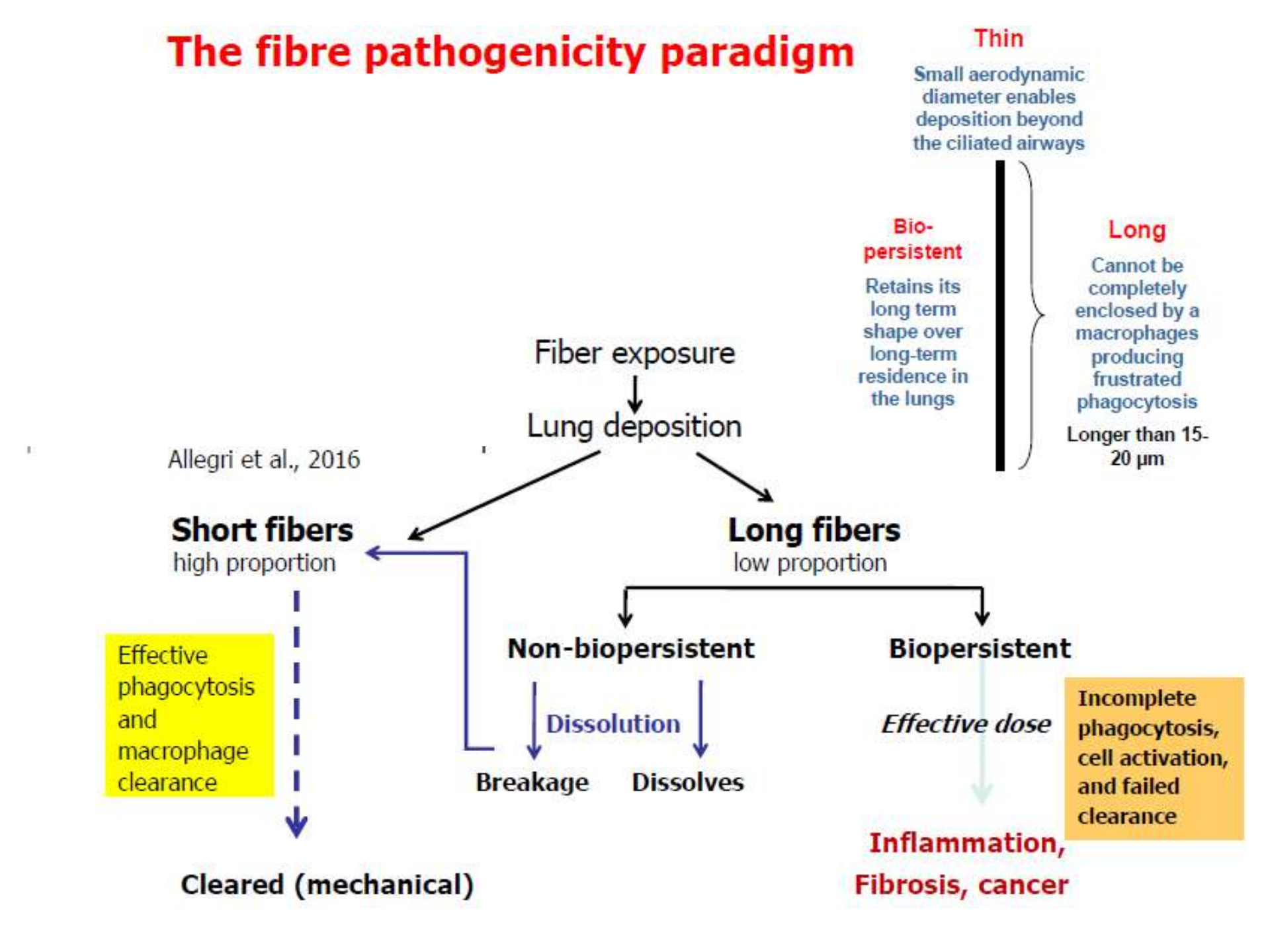
Proprietà tossicologiche dei CNT

- sono NM e quindi possiedono attività biologica superiore a quella di particelle di maggiori dimensioni;
- Presentano elevato rapporto lunghezza:diametro e quindi seguono il paradigma delle fibre;
- sono costituiti da grafite non planare e quindi sono altamente idrofobici, resistono alla frammentazione in fibre di minori dimensioni, permangono a lungo nei tessuti (es. polmonare) – mesi - al pari degli anfiboli ("biopersistenza")
- **Le ragioni della eventuale tossicità o biocompatibilità dei CNTs vanno ricercate nelle caratteristiche fisico-chimiche**

Proprietà fisico-chimiche dei CNTs associate con l'attività biologica



The fibre pathogenicity paradigm



Importanza della lunghezza dei CNT per la clearance dei macrofagi alveolari.

I CNT corti (< 15 μm) vengono fagocitati facilmente dai macrofagi, mentre quelli lunghi (> 15 μm) non vengono inglobati (fagocitosi incompleta)

Mechanisms of lung fibrosis induced by carbon nanotubes: towards an Adverse Outcome Pathway (AOP)

Vietti *et al. Particle and Fibre Toxicology* (2016) 13:11
DOI 10.1186/s12989-016-0123-y

Giulia Vietti*, Dominique Lison and Sybille van den Brule

STUDI PER ISTILLAZIONE INTRATRACHEALE

Mechanisms of lung fibrosis induced by carbon nanotubes: towards an Adverse Outcome Pathway (AOP)

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DOI 10.1186/s12989-016-0123-y

STUDI PER VIA INALATORIA

Hypothetical model of SWCNT- or MWCNT-induced pulmonary and pleural inflammation

- Short SWCNTs induced persistent pulmonary inflammation over a 90-day period following instillation.
- Pulmonary inflammation after short MWCNT instillation decreased in a time-dependent manner.
- MWCNT instillation induced greater levels of pleural inflammation than did short SWCNTs.

- Short SWCNTs and MWCNTs underwent lymphatic drainage to the mediastinal lymph nodes after pleural penetration.
- The extent and time-dependent changes of pulmonary and pleural inflammation differed following SWCNT and MWCNT instillations.

Fujita et al., Toxicology Letters 257 (2016) 23–37

Chemical Research in Toxicology

Article

pubs.acs.org/crt

Thickness of Multiwalled Carbon Nanotubes Affects Their Lung Toxicity

Ivana Fenoglio,*[,†,⊥,#] Elisabetta Aldieri,[†,⊥] Elena Gazzano,[†,⊥] Federico Cesano,[†,#] Massimiliano Colonna,[†,⊥] Domenica Scarano,[†,#] Gianna Mazzucco,[∥] Angelo Attanasio,[∥] Yousof Yakoub,[§] Dominique Lison,[§] and Bice Fubini[†,⊥,#]

Two samples of highly pure MWCNTs similar in hydrophobicity and surface reactivity were prepared with length < 5 μm but different diameter (9.4 vs 70 nm)

Both samples were internalized in MH-S cells. However, thin MWCNTs appeared significantly more toxic than the thicker ones, both in vitro and in vivo, when compared on a mass-dose basis

Cite this: *Chem. Commun.*, 2011, **47**, 10182–10188

www.rsc.org/chemcomm

Making carbon nanotubes biocompatible and biodegradable

Alberto Bianco,*[a] Kostas Kostarelos*[b] and Maurizio Prato*[c]

Il tipo ed il grado di funzionalizzazione dei CNT ha differenti conseguenze:

a) Consente la degradazione *in vitro* e *in vivo*;
b) Influenza la biodistribuzione e la tossicità

Functionalised CNT biodistribution in mice (with low and high degree of functionalisation). Chemical structure, transmission electron microscopy images of the f-MWCNTs and SPECT/CT images of live animals injected with radiolabelled f-CNTs indicate that high liver accumulation (left) can be modulated leading to increase in urinary excretion (high bladder signal) as degree of functionalisation increases (right)

Carbon Nanotube Degradation in Macrophages: Live Nanoscale Monitoring and Understanding of Biological Pathway

Dan Elgrabli,[†,#] Walid Dachraoui,[‡,#] Cécilia Ménard-Moyon,[§] Xiao Jie Liu,[⊥] Dominique Bégin,[⊥] Sylvie Bégin-Colin,[⊥] Alberto Bianco,[§] Florence Gazeau,[*,†] and Damien Alloyeau[*,‡]

Figure 6. Schematic representation of the biological pathways of MWCNT degradation in macrophages. (1) Intracellular oxidative degradation: after engulfment of MWCNTs, NOX_2 complex is activated on cytosolic and phagosomal membranes. Active NOX_2 complex induced $O_2^{\bullet-}$ production and then cytoskeleton reorganization. Into phagosome, $O_2^{\bullet-}$ is turned into H_2O_2 by SOD and H_2O_2 is turned in the presence of Fe^{3+} into $OH^{\bullet}$ (Haber-Weiss reaction). $OH^{\bullet}$ could attack MWCNT defects and unsaturated carbon bonds on the sidewalls of CNTs to generate carboxylic acids creating holes in the graphitic structure. (2) Extracellular oxidative degradation: MWCNT outside the cells can also be degraded by two routes: (i) production of $OH^{\bullet}$ *via* NOX_2 complex and MPO, or (ii) peroxynitrite attack as described by Kagan *et al.*[16]

2020 SCIENCE

Carbon nanotubes: the new asbestos? Not if we act fast.

May 21, 2008

Andrew Maynard

Mix carbon nanotubes and asbestos together (metaphorically) and you get an explosive mix—at least if news coverage of the latest publication coming out of Professor Ken Donaldson's team is anything to go by. The research—published on-line today in Nature Nanotechnology—is the first to explicitly test the hypothesis that long carbon nanotubes behave like long asbestos fibres in the body.

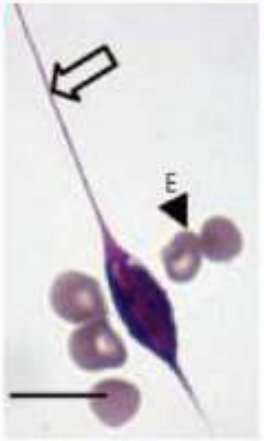
Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study

CRAIG A. POLAND[1], RODGER DUFFIN[1], IAN KINLOCH[2], ANDREW MAYNARD[3], WILLIAM A. H. WALLACE[1], ANTHONY SEATON[4], VICKI STONE[3], SIMON BROWN[1], WILLIAM MacNEE[1] AND KEN DONALDSON[1]*

Effect of fibre length on phagocytosis by peritoneal macrophages *(Poland et al., 2009)*

"frustrated phagocytosis" of long Amosite fibres

Long CNT also leads to frustrated phagocytosis

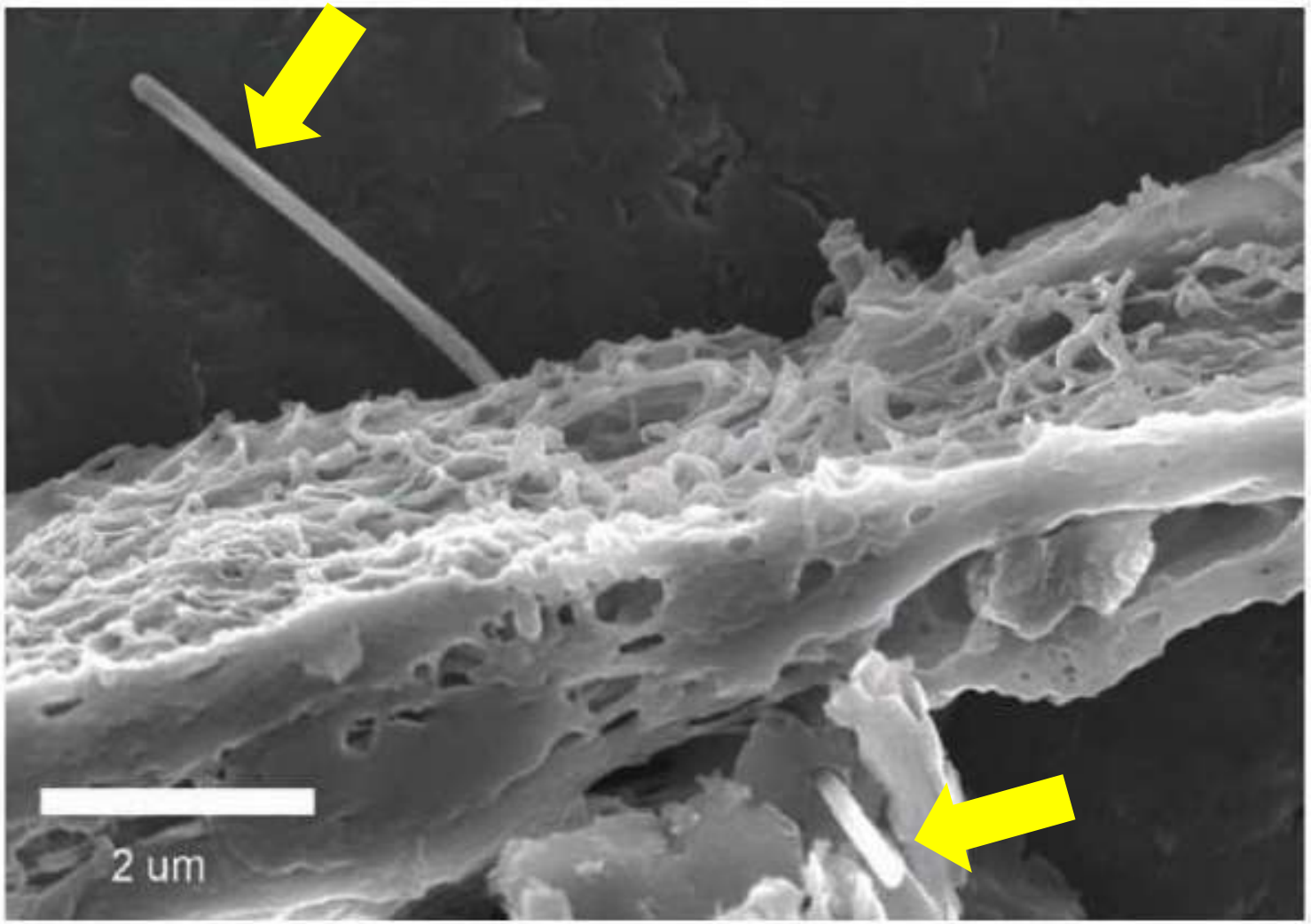


Phagocytosis of short Amosite

Foreign body giant cells (FBGCs)

NTtang1 phagocytosed

All images are shown at 1,000 magnification with a 5 μm scale bar



Penetration of visceral pleura by multi-walled carbon nanotube

Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers (NIOSH Pub. 2013-145)

The carcinogenic effect of various multi-walled carbon nanotubes (MWCNTs) after intraperitoneal injection in rats

Susanne Rittinghausen[1*], Anja Hackbarth[1], Otto Creutzenberg[1], Heinrich Ernst[1], Uwe Heinrich[1], Albrecht Leonhardt[2] and Dirk Schaudien[1]

Substance	Length (µm) WHO fibers*	Diameter (µm) fibers*	Rats with mesothelioma	Mean survival time of mesothelioma-bearing rats (days)	Month of first detection of a morbid mesothelioma-bearing rat
Medium control	-	-	1 (2%)	739	24
MWCNT A low	8.57 ± 1.51	0.085 ± 1.60	49 (98%)	213	5
MWCNT A high			45 (90%)	194	5
MWCNT B low	9.30 ± 1.63	0.062 ± 1.71	46 (92%)	294	6
MWCNT B high			45 (90%)	207	5
MWCNT C low	10.24 ± 1.64	0.040 ± 1.57	42 (84%)	415	10
MWCNT C high			47 (94%)	265	6
MWCNT D low	7.91 ± 1.40	0.037 ± 1.45	20 (40%)	666	20
MWCNT D high			35 (70%)	585	11
Long amosite asbestos	13.95 ± 2.10	0.394 ± 1.83	33 (66%)	623	14

Sargent et al. Particle and Fibre Toxicology 2014, 11:3
http://www.particleandfibretoxicology.com/content/11/1/3

PARTICLE AND FIBRE TOXICOLOGY

Promotion of lung adenocarcinoma following inhalation exposure to multi-walled carbon nanotubes

Table 3 Incidence of lung tumors in early euthanized mice and mice 17 months after exposure

Euthanasia	Early sacrifice				Terminal sacrifice			
	Air	MCA	MWCNT	MCA + MWCNT	Air	MCA	MWCNT	MCA + MWCN
Number of animals	4	6	6	13	56	54	49	42
Number of Bronchiolo-alveolar adenomas	0	1	0	4	6	18*	9	32*
% of mice with one or more Bronchiolo-alveolar adenoma	0%	17%*	0%	31%*	11%	33%*	18%	76%*
Number of Bronchiolo-alveolar adenocarcinomas	0	1	0	7	7	12	7	26
% of mice with Bronchiolo-alveolar Adenocarcinomas	0%	17%*	0%	54%*	13%	22%*	14%	62%*
Number of mice with one or more– Bronchiolo-alveolar adenoma and/or Bronchio-alveolar adenocarcinomas	0	2	0	8	13	28*	13	38*
Percent of mice with lung tumors	0%	33%	0%	62%*	23.2%	51.9%*	26.5%	90.5%*

*Indicates statistical significance at $p < .0001$.

CONCLUSIONS: ...some **MWCNT exposures promote the growth and neoplastic progression of initiated lung cells** in B6C3F1 mice. ....the mouse MWCNT lung burden of 31.2 µg/mouse approximates feasible human occupational exposures. Therefore, caution should be used to limit human exposures to MWCNT.

International Agency for Research on Cancer

World Health Organization

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

Carcinogenicity of fluoro-edenite, silicon carbide fibres and whiskers, and carbon nanotubes

THE LANCET Oncology Volume 15, Issue 13 – December 2014, 1427-28

Regarding **carcinogenicity in experimental animals**, the Working Group concluded that there was **sufficient evidence for MWNT-7** (*) (**possibly carcinogenic to humans: Group 2B**), **limited evidence for the two other types of MWCNTs with dimensions similar to MWNT-7, and inadequate evidence for SWCNTs.**

MWNT-7 caused peritoneal mesotheliomas in rats, and in male *p53*$^{+/-}$ mice in two intraperitoneal injection studies. **Inhalation** of MWNT-7 promoted **bronchioloalveolar adenoma and carcinoma** in male mice. In one i.p. study, two other types of MWCNTs with physical dimensions similar to those of MWNT-7 (length 1–19 μm; diameter 40–170 nm) caused mesotheliomas.

CNTs induce genetic lesions such as **DNA strand breaks, oxidised DNA bases, mutations, micronucleus formation, and chromosomal aberrations**. SWCNTs and MWCNTs also perturb the cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells; **the above mechanisms are all relevant to humans**

(*) *Mitsui & Co. (MWNT-7, lot 061220-31; Ibaraki, Japan)*

MWNT-7: the average width of the particles was between 40 and 90 nm, while the length varied up to several micrometers

Are we ready for spray-on carbon nanotubes?

As artists and manufacturers explore the use of spray-on carbon nanotube coatings, Andrew Maynard explores the state of the science around nanotube safety.

Andrew D. Maynard

Director, Risk Innovation Lab, School for the Future of Innovation in Society, Arizona State University, Tempe AZ, USA. Email: Andrew.maynard@asu.edu

In 2014, the International Agency for Research on Cancer (IARC) published an evaluation of the carcinogenic potential of carbon nanotubes. Unfortunately, because of the variety of carbon nanotube types used in published toxicity studies, the review panel could not draw generalizable conclusions that satisfied IARC's rigorous standards. The panel concluded that carbon nanotubes "cannot be classified due to a lack of data" (group 3 carcinogen).

All, that is, apart from MWNT-7. Because MWNT-7 had been studied so extensively, there was sufficient evidence for the panel to place this one specific form of carbon nanotubes into group 2B – "possibly carcinogenic to humans".

Structure-effect relationship Ranking

1) Cytotoxicity order: Asbestos, recognized as carcinogenic to humans, has less toxicological effects than single-walled CNTs (SWCNTs) but is more toxic than multi-walled CNTs (MWCNTs) [Inoue et al. 2008; Jia et al. 2005; Murr et al. 2005; Tian et al. 2006].

2) CNT purification: Purified CNTs are more toxic than their unrefined counterparts [Carrero-Sánchez et al. 2006; Wick et al. 2007]. Moreover, the cytotoxicity of purified MWCNTs can be increased to be more toxic than asbestos [Muller et al. 2005].

3) Surface area and surface chemistry of CNTs: Tian et al. [2006] has found that the material with the smallest surface area (SWCNTs in this case) is more toxic than other tested materials. Their results also give a good explanation for the effect of CNT purification: the refining process changes the aggregation state of CNTs and then modifies the surface chemistry.

4) CNT structure: Long MWCNTs exhibit asbestos-like hazards, but short and tangled MWCNTs do not show any significant toxicity [Poland et al. 2008]. The presumption of the risk associated with long CNTs is that macrophages cannot completely engulf (or phagocytose) long fibers to clear them from tissues; however, effective phagocytosis is completed for short or tangled CNTs to clear them through the lymphatic system [Kostarelos 2008].

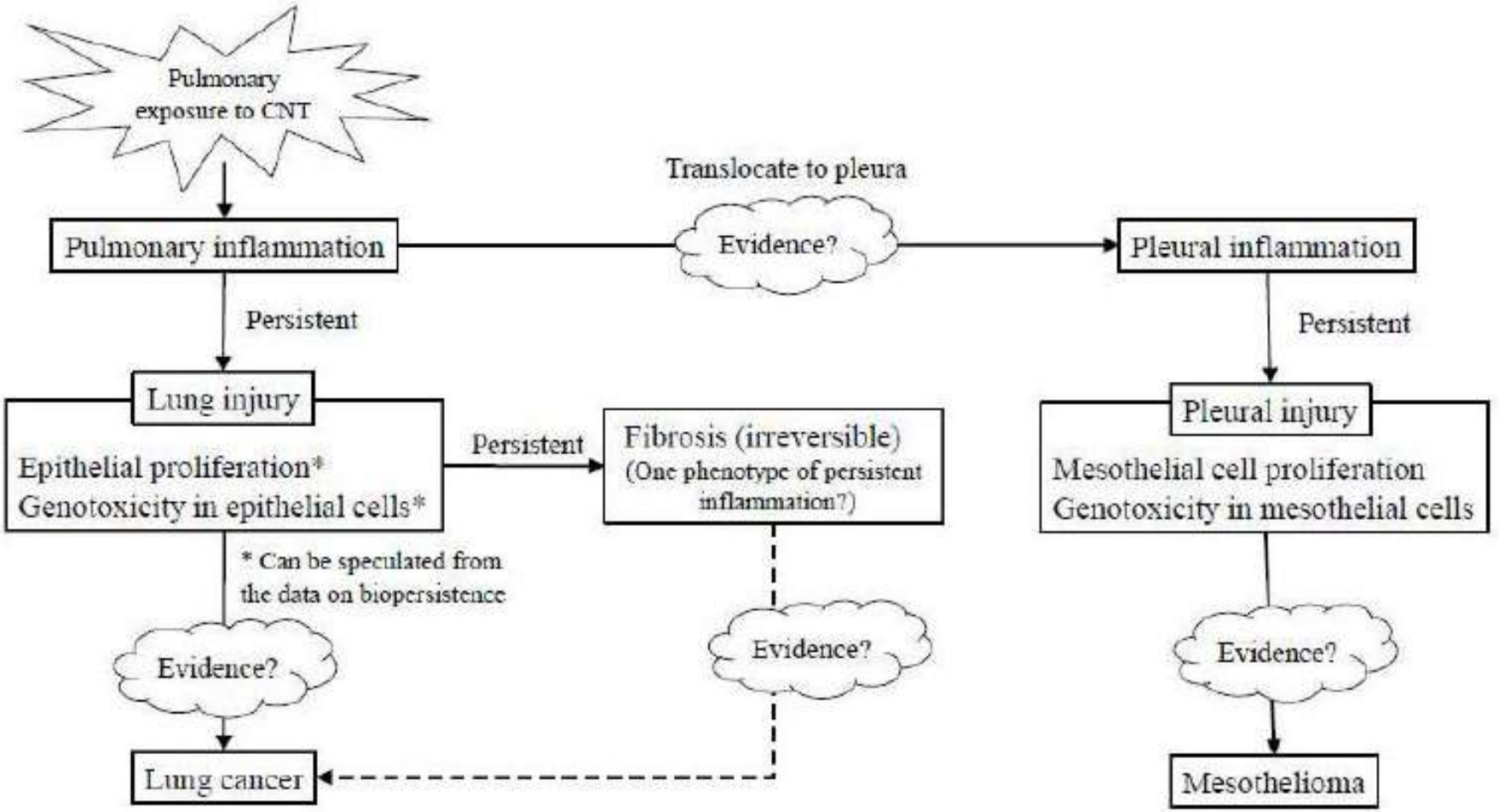
Mercer et al. Particle and Fibre Toxicology 2013, 10:38
http://www.particleandfibretoxicology.com/content/10/1/38

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Extrapulmonary transport of MWCNT following inhalation exposure

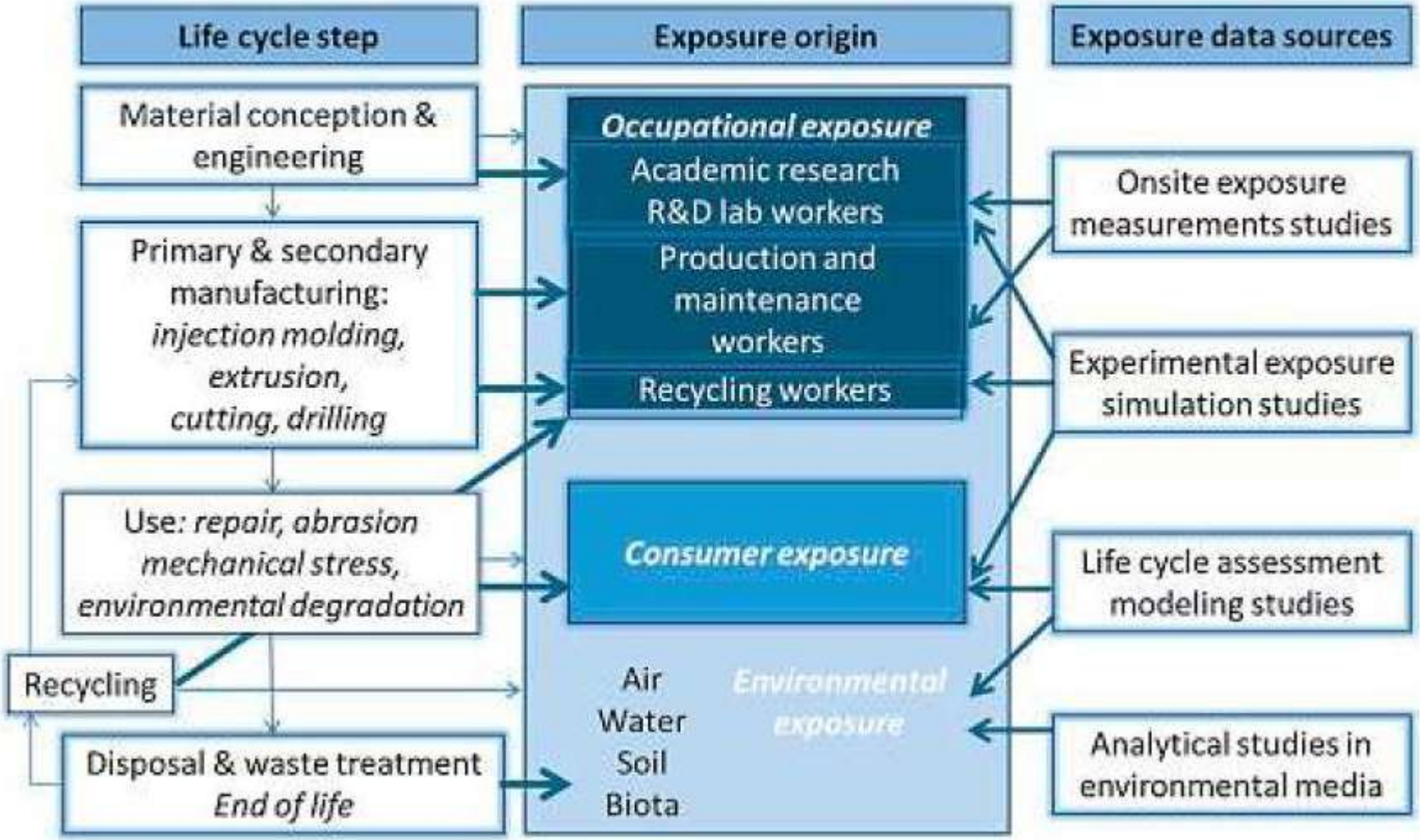
Meccanismi di danno polmonare dei nanotubi di carbonio



CNT, carbon nanotubes
Compiled by the Working Group with data from Morimoto et al. (2014)

IARC MONOGRAPHS – 111

L'esposizione a CNTs e a materiali contenenti CNTs è possibile lungo tutto il ciclo di vita dei materiali



Guseva Canu et al., 2016

Formation of inhalable aerosols during CNTs handling and possible skin contamination

Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V [2004]. Exposure to carbon nanotube material during the handling of unrefined single walled carbon nanotube material. J Toxicol Environ Health Part A 67:87–107.

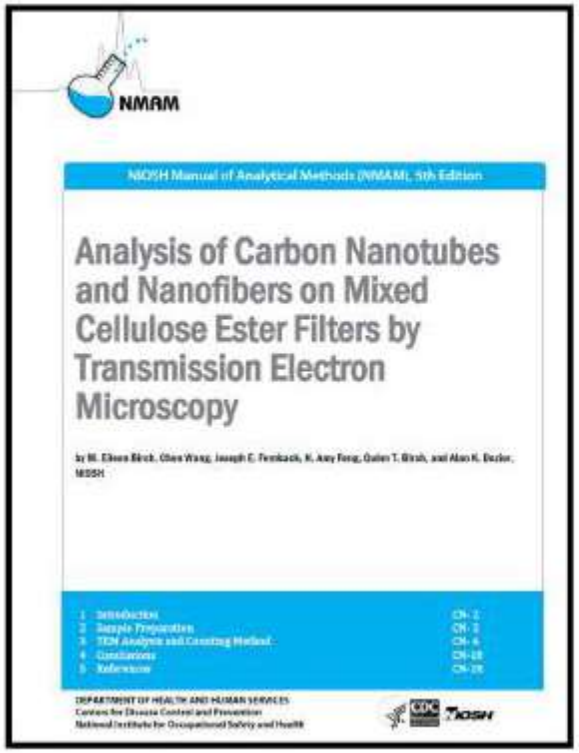
Concentrazioni aerodisperse di CNTs prodotte da specifiche operazioni (fonte: NIOSH)

Specific Task Evaluation: Dry Powder Handling

Process: Extrusion
Task: Weighing MWCNT
Volume: 1 kg
Duration of Sample: 112 min
Exposure Concentration=
3.19 µg/m^3

Process: Wet Shipping
Task: Weighing MWCNT
Volume: 7.7 kg
Duration of Sample: 269 min
Exposure Concentration=
0.3 µg/m^3

Process: Resin Formulation
Task: Weighing CNF/MWCNT
Volume: 100-200 g
Duration of Sample: 178 min
Exposure Concentration=
7.54 µg/m^3


NMAM
NIOSH Manual of Analytical Methods (NMAM), 5th Edition
Analysis of Carbon Nanotubes and Nanofibers on Mixed Cellulose Ester Filters by Transmission Electron Microscopy
by M. Eileen Birch, Chen Wang, Joseph C. Fernback, K. Amy Feng, Quinn T. Birch, and Alan K. Ducler, NIOSH
1 Introduction CN-1
2 Sample Preparation CN-3
3 TEM Analysis and Counting Method CN-4
4 Conclusions CN-18
5 References CN-19
DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health
CDC
NIOSH

Life cycle stage	Engineering	Raw material production	Transformation and composite materials production	Final products production
Target population	**Academic and industrial R&D lab workers**	**Primary manufacture & maintenance workers**	**Secondary manufacture & maintenance workers**	**Secondary manufacture workers/professional users**
Exposure pattern	Accidental: acute, potentially high rate	Chronic, low rate	Chronic, low rate	Chronic, low rate
Available data source	Onsite studies	Onsite studies & experimental studies	Onsite studies & experimental studies	Onsite studies & experimental studies
Data completeness	Partial	Partial	Partial	Limited
Exposure metric or proxy	Mass concentration and number concentration of CNT/CNF structures	Mass concentration and number concentration of CNT/CNF structures	Mass concentration and number concentration of CNT/CNF structures	Mass concentration and number concentration of CNT/CNF structures
Exposure potential	**+++**	**++**	**++**	**++**
Relevance for risk assessment	**Relevant**	**Relevant**	**Insufficient**	**Insufficient**

Modificata da: Guseva Canu et al., 2016

Criticità nella valutazione dell'esposizione

diverse strutture hanno la stessa pericolosità e potenziale tossicità?

ACSNANO

In Vivo Toxicity Assessment of Occupational Components of the Carbon Nanotube Life Cycle To Provide Context to Potential Health Effects

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ARTICLE

Understanding toxicity beyond the as produced, or pure native material, is critical…while the number of workers and consumers increases along the life cycle, toxicity and/or potential for exposure to the as-produced material may greatly diminish

Transmission electron micrographs illustrate consistency between personal breathing zone (PBZ) collections and material preparation in physiologic dosing medium (DM) for in vivo dosing.

A representative image of the material obtained from the lung by bronchoalveolar lavage (BAL) after exposure showed that the particle morphology was maintained.

Currently available studies of workers exposed to CNTs

Bergamaschi et al., 2018

Ref.	EC μg/m³	N (exp/ unexp)	Main findings
Lee et al. (2015)	CNT **6.2-9.3**	9/4	↑ malondialdehyde (MDA), 4-hydroxy-2-hexenal (4-HHE) and n-hexanal in EBC of exposed workers
Shvedova et al. (2016)	MWCNT **2.8 R 14.4 I**	8/7	Dysregulated ncRNA and mRNA expression profiles (miRNAs and their target genes with roles in cell cycle regulation/progression/control, apoptosis, cell proliferation and carcinogenetic pathways) in exposed workers
Fatkhutdinova et al. (2013 & 2016)	MWCNT **0.7-2.8 R**	10/12	In exposed workers: ↑IL-1β, IL-4, IL-5, IL-6, IL-8 and TNF-α in sputum; ↑KL-6 (sputum); ↑IL-1β, IL-4, IL-10 and TNF-α in serum; ↑TGF-β1 in serum (only in workers < 30 year old);
(Vlaanderen et al., 2017)	MWCNT **45-57 I**	22/39	↑ C-C motif ligand 20, soluble IL-1 receptor II
Schubauer-Berigan et al., 2018	CNT/CNF **1.0 R 6.2 I**	108	Positive association with respiratory allergy with inhalable EC and number of years worked; association of systolic BP with PM, RHR with EC

Assessment of the Health Risk and Recommended Exposure Limit

- The best data to use for a quantitative risk assessment and as basis for a recommended exposure limit (REL) are the **non malignant pulmonary data from animal studies**
- NIOSH considers the pulmonary responses of inflammation and fibrosis observed in **short-term and sub-chronic studies** in animals to be **relevant to humans**, as inflammatory and fibrotic effects are also observed in occupational lung diseases associated with workplace exposures to other inhaled particles and fibers
- These **fibrotic lung effects** observed in some of the animal studies **developed early** (e.g., 28 days after exposure) in response to **relatively low-mass lung doses**, and also persisted or progressed after the end of exposure

Estratto da: NIOSH CIB 65 Carbon Nanotubes and Nanofibers

Comparison of approaches and calculation methods for CNF and CNTs. Occupational Exposure Limits (OELs)

Assessment method	NIOSH	Paululn 2010b	Nanocyl	Aschberger 2010	NEDO Project
Material	CNTs & CNF	MWCNTs (Baytubes)	Nanocyl MWCNTs (5-25 nm; 0,1 to 10 μm)	Baytubes and Nanocyl CNTs	SWCNTs & MWCNTs
NOAEL, etc, obtained in animal test/analysis	NOAEL (0,1mg/m^3) Paululn 2010a LOAEL (0,1 mg/m^3) Ma-Hock et al. 2009	NOAEL (0,1 mg/m^3)	LOAEL (0,1 mg/m^3)	NOAEL (0,1 mg/m^3) for MWCNT; LOAEL (0,1mg/m^3) for SWCNT	NOAEL (0,13 mg/m^3) for SWCNT NOAEL (0,37 mg/m^3) for MWCNT
Animal test	Short term (28 day) studies. Shvedova et al. 2005,2008. Muller et al. 2005 & Lam et al 2004. Subchronic studies in rodents. Ma-Hock et al 2009. Paululn 2010a	Rat subchronic (13-wk) inhalation study	Subchronic (13wk)inhalation study in rat. Ma-Hock et al. 2009	MWCNT: Rat subchronic (13-wk) inhalation study. Paululn 2010. SWCNT: Rat subcronic (13-wk) inhalation study. Ma-Hock et al 2009	Short term (28 day) inhalation studies (Morimoto et al 2012 a,b)
Assessment endpoint	Early-stage noncancer lung effects	Pulmonary inflammation, granulomas and alveolar hyperplasia	Granulomatous inflammation and inflammatory biomarkers in lung	Inflammatory biomarkers, granulomas and alveolar hyperplasia in lung	Histopathological findings and inflammatory biomarerks in BALF
Uncertainty factors and/or assessment factors	Species-specific differences in alveolar deposition, differences in ventilation, and time-dependent particle accumulation	Not applied	Adjusted LOAEL to workers and applied assessment factor of 40	Adjusted NOAEL & LOAEL for worker exposure day and air intake; assesment factor of 25 & 50.	Adjusted for worker exposure day, air intake, deposition fraction, and body weight; uncertainty factor of 6.
Worker exposure limit (OEL; 8h TWA)	0,001 mg/m^3	0,05 mg/m^3	0,0025 mg/m^3	0,002 mg/m^3 for MWCNTs 0,001 mg/m^3 for SWCNTs	0,03 mg/m^3 (Period limited)

Assessment of the Health Risk and Recommended Exposure Limit (NIOSH, 2013)

Table A–5. Benchmark dose estimates and associated working lifetime airborne concentrations—grade 1 or higher severity of lung responses in rats after subchronic inhalation of MWCNT (dose metric: estimated deposited or retained dose in lungs)

	Rodent		Human		Human working lifetime airborne concentration[‡] (μg/m³)	
Rodent study and response[*]	BMD[†] (μg/lung)	BMDL (μg/lung)	BMD (mg/lung)	BMDL (mg/lung)	BMC	BMCL
Deposited lung dose (assuming no clearance)						
Ma-Hock et al. [2009] Granulomatous inflammation	21	8.1	5.4	2.1	0.51	0.19
Pauluhn [2010a] Alveolar septal thickening	28	14	7.2	3.5	0.77	0.38
Retained lung dose (assuming normal clearance)						
Ma-Hock et al. [2009] Granulomatous inflammation	11	3.8	2.7	0.97	2.7	1.0
Pauluhn [2010a] Alveolar septal thickening	14	6.5	3.6	1.7	4.2	1.9

[*] Histopathology grade 1 (minimal) or higher severity. Benchmark response level—10% excess (added) risk in exposed animals.

[†] BMD: estimated benchmark dose (maximum likelihood estimate). BMDL: estimated 95% lower confidence limit of the BMD; multistage (polynomial degree 2) [US EPA 2010]. *P* values for the rodent dose-response models: 0.99 for Ma-Hock et al. [2009] and 0.88 for Pauluhn et al. [2010a] (deposited dose); 1.0 for Ma-Hock et al. [2009] and 0.94 for Pauluhn [2010a] (retained dose).

[‡] 8-hr time weighted average (TWA) concentration associated with the human-equivalent BMD(L)s. BMC: maximum likelihood estimate of the benchmark concentration; BMCL: 95% lower confidence limit of the BMC.

Estratto da: NIOSH CIB 65 Carbon Nanotubes and Nanofibers

Valori limite e limiti raccomandati per CNTs

(Bergamaschi et al., 2018)

Institution	Concentration (number/mass)	Interpretation	Year
British Standards Institution (WEL)	**0.01 fibres/mL**	Fibrous nanomaterials with high aspect ratios (> 3:1) and **length > 5000 nm (> 5 μm)**	2007
Nanocyl	**2,5 μg/m^3**	8-hr TWA	2009
Bayer	**50 μg/m^3**	8-hr TWA (Baytubes™)	2010
US NIOSH (REL)	**7 μg/m^3**	8-hr TWA	2010
Dutch Social and Economic Council (OEL)	**0.01 fibres/cm^3** (10.000 f/m^3)	SWCNT or MWCNT or metal oxide fibres **for which asbestos like effects are not excluded**	2012
US NIOSH (REL)	**1 μg/m^3 (r EC)**	8-hr TWA	2013
US OSHA (recommendation)	**1 μg/m^3 (r EC)**	8-hr TWA	2013
Nakanishi (OEL)	**30** (SW) **- 80** (MW) **μg/m^3**	8-hr TWA	2015
Swiss Accident Insurance Funds (SUVA)	**0.01 fibres/mL**	8-hr TWA	2018

Provisional nano reference values (NRV) for engineered nanomaterials

**(German Institute for Occupational safety and Health (IFA)
Social and Economic Council – The Netherlans)**

Class	Description	Density	NRV (8-hr TWA)	Examples
1	Rigid, biopersistent nanofibres for which effects similar to those of asbestos are not excluded	–	0.01 fibres/cm3 (= 10,000 fibres/m^3)	SWCNT or MWCNT or metal oxide fibres for which asbestos?like effects are not excluded by manufacturer.
2	Biopersistent granular nanomaterial in the range of 1 and 100 nm	> 6000 kg/m^3	20,000 particles/cm^3	Ag, Au, CeO_2, CoO, Fe, Fe_xO_y, La, Pb, Sb_2O_5, SnO_2
3	Biopersistent granular and fibre form nanomaterials in the range of 1 and 100 nm	< 6000 kg/m^3	40,000 particles/cm^3	Al_2O_3, SiO_2, TiN, TiO_2, ZnO, nanoclay Carbon Black, C60, dendrimers, polystyrene Nanofibres for which asbestos-like effects are excluded
4	Non-biopersistent granular nanomaterials in the range of 1 and 100 nm	–	Applicable OEL	e.g. fats, common salt (NaCl)

Assessment of the Health Risk and Recommended Exposure Limit (NIOSH, 2013)

In sintesi:

- Le stime di rischio basate sulla dose di riferimento (BMD) indicano un eccesso di rischio del 10% di sviluppare alterazioni polmonari iniziali (infiammazione e fibrosi polmonare) per esposizioni di 8 ore/die, 40 settimane/anno, per 45 anni di vita lavorativa, comprese fra 0,2 e 2 $\mu g/m^3$;

- L'integrazione di queste stime con i fattori di incertezza (dosimetria) indicherebbero un LOAEL compreso fra 4-18 $\mu g/m^3$ come TWA per 8 ore ed un NOAEL compreso fra 1-4 $\mu g/m^3$ come TWA per 8 ore;

- Di conseguenza, l'ipotetica soglia di rischio (R=0) sarebbe da considerarsi una concentrazione < 1 $\mu g/m^3$ come TWA per 8 ore

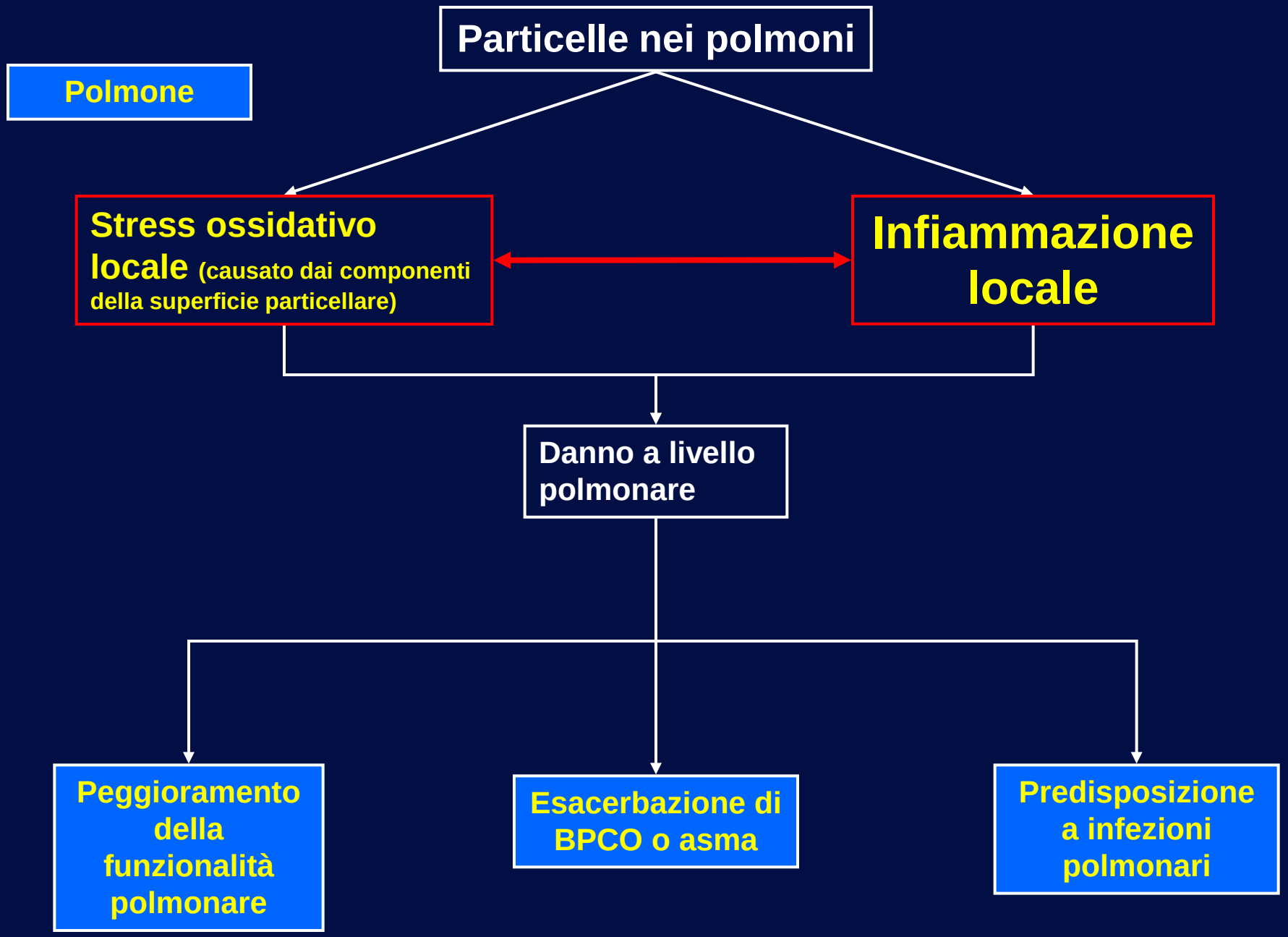
Until the results from animal research studies can fully explain the mechanisms (e.g., shape, size, chemistry, functionalized) that potentially increase or decrease their toxicity, all types of CNT and CNF should be considered a respiratory hazard and occupational exposures controlled at the REL of 1 $\mu g/m^3$

Summary of Epidemiological and Animal Data for ENMs by commercial volume

Nanomaterial	Commercial Tonnage (Tons)	Epidemiologic findings pathologic effects in workers	Potential biomarkers of adverse effects in epidemiological studies of workers	Adverse effects in animals
Carbon black	9.600.000			
Synthetic amorphous silica	1.500.000			
Aluminium oxide	200.000			
Bariun titanate	15.000			
Titanium dioxide	10.000			
Cerium dioxide	10.000			
Zinc oxide	8.000			
Carbon nanotubes/ nanofibers	100 - 3000			
Silver nanoparticles	20			

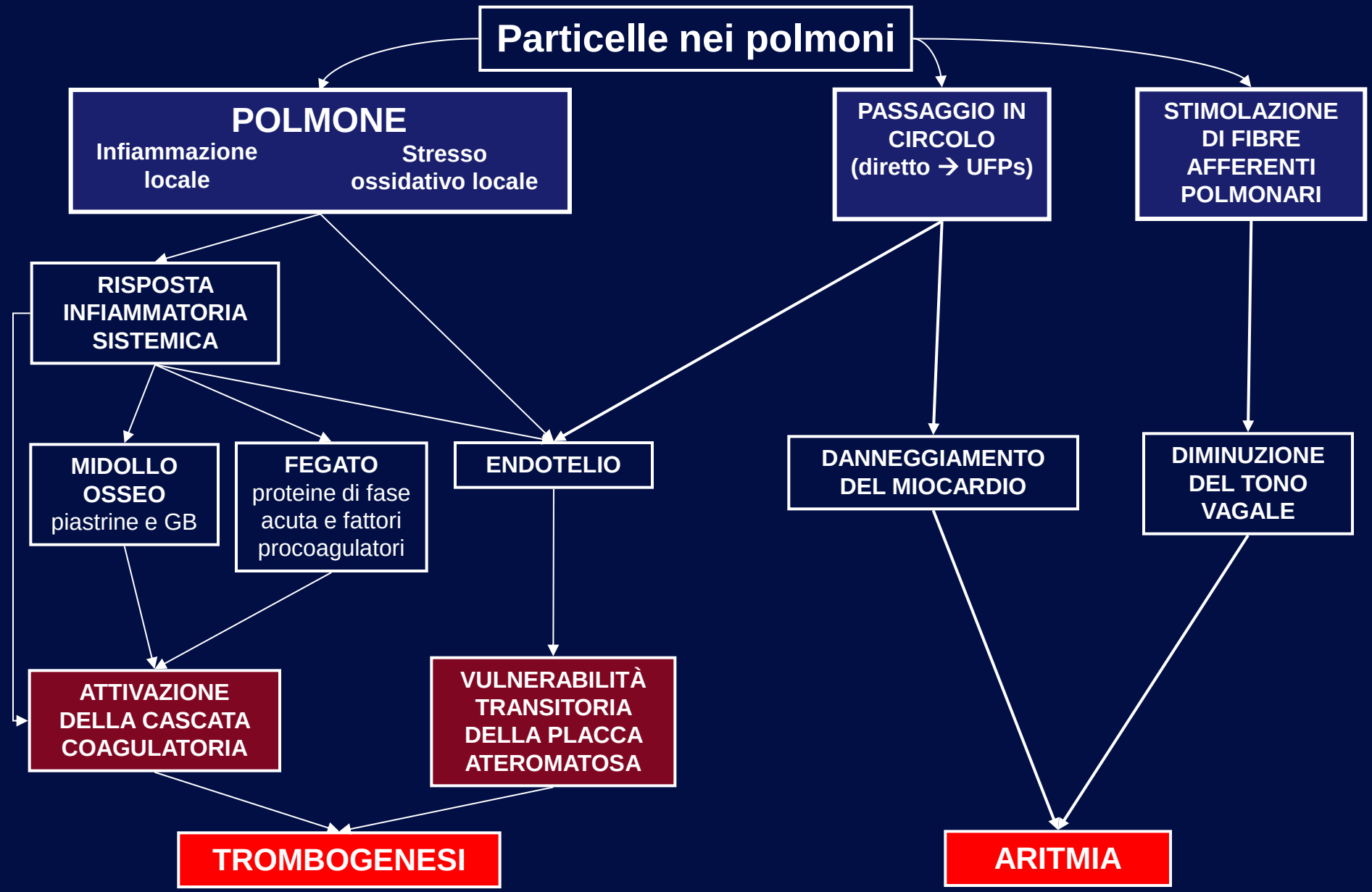
Based on the WHO report 2017

MECCANISMI PM



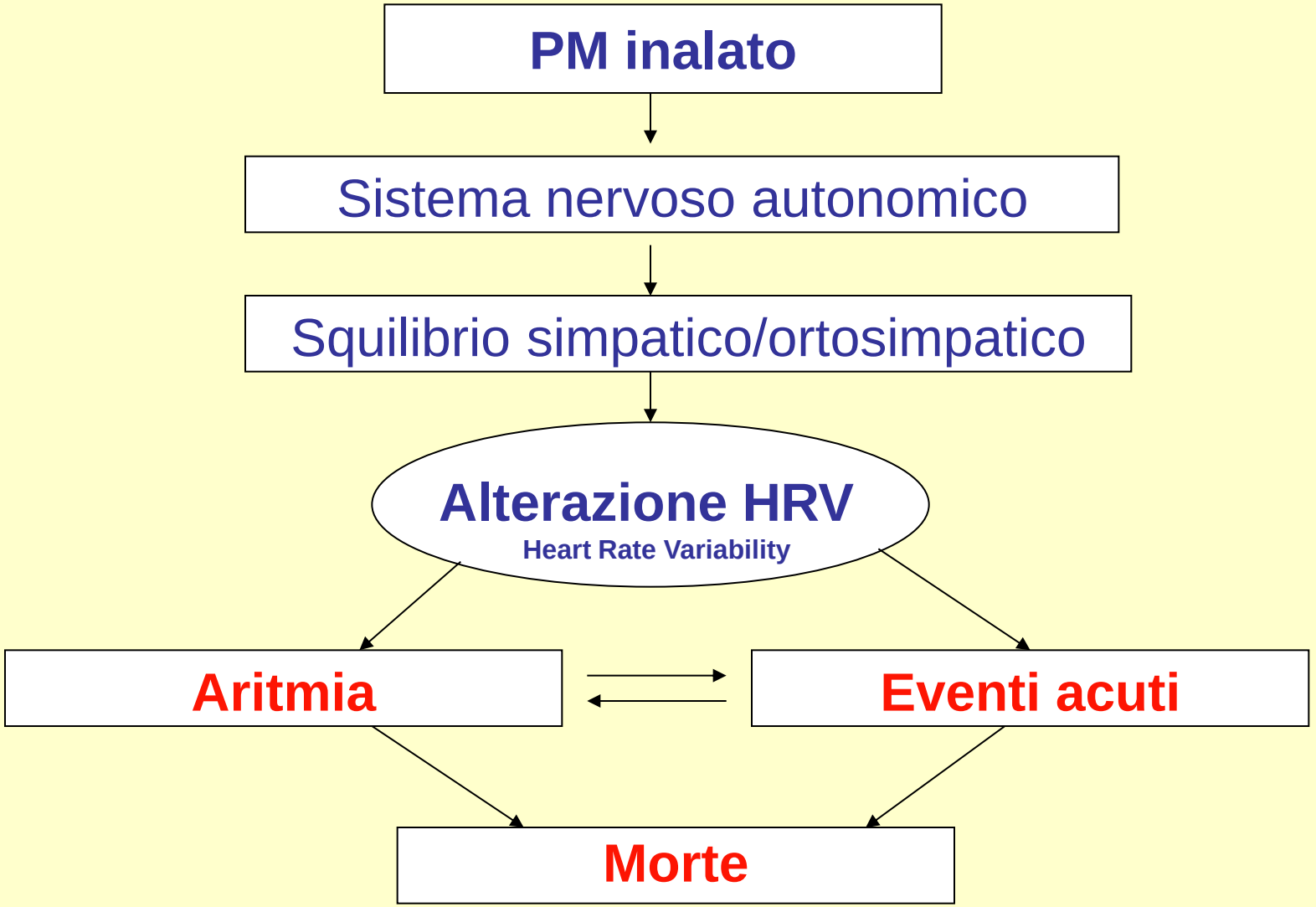
Adattata da Donaldson and Stone 2003; van Eeden et al 2004; Rückerl et al 2006

MECCANISMI PM



Adattata da Donaldson and Stone 2003; van Eeden et al 2004; Rückerl et al 2006

Effetti cardiovascolari acuti PM



EFFETTI DEL PM SULLA SALUTE

Cronici

Tumore del polmone

Examples of Epidemiological and Animal Data for ENMs by commercial volume

Nanomaterial	Commer-cial Tonnage (Tons)	Epidemiologic findings pathologic effects in workers	Potential biomarkers of adverse effects in epidemiological studies of workers	Adverse effects in animals
Carbon black	9.600.000	++ Non Malignant Resp. Diseases	++ pulmonary function ++ fulmonary inflammation	++ Lung cancer +++ pulmonary inflammation +++ cardiovascular
Synthetic amorphous silica	1.500.000	NA	NA	++ NMRD +++ fumed silica + pulmonary inflammation biomarkers
Aluminium oxide	200.000	NA	NA	++ pulmonary inflammation
Bariun titanate	15.000	NA	NA	NA
Titanium dioxide	10.000	+ lung cancer +NonMalignant Resp. Diseases	++ inflammatory and oxidative stress +++ pulmonary diseases ++ cardiovascular diseases	++++ RDS pulmonary inflammation + Genotoxicity + Lung cancer (NIOSH)
Cerium dioxide	10.000	NA	NA	+++ pulmonary inflammation fibrosis
Zinc oxide	8.000	+++ (metal fume fever)	NA	+++ acute inflammatory change
Carbon nanotubes/ nanofibers	100 - 3000	NA	++ pulmonary immunological Cardiovascular + Gene-specific DNA methylation	++++ pulmonary inflammation ++++ fibrosis +++ cardiovascular +++/++++ Cancer (MWCNTs-7 IARC 2B)
Silver nanoparticles	20	NA	NA	+++ pulmonary inflammation +++ bile duct hyperplasia

Modified from P.Schulte (NIOSH) – ICOH 2018 - Dublin

Danno renale

Nanoparticelle di Palladio instillate e.v.

↓

Azione nefrotossica tubulo renale dopo 14 gg

↓

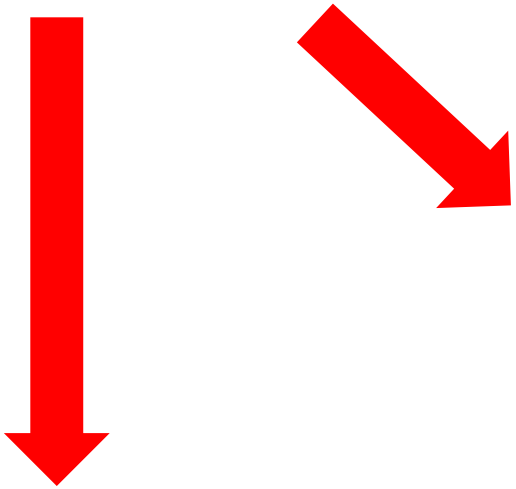
↑ retinol binding protein

↑ β2 microglobulina

Danno al tessuto nervoso

Nanoparticelle di TiO_2

(instillazione intra-nasale)

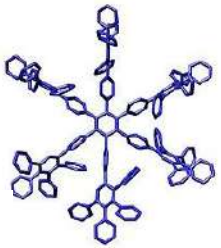


Trasporto trans-sinaptico e accumulo nell'ippocampo e nel bulbo olfattorio (alterazioni morfologiche dei neuroni)

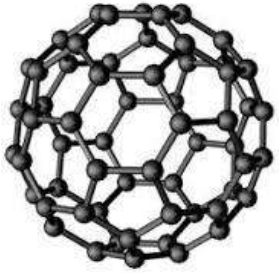
Alterazioni dell'omeostasi enzimatica dei neurotrasmettitori del sistema dopaminergico, stress ossidativo e reazioni infiammatorie, alterazione dell'espressione genica

Altri ENM interessanti

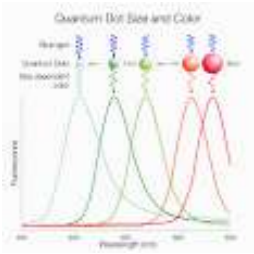
- Dendrimeri



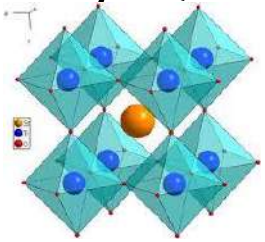
- Fullereni



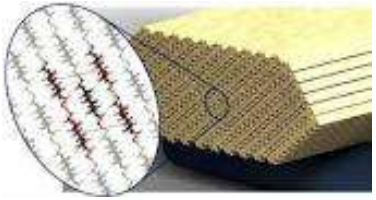
- NanoArgille
- Punti Quantici / Quantum Dots



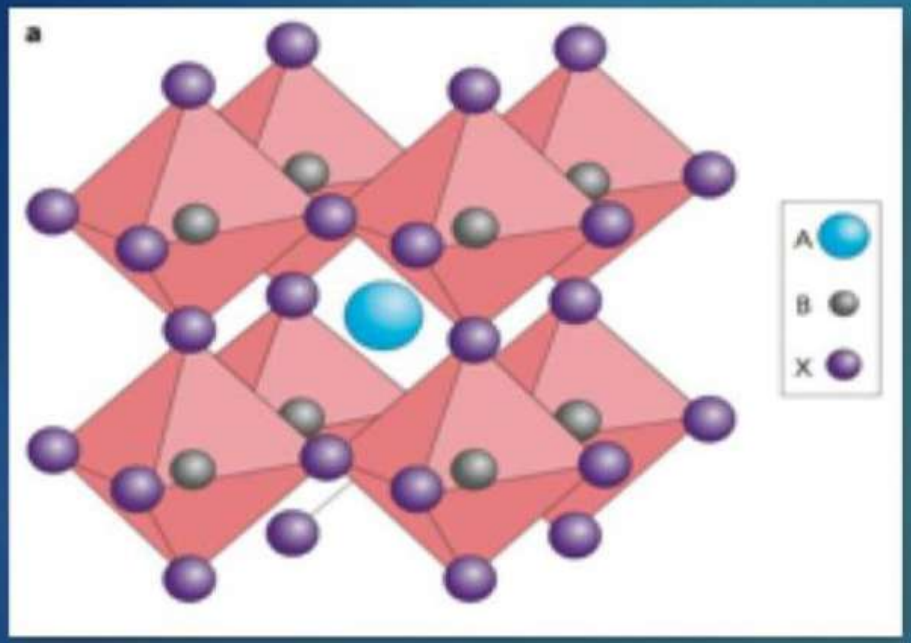
- Perovskite



- Nanocristalli di Cellulosa



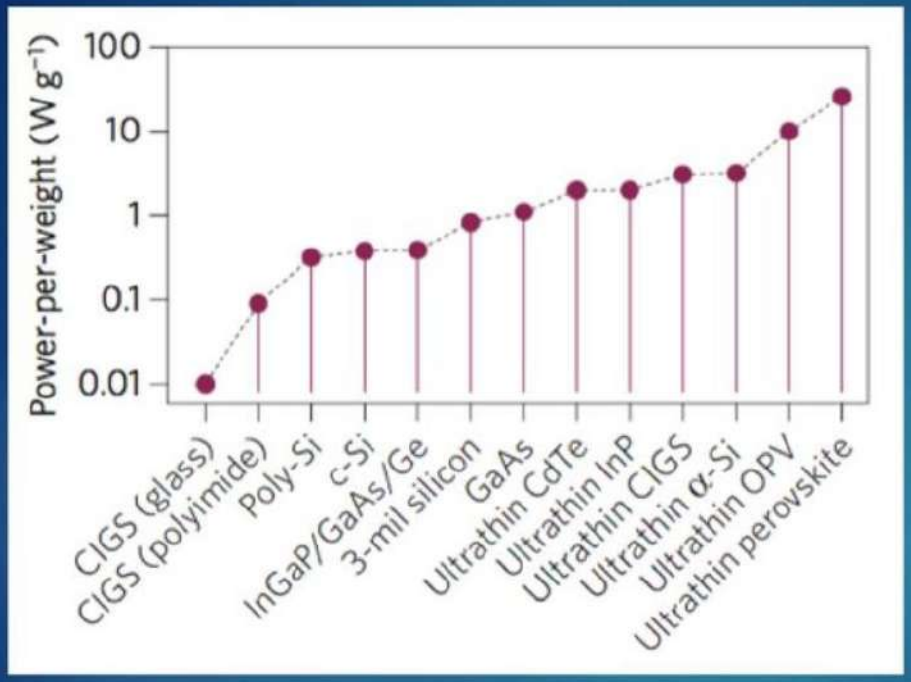
- Materiale carbonaceo non fibroso



Barium Titanate

Cubic Perovskite Structure

- Cation A is CH_3NH_3
- Cation B is Lead (Pb)
- Anion X is a halogen, usually Iodine (I)

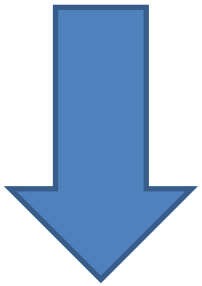


Power-Per-Weight
(Watts per gram)

Conclusioni

Incertezze su molti versanti

- Tossicità verificata solo per poche sostanze
- Rapida comparsa di nuove sostanze
- Basso tonnellaggio
- Piccole aziende molto specialistiche



Necessità di fare RETE

Regione Lombardia ha il vantaggio di poter aggregare molte aziende, molti operatori, molte università

RINGRAZIAMENTI

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