JORNADAS DE PREVENCIÓN DE RIESGOS LABORALES
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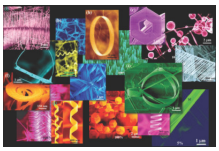
Nanomateriales
Nanomaterials

Human biomonitoring and epidemiological studies: opportunities for risk assessment and management of nanotechnology workers

Prof. Enrico Bergamaschi

Dept. of Clinical and Experimental Medicine - University of Parma, Italy



Anticipating "new" and "emerging" risks

Any occupational risk that is both new and increasing

- ***New*** , i.e. the risk did not previously exist and is caused by new processes, new technologies, new types of workplace, or social or organisational change; or, a long-standing issue is newly considered as a risk due to a change in social or public perceptions; or, new scientific knowledge allows a long-standing issue to be identified as a risk.
- ***Increasing*** if the number of hazards leading to the risk is growing; or the exposure to the hazard leading to the risk is increasing (exposure level and/or the number of people exposed); or the effect of the hazard on workers' health is getting worse (seriousness of health effects and/or the number of people affected).

European Agency for Work

The European Network on the Health and Environmental Impact of Nanomaterials

NanoImpactNet

Strategies for Assessing Occupational Health Effects of Engineered Nanomaterials

Rosemary M Gibson[1*], Anil Adisesh[1], Enrico Bergamaschi[2], Markus Berges[3], Daniel Bloch[4], Steve Hankin[5], Iseult Lynch[6] & Michael Riediker[7]

[1]HSL, Buxton, UK; [2]University of Parma, Parma, Italy; [3]BGIA, St Augustin, Germany; [4]CEA, Grenoble, France; [5]IOM, Edinburgh, UK; [6]CBNI, Dublin, Ireland; [7]IST, Lausanne, Switzerland.

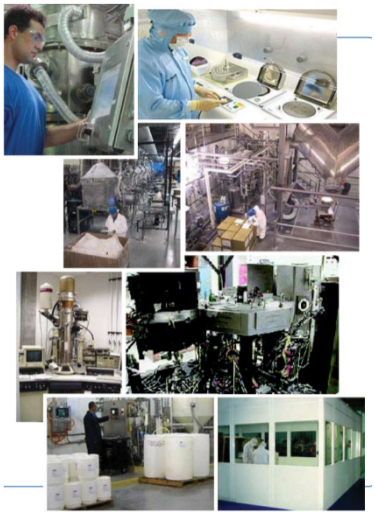
Strategic Research Agenda for Nanosafety Vision 2015-2025

WG 12. Human field studies and epidemiology

Enrico Bergamaschi (Chair)[1], Anjoeka Pronk[2], Derk Brower[2], Daniel Bloch[3], Odile Boutou-Kempf[4], Michael Riediker[5], Gudrun Koppen[6], Erik Tielemans[2].

[1]University of Parma, Parma, Italy; [2] TNO, Zeist, The Netherlands; [3] CEA, Grenoble, France; [4] Institut de veille sanitaire, St Maurice, France; [5] IST, Lausanne, Switzerland; [6] VITO, Belgium.

The many faces of Nanotechnology at work



Outline

- ✓ What we know about health hazard and possible health effects in nanotechnology workers
- ✓ Findings from initial epidemiological studies: strengths and weaknesses
- ✓ The need of an harmonized approach in occupational health assessment
- ✓ Medical surveillance vs Epidemiological surveillance
- ✓ The biological monitoring in health surveillance and epidemiology

Exposure to nanomaterials is not new

JOEM • Volume 48, Number 12, December 2006

CME Available for this Article at ACOEM.org

Integrating Studies on Carcinogenic Risk of Carbon Black: Epidemiology, Animal Exposures, and Mechanism of Action

Arch Toxicol (2002) 75: 625–634
DOI 10.1007/s00204100266

REVIEW ARTICLE

R. Merget · T. Bauer · H. U. Küpper · S. Philippou
H. D. Bauer · R. Breitstadt · T. Bruening

Health hazards due to the inhalation of amorphous silica

JOEM • Volume 48, Number 12, December 2006

Lung Cancer Mortality and Carbon Black Exposure: Uncertainties of SMR Analyses in a Cohort Study at a German Carbon Black Production Plant

Hazard from occupational exposures to UFP

AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 43:350–360 (2003)

Pulmonary Effects of Welding Fumes: Review of Worker And Experimental Animal Studies

James M. Antonini, PhD,[1]* Anthony B. Lewis,[2] Jenny R. Roberts,[1] and David A. Whaley, PhD[2]

JOEM • Volume 51, Number 1, January 2009

Inflammatory Responses and Oxidative Stress From Metal Fume Exposure in Automobile Welders

Human toxicity of NM: what disease endpoint?

➢ Unintentionally produced NP

- **Findings from air pollution epidemiological studies**

UFP have been associated with respiratory irritation, endothelial dysfunction (impaired vasodilation) with mild systemic inflammation, hearth arrythmias and accelerated atherosclerosis, lung cancer…

- **Findings from occupational studies** (*welding fumes and diesel exhausts*)

WF exposure has been associated with lung cancer, metal fume fever, susceptibility to pulmonary infection, obstructive lung disease, and possible neurologic changes…

➢ Intentionally produced NP

- Findings from epidemiological studies of **manufactured NP already on the market** (nano-CB, CNTs,TiO_2, SiO_2, Al_2O_3, nano-Ag,…) **are lacking or very preliminary….**

Zhang et al. Particle and Fibre Toxicology (2014) 11:73
DOI 10.1186/s12989-014-0073-1

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Reduced pulmonary function and increased pro-inflammatory cytokines in nanoscale carbon black-exposed workers

Table 2 The distribution with different size of CB particles in the workplace (particles/cm^3)

Size	Mean	Maximum	Minimum	SD	Percentage (%)
<0.523 μm	233.77	270.6	208.2	24.32	50.77
0.523-1 μm	211.33	220.86	206.52	5.49	45.90
1-2.5 μm	13.24	13.71	12.27	0.58	2.88
2.5-20 μm	2.08	2.24	1.95	0.13	0.45

mean concentration of CB was 14.90 mg/m^3 - TLV of 3.5 mg/m^3

Table 4 The pulmonary function indexes in control and CB-exposed groups (mean ± SD)

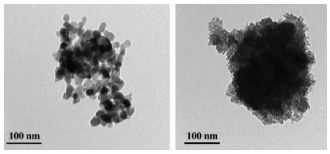
Variables	Control group (n = 104)	CB-exposed group (n = 81)	*P*-value
FEV1 (%)	103.61 ± 14.52	98.07 ± 13.53	0.019[a]
FVC (%)	104.67 ± 14.64	100.12 ± 13.47	0.071[a]
FEV1/FVC	0.87 ± 0.05	0.84 ± 0.05	0.001[a]
PEF (%)	93.76 ± 17.86	78.50 ± 16.80	<0.001[a]
MMF (%)	96.45 ± 23.18	86.86 ± 23.06	0.001[a]

Table 5 The cytokines levels of control and CB workers

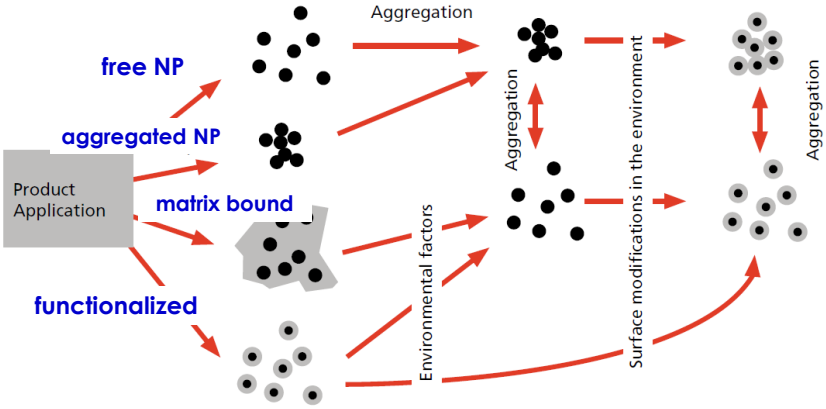
Cytokines[1]	Control group (n = 104) pg/mL, median (5%-95%)	CB-exposed group (n = 81) pg/mL, median (5%-95%)	*P* value
IL-1β	4.16 (0.00-17.75)	11.88 (1.98-38.08)	<0.001[a]
IL-6	27.51 (2.16-180.18)	188.32 (46.13-643.16)	<0.001[a]
IL-8	746.30 (163.55-1879.01)	1117.10 (369.36-3737.82)	<0.001[a]
MIP-1β	804.09 (225.35-2888.59)	2694.52 (1136.97-10074.81)	<0.001[a]
TNF-α	47.75 (0.00-191.33)	232.36 (76.47-572.05)	<0.001[a]
MCP-1	254.75 (94.29-428.72)	238.76 (92.39-438.51)	0.242[a]

Link between human exposure to CB and long-term pulmonary effects

First issue: Toxicokinetics



In relevant concentrations NM generally do NOT appear as free primary particles



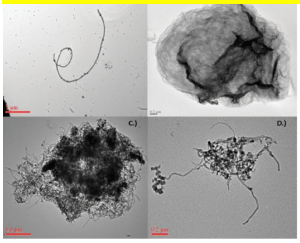
(from Nowack & Bucheli, 2007)

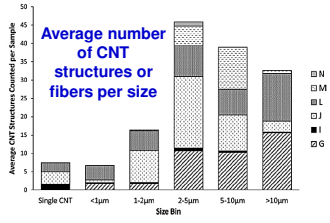
Ann. Occup. Hyg., 2015, 1–19
doi:10.1093/annhyg/mev020

Carbon Nanotube and Nanofiber Exposure Assessments: An Analysis of 14 Site Visits

Matthew M. Dahm[1*], Mary K. Schubauer-Berigan[1], Douglas E. Evans[2], M. Eileen Birch[2], Joseph E. Fernback[2] and James A. Deddens[1]

TEM images of single fibers and CNT agglomerates from personal breathing zone samples.





Ann. Occup. Hyg., 2015, Vol. 59, No. 6, 681–704
doi:10.1093/annhyg/mev023
Advance Access publication 6 April 2015

Occupational Exposure to Nano-Objects and Their Agglomerates and Aggregates Across Various Life Cycle Stages; A Broad-Scale Exposure Study

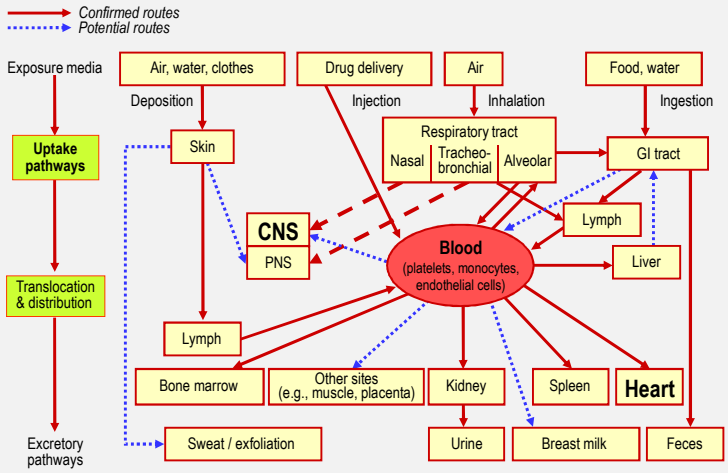
Cindy Bekker[1,2*], Eelco Kuijpers[2], Derk H. Brouwer[2], Roel Vermeulen[1] and Wouter Fransman[2]

Table 2. Overview of measured exposure situations including detailed information about the NOAA type and size, results of the SEM/EDX analysis, direct-reading devices, contextual information, and overall conclusion on the likelihood of significant exposure according to the proposed decision logic.

#	Measurement information: Exposure scenario, NOAA type, and primary particle size	Industrial sector	Measurement type	Results: Qualitative results: Presence of NOAA on filter (SEM/EDX)	Results: Quantitative results: Model estimates: GM (# cm^{-3})	90% CI (# cm^{-3})	P value	R^2	Particle size distribution (nm): GM	GSD	Conclusion: Likelihood of significant exposure to NOAA
Source domain 1: production of nanomaterials											
1.	Laser ablation Ag_2O: <100 nm	Academic and research	BG	NOAA not present	6929	6816–7044	<0.01	0.74	n.a.	n.a.	Unlikely
			PBZ		6404	6162–6655					
Source domain 2: handling/transfer bulk powders											
2	Big bag replacement: Carbon black: 30–50 nm	Toner/ink production	BG	Clusters: 50 nm–20 μm, no free CB particles	15 578	12 103–20 051	<0.01	0.91	44	1.65	Presumable
			PBZ/NF		91 711	64 489–130 424			52	2.16	
3	Big bag replacement: Carbon black: 50 nm		BG	Clusters: 50 nm–5 μm, no free CB particles	11 099	7061–17 445	<0.01	0.62	47	2.15	Presumable
			PBZ/NF		48 771	28 706–82 861			53	2.15	
4	Big bag replacement: SiO_2: 20–40 nm		BG	NOAA not present	8343	7944–8762	<0.01	0.82	60	2.16	Likely
			PBZ/NF	Clusters: 1–5 μm, no free SiO_2 particles	12 769	11 816–13 800			84	1.61	
5	Big bag replacement: ZnO: 10 nm	Material development/ production	BG	Clusters: 50 nm–10 μm, no free ZnO particles	17 452	17 375–17 529	<0.01	0.78	20	2.1	Possible/not excluded
			PBZ/NF		14 814	14 637–14 992			23	2.4	
6	Big bag replacement:* Carbon black: 30–60 nm	Toner/ink production	BG*	NOAA not present	10 312	9081–8762	<0.01	0.75	n.a.	n.a.	Likely
			PBZ/NF	Clusters: 50 nm–50 μm, no free CB particles	18 732	16 188–21 677			41	2.71	
7	Dumping powder manually: SiO_2: 20–50 nm		BG	Clusters: 100 nm–100 μm, no free SiO_2 particles	15 508	13 603–17 679	<0.01	0.8	52	1.93	Presumable
			PBZ/NF		67 488	54 573–83 459			58	1.79	

Systemic distribution and toxicity of persistent NM

Long-term accumulation?



Oberdörster et al., 2005

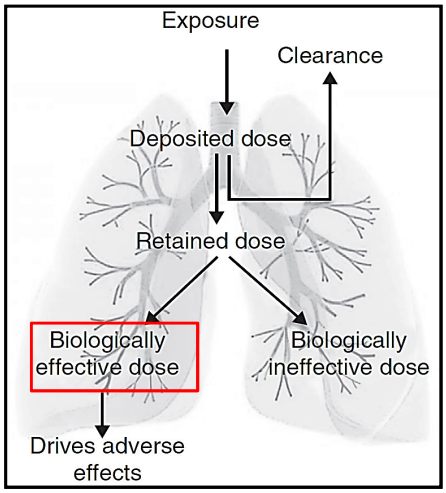
NPs translocation to vital target organs can potentially induce several adverse health effects

- **Blood system**: increased coagulability, platelet aggregation, microthrombi and artheromatous plaque formation
- **Lung**: Acute inflammatory response, granuloma formation, oxidative stress, asthma, COPD
- **Heart**: changes in heart rate and heart rate variability, manifestation of adverse cardiovascular effects
- **Lymph system**: impaired immune response, spleen and bone marrow: reticulo endothelial system damage
- **Reproductive system, placenta, breast**: impaired foetus/child development
- **Central and peripheral nerve system**: neurological disorders, and pathological changes in kidney, liver, pancreas

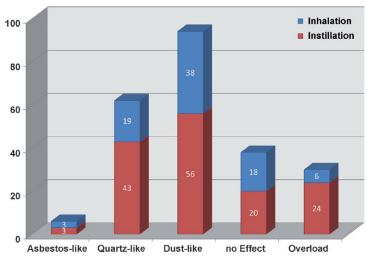
Available online at www.sciencedirect.com
SciVerse ScienceDirect
Current Opinion in Biotechnology
2013, 24: 724-734

Nanotoxicity: challenging the myth of nano-specific toxicity

Ken Donaldson[1,2] and Craig A Poland[2]



- ✓ **Mass cannot be the entity that is toxic; effects depend on molecular effects affected by P-Chem of particles**
- ✓ **Mechanisms of NP toxicity need to be considered in relation to conventional particles**
- ✓ **Recognition of similar mechanisms would aid in benchmarking the NP hazard**
- ✓ **The Biologically Effective Dose (BED) is defined as "the entity within any mass dose of particles that drives a critical pathophysiologically relevant form of toxicity, such as inflammation, genotoxicity or cellular proliferation"**
- ✓ **Currently known NP BEDs include surface area, soluble species, charge and shape (aspect ratio)**
- ✓ **All of these BEDs also drive conventional particle toxicity so, whilst nano-relevant, they are not nano-specific**

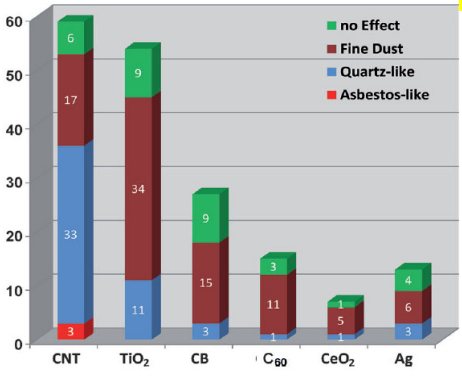


Angewandte Reviews
Nanotoxicology
2014, 53, 12304-12319

Nanosafety Research—Are We on the Right Track?

*Harald F. Krug**

A very important, result is the recognition that the **type of effects caused by ENMs in the lungs** are fundamentally not different to those arising from other forms of particulate exposure (results from 317 studies)

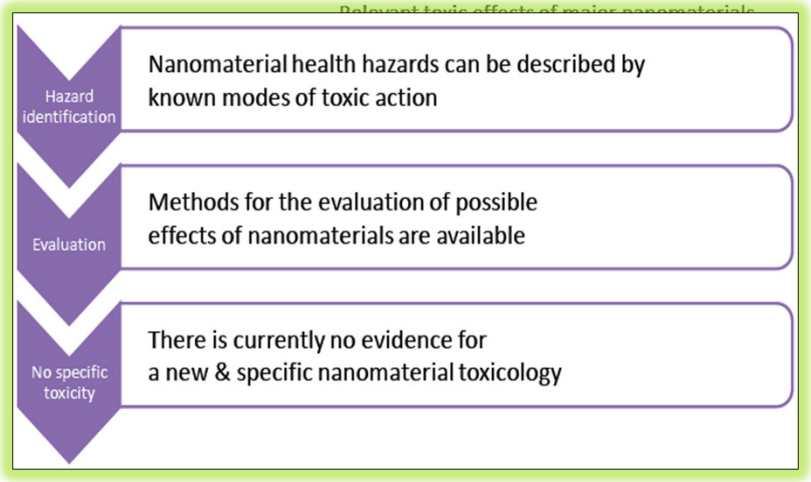


- **asbestos-like**= tumour induction comparable to mesothelioma;
- **quartz-like**= inflammation, oxidative stress, fibrosis, granuloma formation;
- **dust-like**= effects as for "normal" fine-particle exposure, transient inflammatory processes

Available online at www.sciencedirect.com
SciVerse ScienceDirect
Current Opinion in Biotechnology
2013, 24: 724-734

Nanotoxicity: challenging the myth of nano-specific toxicity

Ken Donaldson[1,2] and Craig A Poland[2]



NEWS AND OPINIONS
(**2014**) **9**: 266-70

A strategy for grouping of nanomaterials based on key physico-chemical descriptors as a basis for safer-by-design NMs

Iseult Lynch[a,*], Carsten Weiss[b], Eugenia Valsami-Jones[a,c]

Modes of action for NM toxicity currently recognised:

(i) the **release of toxic chemical constituents** from NMs (e.g. Cd from quantum dots, ionic silver from Ag NPs)— i.e. ***NM dissolution***;

(ii) direct effects from **physical contact with** NMs, influenced by their size, shape and surface properties, and which produce interferences with important biological functions for example by altering conformation of biomolecules - i.e. ***NM surface effects***;

(iii) the **inherent properties** of the material, such as photo-chemical and redox properties resulting from bandgap or crystalline form - i.e. ***NM structure effects***;

(iv) the capacity of NMs **to act as vectors for the transport of other toxic chemicals** to sensitive tissues - i.e. ***NM Trojan horse effects***.

NANOSAFETY

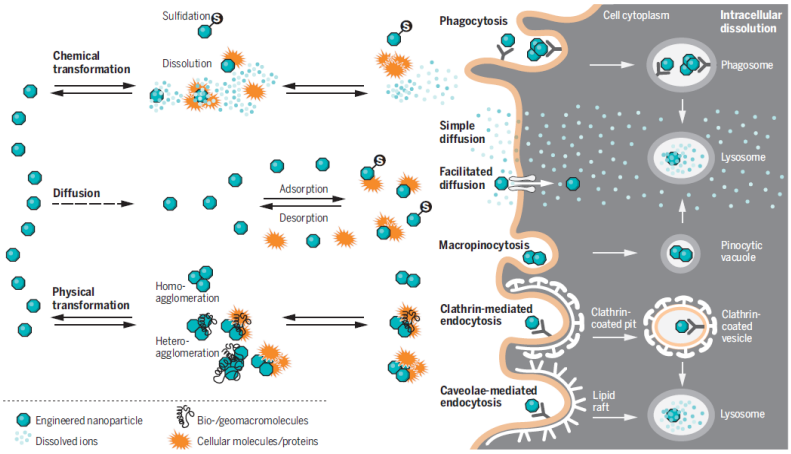
Science 350(6259):388-9, **2015**

How safe are nanomaterials?

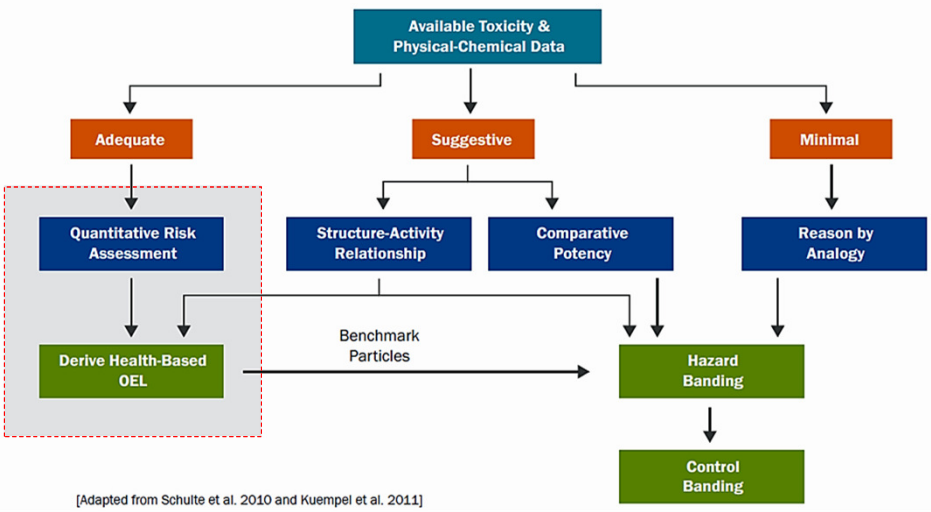
There is still no consensus on the toxicity of nanomaterials

By **Eugenia Valsami-Jones** *and* **Iseult Lynch**

Nanoparticle complexity. Schematic illustration of the competing environmental transformation and organismal uptake processes that occur for a NM in aquatic environments, illustrated using a **Ag NP**.

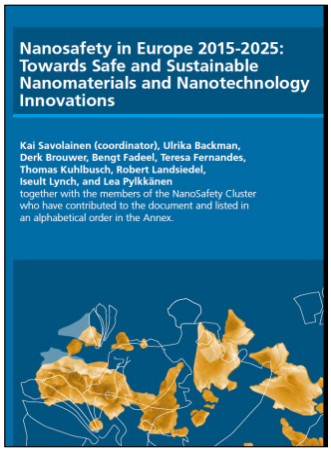


Impact of the evidence on the development of Occupational Exposure Limits and Exposure Bands



[Adapted from Schulte et al. 2010 and Kuempel et al. 2011]

The principal challenges in RA



(1) introduction or establishment of a systematic and standardized metrology for physically characterizing NM (*multiple metrics needed*);

(2) uncertainty in the nature of the dose-response relationship between exposure of NM and biological effects, whether they are - or not - "**nano-specific**" (*hazard characterization*);

(3) the difficulties associated with measuring exposure to NM and surveillance once they are introduced into the environment (*Life-cycle assessment*).

There are inadequate data to inform quantitative risk assessments on current and emerging NM. At most, only qualitative risk assessments are feasible, given the current state of knowledge

Research needs and priorities for the coming 10 years

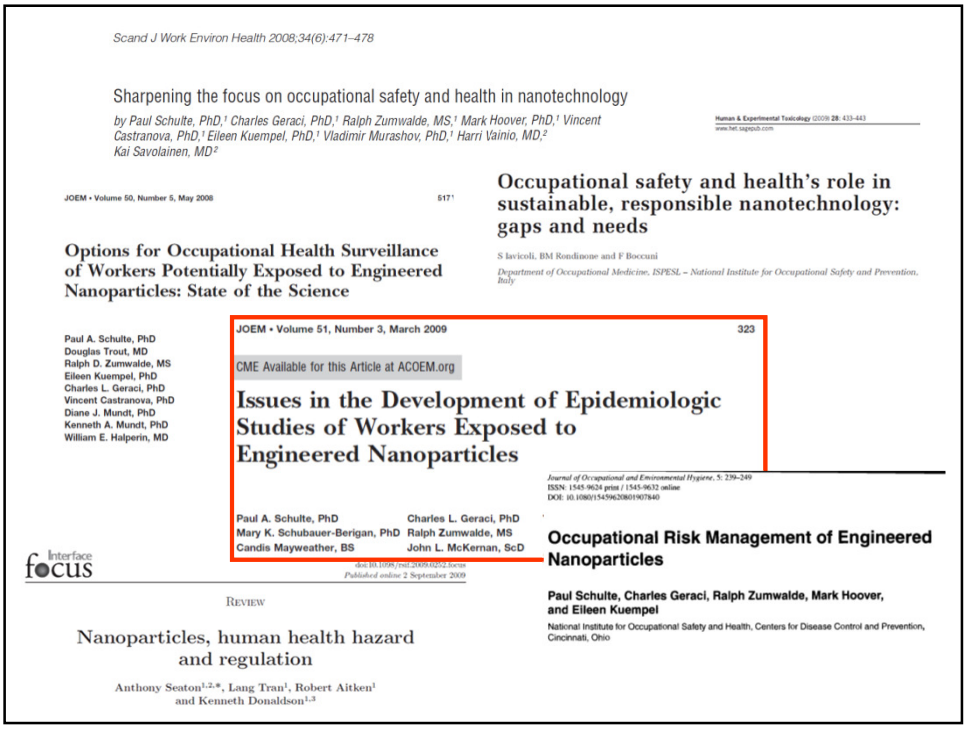
1. Nanomaterial identification and classification (*synthetic identity – biological identity*);
2. Nanomaterial exposure and transformation (*changes along the lyfe-cycle*);
3. Hazard mechanisms related to effects on human health and the environment;
4. Tools for the Predictive Risk Assessment and Management (***biomarkers studies***, *databases and ontology*).

NanoSafety Cluster

The risk prediction and management tools

Milestone	Topic	By 2015	By 2020	By 2025
Epidemiology & health surveillance	*Health effect*	Markers for short term effect identified	Markers for long term effect identified	Implementation of the markers
	Register	Health surveillance registries developed Exposure registries developed	Using registries for research	Implementation of results for regulations
	Study design	Pilot panel studies completed	Case-control studies completed	Longitudinal studies started

Databases and epidemiological or health studies can be considered as "enabling tools" supporting the processes of RA and RM.



Scand J Work Environ Health 2008;34(6):471–478

Sharpening the focus on occupational safety and health in nanotechnology

by Paul Schulte, PhD,[1] Charles Geraci, PhD,[1] Ralph Zumwalde, MS,[1] Mark Hoover, PhD,[1] Vincent Castranova, PhD,[1] Eileen Kuempel, PhD,[1] Vladimir Murashov, PhD,[1] Harri Vainio, MD,[2] Kai Savolainen, MD[2]

Human & Experimental Toxicology (2009) 28: 433–443
www.het.sagepub.com

JOEM • Volume 50, Number 5, May 2008 517[1]

Options for Occupational Health Surveillance of Workers Potentially Exposed to Engineered Nanoparticles: State of the Science

Paul A. Schulte, PhD
Douglas Trout, MD
Ralph D. Zumwalde, MS
Eileen Kuempel, PhD
Charles L. Geraci, PhD
Vincent Castranova, PhD
Diane J. Mundt, PhD
Kenneth A. Mundt, PhD
William E. Halperin, MD

Occupational safety and health's role in sustainable, responsible nanotechnology: gaps and needs

S Iavicoli, BM Rondinone and F Boccuni

Department of Occupational Medicine, ISPESL – National Institute for Occupational Safety and Prevention, Italy

JOEM • Volume 51, Number 3, March 2009 323

CME Available for this Article at ACOEM.org

Issues in the Development of Epidemiologic Studies of Workers Exposed to Engineered Nanoparticles

Paul A. Schulte, PhD
Mary K. Schubauer-Berigan, PhD
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John L. McKernan, ScD

Journal of Occupational and Environmental Hygiene, 5: 239–249
ISSN: 1545-9624 print / 1545-9632 online
DOI: 10.1080/15459620801907840

Occupational Risk Management of Engineered Nanoparticles

Paul Schulte, Charles Geraci, Ralph Zumwalde, Mark Hoover, and Eileen Kuempel

National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio

Interface focus

doi:10.1098/rsif.2009.0252.focus
Published online 2 September 2009

REVIEW

Nanoparticles, human health hazard and regulation

Anthony Seaton[1,2,*], Lang Tran[1], Robert Aitken[1] and Kenneth Donaldson[1,3]

Critical issues in designing epidemiological studies of ENM workers

1. **Heterogeneity** of nanoparticles;
2. Define the **disease endpoint**;
3. **Temporal factors** (likelihood to observe the outcome; shot-term vs. long-term effects)
4. **Exposure** duration and intensity (effectiveness)
5. Identify the **target population**/study population

Critical issues in designing epidemiological studies of ENM workers

1. **Heterogeneity** of nanoparticles

The variability of particle types and/or in toxic potential can make it difficult to identify adequately large cohorts with exposure to the same materials

Failure to account for particle heterogeneity can lead to misclassification on exposure and bias measures of association toward the null hypothesis.

- Provide a choice of ENM which are eligible for the study
- Classification by NM classes: Carbon-based, Metal(Ox) NP, HARNs
- Size (sub-classes) may not be the right descriptor
- ***Biological activity ?*** *(e.g. ROS generation in vitro)*

Critical issues in designing epidemiological studies of ENM workers

2. Define the **disease endpoint**

In association studies, **the starting hypothesis** should consider **the likelihood of the disease** and know **what diseases** (disturbances, biochemical changes…) are included in the hypotheses.

From studies on incidental particles, air pollution and experimental animal studies, malignant and non-malignant respiratory disease and cardiovascular disease are thought to be primary health effects of concern for exposed workers, although it is clear that not all incidental nanoparticles give the same biological responses.

However, consistencies between the agent/chemical involved, the effects observed and some putative biological mechanism have been shown.

Critical issues in designing epidemiological studies of ENM workers

2. Define the **disease endpoint**

Exposure to incidental nanoparticles (e.g., generated from combustion or hot processes) has been associated with various adverse health effects in workers:

- diesel exhaust has been associated with eye and respiratory irritation, endothelial dysfunction (impaired vasodilation) with mild systemic inflammation, and lung cancer;
- welding fume exposure has been associated with lung cancer, metal fume fever, susceptibility to pulmonary infection, obstructive lung disease, and possible neurologic changes

Environmental/incidental/engineered NP and C-BNM share some characteristics, such as the capacity to cause Ox-stress and inflammation in the lung, affect the cardiovascular system, to enhance atherotrombosis and modulate heart-rate variability.

Critical issues in designing epidemiological studies of ENM workers

3. **Temporal factors** (likelihood to observe the outcome; latency time)

Currently, it is unlikely that a population of workers with long-term exposure to nanomaterials large enough for epidemiological study exists at this time

➡ the number of people actually exposed for some period that could significantly put them at risk of **chronic effects** may not be large enough to form an adequate recruitment pool or sampling frame for conducting epidemiologic studies for many years

Nonetheless, it is worth noting that some of the cardiovascular effects associated with UFP exposure (e.g., increased daily cardiovascular disease mortality within 1 or 2 days of high particulate air pollution exposure) suggest that cardiovascular effects of NP might not require long periods of follow-up.
This might particularly be true in middle-aged or older workers with pre-existing cardiovascular disease or risk factors.

Critical issues in designing epidemiological studies of ENM workers

4.Exposure **duration and intensity**

If workers are minimally exposed, due to enclosed processes or handling of materials in which NP are embedded, such studies may be uninformative.

If there is sufficient exposure to cause acute and chronic health effects, epidemiological studies may be able to be conducted.

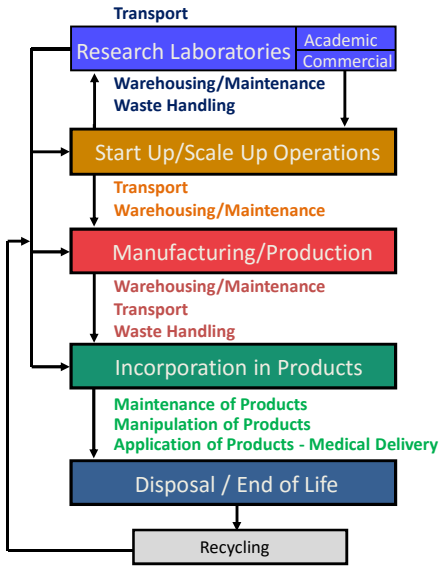
A key requirement for all studies is **good quality exposure data** for workers (in reality, this may not always be possible).

Critical in the assessment of exposure-response relationships is the distribution of exposures and exposure variability. Epidemiologic studies require sufficient variability in exposures and sufficient distribution of exposures to provide contrasts necessary to estimate such relationships reliably

Exposure Assessment

- Critical component of risk management
- **Identifies populations at risk**
- Characterize the exposure, therefore better understanding of risk
  - Nature of exposure: low v high, short v long
  - Extent of exposure: few or many
  - Complexity of the exposure
  - Place the exposure on the life cycle
- Verify controls

1. Nanomaterial Life Cycle



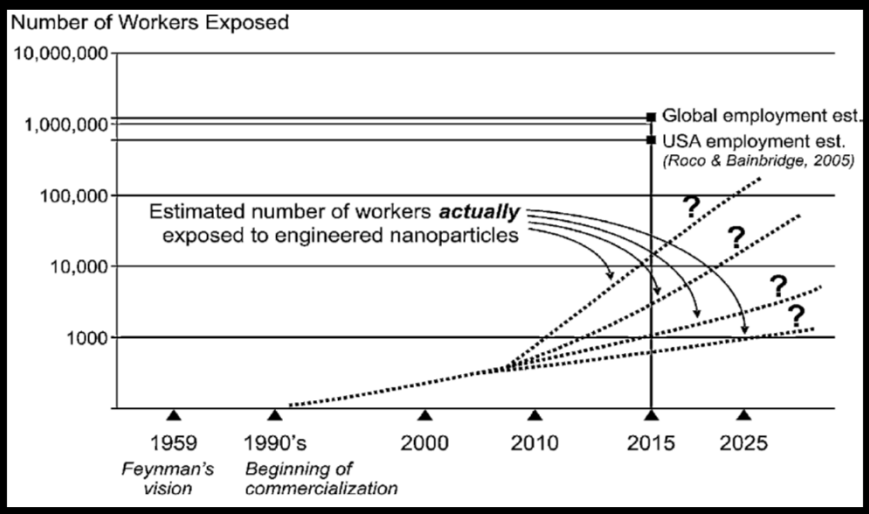
Schulte et al. [2008]

Examples of NIOSH Field Investigations

Type of Facility	Type of Particle, Morphology	Size of Particle	Range of "Potential" Exposure Concentrations
University Research lab	Carbon Nanofibers	Approx. 100 nm diameter, 1-10 microns long	60-90 µg/m^3
Metal Oxide Manufacturer	TiO_2, Lithium Titanate, powder	100-200 nm	<100 nm: 1.4 µg/m^3 (TiO_2) Total dust: 4-149 µg/m^3 (TiO_2) <100 nm: ND (Li) Total dust: ND -3 µg/m^3 (Li)
Manufacturer	Carbon Nanofibers	Approx. 100 nm diameter, 1-10 microns long	15 - 1800 µg/m^3
Research and Development lab	Quantum Dots, spheres	2 -8 nm	ND
Metal Oxide Manufacturer	Manganese, Silver, Nickel, Cobalt, Iron oxides, spheres	8 -50 nm	67 - 3619 µg/m^3
Research and Development lab (Pilot-Scale)	Aluminum, spheres	50 – 100 nm	40 - 276 µg/m^3
Research and Development lab	Elemental Metals - Silver, Copper, TiO_2	15 – 40 nm	ND
Filter Media Manufacturer	Nylon 6 Nanofiber	70 - 300 nm diameter, continuous length	ND

Critical issues in designing epidemiological studies of ENM workers

5. Dilemmas in identifying the **target population**



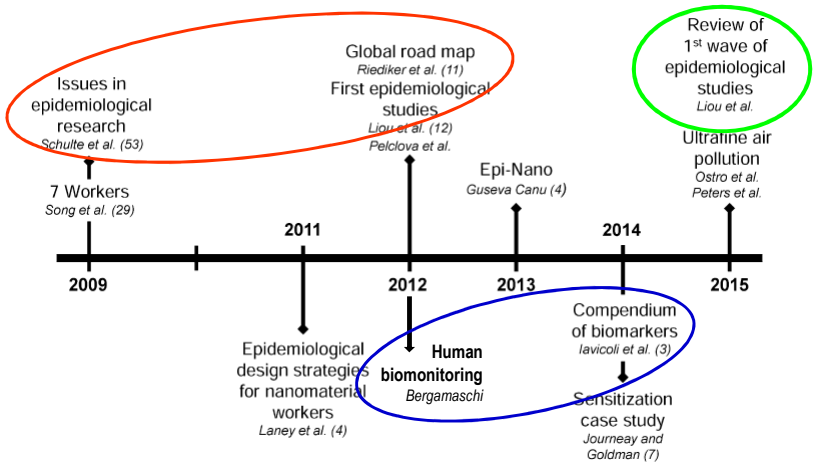
J Nanopart Res (2016)18:159
DOI 10.1007/s11051-016-3459-1

REVIEW

Taking stock of the occupational safety and health challenges of nanotechnology: 2000–2015

P. A. Schulte · G. Roth · L. L. Hodson · V. Murashov · M. D. Hoover · R. Zumwalde · E. D. Kuempel · C. L. Geraci · A. B. Stefaniak · V. Castranova · J. Howard

Advances in the field of ENM worker epidemiology



NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
NTRC NANOTECHNOLOGY RESEARCH CENTER

Engineered Carbon Nanomaterials (ECN) Workforce: Industrywide Studies

- **Phase I:** Collected information to determine feasibility of industrywide exposure assessment and epidemiologic studies
  - U.S. workforce size and growth for different types of ECN in companies larger than R&D
- **Phase II:** Conducted industrywide exposure assessment for carbon nanotubes (CNT) & nanofibers (CNF)
  - Optimized methods to measure CNT & CNF exposure in workplaces
- **Phase III:** Conduct epidemiologic studies
  - Evaluate markers of early biological effects in relation to metrics of exposure

Schubauer-Berigan & Dahm [2013]

CDC Workplace NIOSH

Nanosafe 2012: International Conferences on Safe Production and Use of Nanomaterials IOP Publishing
Journal of Physics: Conference Series **429** (2013) 012066 doi:10.1088/1742-6596/429/1/012066

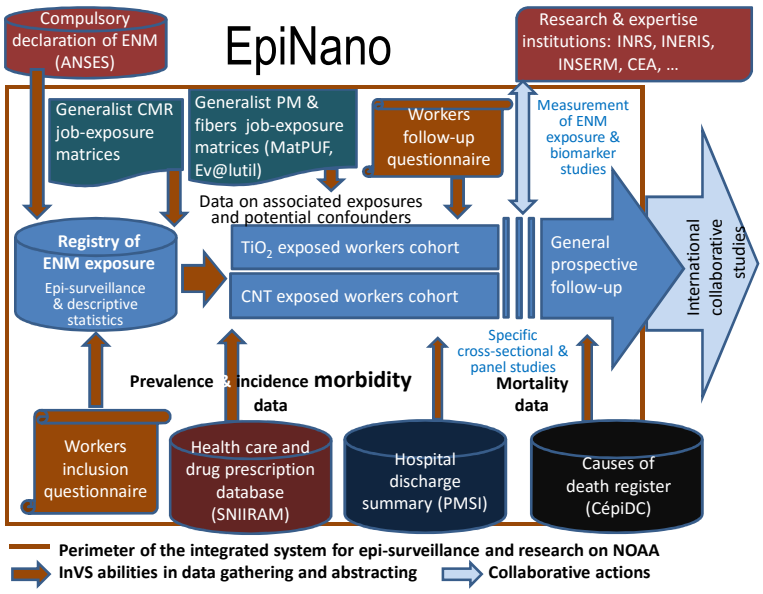
French registry of workers handling engineered nanomaterials as an instrument of integrated system for surveillance and research

I Guseva Canu[1], O Boutou-Kempf[1], L Delabre[1], S Ducamp[1], Y Iwatsubo[1], JL Marchand[1], and E Imbernon[1]

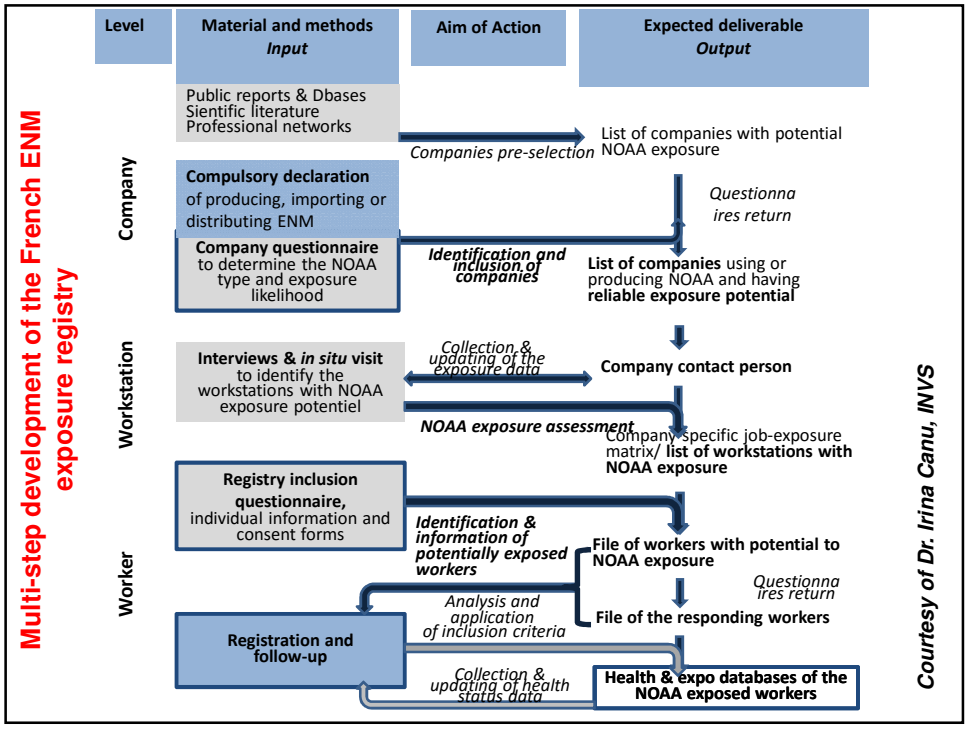
[1]French Institute for Public Health Surveillance (InVS), Occupational health department (DST), 94415 St Maurice, France

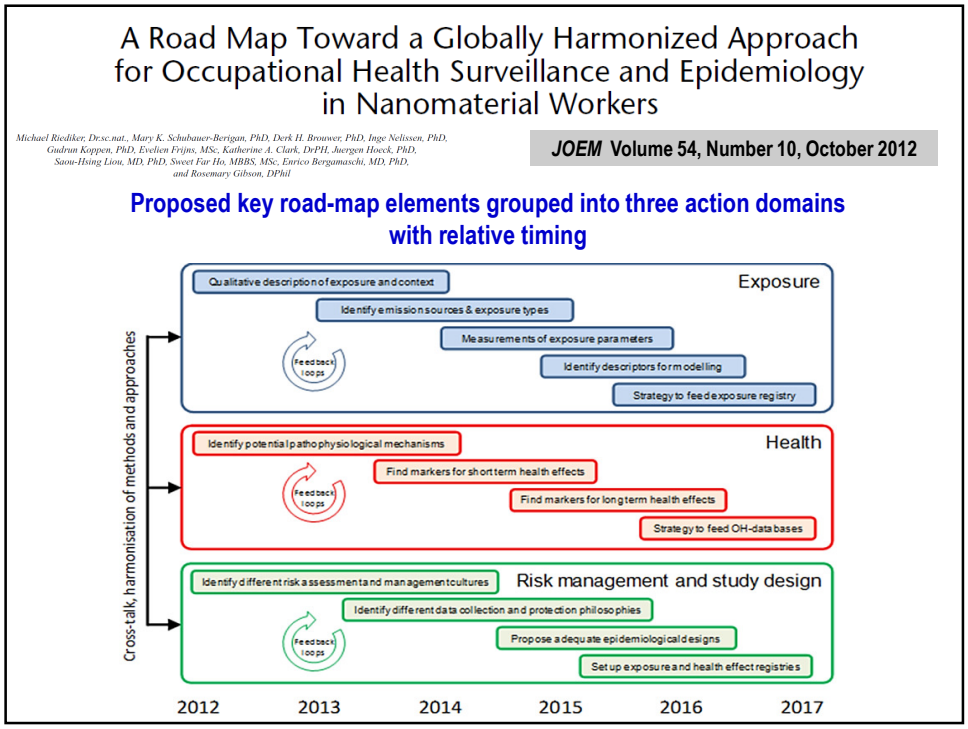
INSTITUT DE VEILLE SANITAIRE

Pattern of the French integrated ENM surveillance system



Courtesy of Dr. Irina Canu, INVS


Multi-step development of the French ENM exposure registry
Level
Material and methods
Input
Aim of Action
Expected deliverable
Output
Company
Workstation
Worker
Public reports & Dbases
Sientific literature
Professional networks
Companies pre-selection
List of companies with potential NOAA exposure
Compulsory declaration
of producing, importing or distributing ENM
Company questionnaire
to determine the NOAA type and exposure likelihood
Identification and inclusion of companies
Questionnaires return
List of companies using or producing NOAA and having reliable exposure potential
Interviews & in situ visit
to identify the workstations with NOAA exposure potentiel
Collection & updating of the exposure data
Company contact person
NOAA exposure assessment
Company specific job-exposure matrix/ list of workstations with NOAA exposure
Registry inclusion questionnaire,
individual information and consent forms
Identification & information of potentially exposed workers
File of workers with potential to NOAA exposure
Questionnaires return
File of the responding workers
Analysis and application of inclusion criteria
Registration and follow-up
Collection & updating of health status data
Health & expo databases of the NOAA exposed workers
Courtesy of Dr. Irina Canu, INVS


A Road Map Toward a Globally Harmonized Approach for Occupational Health Surveillance and Epidemiology in Nanomaterial Workers
Michael Riediker, Dr.sc.nat., Mary K. Schubauer-Berigan, PhD, Derk H. Brouwer, PhD, Inge Nelissen, PhD, Gudrun Koppen, PhD, Evelien Frijns, MSc, Katherine A. Clark, DrPH, Juergen Hoeck, PhD, Saou-Hsing Liou, MD, PhD, Sweet Far Ho, MBBS, MSc, Enrico Bergamaschi, MD, PhD, and Rosemary Gibson, DPhil
JOEM Volume 54, Number 10, October 2012
Proposed key road-map elements grouped into three action domains with relative timing
Cross-talk, harmonisation of methods and approaches
Exposure
Qualitative description of exposure and context
Identify emission sources & exposure types
Measurements of exposure parameters
Identify descriptors for modelling
Strategy to feed exposure registry
Health
Identify potential pathophysiological mechanisms
Find markers for short term health effects
Find markers for long term health effects
Strategy to feed OH-databases
Risk management and study design
Identify different risk assessment and management cultures
Identify different data collection and protection philosophies
Propose adequate epidemiological designs
Set up exposure and health effect registries
Feedback loops
2012
2013
2014
2015
2016
2017

The implementation of epidemiological studies in the workforce exposed to ENMs is currently hampered by:

(1) few standardized, accurate, and reliable approaches for **estimating exposure**;

(2) large variability in nanomaterial metrics and **coexposures**;

(3) lack of information about **health effects and biomarkers**;

(4) lack of large **production forces** in this emerging industry, rather a considerable proportion in research and development;

(5) questionable statistical power related to currently **small workforce sizes** and **short latency** for disease occurrence;

(6) a changing **regulatory framework** without harmonized registration systems for workers used in these industries

Riedeker et al., 2012

Information that should be collected for epidemiological studies in NM workers to address the following issues:

- **Exposure data** measurements and models need to be evaluated and validated so that they are linkable to worker activities, task profiles, job titles, job descriptions, and industries on the one hand and to production processes on the other.
- Potential **health concerns** need to be identified and biomarkers of effects need to be assessed for detecting short- and long-term effects.
- To facilitate global harmonization, not only **differences in risk assessment and management** but also **data protection** philosophies are important while designing epidemiological studies, identifying suitable cohorts, and setting up exposure and health effect registries.

Riedeker et al., 2012

The ROAD MAP: many steps, many actors

Riedeker et al., 2012

1. Assessing and recording exposure

a. How to gather qualitative and quantitative information on exposure (nature of worker tasks, areas of workplaces, materials being handled, control measures in place, field studies, etc)

b. How to record and access this information

2. Assessing and monitoring health or health surveillance

a. How to define a harmonized approach for data recording and access

b. How to identify "effects" to be monitored, preferably early markers of effect or indicators of biological response as opposed to final outcome (disease and death)

3. Information dissemination

a. How to raise awareness among relevant health care professionals

b. How to disseminate the results of the monitoring to workers and management

c. How to provide support to policymakers and other decision takers

B] Occupational Health Effect Assessment

- **Identify Potential Health Concerns and Mechanisms**

There is already a wealth of information in the epidemiological literature about exposure to "traditional" or "classic" particles and other (non engineered) materials that fall within the European Commission's definition of nanomaterials.

- **Strategy to Identify Short- and Long-Term Health Effect Markers**

Pulmonary and cardiovascular diseases (leading to increased morbidity and mortality among vulnerable groups) have been linked to pollution and levels of ultra-fine particles, but their use as health endpoints for workers exposed to MNM have limitations: they are nonspecific (and certainly not nano-specific), have a high prevalence in the general population, and share multiple non occupational risk factors.

Riedeker et al., 2012

B] Occupational Health Effect Assessment

- **Strategy to Identify Short- and Long-Term Health Effect Markers**

A variety of other potential short- or long-term effect parameters have been proposed for a targeted assessment of personnel exposed to NMs. These include heart rate variability, blood-clotting parameters, proinflammatory cytokines, upregulation of adhesion momolecules or antioxidant capacity, and biomarkers of pulmonary fibrosis.

Although promising in epidemiological research as putative biomarkers of effect, these parameters are still not assessable for their predictive value of health risk at an individual level; they are not routinely applicable and need to be further validated.

Riedeker et al., 2012

Possible health monitoring endpoints include the following:

- ✓ **Biomarkers of exposure** (eg, presence of chemicals in the blood or the urine; this can readily be done for chemicals such as metals).
- ✓ **Chemical changes in exhaled air or exhaled breath condensate** suggested to reflect not only abnormalities of the airway lining fluid and lung inflammation, but also potential exposure.
- ✓ **Local effects**: inflammatory changes, short-term respiratory changes, respiratory, eye, or skin irritation, depending on the route of exposure/site of uptake (with special tests to study biopersistent long fibers such as some forms of carbon nanotubes).
- ✓ **Systemic effects** to confirm cardiovascular changes and inflammatory mechanisms: heart-rate variability, platelet aggregation, and other prothrombotic effects, as well as cytokines and differential blood cell counts.
- ✓ **Medical tests** for early detection of health effects at a preclinical stage

C] Harmonized Study Designs and Data Collection

Ethical, Cultural, and Regional Differences (e.g., in data collection and protection…) should be identified, considered and harmonized (e.g., by WHO, OECD)

Define Epidemiological Designs

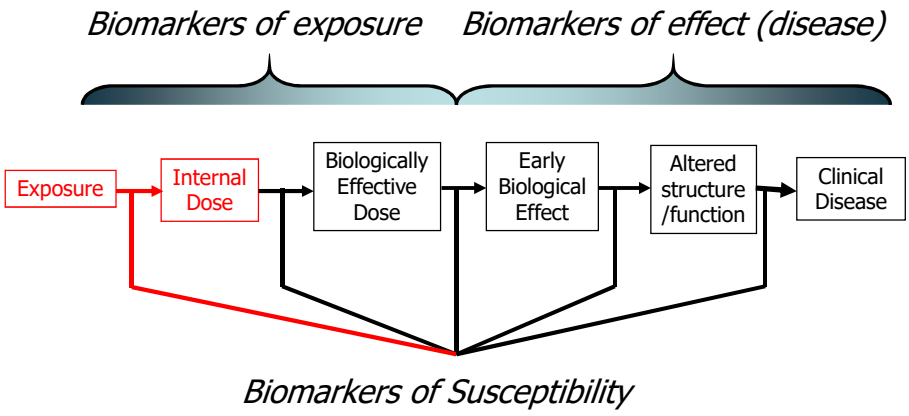
Major challenges in conducting prospective cohort studies of nanomaterial workers include developing a large cohort size and the long periods of time required to draw firm conclusions regarding chronic health effects.

In the interim, small-scale studies of 50 to 100 workers could be conducted within the next years to assess biomarkers of exposure or of early effect.

Thus, use should be made of biological monitoring (*where applicable*).

Riedeker et al., 2012

Biomarkers to Pick-out Events Linking Chemical Exposure(s) with Outcome(s)



Issues in the development of biomarkers for NM

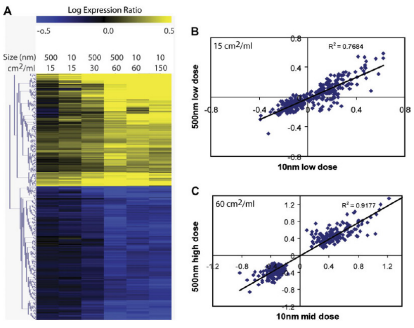
- **Heterogeneity** of nano-objects and their agglomerates and aggregates;
- **Interaction with biological media** and crossing biological membranes can dramatically change surface properties ("corona");
- Behaviour in biological media is affected by chemical composition (**dissolution**, ions release, slow clearance, **biopersistence**);
- Knowledge of t**oxicokinetic/toxicodynamic** data are lacking for many classes of NM;
- Biomarkers in accessible media can be either correlated with or diverge from target organ levels;
- The **health significance of low doses** – as compared to traditional chemicals - needs to be assessed;
- Difficulty to determine whether biochemical and/or functional changes can be **material-specific** or **exposure-route specific**.

Bergamaschi E., J. Nanomaterials, 2012

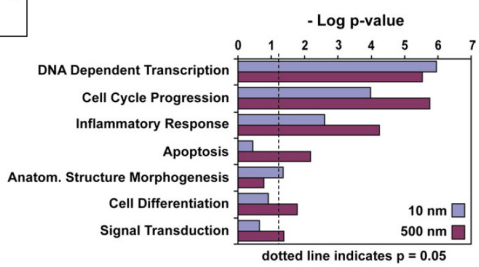
Macrophage Responses to Silica Nanoparticles are Highly Conserved Across Particle Sizes

TOXICOLOGICAL SCIENCES **107(2)**, 553–569 (2009)
doi:10.1093/toxsci/kfn250
Advance Access publication December 10, 2008

Katrina M. Waters,*·†·1 Lisa M. Masiello,*·‡·1 Richard C. Zangar,*·‡ Barbara J. Tarasevich,*·§ Norman J. Karin,*·‡ Ryan D. Quesenberry,*·‡ Somnath Bandyopadhyay,*·† Justin G. Teeguarden,*·¶ Joel G. Pounds,*·‡ and Brian D. Thrall*·‡·2



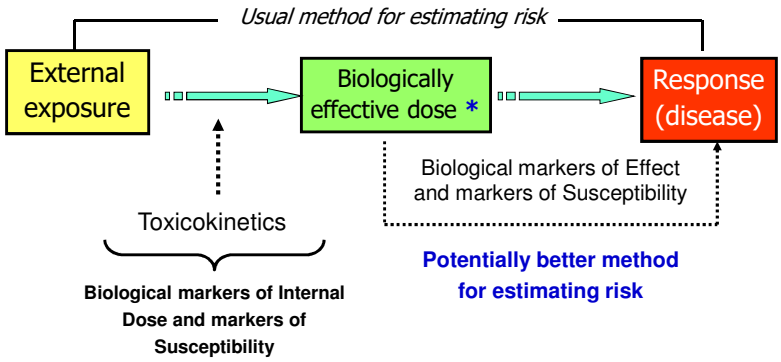
Relationship between mRNA abundance changes and particle surface area dose for 404 genes regulated in response to 10 and 500 nm AS.
(A) Unsupervised cluster analysis of all pattern A genes orders the gene expression profiles according to surface area dose. The pseudocolor scale indicates the expression ratio (log_{10}).
(B and C) Scatter plots comparing the magnitude of expression change between 10- and 500-nm particles at equivalent surface area doses, as indicated.



Cell processes perturbed by AS particles identified by GSE. Each of the major nonredundant cell processes identified is plotted as a function of significance (log p value). The dotted line indicates the threshold for p > 0.05.

Rationale for using biomarkers in risk assessment

IPCS, 1998

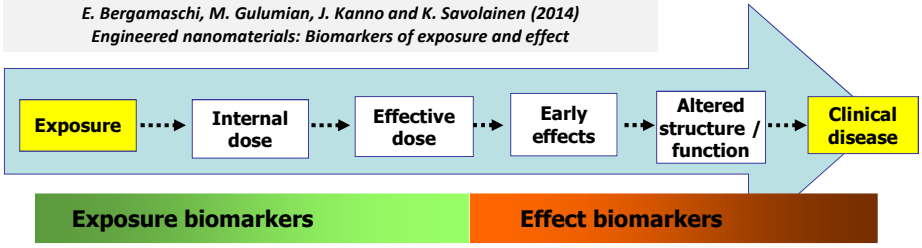


Biomonitoring in health surveillance and epidemiology

- BM is an important component of the occupational and environmental health surveillance, along with clinical monitoring that corresponds to the continuous systematic collection, analysis and interpretation of specific health effects.
- On one hand, BM based on the effects biomarkers can be helpful in providing the correct interpretation of doubtful clinical tests and a better objectivity of the clinical judgment.
- On the other hand, BM based on the exposure biomarkers can be helpful especially when occupational and/or environmental exposure monitoring data are unavailable or difficult to obtain.

An appraisal of available biomarkers associated with exposure to UFP & NMs (manufactured/engineered)

E. Bergamaschi, M. Gulumian, J. Kanno and K. Savolainen (2014)
Engineered nanomaterials: Biomarkers of exposure and effect



Exposure biomarkers | **Effect biomarkers**

- **Exhaled particles and/or elements in EBC** (estimate of the "deposited dose")
- *Particles/break down products in biological media*
- **Elements analysis** in biological fluids (excretion, body burden)
- Protein modification ("corona")

- **Lipid peroxidation products** in EBC or blood (MDA, T-BARS, conjugated dienes, LTB_4, F2- and 8-isoprostane)
- **DNA excision base products** (8-OH-dG, 8-oxo-Gua)
- **Exhaled NO (FeNO)** and **nitrosative stress** products (3-nitrotyrosine)
- **Carbonyl compounds (4-HNE) in EBC**
- Serum pneumoproteins (CC16)
- Platelet activation/aggregation; pro-thrombotic changes
- **Acute phase proteins** (hsCRP), **Haptoglobin**
- **IL-6, IL-8, TNFα and sTNF-RII**
- **Clotting factors** (fibrinogen, **PAI-1**)
- **Vascular adhesion molecules** (V-CAM-1)

- Fibrogenic markers (osteopontin)
- Cell transformation
- **Micronucleus**
- **DNA strand breaks** (Comet assay + FPG-ENDO III)
- **DNA (hypo)methylation**
- **MicroRNAs** (miRNAs)

Characteristics and findings of six published epidemiological studies on nanomaterial-handling workers

S-H Liou, **J Nanopart Res** (2015) 17:413 *DOI 10.1007/s11051-015-3219-7*

Descriptive				
Lee et al. (2012)	Korea	Nanosilver	Blood and urine levels of silver were low in 2 workers. No abnormality was found in hematological data and blood chemistry values	2
Cross-sectional				
Liou et al. (2012)	Taiwan	Various[a]	Decreased antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) and increased cardiovascular markers, fibrinogen, intercellular adhesion molecule (ICAM), and interleukin 6 were noted in exposed workers	227/137
Liao et al. (2014a)	Taiwan	Various[a]	The only symptom identified as significantly work-related was sneezing. The only disease significantly worsened by work was allergic dermatitis	258/200
Wu et al. (2014)	Taiwan	Various[a]	A significant association between risk level 2 of NP exposure and FENO. When the multivariate logistic regression model was adjusted for confounders, nano-TiO_2 in all of the nanomaterial-exposed categories had a significantly increased risk in FENO > 35 ppb	241/196
Lee et al. (2015)	Korea	MWCNTs	The malondialdehyde (MDA), 4-hydroxy-2-hexenal (4-HHE), and n-hexanal levels in the EBC of MWCNT manufacturing workers were significantly higher than those in the office workers	9/4
Longitudinal				
Liao et al. (2014b)	Taiwan	Various[a]	Changes in the antioxidant enzymes (decreased SOD and GPX), cardiovascular markers (increased VCAM, decrease of paraoxonase), the small airway damage marker (decreased Clara cell protein 16), and lung function parameters (decreased MMF, PEFR, and FEF25 %) were significantly associated with nanomaterial-handling	124/77

[a] Carbon nanotubes, silica dioxide, titanium dioxide, nanosilver, and nanoresin

S-H Liou, **J Nanopart Res** (2015) 17:413

Biomarkers found in 11 cross-sectional epidemiological studies on nanomaterial-handling workers

The bio-effects are categorized into four organs/systems

- **Lung**: increased lung fibrosis markers (serum TGF-b1 and sputum KL-6) (Fatkhutdinova 2013), increased lung inflammation markers (sputum IL-1b and IL-8, FENO) (Wu 2014; Cui 2013), and increased EBC LTB_4, C_4, and E_4 (Pelclova 2012, 2013, 2014);
- **Cardiovascular**: increased cardiovascular effect markers (fibrinogen, intercellular adhesion molecule [ICAM], and interleukin 6) (Liou 2012), increased LF/HF in HRV (Ichihara 2013, 2015);
- **Immunological**: increased immunological cytokines (Vermeulen et al. 2014);
- **Oxidative damage**: increased EBC markers of lipid oxidation (MDA, HNE, HHE, 8-isoprostane, n-hexanal, and C_6–C_{12}); markers of oxidation of nucleic acids and proteins (8-OHdG, 8-OHG, 5-OHMeU, 3-ClTyr, 3-NOTyr) (Pelclova 2012, 2013, 2014); and urine C_6, C_7, C_{10}, C_{12}, HHE, 8-OHG, and 3-ClTyr (Pelclova 2012, 2013, 2014); and decreased antioxidant enzymes [superoxide dismutase (SOD)] and glutathione peroxidase (GPX) (Liou 2012).

J Nanopart Res (2015) 17:413
DOI 10.1007/s11051-015-3219-7

REVIEW

Saou-Hsing Liou · Candace S. J. Tsai · Daniela Pelclova · Mary K. Schubauer-Berigar Paul A. Schulte

Assessing the first wave of epidemiological studies of nanomaterial workers

Analysis of four longitudinal studies

Two longitudinal panel studies (Cui 2013; Liou et al. 2013) yielded negative findings (**no association** between nanoparticle exposure and biomarker change)

Table 2 continued

Study type, authors, year (presentation)	Country	Nanomaterials	Major findings	Sample size (no. exposed/no. controls)
Longitudinal				
Liou et al. (2013) (2013 EPICOH)	Taiwan	Various (carbon nanotubes, silica dioxide, titanium dioxide, nanosilver, nanoresin)	No significant difference was revealed between exposed workers and controls in the changes of all markers, including lung injury markers, cardiovascular disease markers, heart rate variability (HRV), inflammation markers, oxidative stress and lipid peroxidation markers, comet assay, pulmonary function test, and neurobehavioral test, in this 4-year follow-up study	206/140 followed up no less than 2 times
Cui (2013) (PhD thesis)	China	Calcium carbonate	No significant cross-shift effect for FEV1, BP, and FENO	66–102/0
Pelclova et al. (2014b) (EUROTOX Conference, Edinburgh, UK)	Czech Republic	TiO_2 (70–90 % of particles <100 nm)	In 2013, all markers of oxidation of lipids, nucleic acids, and proteins (MDA, HNE, HHE, C_6–C_{11}, 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, 3-Cl-Tyr, 3-NOTyr, o-Tyr), and LTB_4 in EBC were elevated ($p < 0.001$); C_{12} p value was < 0.05 in 2013 versus unexposed controls. The following post-shift markers in 2013 were elevated versus post-shift markers in 2012: C_9, C_{12}, 8-isoprostane, 8-OHdG, 5-OHMeU, and 3-NOTyr. There was no difference in FENO, pH, and LTs, except for LTC_4. No markers were increased in analyzed urine specimens in 2013, when exposure to TiO_2 decreased	14/25

J Nanopart Res (2015) 17:413
DOI 10.1007/s11051-015-3219-7

REVIEW

Saou-Hsing Liou · Candace S. J. Tsai · Daniela Pelclova · Mary K. Schubauer-Berigar · Paul A. Schulte

Assessing the first wave of epidemiological studies of nanomaterial workers

Strengths and weaknesses of current epidemiological studies of nanomaterial workers

Weaknesses

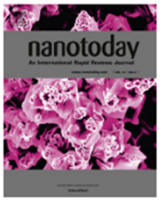
- Most of the studies were cross-sectional; inherently they could not demonstrate causality
- The few longitudinal studies lacked rigorous exposure assessment (e.g. different sampling strategies to measure elemental carbon…)
- Most of the studies lacked time-weighted averaged personal breathing zone exposure data
- Qualitative risk assessment approaches (*Control Banding* tools)
- The small size of the study populations hinders subgroup assessments and restricts the generalizability of the results
- Most of studies measured nanoparticle mass concentrations instead of nanoparticle counts or surface area or other P-Chem parameters relevant for health effects
- Most studies used area sampling instead of personal sampling (which represents actual exposure of nanomaterial workers) to measure nanoparticle concentrations
- Selection bias in identification of the study population; company workers who are more concerned about their health condition may be prone to participate in health examinations and report more negative health effects, which could lead to a biased evaluation

NEWS AND OPINIONS

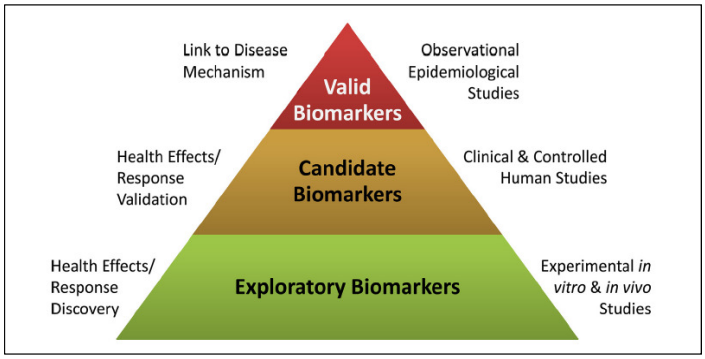
The role of biological monitoring in nano-safety

(2015) 10: 274-77

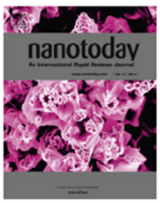
Enrico Bergamaschi[a,*], Craig Poland[b,1], Irina Guseva Canu[c,2], Adriele Prina-Mello[d,3]

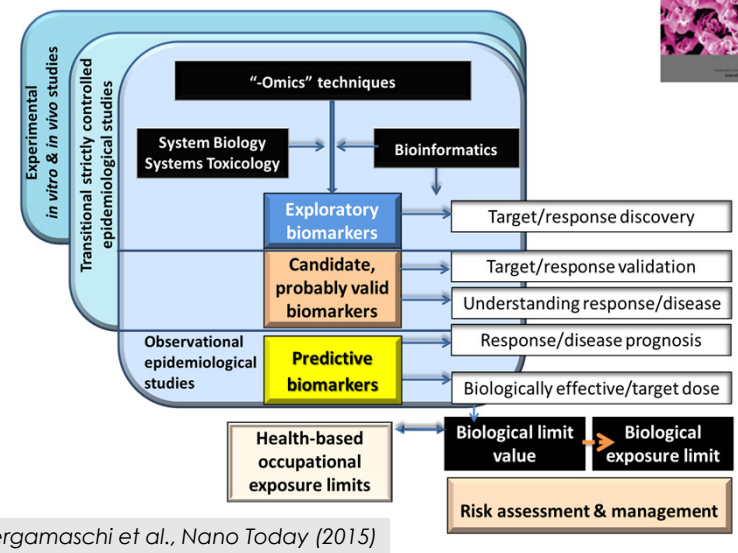


Layout of biomarkers research as condition of the responsible development of nanotechnologies and safety of workers exposed to ENM



Layout of biomarkers research as condition of the responsible development of nanotechnologies and safety of workers exposed to ENM





E. Bergamaschi et al., Nano Today (2015)

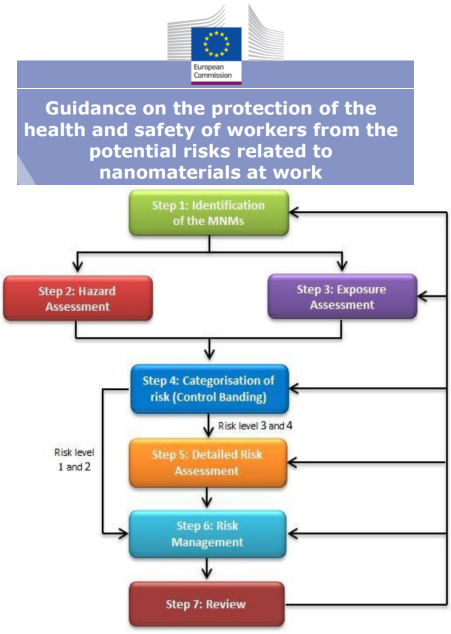


Diagram for Risk Assessment

Symposium on the health protection of the Nanomaterial Workers
International Commission on Occupational Health

Risk Management for ENMs

Schulte et al., 2009

Take-home message

- ✓ There is a pressing need to overcome pitfalls in risk assessment (RA) for engineered nanomaterials (ENM)
- ✓ Inherent properties of ENM are subject to changes in the environmental settings
- ✓ Similar paradigms for particle/nanoparticle hazard do not support "nano-specificity"
- ✓ The issue of biomarker specificity for ENM is challenging but should not hamper their use in epidemiological research
- ✓ Candidate biomarkers validated in epidemiological studies should consistently support the RA