Nanomaterials and Biosafety

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U.S. National Institute for Occupational Safety and Health

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Content

1. Definitions, properties and applications
2. Hazards
3. Exposures
4. Risk assessment
5. Risk mitigation
6. Resources

1. Definitions, properties and applications

Definitions

Nanomaterials can be defined as materials composed of structures in the nanoscale size range.

Proposed definitions of the nanoscale size range vary depending on their intended use.

- Health and safety advocates, who look at interactions of nanomaterials with biological systems, tend to extend the upper boundary to include larger sizes up to 1000 nm (e.g. the largest pore size that will yield a sterile filtrate is 200 nm).
- On the application side, electronic properties change qualitatively at around 30 nm.

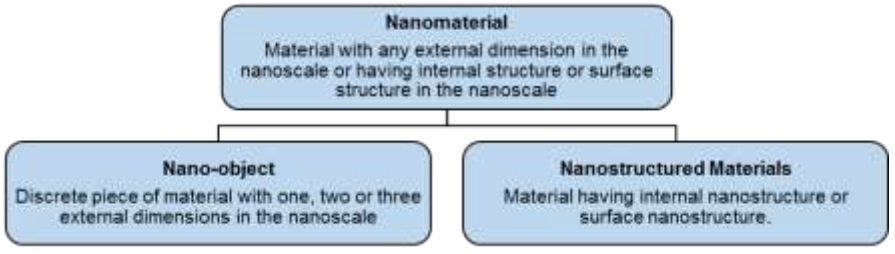
As a compromise, the International Organization for Standardization (ISO) and many other organizations agreed to define **nanoscale size range as approximately from 1 nm to 100 nm**

U.S. FDA Guidance for Industry, 2014

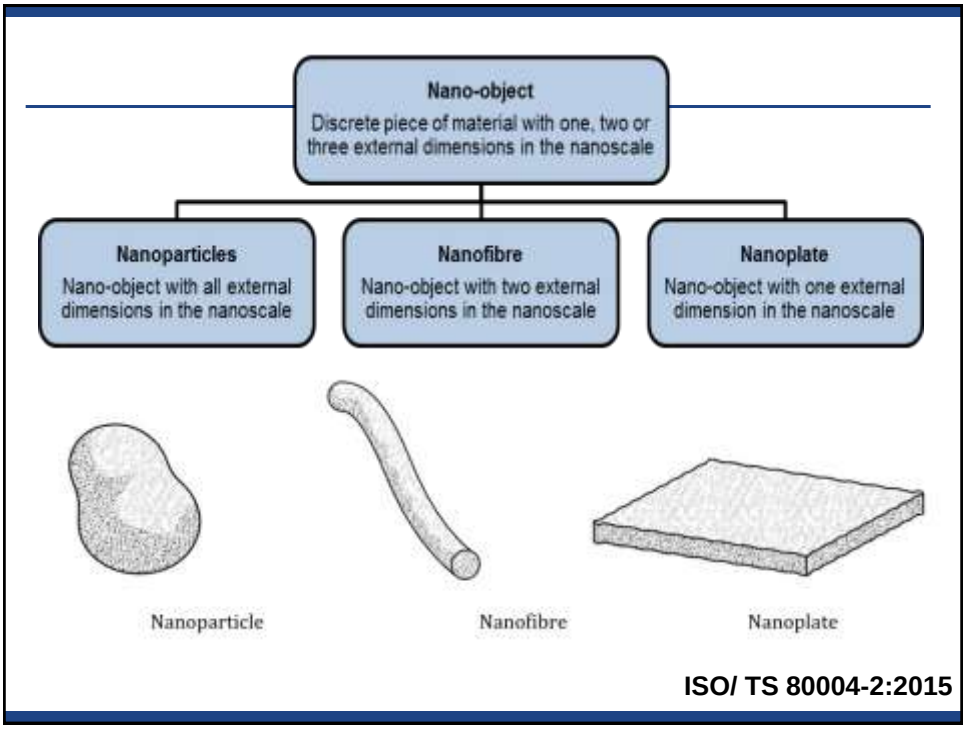
- "the application of nanotechnology" includes "a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm)," because materials or end products can also exhibit related properties or phenomena that are relevant to evaluations of safety, effectiveness, performance, quality, public health impact, or regulatory status of products.

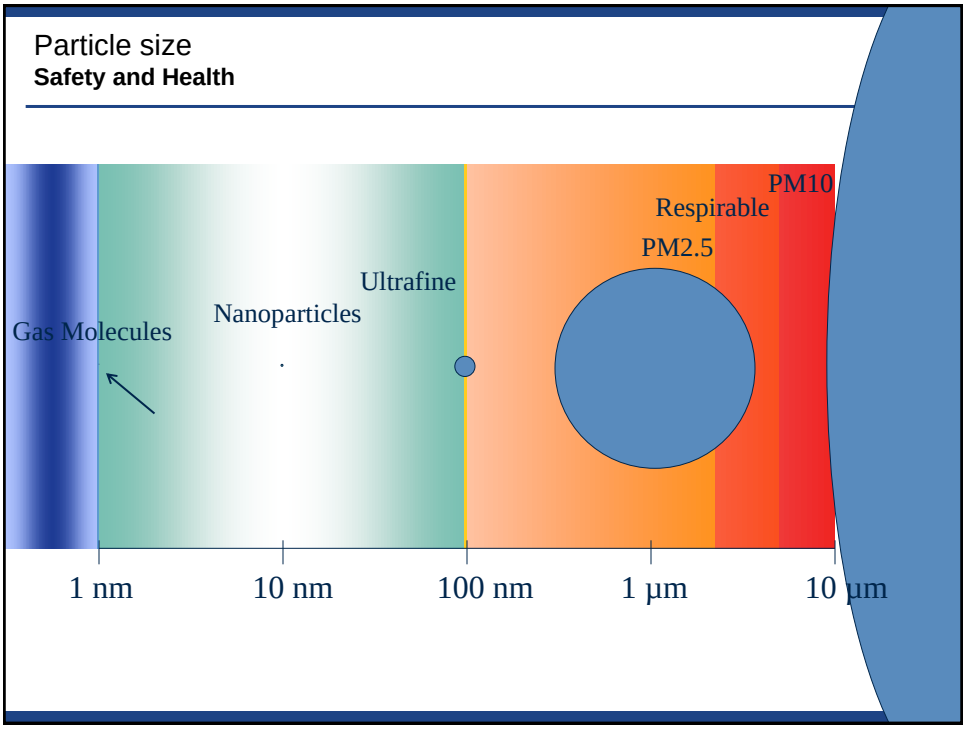
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considering-whether-fda-regulated-product-involves-application-nanotechnology

Definitions

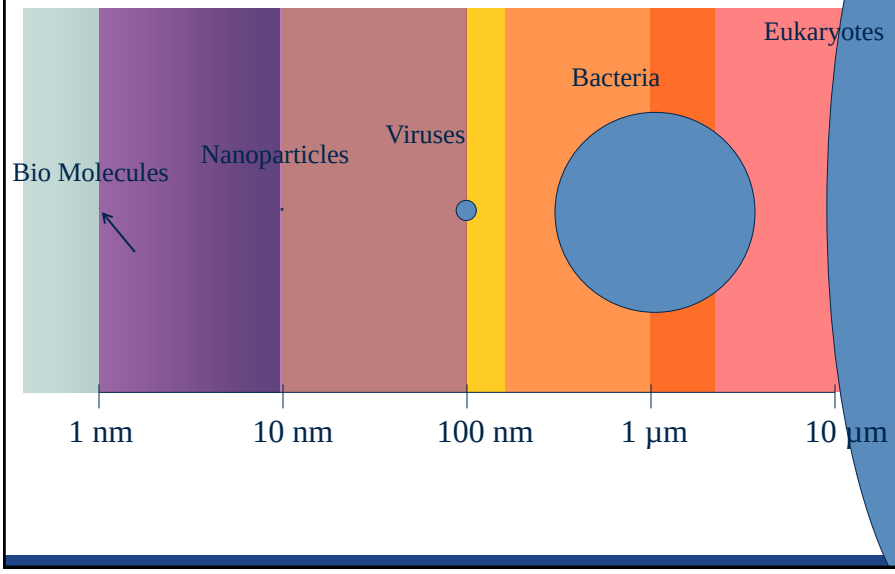


ISO/TS 80004-1:2015

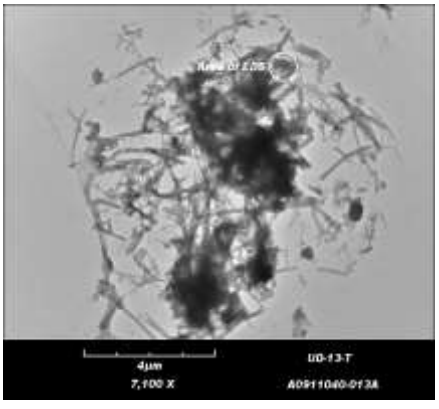

Nano-object
Discrete piece of material with one, two or three external dimensions in the nanoscale
Nanoparticles
Nano-object with all external dimensions in the nanoscale
Nanofibre
Nano-object with two external dimensions in the nanoscale
Nanoplate
Nano-object with one external dimension in the nanoscale
Nanoparticle
Nanofibre
Nanoplate
ISO/ TS 80004-2:2015


Particle size
Safety and Health
PM10
Respirable
PM2.5
Ultrafine
Nanoparticles
Gas Molecules
1 nm
10 nm
100 nm
1 µm
10 µm

Particle size
Biology



Nanomaterials in Personal Breathing Zone sample: Nano-Objects and their Agglomerates and Aggregates (NOAA)



Courtesy of NIOSH

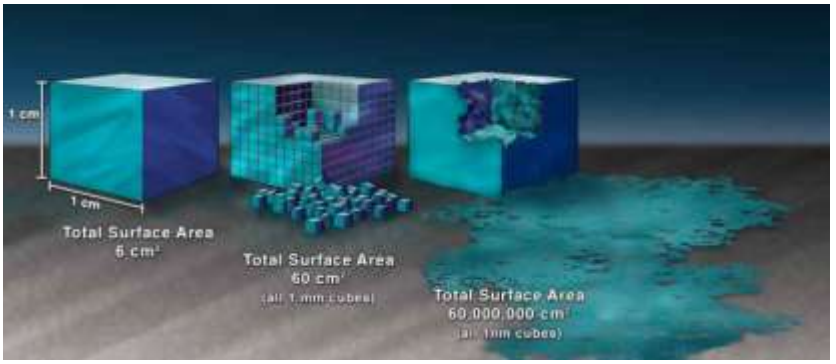
Properties affecting biological activity

- Internalized by cells through endocytosis
  - Can affect intracellular pathways
- Dissolve into molecular/ionic species
  - Can exert toxicological activity through traditional chemical mechanisms
- Move randomly in fluids according to the laws of Brownian dynamics
  - Diffuse through the air
  - Transport in body fluids and reach distal organs and biological sites

Properties affecting biological activity

- High surface area
  - Higher biological activity and higher adsorption

Small size ⇨ **Large surface area**



Properties affecting biological activity

- High “stickiness”
  - “Trojan horse” toxicity mechanism in biological systems
  - Quick agglomeration
  - High capture efficiency of filters

Nanomaterial transport abilities can be enhanced

- To optimize performance for certain applications (e.g. medical), nanomaterials can be designed to remain non-agglomerated.
- Non-agglomeration of nanomaterials can enhance their bioavailability, which can increase their hazard and exposure potentials.
- Smaller nanoparticles, which are deliberately stabilized to remain non-agglomerated in dispersions, have been shown to penetrate intact skin, while mostly agglomerated and aggregated nanomaterials were unable to penetrate intact skin.

Nano-enabled products: history

- 300 AD Lycurgus Cup with nano gold
- 8th to 15th century Maya blue pigment – organoclay composed of indigo extract and palygorskite
- 13th to 18th century Damascus steel blades contain carbon nanofiber
- 2000's Beginning of nanomaterial applications & nano-enabled consumer products

Nano-enabled Drug Product Submissions to FDA

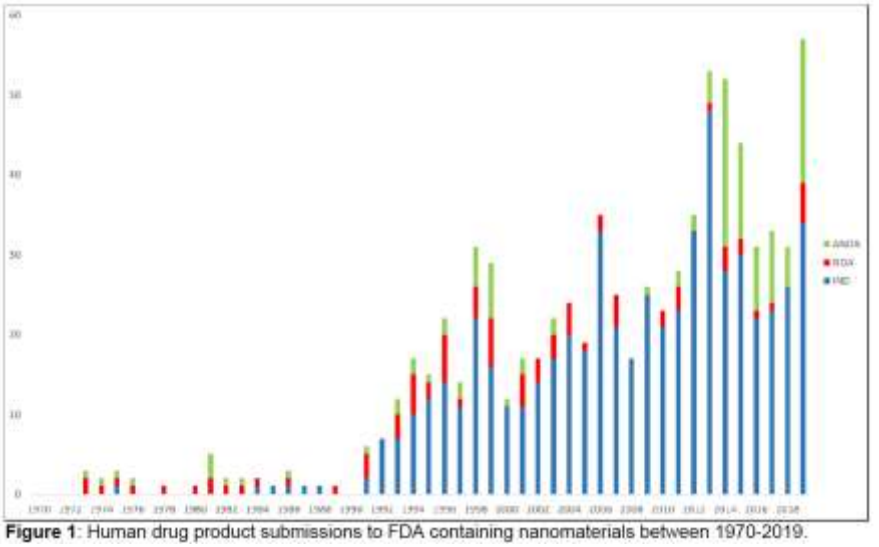


Figure 1: Human drug product submissions to FDA containing nanomaterials between 1970-2019.

- ANDA – Abbreviated New Drug Application; NDA – New Drug Application; IND – Investigational New Drug
- Ref.: FDA. Nanotechnology—Over a Decade of Progress and Innovation, 2020.

Common Nanomaterials on the Market

Categories of Nanomaterials in OECD Toxicity Testing Sponsorship Program

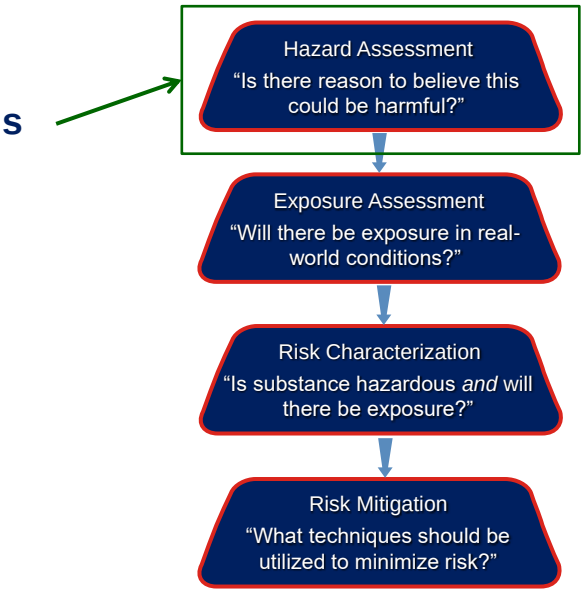
Major Category	Specific Nanomaterials
Carbon-based	Single-wall carbon nanotubes, multiwall carbon nanotubes, fullerenes
Oxides	Titanium dioxide, silicon dioxide, cerium dioxide, zinc oxide
Metals	Silver, gold
Dendrimers	Poly(amidoamine) dendrimers
Nanoclays	Bentonite

Source: OECD, http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm

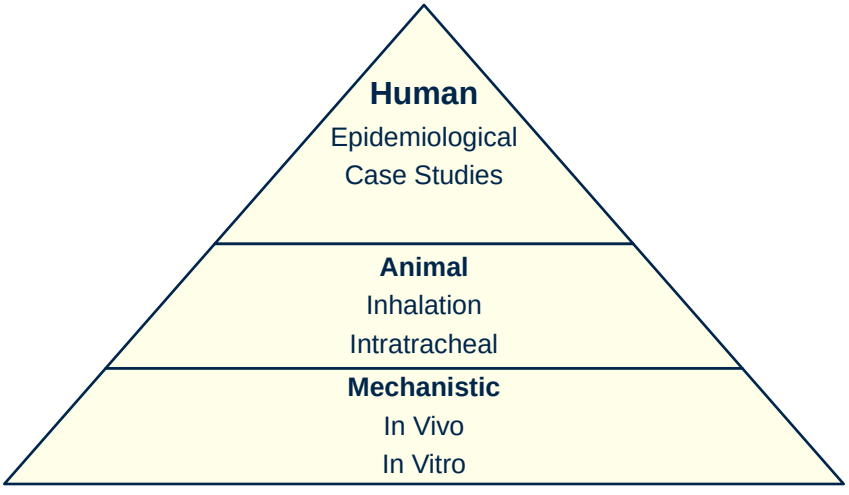
2. Hazards

Health Risk Model

Are nanomaterials hazardous?



Hierarchy of information related to human health effects from nanomaterial exposures



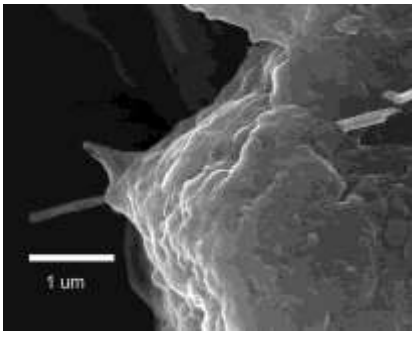
Health Effects of Nanomaterials: Human Epi Studies

- **Findings from air pollution epidemiology**
  - Particles < 2.5 µm associated with respiratory and cardiovascular effects
- **Studies of industrial fumes (e.g., welding fumes) and combustion (e.g., diesel) products**
  - Wide range of effects: pulmonary and eye irritation, fever, lung cancer
- **Studies of legacy manufactured nanomaterials (carbon black, titania, silica, etc.)**
  - Nonmalignant respiratory morbidity/pulmonary function decrement, symptoms of chronic bronchitis (carbon black); metal fume fever (zinc oxide)

Health Effects of Nanomaterials: Animal Studies

Certain nanomaterials can

- Cause rapid and persistent pulmonary fibrosis, granulomas, inflammation, lung cancer, and mesothelioma-like effects
- Cause cardiovascular dysfunction: oxidative stress, plaque formation
- Migrate along the olfactory nerve into the brain



Alveolar Epithelial Penetration by Multiwall Carbon Nanotube

Courtesy of R. Mercer, NIOSH

Mechanism of Toxicity: Low Solubility Nanomaterals

As a solid particle, nanomaterials could

1. pose a hazard due to physical shape (e.g. toxicity of long, rigid fibers)
2. elicit immunologic responses (e.g. foreign body reaction leading to fibrosis)
3. act as carriers due to high adsorptive properties delivering hazardous chemicals present in the environment to biological compartments which would otherwise be inaccessible to those chemicals (e.g. air pollution)
4. exert biological activity through chemical entities present on the surface as a result of the manufacturing process (intended and unintended surface functionalization) and through deposition in biological environments (e.g. protein corona)
5. create superoxides and catalyze electron transport in redox regulators

Mechanism of Toxicity: Soluble Nanomaterals

As a soluble particle, nanomaterials could

- pose a hazard due to dissolving ions (e.g. toxicology of silver nanoparticles due to silver ions) characterized by
  - immunological functions,
  - genotoxic action,
  - catalytic potential.

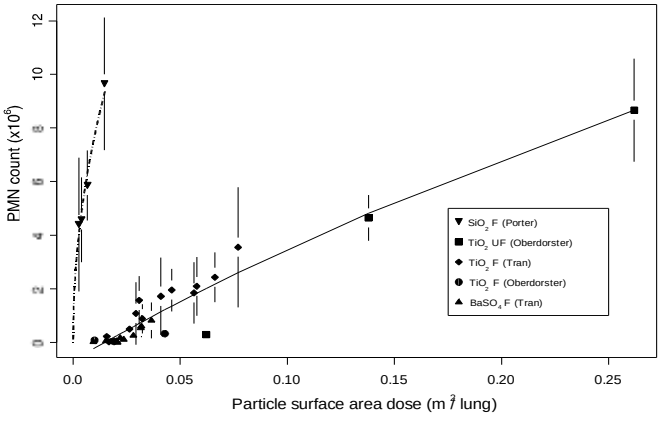
Metrics of dose and exposure

The mechanism of toxicity determines the most appropriate metric for dose and exposure.

- The inability of macrophages to engulf and clear long rigid fibers leads to adverse health effects ("fiber pathogenicity paradigm")
  - the most appropriate metric for dose is the total number of fibers and the metric for exposure is the fiber number concentration
- Soluble nanomaterials exert toxicity through dissolved ions and molecules
  - the most appropriate metric for dose and exposure is mass concentration, as mass concentration represents the amount of ions produced during dissolution of nanomaterials

Metrics of dose and exposure (cont.)

- Insoluble nanoparticles due to their higher surface area per mass are more potent than larger particles of similar composition in causing pulmonary inflammation, tissue damage, and lung tumors ⇒ the most appropriate metric for dose is surface area concentration



Maynard & Kuempel, J. Nanopart. Res. 2005, 7, 587

Metrics of dose and exposure (cont.)

In sufficiently well characterized nanomaterials, different metrics can be converted from one into another using mathematical formula.

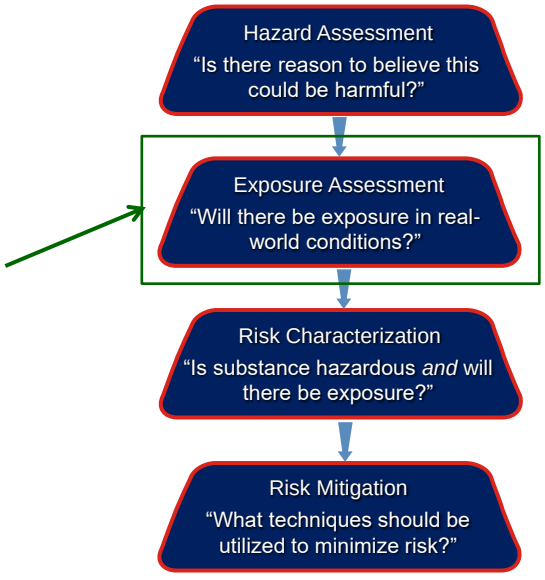
E.g. for 10 µg/m^3 mass concentration:

Diameter (nm)	Number concentration (cm^{-3})	Surface area concentration ($\mu m^2/cm^3$)
2500	1.2	24
1000	19	60
500	153	120
100	19 100	600
20	2 400 000	3 000

3. Exposures

Health Risk Model

Can exposure be measured and is there actual exposure?



Routes of Exposure

Route	Thickness of blood barrier	Total surface area
Inhalation	< 0.001 mm	143 m^2
Dermal	0.03 mm up to several mm (epidermis)	1 to 2 m^2
Gastro-intestinal	0.02 – 0.8 mm (epithelium)	30 to 40 m^2
Parenteral	0	1 to 2 m^2

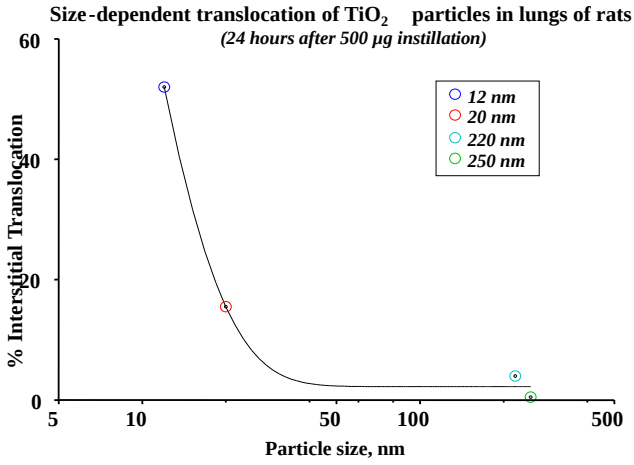
Inhalation Exposure: The most common route of exposure to any aerosol particle in the workplace

Courtesy of NIOSH

Inhalation Exposure

- The deposition of particles in the respiratory tract is determined by the aerodynamic diameter of the particles.
- Discrete nano-objects are deposited in all regions of the respiratory tract, including the deep alveolar region, to a greater extent than larger respirable particles.
- Deposition increases with exercise due to increase in breathing rate and change from nasal to mouth breathing.
- Deposition increases among persons with existing lung diseases or conditions.

After deposition, discrete nanoparticles and nanofibers may enter the bloodstream from the lungs, translocate to distant sites and interact with cells, nucleic acids, and proteins in other organs of the body



Translocation varies inversely with particle size (*G. Oberdorster, EHP, 1992, 97, 193*)

Dermal Exposure

Several exposure factors can affect dermal absorption. They include

- the type of work process or task being conducted,
- the duration of the work,
- the surface area of the exposed skin,
- the type and extent of personal protective clothing,
- the concentration of the chemical, and
- hygiene practices such as hand washing and eating.

Dermal Exposure

Available data are limited and often conflict;
Skin cannot be ruled out as a potential route of exposure

- Several studies show little to no penetration of nanoscale oxides beyond surface skin layers
- Polysaccharide and metal nanoparticles have been shown to penetrate flexed, damaged or diseased skin
- Quantum dots were found to penetrate intact pig skin within 8-24 hours at occupationally relevant doses
- Penetration of intact skin can occur through a number of pathways, e.g., sweat ducts, stratum corneum via inter- or intracellular modes, and hair follicles
- Chemicals that are irritating to the skin can facilitate absorption by opening portals of entry.
- Chemical properties of solvents used to disperse nanomaterials can determine dermal absorption, especially if the solvent is lipophilic.

Gastro-intestinal Exposure

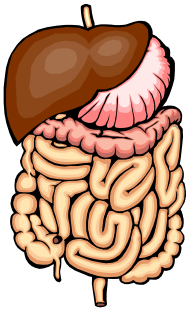
Ingestion is a potential route of exposure;
Ingested nanoparticles can translocate throughout the body

- Ingestion may occur after inhalation exposure when mucus is brought up the respiratory tract and swallowed.
- Poor work practice can result in hand-to-mouth transfer and ingestion of contaminated food or water.
- Ingested nanoparticles can translocate to other organ systems
  - Ingestion of colloidal silver can result in permanent discoloration of skin, nails and eyes

Gastro-intestinal penetration

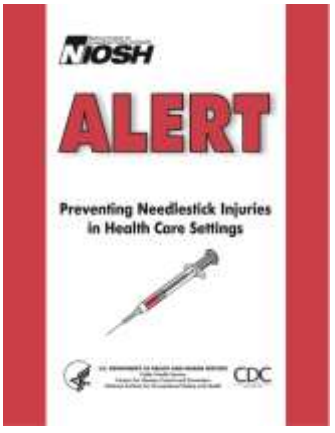
It has been shown that inorganic particles such as 500 nanometer titanium dioxide and nanoscale gold have the potential to cross the digestive tract lining and translocate to systemic organs such as the liver, spleen, lung and peritoneal tissues (*Froehlich E, Roblegg E. Tox, 2012, 291, 10*).

There is some evidence that smaller particles can be transferred more readily than their larger counterparts across the intestinal wall (*Behrens I, Pena AIV, Alonso MJ, Kissel T. Pharm Res, 2002, 19, 1185*).



Parenteral Exposure

Nanomaterials can be introduced into human bodies via the parenteral route either incidentally through cuts and other damage to intact skin or intentionally for drug delivery, medical imaging, or other applications.



Characterizing Exposure

- Concentration (in the form of particle number, mass, and surface area as discussed earlier)
- Physical form of their formulation (for example, dry powder or liquid suspension)
- Route of absorption
- Frequency and duration of exposure
- Distribution of nanomaterials

Exposure characterization in laboratories

- Identify tasks that may expose workers to NM and researchers conducting those tasks
- Include information on the determinants of potential exposure such as dustiness of materials, type of processes, magnitude, duration, and frequency of exposure during different job tasks and the amounts of materials being used

Exposure Measurements

- Since there is no single exposure metric for nanomaterials, a multifaceted approach incorporating several sampling techniques focusing on different parameters characterizing exposure is recommended.
- Regardless of the metric and method selected for exposure monitoring, it is critical that measurements be conducted before production or processing of a nanomaterial to obtain background exposure data.

Background nanoparticles

The presence of background nanoparticles unintentionally generated by sources within the workplace (e.g. diesel exhaust, combustion products, electrical motors, photocopiers) and introduced from the outdoor environment further complicates interpretation of workplace exposure measurements.

Courtesy of NIOSH

Background nanoparticles

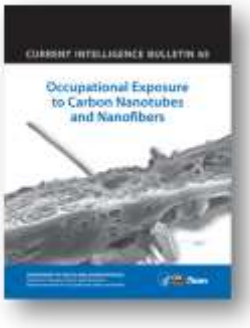
It is especially challenging to distinguish between intentionally produced carbonaceous particles (such as carbon nanotubes) and background carbonaceous particles.

One approach is to measure residual metals as a surrogate for carbon nanotubes.

Occupational Exposure Limits

NIOSH RELs:

300 µg/m^3	for nano TiO_2
1 µg/m^3	for CNT and CNF
0.9 µg/m^3	for nano Ag



Stepwise approaches for inhalation exposure

- OECD Harmonized Tiered Approach

- U.S. NIOSH Nanomaterial Exposure Assessment Technique (NEAT 2.0)

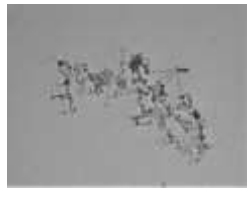
Key Elements of NEAT 2.0

- Pre-assessment prioritization
- Field measurements
- Risk mitigation
- Routine monitoring (confirmation)

Elemental Analysis

Particle Counters and Size Analyzers

Electron microscopy



Eastlake et al. J. Occ. Env. Hyg. 2016, 13(9), 708-717

Pre-assessment Prioritization

- Process description and flow
- Number of employees and job description
- Safety Data Sheets or other information
- Walk about to look at process, identify possible exposure potentials
- Review occupational exposure limits and health effects
- Identify and review pertinent literature

Courtesy of A. Eastlake, NIOSH

Field Measurements

- Filter-cassette based
  - Elements and Electron Microscopy (EM)
  - PBZ, Source/ Area, Background
  - Full shift and task specific
  - With and without cyclones
  - Various filter media
- Data logging with Direct Reading Instruments (DRI's)
  - Source/Area and Background

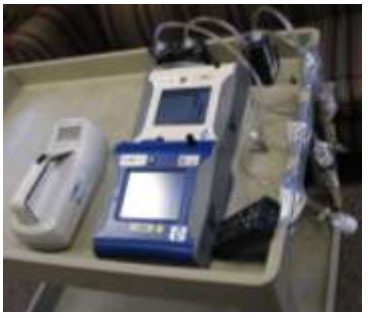
Courtesy of A. Eastlake, NIOSH

Integrated Sampling

- Personal breathing zone
  - "True" indicator of worker's exposure
  - Determines levels of exposure throughout workday
  - Can be compared to OELs
- Area
  - Survey sources of contaminant
  - Evaluate engineering controls
- Background
  - Other contributions not related to the process

Direct Reading Instruments

- It's not just nano, wide size range of particles measured 0.01 – 10 µm; no material identification
- Data, in conjunction with integrated data, can be used to show deficiencies in worker practices or engineering controls
- Use data with caution since there are many interfering incidental fine particles



In-depth assessments

- Wipe sampling
- Concentration mapping
- Use of advanced aerosol sampling equipment (SMPS, FMPS, ELPI, MOUDI, Surface Area)
- Complete industrial hygiene sampling (VOCs, acids, IAQ, etc.)

Wipe Sampling

- Surface contamination
- Assess worker hygiene practice, no correlation with worker inhalation exposures
- Qualitative or quantitative
  - NMAM 9100 (Lead in surface wipe samples)
  - NMAM 9102 (Elements on wipes)
  - Gauze, filter paper, premoistened wipes
  - Analysis by inductively coupled argon plasma, atomic emission spectroscopy
- How clean is clean enough?
  - Brookhaven National Laboratory
  - www.bnl.gov - Surface wipe criteria for several elements

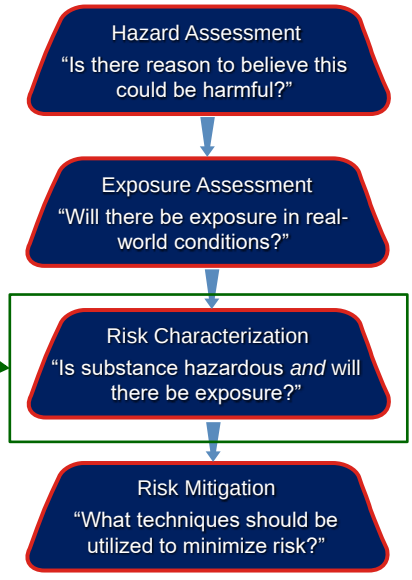
Internal exposure or Dose

- Dose assessment involves analysis of biological specimens such as tissue, body fluids, and exhaled air. In the occupational setting, less invasive methods such as collection of urine, hair, and exhaled air are used most commonly.
- The dose can be determined by measuring the amount of nanomaterials of interest, and/or their metabolites in living organisms.
- Biomarkers of exposure to nanomaterials are in the early development stage; this development is complicated by the great variety of nanomaterial chemical and physical properties resulting in a wide range of biological responses.

4. Risk assessment

Health Risk Model

Risk =
Hazard x Exposure



Risk

- The risk of a substance is commonly defined as the likelihood that the substance with hazard potential will cause harm, taking into account wider considerations of exposure and uncertainty

- Risk assessment requires information on both the potential hazard, the release of the substance into the environments, and the likelihood and/or degree of resulting short- and long-term exposure

European Agency for Safety and Health at Work

OiRA: free and simple tools for a straightforward risk assessment process

Risk assessment is the essential first step in the prevention of occupational accidents and ill health. OiRA — Online interactive Risk Assessment — makes this process easy.

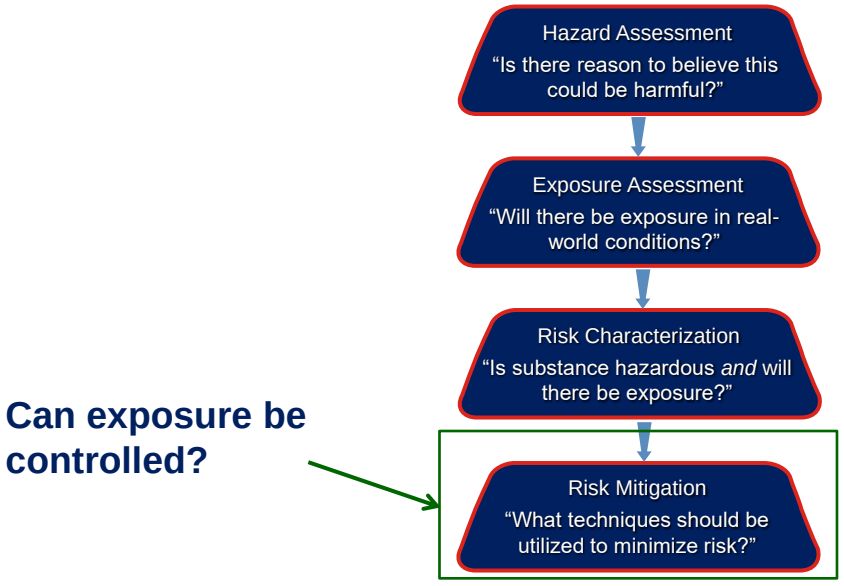
It provides the resources and know-how required to enable micro and small organisations to assess their risks themselves. Available free on the web, OiRA tools are easily accessible and easy to use.

OiRA offers a step-by-step approach to the risk assessment process, beginning with the **identification of workplace risks, then taking the user through the process of implementing preventive actions, and finally to monitoring and reporting risks.**

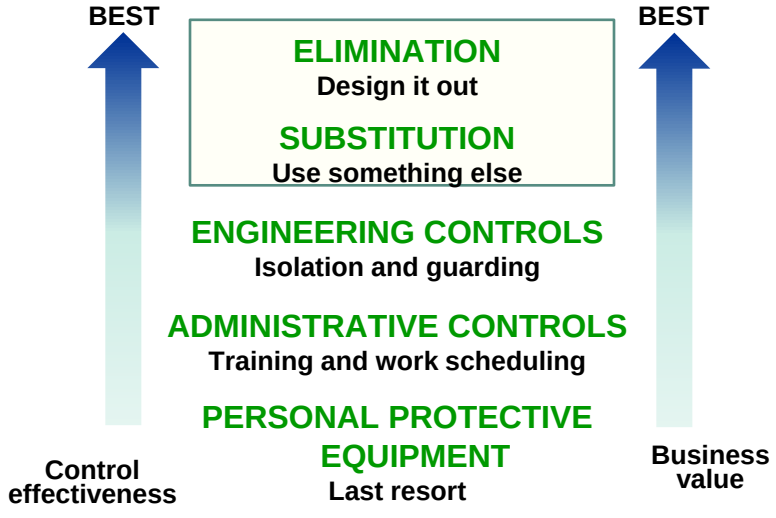
https://osha.europa.eu/en/tools-and-resources/oira

5. Risk mitigation

Health Risk Model



Hierarchy of Controls



Substitution: composition

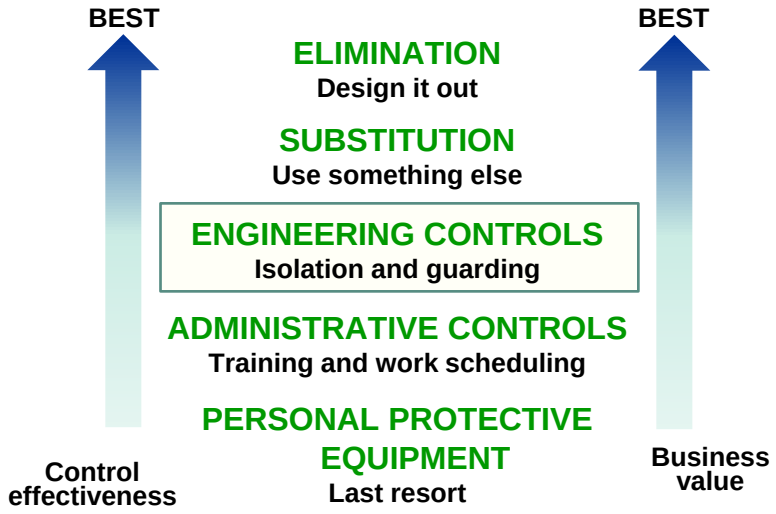
- Less toxic precursor gases, catalysts, solvents
- Identify safer nanomaterials with same functionality: size, shape, functionalization, surface charge, solubility, agglomeration all effect toxicologic properties
  - e.g. Toxicity testing of functionalized MWCNT with –COOH (carboxylated) group showed less cytotoxicity and inflammasome activation
  - Silver nanoparticle toxicity depends on release of silver ions: suppressing Ag^+ release may compromise antibacterial function

Functionality  Health and Safety

Substitution: formulation

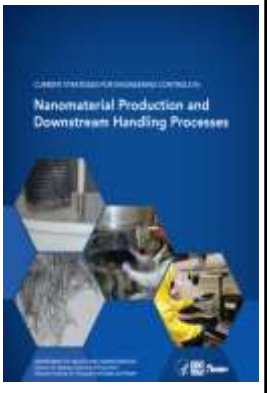
- Nanoparticles are often provided and worked in a wet state to reduce the risks of exposure.
- However, dispersing nanomaterials in liquids does not necessarily reduce the potential for exposure to zero.
- Nanomaterials can become airborne when mixed in solution by sonication, especially when nanomaterials are functionalized or in water containing natural organic matter.

Hierarchy of Controls



Engineering Controls

- NIOSH Current Strategies for Engineering Controls in Nanomaterial Production and Downstream Handling Processes
  - Provides guidance regarding approaches and strategies to protect workers by using available engineering controls for manufactured nanomaterials in the workplace.
  - Covers common processes including material weighing and handling, reactor harvesting and cleaning, bag dumping and large-scale material handling/transfer
  - http://www.cdc.gov/niosh/docs/2014-102/
- Summary of control evaluations at three carbonaceous nanomaterial plants
  - Heitbrink, WA. J Occup Environ Hyg. 2015;12(1):16-28.



Estimated average nano-specific efficacy values: $C_{control\ on}$ / $C_{control\ off}$

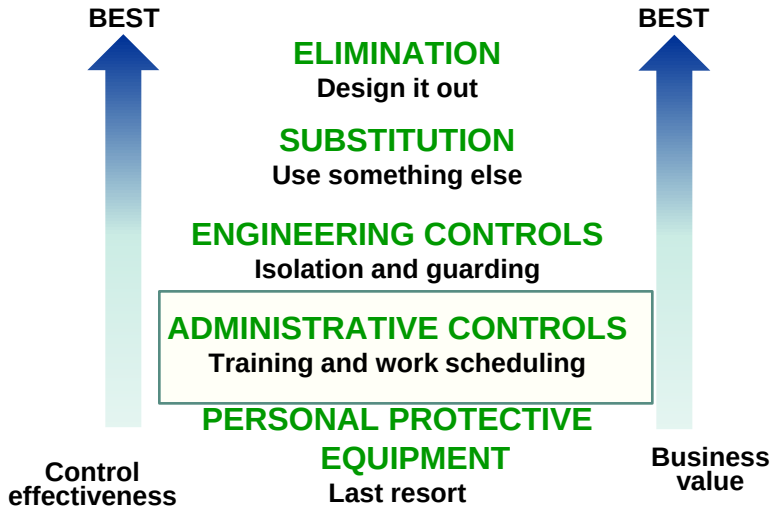
Engineering control	Number of studies	Nano-specific efficacy (%)
Containment	21	99.5
High level	14	99.7
Medium level	7	98.0
LEV – capturing hoods	46	95.2
Fixed hood	29	97.5
Movable hood	17	90.0
LEV – enclosing hoods	6	76.9
Fume cupboard	5	77.3
Glove boxes	4	93.4
Suppression techs	3	94.6
Unidirectional room airflow system	3	90.1

Goede et al. Ann. Work Expos Health, 2018, 907-922

NIOSH Safe Lab Practices: Controls

- General exhaust ventilation alone is not an appropriate control for nanomaterials
- Local exhaust ventilation, such as the standard laboratory chemical hood, captures emissions at the source and is appropriate for control of nanomaterials
- Control verification with traditional industrial hygiene sampling is essential to ensure that implementation tools are performing as specified

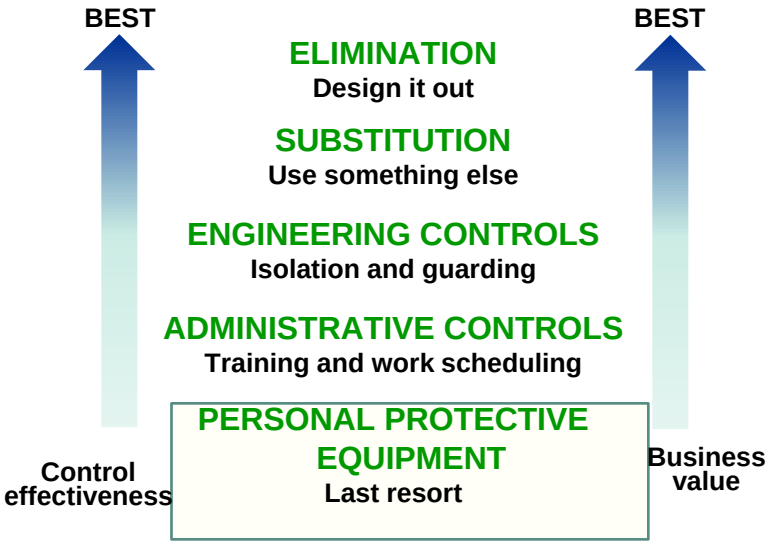
Hierarchy of Controls



Adminstrative Controls

- Limiting access, pass keys only after completion of training
- Procedural Controls
  - transport in secondary containment
  - transport in access-controlled areas
- Work practices, housekeeping, labeling, storage

Hierarchy of Controls

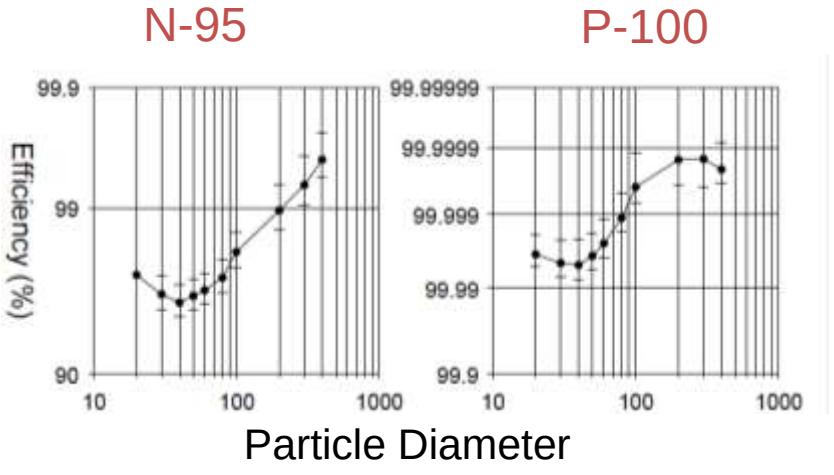


Personal Protective Equipment

- Provide respiratory protection when exposures cannot be controlled below OELs.
  - N95, P100 filtering devices are effective at capturing nanomaterials
- Provide clothing and gloves when there is potential for contact with contaminated surfaces. Could be task specific:
  - Maintenance
  - Emergencies

Courtesy of NIOSH

Respirator Use: With nanoparticles between 20 - 400 nm, 40 nm is the most penetrating size

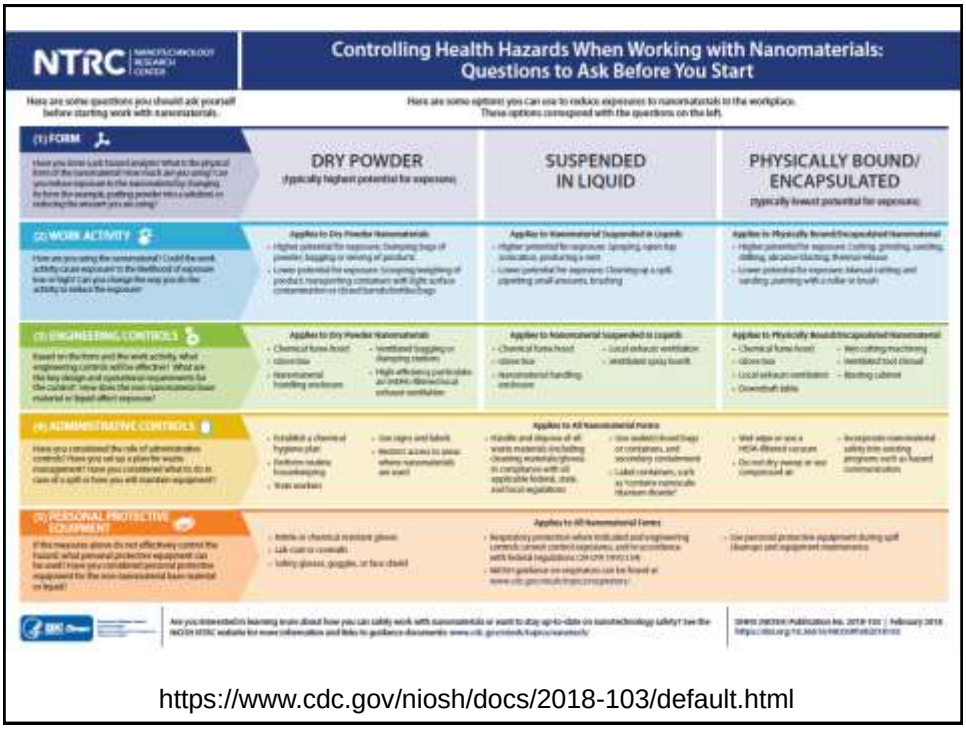


Courtesy of NIOSH

Wearing hand protection when working with nanoparticles

- Nitrile (most generally used)
- Neoprene
- Polyvinyl chloride (PVC)

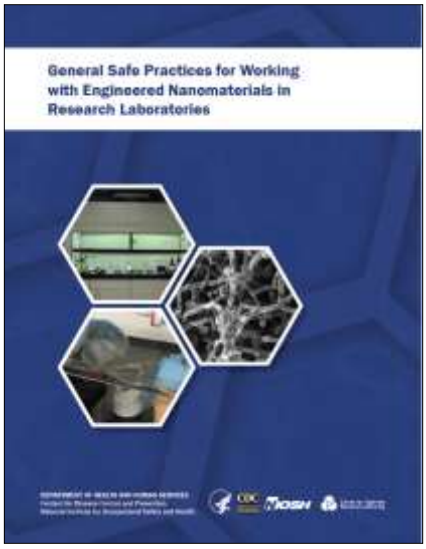
Nitrile gloves



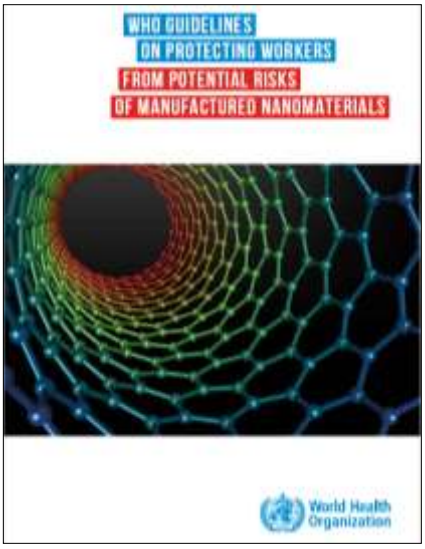
https://www.cdc.gov/niosh/docs/2018-103/default.html

6. Resources

Nanomaterial Safety Guidelines



www.cdc.gov/niosh/topics/nanotech/

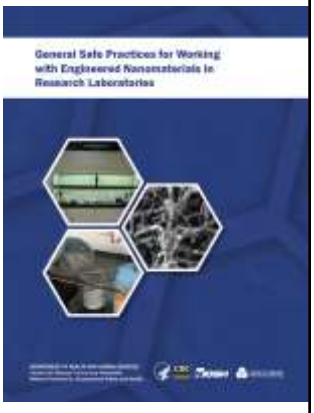


www.who.int/occupational_health/topics/nanotechnologies/

General Safe Practices for Working with ENM in Research Laboratories (NIOSH)

Flexible & adaptive risk management program:

1) Anticipate the emergence of NMs into laboratory settings
2) Recognize the potential hazards
3) Evaluate the exposure to NM
4) Develop controls to prevent or minimize exposure
5) Confirm the effectiveness of those controls




Thank you!
NIOSH
World Health Organization
OECD
unitar