NANOMATERIAL TOXICITY

By:
Anshika Agarwal
Anant Raheja
Saranath P.
Upender G.

Introduction:

Nanotechnology is the convergence of engineering and molecular biology, leading to the development of structures, devices and systems that have novel functional properties with size ranging between 1 and 100 nm (Seetharam and Sridhar, 2007).The nanotechnology industry is rapidly growing with promises of substantial benefits that will have significant economic and scientific impacts, applicable to a whole host of areas ranging from aerospace engineering and nano-electronics to environmental remediation and medical healthcare. Nanotechnology has tremendous potential to change and improve many sectors of the economy, including consumer products, healthcare, transportation, energy and agriculture.

Unusual physicochemical properties of engineered nanomaterial (NM) are attributable to their small size, chemical composition, surface structure, solubility, shape, and aggregation (Nel *et al*, 2006).

Yet concerns have been raised that the very properties of nanostructured materials that make them so attractive could potentially lead to unforeseen health or environmental hazards. Engineered nanomaterials have all the traits that should raise eyebrows with regard to health assessments of any particulate: novelty in both form and function, unique chemistry and physics by design, complex interactions with biological and environmental milieu, biopersistence (both organismal and within the food chain), ready dispersibility and possible bioaccumulation, tissue penetration, and/or irreversible biochemical and materials activities. These types of properties have history in case studies of toxicities resulting from newly introduced substances.

The spectre of possible harm — whether real or imagined — is threatening to slow the development of nanotechnology unless sound, independent and authoritative information is developed on what the risks are, and how to avoid them. Currently, a complete understanding of the size, shape, composition and aggregation-dependent interactions of nanostructures with biological systems is lacking and thus it is unclear whether the exposure of humans, animals, insects and plants to engineered nanostructures could produce harmful biological responses (Fischer and Chan, 2007).

Characteristics of nanomaterials that can give rise to toxicity:

Particle size and surface area are important material characteristics from a toxicological perspective. As the size of a particle decreases, its surface area increases and also allows a greater proportion of its atoms or molecules to be displayed on the surface rather than the interior of the material. The change in the physicochemical and structural properties of engineered Nano material with a decrease in size could be responsible for a number of material interactions that could lead to toxicological effects.

These new materials could have a number of possible causes of toxicity:

(1) Nanostructures have been demonstrated to have electronic, optical, and magnetic properties that are related to their physical dimensions, and the breakdown of these nanostructures could lead to a unique toxic effect that is difficult to predict, an example of which is provided in the introduction,

(2) Nanostructure surfaces are involved in many catalytic and oxidative reactions. If these reactions induce cytotoxicity, the toxicity could be greater than a similar bulk material because the surface area-to-volume ratio for nanoscale material is much greater

(3) Some nanostructures contain metals or compounds with known toxicity and thus the breakdown of these materials could elicit similar toxic responses to the components themselves

Toxic effects:

The toxicity of the nanomaterials can broadly be divided into two categories biological toxicity and environmental toxicity.

Biological toxicity:

Though the nanoparticles may primarily target at respiratory organs, however, other organs, e.g. gastrointestinal tract, also need to be considered. Because nanoparticles could get into gastrointestinal tract by many ways, such as indirectly via mucociliary movement, directly via oral intake of water, food, cosmetics, drugs and drug delivery system in nanoscale (Meng *et al*, 2007).

Nanotoxicology refers to the study of the interactions of nanostructures with biological systems with an emphasis on elucidating the relationship between the physical and chemical properties (e.g. size, shape, surface chemistry, composition, and aggregation) of nanostructures with induction of toxic biological responses (Maynard, 2006).

The overall behavior of nanostructures inside body could be summed as follow:

1) Nanostructures can enter the body via six principle routes: intra venous, dermal, subcutaneous, inhalation, intraperitoneal, and oral,

(2) Absorption can occur where the nanostructures first interact with biological components (proteins, cells),

(3) Afterward they can distribute to various organs in the body and may remain the same structurally, be modified, or metabolized

(4) They enter the cells of the organ and reside in the cells for an unknown amount of time before leaving to move to other organs or to be excreted (Fischer and Chan, 2006).

Interaction with biological systems can give rise to either of the following toxic effects:

- Allergy (Maynard, 2006)
- Fibrosis (Nel *et al*, 2006)
- Deposition in different organs : can lead to organ failure (Nel *et al*, 2006)
- Inflammation .(Nel *et al*, 2006)
- Cytotoxicity .(Nel *et al*, 2006)
- Tissue damage (Singh *et al*, 2009)
- ROS generation (Meng *et al*, 2007)
- DNA damage (Singh *et al*, 2009)

Table 1: Showing possible NM effects as the basis for pathophysiology and toxicity (Nel *et al*, 2006).

Experimental NM effects	**Possible pathophysiological outcomes**
ROS generation	Protein, DNA and membrane injury, oxidative stress
Oxidative stress	Phase II enzyme induction, inflammation, mitochondrial perturbation
Mitochondrial perturbation	Inner membrane damage, permeability transition (PT), pore opening, energy failure, apoptosis, apo-necrosis, cytotoxicity
Inflammation	Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system	Asymptomatic sequestration and storage in liver, spleen, lymph nodes, possible organ enlargement and dysfunction
Protein denaturation, degradation	Loss of enzyme activity, auto-antigenicity
Nuclear uptake	DNA damage, nucleoprotein clumping, autoantigens
Uptake in neuronal tissue	Brain and peripheral nervous system injury
Perturbation of phagocytic function, ‘‘particle overload,’’ mediator release	Chronic inflammation, fibrosis, granulomas, interference in clearance of infectious agent
Endothelial dysfunction, effects on blood clotting	Atherogenesis, thrombosis, stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

Environmental toxicity:

Outburst of nanomaterial research will certainly pump a lot of NPs to the environment, which will ultimately lead to the so-called nanoparticle pollution, by deposition of nanoparticle in ground water and soil (Seetharam and Sridhar, 2006, Colvin, 2007). To date, there are no detailed studies on the mechanism of transport and biodegradation or association of NPs with biological materials that may eliminate nanomaterials (Seetharam and Sridhar, 2006).

The presence of nanomaterial in environment also affects the ecosystem. In recent study, toxicity of fullerene-C60 in two aquatic species, *Daphnia* and *Pimephales* elevated lipid peroxidation (LPO) in brain, significantly increased LPO in gill, and resulted in significant increase in expression of genes related to the inflammatory response and metabolism. In contact with water, C60 spontaneously forms a stable aggregate (nano-C60) with nanoscale dimensions (d = 25–500 nm). Prokaryotic exposure to these aggregates even at relatively low concentrations is growth-inhibitory (0.4 ppm) and decreases the rate of aerobic respiration (4 ppm) (Seetharam and Sridhar, 2006).

Processes that control transport and removal of NPs in water and wastewater are yet to be investigated to understand the fate of NPs. Studies on the effect of NPs on plants and microbes are also rare. The fate of nanomaterials in aqueous environment is controlled by many biotic/abiotic processes such as solubility/ dispersability, interactions between the nanomaterials and natural/anthropogenic chemicals in the ecosystem. In addition, ecological risk assessment is essential to understand environmental implications of nanomaterials. Before unknowingly dumping a huge amount of dangerous nanomaterials into the environment, we need to investigate the solubility and degradability of engineered NPs in soils and waters, to establish baseline information on their safety, toxicity and adaptation of soil and aquatic life.

Nanomaterial Exposure:

The exposure can be divided into three wide categories: Occupational exposure, Consumer exposure and Environmental exposure. Exposure of nanotechnology workers and consumers using nanoparticle containing products are near time concern, which needs immediate attention.

Occupational exposure is due to constant involvement of the person with nanomaterial manufacturing and research. With the increasing demand of nanomaterials in the market the exposure of workers making these materials and using nanoparticles in the manufacturing plant is increasing.

Consumer exposure to engineered nanoparticles presents another exposure routes for these materials. Engineered nanoparticles are used in personal care products, ranging from cosmetics to sunscreens, where decreasing the size active ingredients, typically

pigments, yields better performance. It is impossible to assess the quantities and types of nanoparticles in such products as such informations are often protected from public disclosure. In recent studies it has been shown that the titania and zinc oxide particles present in sunscreen are active photcatalyst and can generate free radicals under illumination thus can degrade the sunscreen formulation and moreover can damage the biological molecule thus posing some risk to the consumer (Picatonotto *et al*, 2001, Rossatto *et al*, 2003).

Longer term, there is an opportunity for a much wider exposure of the entire ecosystem to engineered nanomaterials through the water and soil. The concentration of unnatural substances in the environment increases in direct proportion to their use in society. If engineered nanomaterial applications develop as projected, the increasing concentrations of nanomaterials in groundwater and soil may present the most significant exposure avenues for assessing environmental risk.

Reasons for toxicity:

The toxicity of Nanoparticles can be attributed to the features below:

- Surface area to volume ratio of the particles, which increases their interaction with the surrounding molecules.
- Chemical composition of the particle which is responsible for its reactivity.
- Surface charge of the particle is responsible for electrostatic interactions.
- Hydrophobicity and sometimes lipophilic groups which may allow the particle to interact with proteins and membranes respectively.
- Complementarity of nanostructure could cause inhibition of enzyme activity either competitive or noncompetitive.
- Accumulation of an inert particle in the body could also trigger tissue formation around the foreign entity and thus leading to formation of a scar tissue.

Nanoparticle toxicity could occur at two levels i.e. cellular and systemic. However, toxicity uncontrolled at any level could be fatal. These points are much clearer from the literature diagrams and images.

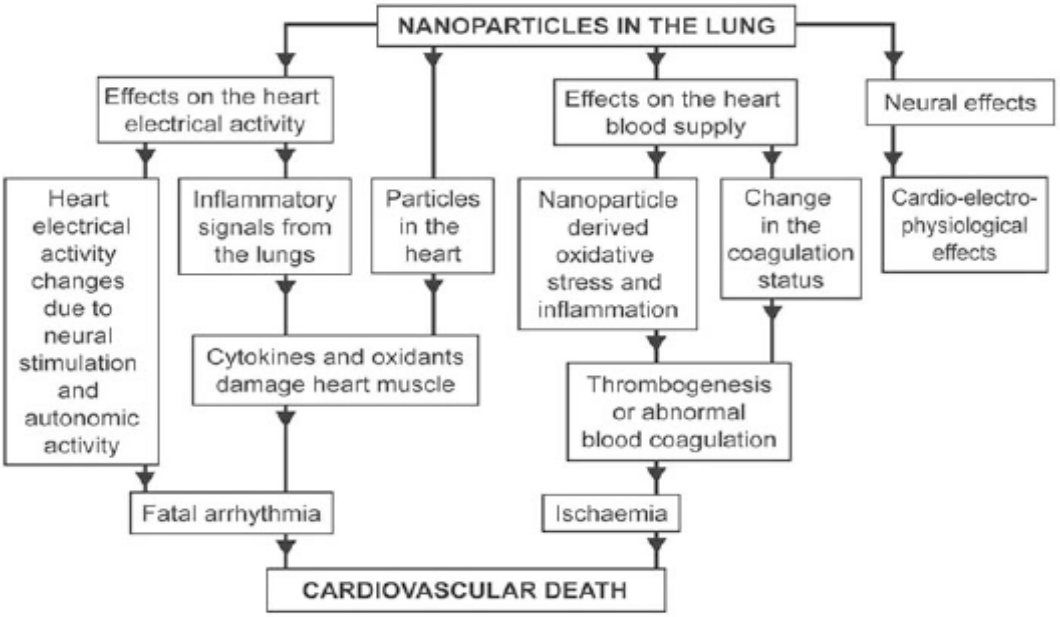
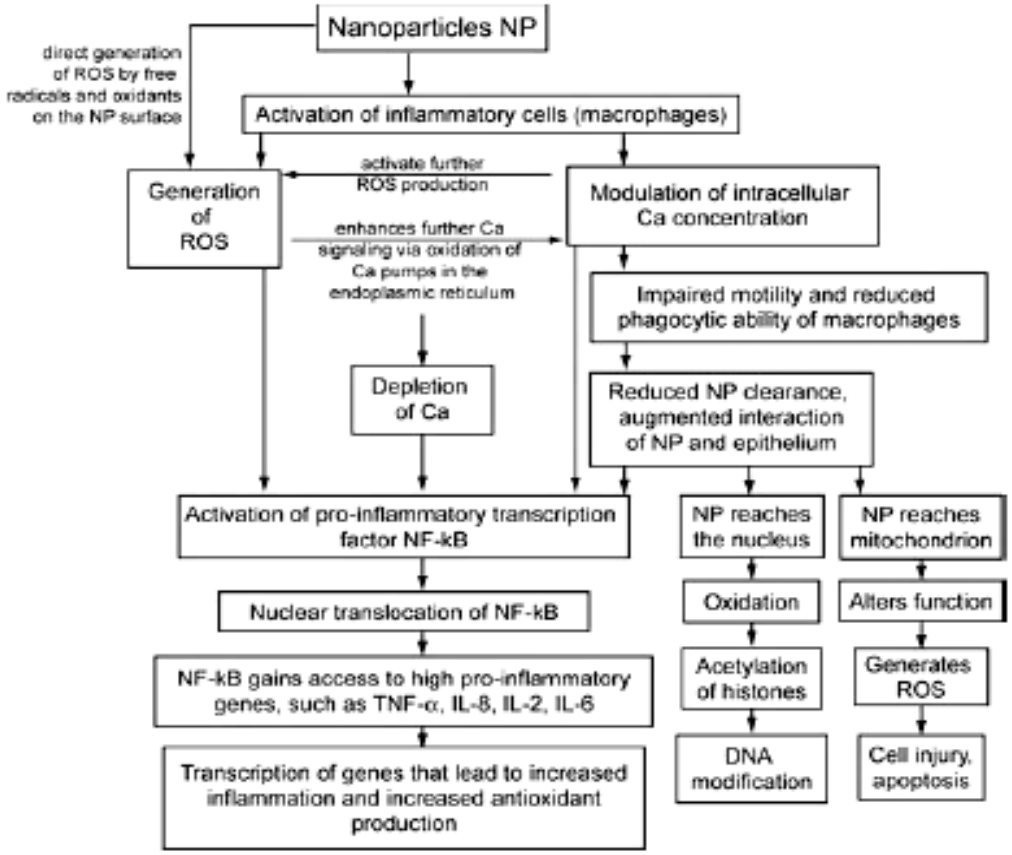


Fig: Scheme of hypothetical mechanisms that link lung with adverse cardiovascular effects as given by Buzea *et al,* 2007.

The following picture depicts the molecular mechanism of toxicity.



As shown above nanotoxic particle could trigger direct or indirect generation of free radicals leading to inflammation, DNA modification or cell injury.

At the organ level, toxicity is mainly due to accumulation of particles as shown below in the example for percentage deposition/accumulation of particles with respect to their sizes.

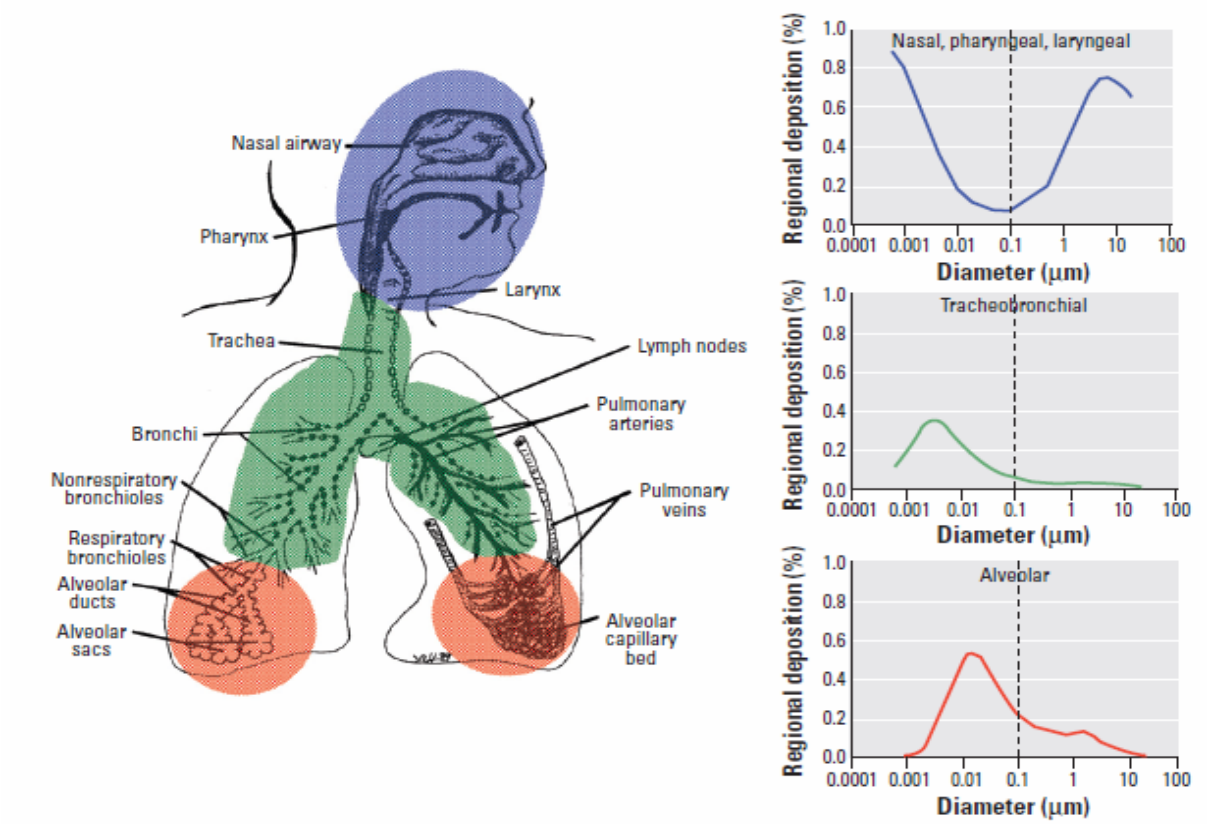


FIG: Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial and alveolar region of the human respiratory tract (Oberdorster *et al,* 2005)

Screening strategies/ toxicity assessment:

Due to expanding use of nanoparticles (NPs) and commercialization of nanotechnology products, exposure of the environment and humans to NPs is bound to increase and an evaluation of their potential toxicity is highly essential. Currently, nanoparticle cytotoxicity testing is based on in vitro methods established for hazard characterization of chemicals. However, screening techniques commonly used for toxicity testing of macro-scale substances may not be appropriate for nanoparticle hazard characterization, but may have to be adapted or modified with regard to their nanospecific properties (Oberdorster *et al*, 2005).

An overview of currently used in vitro cytotoxicity methods is given below (Kroll *et al*, 2006):

1. *Cell Viability*

It is determined by various cellular processes that are used to assess the state of cultured cells *in vitro*.

a) **Detection of mitochondrial activity**

The colorimetric MTT assay is employed to detect mitochondrial activity. It is based on the reduction of the yellow tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) to a purple water insoluble formazan in cells bearing intact mitochondria as shown in MTT assays reported for rat liver cells exposed to nanoparticles of ZnO by Sharma *et al*, 2009.

b) **Detection of LDH release upon necrosis**

The colorimetric lactate dehydrogenase (LDH) assay is based on the oxidation of the yellow tetrazolium salt INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazoliumchloride) to a red formazan has long been used in clinics to evaluate tissue or cell damage. As significant amounts of LDH are released from the cytosol upon cellular necrosis, LDH activity is measured in the cell culture supernatant. This has been applied to assess cytotoxic potential of, for instance, ZnO nanoparticles by Sharma *et al*, 2009.

c) **Annexin V/propidium iodide staining for apoptotic and necrotic cells**

Annexin V (VAC alpha), binds strongly to phosphatidylserine (PS) in a calcium-dependent manner. Phosphatidylserine is normally excluded from the extra cellular side of the plasma membrane, but flips between the inner and the outer side upon the onset of apoptosis. Fluorescently labeled Annexin V can therefore be used to detect apoptotic cells. Necrotic cells will allow Annexin V to bind PS on the inner part of the plasma membrane resulting in false negative results due to cell disintegration. Hence, cells have to be co-stained with propidium iodide (3, 8-diamino-5-[3-(diethylmethyl-ammonio) propyl]-6-phenylphenanthridiniumdiiodide) which will exclusively stain necrotic cells. This has been reported for cells damaged by pure and polyhydroxylated fullerenes by Isakovic *et al*, 2006.

d) **Detection of intact lysosomes via neutral red uptake**

Neutral red (3-amino-7-dimethyl-amino-2-methylphenazinehydrochloride) is weakly cationic, and is thought to be taken up into the cytosol by non-ionic diffusion through the cell membrane to then accumulate in the lysosomes of viable cells, whereas it is excluded from dead cells. The up-take of neutral red

may be detected via fluorescence or absorption measurement. TiO2 nanoparticles in osteoblasts have been tested in NRU assays by Ramires *et al*, 2001.

e) **Detection of the apoptosis marker Caspase-3**

The detection of active Caspase-3 is one of the most commonly used apoptosis assays. Apoptosis may be triggered by different elicitors activating two main signaling cascades that converge in the activation of Caspase-3. The cystein protease Caspase-3 is produced in the cytosol and is activated in the terminal apoptotic cascade by cleavage. As soon as Caspase-3 is activated, cell death is inevitable. Activated Caspase-3 can be detected by measuring the cleavage of a Caspase-3 substrate linked to a chromophore (pNA) or fluorophore (AFC, AMC) that absorbs or emits light when separated from the substrate. As yet, the Caspase-3 assay has been utilized to examine apoptosis in cell culture cells upon exposure, e.g., to quantum dots by Chan *et al*, 2006.

2. *Stress Response*

Cellular stress response is often investigated with H2DCF-DA (2', 7'-dichlorodihydrofluo-
resc (e) in diacetate), which is a widely used probe for the in vitro detection of intra cellular reactive oxygen species (ROS). The acetylated non-fluorescent molecule is taken up by cell culture cells, is presumably trapped in the cytosol by deacetylation and becomes fluorescent upon intracellular oxidation. This has been investigated after cell culture exposure to silica nanoparticles by Deshpande *et al*, 2007.

3. *Inflammatory Response*

In vitro study of inflammatory marker production via enzyme-linked immunosorbent assay (ELISA) enables simple and accurate quantification of inflammatory markers in cell culture supernatants through antibodies and enzymatic detection reactions. ELISA results for lung inflammations caused by exposure to silica nanoparticles have been reported by Rao *et al*, 2004.

Predictive assessment methods:

In order to assess nanomaterial hazards, reliable and reproducible screening protocols are needed to test basic materials as well as consumer products made from them. Achieving this goal has proven to be quite challenging because of the large number of new nanomaterials that are produced continually, their host of novel physico-chemical properties, and uncertainty in how those properties may relate to biological outcomes.

Based on the above assays, some predictive assessment methods have been proposed for screening of toxic nanoparticles. The predictive toxicological approach is defined as establishing and using mechanisms and pathways of injury at a cellular and molecular level to prioritize screening for adverse biological effects and health outcomes in vivo. Specifically, as it relates to nanomaterials, a predictive approach has to consider the physico-chemical properties of the material that leads to molecular or cellular injury and also has to be valid in terms of disease pathogenesis in whole organisms. One such method by Meng *et al*, 2009 proposes that where a link has been established between a mechanistic pathway (such as oxidative stress) at the cellular level and an in vivo outcome (such as allergic airway inflammation or atherosclerosis), in vitro studies can help to predict the hazard potential of a series of ambient particles that differ in composition based on collection site and other
factors.

This concept is encapsulated in the hierarchical oxidative stress paradigm, which posits that ROS production leads to incremental cellular responses that can be classified as antioxidant defense, pro-inflammatory effects, and cytotoxicity.

Thus it is possible to compare the in vitro hazard potential of engineered nanomaterials by conducting cellular assays that reflect each of the tiers of oxidative stress.

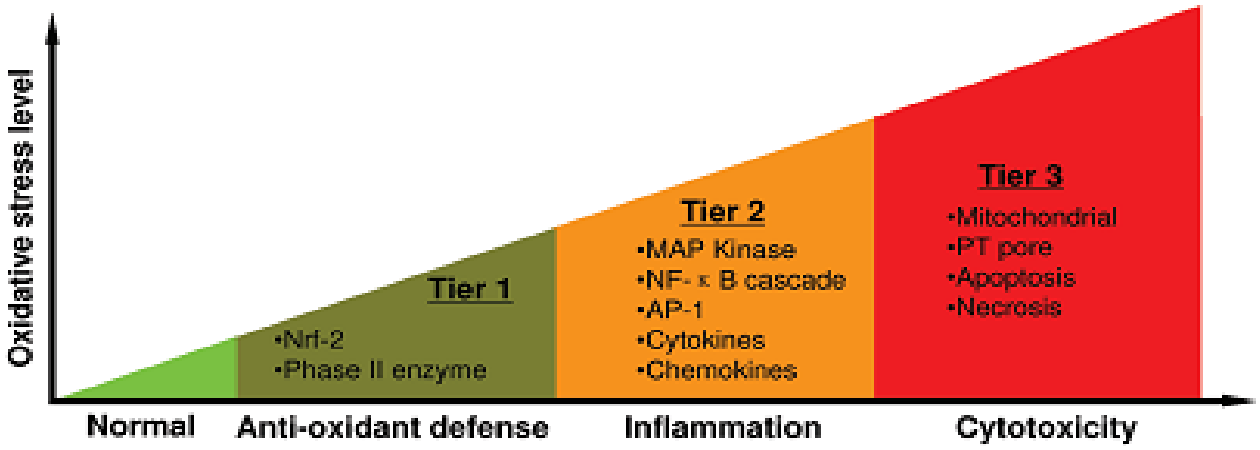


FIG: Use of hierarchical oxidative stress assessment to make predictions about nanomaterial hazards (Meng *et al,* 2009)

Recent advances in cell-based assays allow for toxicity and/or efficacy screening of multiple nanomaterials at multiple concentrations with multiple cell lines, simultaneously. This expansion of experimental design is practically enabled through the miniaturization and multiplexing of the experimental apparatus and method by utilization of either ultra-small 384-well cell culture plates or nanodrop sample chambers on a chip.

Jan *et al*, 2008 demonstrate such a tool based on high-content screening (HCS) technology, a recent advance in the integration and automation of quantitative fluorescence microscopy and image analysis. The researchers test the ability of HCS to identify cytotoxicity in two different systems: CdTe quantum dots in murine neuroblastoma cells, and Au nanoparticles in human hepatocellular carcinoma cells. In the first system, the researchers imaged cells exposed for various time periods and different concentrations of quantum dots capped with thyoglycolic acid (TGA-QDs) and compared this to dots produced in the presence of gelatin (Gelatin-QDs), looking for apoptotic or necrotic cells and effects on neurite outgrowth.

The researchers also used a cocktail of fluorescent probes to visualize other effects on cellular health that could indicate toxicity, including nuclear count, mitochondrial membrane potential, and intracellular free calcium concentration. These same probes were used to evaluate Au nanoparticles. Each test generated a unique "fingerprint" of cytotoxicity that could reliably estimate the hazardous effects of the particles in each cell type. The authors suggest that this tool could eventually be used to screen various nanoparticles to generate a database of toxicity in various cells and tissues.

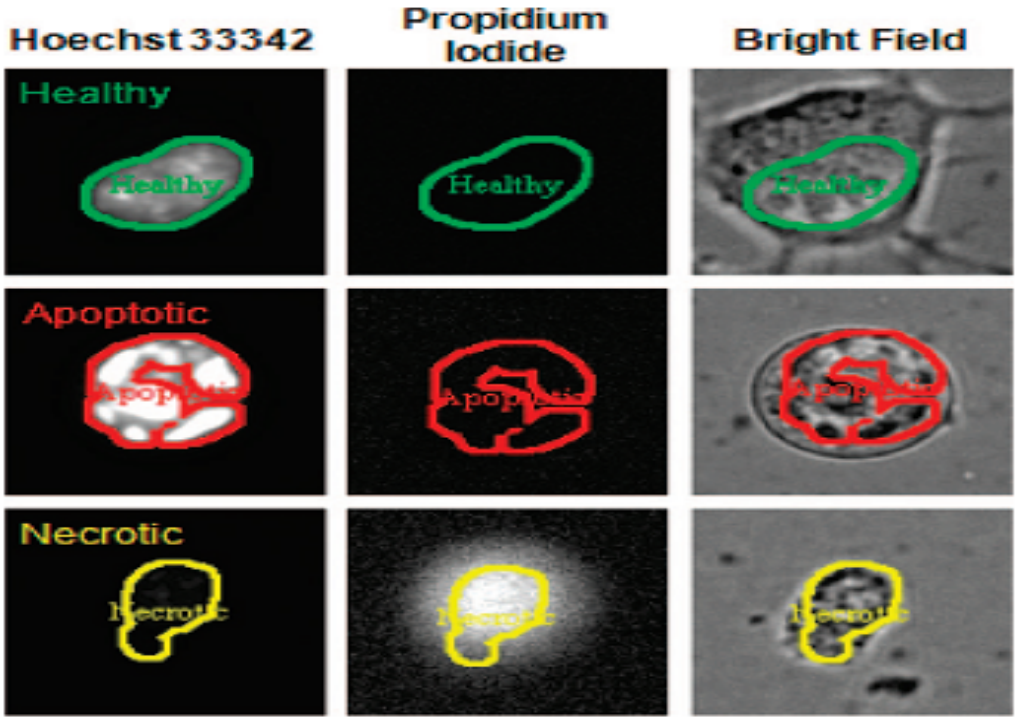


Fig: Representative fluorescence and brightfield images of a healthy (green outline), an apoptotic (red outline), and a necrotic (yellow outline) cell. Cells were stained simultaneously with Hoechst33342 (blue channel, first column) and propidium iodide (red channel, middle column). Outline and classification of cells were generated by the IN Cell Investigator image analysis software using the supervised classification capability (Jan *et al,* 2008).

Fako and Furgeson 2009 report that the small size of zebrafish embryos allows facile, economic medium through-put screening of drugs and drug carriers. Assessment of nanotoxicity can be semi-quantified as sublethal toxicities (viz. survival of the embryo and the severity of phenotypic and gross morphological differences). This nano-toolkit allows several parameters to be varied including concentration, nanomaterial size, chemical composition, density, route of exposure, time of exposure, and the point of embryonic development at which the nanomaterial is administered. To semi quantify these physicochemical metrics and associated nanotoxicity, a modified scoring spectrum is used based on the phenotypic changes of the zebrafish embryos, ranging from 0 (normal phenotype); 1 (minor phenotypic changes); 2 (moderate alterations); 3 (severe embryo deformation); and 4 (embryo death). Unlike traditional biochemical assays that explore specific molecular targets, the zebrafish model relies on the analysis of phenotypic changes. This method allows researchers to bypass several roadblocks commonly associated with current drug discovery efforts, which are based on in vitro biochemical screens followed by in vivo mammalian studies. The zebrafish model serves as a rapid and cost-effective method to conservatively assess the toxicity of novel nanopharmaceuticals, flagging those samples displaying nanotoxicity for closer scrutiny and possible removal from continued drug development.

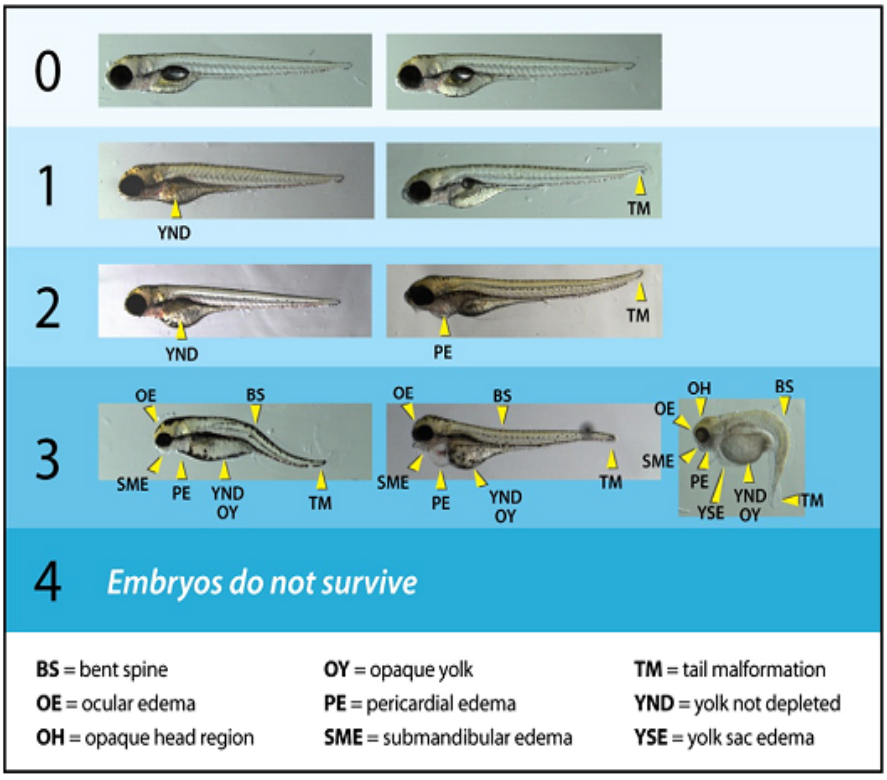


Fig: Semi-quantitative coring system to assess zebrafish nanotoxicity as a function of nanomaterial concentration and exposure time (Fako and Furgeson, 2009)

Table 2
Nanomaterial toxicity as determined by zebrafish testing.

Class of nanomaterial	Zebrafish test	Toxicity reported
Dendrimers	Zebrafish embryos; up to 220 μM G3.5 and G4 ± RGD PAMAM dendrimers	• G3.5—non-toxic • RGD G3.5—non-toxic • G4—dose and time-dependent toxicity • RGD G4—less potent dose and time-dependent toxicity than G4 dendrimers
Fullerenes	Zebrafish embryos; nC_{60} and fullerol ($C_{60}(OH)_{18-18}$) within 96 hpf	• Fullerol—non-toxic at 50 mg/L • nC_{60}—severely toxic at 1.5 mg/L
	Zebrafish embryos; toxic potential of C_{60} following light activation in an antioxidant environment to determine toxicity mechanism	• Toxicity determined to be induced by oxidative stress
Carbon Nanotubes	Zebrafish embryos; SWNT and DWNT	• SWNT—not significantly toxic up to 360 mg/L • DWNT—delayed hatching • Carbon black control—non-toxic
Metal Oxides	96 h zebrafish embryo-larval assay; nZnO, $nTiO_2$, nAl_2O_3, and bulk counterparts	• nZnO and bulk—delayed hatching rate and development • $nTiO_2$, nAl_2O_3 and bulk counterparts—non-toxic
Copper Nanoparticles	Adult zebrafish; 80 nm CuNPs and soluble copper ($CuSO_4$)	• CuNP—48 h LC_{50} of 1.5 mg/L; only 16% of toxicity is caused by dissolved silver • $CuSO_4$—48 h LC_{50} of 0.25 mg/L

Problems faced during cytotoxicity assays:

Although numerous in vitro nanotoxicity studies have already been published, most of the experiments carried out so far have used particles not well characterized regarding their composition and physicochemical properties. However, such a characterization is mandatory since nanoparticles might interact with assay components or interfere with detection systems resulting in unreliable data (Spohn *et al*, 2009).

Due to their large surface per unit mass, nanoparticles display an increased adsorption capacity and biological reactivity as compared to the bulk material. Therefore, many nanoparticles become coated with a set of different proteins when entering a biological fluid. The composition of the resulting protein corona is not only determined by protein identity, but strongly depends on particle surface, size, aggregation state and even on particle concentration. In turn, the coating process determines the effective size, charge and therefore the behavior of nanoparticles under physiological conditions. Fullerenes, for instance, are capable of specific interactions with proteins as shown by the production of fullerene-specific antibodies.

Hence, nanoparticles could potentially confound cytotoxicity data by inducing indirect effects through the adsorption of nutrients and growth factors from culture media. In addition, nanoparticles may directly influence the assay outcome when protein concentration or activity is utilized to evaluate particle toxicity. Furthermore, nanoparticles have been shown to interact with other assay components (e.g., substrates, dyes) thereby introducing artifacts into a number of different in vitro studies. Hydrophobicity of the particles also determines their ability to adsorb proteins and other assay components.

If light absorption or fluorescence detection is used to evaluate particle toxicity, it has to be considered that many nanoparticles display optical properties potentially interfering with the detection system e.g. gold nanoparticles.

Quantum dots or nanoshells, used for medical imaging, can absorb and emit light of different wavelengths and might distort the signal intensity in assays with an optical readout, which is the case for most of the commonly used cytotoxicity methods.

The high surface/mass relationship of nanosized materials results in an excess surface energy enhancing any catalytic activity. A number of different nanoparticles such as metal oxide nanoparticles, fullerenes and silica particles have been shown to produce ROS in cell-free systems. Photoactivated TiO2 and ZnO nanoparticles have been shown to degrade anionic dyes like erythrosine (Hasnat *et al*, 2007), while SWCNTs (Single-walled carbon nanotubes) may interfere with MTT viability tests by oxidizing the substrate MTT (Belyanskaya *et al*, 2007).

Another issue that has to be considered is the pH of nanoparticles in solutions since the most widely used in vitro assays are pH-dependent and may thus be influenced by acidic or basic nanoparticles if the seremain in considerable amounts in the assay mixture. Some metal oxide nanoparticles like Fe2O3 are superparamagnetic and generate strong, local magnetic fields which lead to the production of free radicals that in turn may interfere with cytotoxicity methods based on redox reactions. Nanoparticles that are designed to dissolve in aqueous solutions, like water-soluble QD, or particles that show an intrinsic, size-dependent dissolution in aqueous media, like ZnO, will release metal ions or trace metals when introduced into biological media.

Modification of nanomaterials to make them ecofriendly:

Whether MNMs could be designed to be “safe” and still display the reactivity or properties that make them useful is an outstanding and daunting question that needs urgent attention. Some scientists believe that manipulating MNM structure to suppress the properties that make them toxic might compromise their usefulness and advocate risk management primarily through exposure control. However, the modern chemical industry has demonstrated that a wide range of substances can be reengineered to create safer, greener, and yet effective products and processes.

Encouraging examples include the substitution of branched alkylbenzene sulfonate surfactants, which caused excessive foaming in the environment, with biodegradable linear homologues,5 and the replacement of ozone-depleting chlorofluorocarbons (CFCs) by less harmful and less persistent hydrochlorofluorocarbons (HCFCs).Furthermore, even those MNMs whose value draws from the same chemical activity that may cause adverse biological effects could be amenable to ecoresponsible life-cycle engineering. In these cases, tailored coatings, on-board packaging, or special disposal strategies are worthy of consideration (Alvarez *et al*, 2009).

The most important barrier to achieve environmentally benign design and disposal of MNMs are elaborated below (Alvarez *et al*, 2009):

1) Structure-Activity Relationships for Manufactured Nanomaterials in the Environment.
2) The Nanoparticle Environment Interface.
3) Manufactured Nanomaterial Bioavailability and Sub-lethal Effects
4) Predictive Modeling of Multimedia Fate and Transport
5) Disposal Scenarios and Release Dynamics

Conclusion:

In this new century with increasing demand of nanotechnology apart from the potential benefits, scientist and engineers must also anticipate and characterize potential risk associated with new technology. Although there are currently no conclusive data or scenarios that indicate that these effects will become a major problem or that they cannot be addressed by a rational scientific approach. At the same time, we can no longer postpone safety evaluations of nanomaterials. A proactive approach is required, and the regulatory decisions should follow from there. In addition to facilitating the safe manufacture and implementation of engineered nanoproducts, an understanding of nanotoxicity could also have a positive sequel.

References:

Alvarez P. J. J., Colvin V., Lead J., Stone V.; Research Priorities to Advance Eco-Responsible Nanotechnology; ACS Nano 3:7 (2009): 1616-1619.

Belyanskaya L., Manser P., Spohn P., Bruinink A., Wick P.; The reliability and limits of the MTT reduction assay for carbon nanotubes – cell interaction; Carbon 45 (2007): 2643–2648.

Buzea C., Blandino I. I. P., Robbie K.; Nanomaterials and nanoparticles: Sources and toxicity; Biointerphases 2: 4 (2007): MR17 - MR172.

Chan W. H., Shiao N. H., Lu P. Z.; CdSe quantum dots induce apoptosis in human neuroblastoma cells via mitochondrial – dependent pathways and inhibition of survival signals; Toxicology Letters 167 (2006): 191–200.

Colvin V. L.; The potential environmental effect of nanomaterials; Nature Biotechnology 21:10 (2003): 1166-1170.

Deshpande A., Narayanan P. K., Lehnert B. E.; Silica-induced generation of extracellular factor(s) increases reactive oxygen species in human bronchial epithelial cells; Toxicological Sciences 67 (2002): 275–283.

Fako V. E. and Furgeson D. Y.; Zebrafish as a correlative and predictive model for assessing biomaterial nanotoxicity; Advanced Drug Delivery Reviews 61 (2009): 478–486.

Fischer H. C., Chan W. C. N.; Nanotoxicity: the growing need for *in vivo* study; Current Opinion in Biotechnology 18:6 (2007): 565-571.

Grainger D. W.; Nanotoxicity assessment: all small talk? Advanced Drug Delivery Reviews 61 (2009): 419–421.

Hasnat M. A., Uddin M. M., Samed A. J., Alam S. S., Hossain S.; Adsorption and photocatalytic decolorization of a synthetic dye erythrosine on anatase TiO2 and ZnO surfaces; Journal of Hazardous Materials 147 (2007): 471–477.

Isakovic A., Markovic Z., Todorovic-Markovic B., Nikolic N., Vranjes-Djuric S., Mirkovic M., Dramicanin M., Harhaji L., Raicevic N., Nikolic Z., Trajkovic V.; Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene; Toxicological Sciences 91 (2006): 173–183.

Jan E., Byrne S. J, Cuddihy M., Davies A. M., Volkov Y., Gun'ko Y. K., Kotov N. A.; High-Content Screening as a Universal Tool for Fingerprinting of Cytotoxicity of Nanoparticles; ACS Nano 2 (5) (2008): 928-938.

Kroll A., Pillukat M. H., Hahn D., Schnekenburger J.; Current in vitro methods in nanoparticle risk assessment: Limitations and challenges; European Journal of Pharmaceutics and Biopharmaceutics 72 (2009): 370–377.

Maynard A. D.; Safe handling of Nanotechnology; Nature 444:16 (2006): 267-269.

Meng H., Chen Z., Xing G., Yuan H., Chen C., Zhao F., Zhang C., Zhao Y.; Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles; Toxicology Letters 175:1-3 (2007): 102-110.

Meng H., Xia T., George S., Nel A. E.; A Predictive Toxicological Paradigm for the Safety Assessment of Nanomaterials; ACS Nano 3 (7) (2009): 1620-1627.

Nel A., Xia T., Ma¨dler L., Li N.; Toxic Potential of Materials at the Nanolevel; Science 311 (2006): 622-627.

Oberdorster G., Maynard A., Donaldson K., Castranova V., Fitzpatrick J, Ausman K., Carter J., Karn B., Kreyling W., Lai D., Olin S., Monteiro-Riviere N., Warheit D., Yang H.; Principles for characterizing the potential human health effects from exposure to nanomaterials : elements of a screening strategy; Particle and Fibre Toxicology 2:8 (2005).

Oberdorster G., Oberdorster E., Oberdorster J.; Nanotoxicology : an emerging discipline evolving from studies of ultrafine particles; Environmental Health Perspectives 113:7 (2005): 823–839.

Picatonotto T., Vione D., Carlotti M. E., Gallarate M.; Photocatalytic activity of inorganic sunscreens; Journal of Dispersion Science and Technology 22 (2001): 381-386.

Ramires P. A., Romito A., Cosentino F., Milella E.; The influence of titania/hydroxyapatite composite coatings on in vitro osteoblasts behaviour; Biomaterials 22 (2001): 1467–1474.

Rao K. M., Porter D. W., Meighan T., Castranova V.; The sources of inflammatory mediators in the lung after silica exposure; Environmental Health Perspectives 112 (2004): 1679–1686.

Rossatto V., Picatonotto T., Vione D., Carlotti M. E.; Behavior of some rheological modifiers used in cosmetics under photolytic conditions; Journal of Dispersion Science and Technology 24 (2003): 259-271.

Seetharam R. J. and Sridhar K. R.; Nanotoxicity: Threat posed by nanoparticles; Current Science 93:6 (2007): 769-770.

Sharma V., Shukla R. K., Saxena N., Parmar D., Das M., Dhawan A.; DNA damaging potential of zinc oxide nanoparticles in human epidermal cells; Toxicology Letters 185 (2009) 211–218.

Singh N., Manshian B., Jenkins G. J. S., Griffiths S. M., Williams P. M., Maffeis T. G. G, Wright C. J., Doak S. H.; NanoGenotoxicology: The DNA damaging potential of engineered nanomaterials; Biomaterials 30 (2009): 3891–3914.

Spohn P., Hirsch C., Hasler F., Bruinink A., Krug H. F., Wick P.; C60 fullerene : A powerful anti oxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays; Environmental Pollution 157 (2009): 1134–1139.