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editor@iajavs.com
iajavs.editor@gmail.com

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EXPOSURE, METABOLISM, EVALUATION, AND MODERN PROGRESS IN THE STUDY OF THE TOXICITY OF NANOMATERIALS

M.MANI KUMAR[1],ANUJA. M [2]

Abstract

There has been a rise in both the manufacturing sector and the application of nanomaterials in final consumer items during the last several decades. Nanotoxicology, also known as toxicity assessment, is required in order to comprehend possible adverse effects and the destiny of these manufactured compounds inside the human body. This review is an in-depth examination aimed at delivering up-to-date information on nanotoxicity. It discusses the many toxicities and the research into them, as well as a summary of the many different types of cell death. An entirely new section has been included to detail any and all types of analysis. Latest findings from in vitro, in silico, and in vivo studies of nanomaterials are outlined.

Keywords: Cellular uptake mechanism, physiological endpoint, toxicology, nanotoxicology, human exposure, 2D layered materials.

INTRODUCTION

In the contemporary environment, manufactured goods without a nanomaterial component are quite unusual. Materials with dimensions typically less than 100 nm are known asnanomaterials (NMs)(1), (2). Long before the invention of the diode, renowned scientist Richard Feynman envisioned a quantum universe in which it would be possible to fit the entirety of the Encyclopaedia Britannica onto the tip of a pin. Since then, experimental techniques and the development of analytical tools have given us the tools we need to create such a quantum world(3). All living things are continually exposed to xenobiotics, or foreign substances, like nanoparticles(4). The consumption of manufactured goods containing

1. Assistant professor, Department of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy, Vishakapatnam
2.Assistant professor, Department of Pharmacology, Sri Venkateswara College of pharmacy, Vishakapatnam

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nanomaterials has increased significantly during the past ten years and is anticipated to reach 58000 tonnes by the year 2020. The National Nanotechnology Initiative (NNI), which is planning to spend $1.4 billion on research projects to understand the transport and life cycle of nanoparticles (NPs) in the earth and thereby evaluate its impact on people and the environment, can be used to get an idea of the boom in these nanomaterial industries(5), (6), (7). Metal nanoparticles for groundwater remediation is one application that heavily utilises nanomaterials in all dimensions (0D, 1D, 2D, and 3D);Iron oxide nanoparticles are used to remove arsenic from the ground and treat the water. Titania nanoparticles are used in sunscreens and paints. Fullerene nanotubes are used in tennis rackets and video screens. Silica nanoparticles are used in electronic industries. Zinc oxide nanoparticles are used as potential industrial coatings to block UV rays on wood, plastics, and textiles. Silver nanoparticles are used as potential antimicrobial agents(8), (9).

NANOTOXICOLOGY

Although the name "Nano toxicology" may cause concern in the general public, it is a relatively young field of toxicology that fills the information gap regarding the toxicity caused by nanomaterials. This new area of toxicology, according to Donaldson et al., presentstechniques to access the toxicity caused by nanoparticles. This branch includes fundamental knowledge of the physicochemical effects of nanomaterials and their mechanisms for exposure and uptake for toxicity testing in humans and the environment(10). Although the issue of toxicity assaying is not new, the use of nanomaterials in a rapid score has caused a paradigm shift towards the evaluation of nanotoxicity.The significance of the nano-bio-eco interaction is discussed by He et al. in a more recent review. When nanomaterials are released into the environment, they may experience alteration, which could ultimately change their toxicological profile. Therefore, the environment's dynamics create a degree of uncertainty regarding the fate of the nanomaterials

TYPES OF TOXICITY

BIODEGRADATION

A biologically catalysed process called biodegradation converts complex natural particles into simpler substances. The bacteria take part in the breakdown of complicated organic compounds into simpler substances. The two categories of biodegradation are biotransformation and bio mineralization. The complex organic compounds are broken down into simpler inorganic molecules like carbon dioxide, water, etc. during the bio mineralization process. Contrarily, during the biotransformation process, the molecules go through incomplete breakdown and change into a simpler product that may or may not be an

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inorganic molecule with a variable level of toxicity. Some non-lethal mixtures frequently bio transform into more potently toxic substances. Therefore, biodegradation is one of the likely ways to cause environmental toxicity.(11), (12).

BIOACCUMULATION

Bio concentration is the process of ingesting xenobiotic substances into living creatures by food or body surface, and bio magnification is the process of exchanging the foreign material on organisms found at higher levels of the food chain(13). These xenobiotic compounds typically cause toxicity in living organisms when they are ingested. The best way to illustrate the concept of bioaccumulation is to use the ideal scenario of DDT (Dichlorodiphenyltrichloroethane). DDT is a well-known insecticide with a half-life of 15 years; as a result, even after 100 years, the substance does not totally dissolve. The breakdown of these by-products is significantly even more intense as a result of the biotransformation of DDT, which frequently a result in more harmful exacerbates.As a result,they accumulate in the soil and water sources. These chemicals are absorbed or taken up by microorganisms, fish, and various other living things, which then pass them on to other living things higher up the food chain. Therefore, bioaccumulation is another method of causing environmental toxicity(14), (15).

GENOTOXICITY

Genotoxicity is the term for toxicity that destroys a cell's genetic makeup(16). Genotoxins are substances that compromise the integrity of the genetic material within the cell. The cell nucleus has a variety of issues as a result of genotoxicity. It may result in DNA strand mutations such as duplication, deletion, chromatic aberrations, etc. The DNA damage may cause the cells to become cancerous, and in other cases, it may lead to an aberration withinthe germ cells, which may result in hereditary disorders as diabetes, cystic fibrosis, sickle cell anaemia, haemophilia, etc. To determine and access the danger of diverse materials, numerous in vivo and in vitro genotoxicity techniques have been created(17), (18), (19).

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CYTOTOXICITY

Cytotoxicity is the term for toxicity that results in cell death. It is among the most pervasive toxicological effects in living things. When cells are exposed to hazardous substances, different deadly outcomes, such as total breakdown, rupture of the cell membrane, and destruction of cytosolic components, frequently occur. They might also start the process of "planned cell death" (Apoptosis), which could slow down cell proliferation or cause a decrease in the number of cells(20).

ASSAYS FOR TOXICITY

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CYTOTOXICITY ASSAY

The basic protocol for cell viability quantification using different colorimetric assays: Several enzymes are released as a result of the metabolic activity of the cells, whether they are alive or not. Different colours combine with DNA or enzymes that have been released from cells to form complexes. These complexes produce a variety of colours, and the color's intensity aids in determining whether or not a cell is viable or not(21), (22), (23). In most cases, a little amount of dye is added to a certain amount of cell suspension. A haemocytometer and binocular microscope are typically used to count the cells in a drop of this combination(24), (25). The percentage of viable cells is calculated as follows;

$$viable\ cells\ (\%) = \frac{total\ ko.\ of\ viable\ cells\ per\ ml\ of\ aliquot}{total\ ko.\ of\ cells\ per\ ml\ of\ aliquot} * 100$$

CELL PROLIFERATION ASSAYS

To assess the vitality of cells following exposure to toxins, cell proliferation tests are employed. It even aids in the early diagnosis of a number of cancer forms. The analysis ofcell proliferation can be done using a variety of techniques, including histo-chemical, immune histo-chemical, and flow cytometric procedures. The DNA content of the cellsundergoing viability assessment is directly assessed using the histochemical approach assaying procedure by staining the cells with various fluorescent or radioactive markers, such as [3H] Thymidine and Bromodeoxyuridine. One of the most precise and dependable ways is to measure the DNA content. In this method, a radioactive thymidine analogue replaces thymidine in the DNA of a live cell during mitosis(26).The replication of thymidine uptake within the cell aids in determining the vitality of the cells. Utilizing a scintillation beta- counter, the radioactivity of the DNA isolated from the cells following cell division isquantified, aiding in the estimation of the number of viable cells. It is simple to employ this technique of thymidine uptake in immunohistochemistry and immunocytochemistry tests. There are a few drawbacks to this assaying method, though. The incorporation of radioactive thymidine may result in DNA damage and mutation. Furthermore, the replication process cannot be controlled in vitro when thymidine is added to

dividing cells during mitosis. The addition of radioactive materials is quite expensive, necessitates new infrastructure, and calls for special training.The nonradioactive thymidine analogue bromodeoxyuridine [BrdU], also known as 5-bromo-2'-deoxyuridine (BrdU), is used to synthesise DNA. This marker is used to assess the kinetics of the cell cycle. Anti-BrdU antibodies are utilised in its detection. Although this marker is more effective than [3H] thymidine, it also has some drawbacks. DNA is denaturized and its morphology is destroyed when anti-BrdU antibodies are used for

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analysis. There are a few additional markers, such as 5-iodo-2′-deoxyuridine (IdU) and 5- chloro-2′-deoxyuridine (CldU) that are integrated into newly synthesised DNA and act as thymidine analogues. Similar to BrdU, they have the same set of drawbacks(27).

COMET ASSAY

One of the often used in vitro tests for identifying DNA single strand breaks, alkali labilesites, and crosslinking using the single cell approach typical of cytogenetic assays is theSingle Cell Gel Electrophoresis assay (SCGE), also known as the Comet Assay. It is one ofthe accurate, trustworthy, and affordable techniques for determining DNA damage. The basisfor this test is the separation of DNA fragments using gel electrophoresis. An electric fieldcauses negatively charged DNA fragments to be pulled through an agarose gel. Usingdetergents and salts, cells that have been encased in an agarose solution are further lysed. Complete digestion of the cytoplasm, membrane, mitochondria, and other cell componentsresults from the lysis of the cell with non-ionic detergent and high-molarity sodium chloride.

AMES TEST

It is yet another in vitro test that is used to determine a material's genotoxicity. Any organismor animal can be used to assess the toxicity of any source because DNA in all animals has thesame chemical makeup. This concept was used by Bruce Ames and colleagues to create aquick and accurate process for genotoxicity assessment. To test the toxicity of any source, mutant strains of the bacterium *Salmonella Typhimurium* (*S. Typhimurium*) are utilised. This bacterium already lacks the ability to produce histidine due to a mutation in the gene that codes for the histidine enzyme, despite the fact that histidine is necessary forgrowth.Therefore, if the bacteria suffer a second mutation (reverse mutation) of the geneencoding for histidine enzyme to enable its production in response to a potentially harmfulassault. The identification of possible

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mutagens or carcinogens can be aided by the reversemutation process. The use of a bacterium strain (a prokaryote cell) cannot be precisely usedas a model for human cell metabolism, despite the fact that this method has drawbacks of itsown. Rat liver cells are so frequently utilised to replicate the architecture of human cells(28).

RECENT ADVANCES IN TOXICOLOGICAL ASSESSMENT OF NANOMATERIALS

Engineered nanoparticles are frequently transformed into extremely hazardous components during industrial manufacturing processes. However, the likelihood and degree of these components' contact with live cells determines their risk of exposure. As a result, toxicology studies (both in vitro and in vivo) aid in determining the potential for toxicity. The hazardous potential of some nanomaterials depends on a variety of complex aspects that were covered in

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the preceding section; therefore screening techniques are unquestionably the key element. As a result, the current part seeks to cover an overview of recent developments in toxicological assessment over the past few years and offers a concise synopsis of those works.

METAL NANOPARTICLES

Toxicology studies on metal nanoparticles are urgently needed due to their widespread use. (29)investigated how solubility affected the toxicity's effect on different types of live cells. The toxicity of copper nanoparticles (NPs) with distinctly varied chemical and physical characteristics was compared by the authors. Copper coated with carbon and copper oxides are both tested for cytotoxicity. Due to their surface characteristics, the carbon-coated CuO NPs exhibited regulated toxicity, but the Cu NPs exhibited a Trojan horse-like mechanism and produced considerable toxicity. This study further established the value of physicochemical properties. (30)The study discovered that a Clathrin-dependent mechanism was used to uptake the less soluble copper oxide NPs inside the cell. However, they become partially soluble in the lysosomes' low pH environment, releasing Cu ions that contribute to their cytotoxicity even more. (30) In a different investigation, the scientists assessed the impact of media on the uptake of Au nanoparticles by cells and their cytotoxicity. When NPs enter the cellular medium, they form an NP-protein complex; hence, altering the medium causes the complex to form differently. Both Roswell Park Memorial Institute medium (RPMI) and Dulbecco Modified Eagle's medium (DMEM) were employed. In comparison to DMEM, the RPMI and NP created a combination that demonstrated increased ingestion and higher levels of toxicity.(31)Examined the toxicities of two various consumer creams that included Au NPs. Even after 24 hours, it was seen that the exposed cell lines were not toxicated by the NPs. The toxicity profile of the Au NPs in the rat brain was investigated by (32). In the rat

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brain, Au NPs induced oxidative stress and decreased the activity of antioxidant enzymes like glutathione peroxidase. Additionally, there was an increase incaspase-3, heat shock protein 70, and 8-hydroxydeoxyguanosine, which raised the risk of DNA damage and cell death.

The cytotoxicity of silver nanoparticles on mouse peritoneal macrophage cell lines was investigated by (33). (RAW264.7) exposed for 96 hours at a dosage of up to 1.6 ppm.Ag nanoparticles were internalised through phagocytosis and caused toxicity using a process akin to a Trojan horse. Cellular viability reduced over time and in relation to dose. Furthermore, the increased ROS levels and inflammation were blamed for the Ag NPs' toxicity(33). The difference in the cytotoxic effects of Ag nanoparticles and Ag ions was examined by the authors of another report. The exposed cells experienced high

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degrees of toxicity due to the Ag nanoparticles. The added nanoparticles altered histone methylation, which further decreased the quantities of haemoglobin.

SAFETY GUIDELINES FOR HANDLING NANOMATERIALS

As studies on novel characteristics and their impact on toxicity are inconclusive, the toxicological profile of nanoparticles is a chapter that is constantly changing. 409, 410 In recent years, institutes and industry have adopted guidelines for the safe and protective handling of nanomaterials(34), (35). While these recommendations do not offer specific measures to get around the issue, they do serve as the gold standard for best practises for mitigating risk(36). The fundamental structure for all of these recommendations is outlined asfollows;

1. Evaluating dangers and locating uncertainties in the production and application of nanomaterials.
2. Developing and implementing a successful strategy to address and manage the risks
3. Preventing and limiting the required exposure
4. Requiring the continuation of the current process and the application of the requiredcontrols.
5. Assessing exposure levels and carrying out appropriate surveillance.
6. starting a sufficient health analysis
7. Procedures and measures that must be started in preparation in case of emergencies ormishaps.
8. Ensuring that students or employees of the institution or industries are properly trained, informed, and supervised.
9. In addition to these recommendations, the serious threat that nanoparticles offer is the subject of serious debate among academics and policymakers. In a recent book chapter, the global steps made to address health and safety concerns were highlighted(37).

Organization	**Objectives**
The Organisation for Economic Cooperation and Development (OECD)	• Developing and implementing a successful strategy to address and manage the risks. • Preventing and limiting the required exposure. • Analysing risk assessment methods. • We produced nanomaterials using sharing of

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	information and identification opportunity to improve and strengthen capability for risk assessment.
National Institute for Occupational Safety and Health (NIOSH)	• Strategic planning and research are conducted to advance the commercialization of nanotechnology and to serve as a global and national resource for implementing the results of studies on the uses and implications of nanotechnology into best practises for occupational safety and health.
EU NanoSafetyCluster	• It focuses on initiatives that deal with toxicology, ecotoxicology, and exposure assessment, mechanisms of interaction, risk assessment, and standardisation, among other elements of nanosafety. • Holds workshops and seminars to inform people, especially those working in nanotechnology.
Modena Cost	• Studying the synthesis of engineered nanomaterials (ENM) with regulated composition, size, area, and nanotexture is one of the specific objectives. • To create methods for immobilising ENM in matrices and on substrates that have the least impact possible on the desired characteristics and surface reactivity, and to find the pertinent datasets for modelling quantitative nanostructure-toxicity relationships (QNTR).
Federal Research Institute of Nutrition and Food (Max Rubner-Institut, MRI)	• Discovering and analysing nanoparticles in complicated matrices, like food, etc. • study of nano-sized carriers for bioactive substances • Nanomaterials and food matrix components

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	interacting
Federal Institute of Occupational Safety and Health (BAuA)	• Grouping of nanostructured materials for reducing risk and protecting consumers, workers, and the environment
Federal Institute for Materials Research and Testing (BAM)	BAM is active in FP7 initiatives that are supported by the EU: • (FP7) NanoDefine: By addressing the issues regarding the availability of suitablemeasuring techniques, reference material, validated methods, acceptable for all stakeholders, and delivering an integrated and interdisciplinary approach, NanoDefine seeks to support the governance challenges associated with the implementation of the nanomaterial legislation. • FP7 NanoValid The development of new reference methods and certified reference materials, such as those for characterization, detection/quantification, dispersion control, and labelling, as well as those for hazard identification, exposure, and risk assessment of ENs, is the primary goal of NanoValid.
Federal Ministry of Education and Research (BMBF)	• Safe handling of manufactured nanomaterials: Nanocare investigating how ENM affects both people and the environment
Federal Environment Agency (Umweltbundesamt, UBA)	• Safe Handling of Nanoparticles • Nanomaterials produced in a lab • Examining the Effects on Health and the • Environment
Federal Institute for Risk Assessment (Bundesinstitut fur Risikobewertung, BfR)	• To establish new concepts and principles using information from value chain implementation studies. • To make Safe-by-Design a cornerstone in the

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	verification of an unique produced nanomaterial • establishing methodologies for the grouping and classification of nanomaterials in accordance with their toxicity and biological impacts to aid risk assessment • grouping of nanostructured materials for reducing risk and protecting consumers, employees, and the environment

COMMERCIAL APPLICATIONS OF NANOMATERIALS

Although the usage of synthetic nanomaterials in commonplace products has undoubtedly improved our quality of life, it has also raised questions among regulatory bodies and academic scholars about potential negative impacts. The usage of modified NMs has increased dramatically over the past ten years, but policymakers and regulators are concerned about it. Even without the knowledge of the authorities, there is a potential that untested materials will find their way onto the consumer market. Food, personal care products, and other household goods are under the regulatory authorities' and environmental advocacy groups' constant surveillance(38).

DISCUSSION

Despite all the hype surrounding nanomaterials, its physicochemical impact on living things is still very unclear(39). Analyzing the impact of nanomaterials on the environment is difficult because it depends on a complex range of factors, including the size, shape, surface properties, (31)and charge, and so on of the nanomaterial. However, just like with any other contaminant, the impact on the environment depends on its physicochemical properties, which influence the environment.Globally, intensive research is being done on various nanomaterials to change their behaviour in any way (morphologically, optically or chemically). However, the scientific knowledge of how they affect living beings is not

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included. The behaviour of the nanomaterial and the way it affects living cells can both completely change as a result of a single adjustment in the material. The complicated material characterization and continually changing process of material synthesis present a significant challenge even in the presence of state-of-the-art toxicity evaluation tools. According to certain studies, nanomaterials might cause diseases like dermatitis, rhinitis, pleural, interstitial lung disease, lung infections, TB, respiratory embolism, breast cancer, lung growth, immune

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system disorders, and more.Therefore, in the present situation, a thorough understanding of nanomaterial toxicology (Nanotoxicology) is essential. A methodical understanding of nanotoxicity can assist researchers in selecting materials that are environmentally friendly and in setting priorities for research to reduce real risks to the environment and human health(40).

CONCLUSIONS

In retrospect, there is a definable lack in our understanding of toxicity and its effects on the human world, despite the existence of numerous research and reviews in the literature. As was previously said, the toxicity of nanomaterials depends on a number of physicochemical characteristics. Changing any one of these characteristics would have an impact on the toxicity pattern and lead to a different physiological endpoint. Different nanomaterials show a lack of association between in vitro, in vivo, and in silico data.As a result, during the past few decades, the demand for toxicity libraries of nanomaterials has gradually increased. The toxicity of some novel manufactured nanomaterials can be predicted and prevented by combining the various toxicity assaying approaches and creating a hub for highlighting the possible toxicity of the materials. Therefore, in-depth research is necessary to match the volume of various nanomaterials generated and heavily utilised in industrial applications. Additionally, this will contribute to the expansion of our fundamental knowledge of toxicology and its effects on both individuals and the environment.

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