An insight into impact of nanomaterials toxicity on human health (#82973)

First submission

Guidance from your Editor

Please submit by **30 Jul 2023** for the benefit of the authors (and your token reward) .

Literature Review article
This is a Literature Review article, so the review criteria are slightly different.
Please write your review using the criteria outlined on the 'Structure and Criteria' page.

Image check
Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

Files

Download and review all files from the materials page.

2 Figure file(s)
2 Table file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **STUDY DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Is the review of broad and cross-disciplinary interest and within the scope of the journal?
- Has the field been reviewed recently? If so, is there a good reason for this review (different point of view, accessible to a different audience, etc.)?
- Does the Introduction adequately introduce the subject and make it clear who the audience is/what the motivation is?

STUDY DESIGN

- Article content is within the Aims and Scope of the journal.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.
- Is the Survey Methodology consistent with a comprehensive, unbiased coverage of the subject? If not, what is missing?
- Are sources adequately cited? Quoted or paraphrased as appropriate?
- Is the review organized logically into coherent paragraphs/subsections?

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Meaningful replication encouraged where rationale & benefit to literature is clearly stated.
- Conclusions are well stated, linked to original research question & limited to
- Is there a well developed and supported argument that meets the goals set out in the Introduction?
- Does the Conclusion identify unresolved questions / gaps / future directions?

supporting results.

Standout reviewing tips

3

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. ...* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be*

improved upon before Acceptance.

An insight into impact of nanomaterials toxicity on human health

Wajhul Qamar [1], **Shweta Gulia** [2], **Mohammad Athar** [3,4], **Razi Ahmad** [5], **Mohammad Tarique Imam** [6], **Prakash Chandra** [2], **Bhupendra Pratap Singh** [7], **Rizwanul Haque** [8], **Md. Imtaiyaz Hassan** [9], **Shakilur Rahman** [Corresp. 10]

[1] Department of Pharmacology and Toxicology and Central Laboratory, King Saud University, Riyadh, Saudi Arabia

[2] Department of Biotechnology, Delhi Technological University, New Delhi, India

[3] Department of Medical Genetics, Umm Al-Qura University, Makkah, Saudi Arabia

[4] Science and Technology Unit, Umm Al-Qura University, Makkah, Saudi Arabia

[5] Department of Chemistry, Indian Institute of Technology, Delhi, New Delhi, India

[6] Department of Clinical Pharmacy, College of Pharmacy, Prince Sattam Bin Abdul Aziz University, Al Kharj, Saudi Arabia

[7] Department of Environmental Studies, Deshbandhu College, University of Delhi, New Delhi, India

[8] Department of Biotechnology, Central University of South Bihar, Gaya, Bihar, India

[9] Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia University, New Delhi, India

[10] Department of Medical Elementology and Toxicology, Jamia Hamdard University, New Delhi, India

Corresponding Author: Shakilur Rahman
Email address: rshakilur25@gmail.com

In recent years, advances in nanotechnology have significantly influenced electronics manufacturing, industrial processes and medical research. Various industries have seen a surge in the use of nanomaterials. However, several researchers have raised the alarm about the toxicological nature of nanomaterials, which appear to be quite different from their crude forms. This altered nature can be attributed to their unique physico-chemical profile. They can adversely affect human health and the environment. Nanomaterials that have been released into the environment tend to accumulate over time and can cause a significant impact on the ecosystem and organisms with adverse health effects. Increased use of nanoparticles has led to increased human exposure in their daily lives, making them more vulnerable to nanoparticle toxicity. Because of their small size, nanomaterials can readily cross biological membranes and enter cells, tissues, and organs. Therefore, the effect of nanomaterials on the human environment is of particular concern. The toxicological effects of nanomaterials and their mechanisms of action are being researched worldwide. Technological advances also support monitoring new nanomaterials marketed for industrial and household purposes. It is a challenging area because of the exceptional physiochemical properties of nanomaterials. In this updated review, we have discussed the diverse toxicological perspective of nanomaterials, including the use of different types of nanoparticles and their physiochemical properties responsible for toxicity, their potential routes of exposure in humans, their bio- distribution and mechanism of toxicity, and

various *in vivo* and *in vitro* methods of assessing the toxicity of nanomaterials. Finally, this review will provide a detailed insight into nonmaterial's toxicological response, which can be beneficial in designing safe and effective nanoparticles.

Reviewer comments:
Dear authors, hope you understand my notes, comments, and suggestions. All observed issues and suggestions were made considering the improvement of the manuscript, which does not invalidate the submitted work.

General aspects of the article:
It is written in adequate English.

Title: An insight into impact of nanomaterials toxicity on 1 human health
Abstract: the summary is appropriate to the article.
Keywords: Some keywords are already present in the title. I recommend changing them for others, enhancing the paper's visibility.

General comments:

The article is presented in good condition, with proper English language.

It is possible to see a vast quantity of references read by the authors to write the submitted manuscript. Most of the references are recent and is possible to see an effort of the authors in searching updated articles. However, old references are still being used and could be replaced by newer ones.

Short sentences must be used in order to make information clear.

In the entire article, the authors should only use the toxicity on non-human species to clearly support or relate the effects on human health - the main subject of the text. If this is not the case, avoid using the information.

Some words and expressions must be standardized, e.g. 'physicochemical' without the hyphen, 'in vivo' and 'in vitro' without the hyphen and in italic form.

This paper has great potential and could be improved upon before Acceptance.

Detailed observations/suggestions:

☑ **1. Introduction:**
This section is well presented, making the reader aware of the subject that will be addressed in the article and the purpose of the literature review.
1.1. The rationale of the study: Important part of the manuscript, as it presents the purpose of the review.
1.2. Search methodology: It describes how the article was constructed. As an exploratory study, the bibliography search was quite comprehensive.

☑ **2. Classes of nanomaterials:**
I understand this section as basic information on which classes of nanomaterials are divided, being responsible to support some terms used in the article. Then, it must have a very clear explanation.

In addition, I missed an indication of other studies for each subitem, in case the reader desires to go deep into specific knowledge. Perhaps a figure could illustrate the different classes of nanomaterials.

☑ **3. Physico-chemical properties of nanomaterials responsible for toxicity:**

There is a good introduction part of this section, followed by subsection '3.1. Effects of physicochemical properties of nanomaterials on a cellular level'. This subsection is quite interesting but very concise. It could be more explored.

Table 1: It comprehends some examples of nanotoxicity towards physicochemical properties of nanomaterials. In the text caption, it is mentioned that contains information about damage to mammal cells, i.e., by in vitro testing. However, Demir's study is a review paper and not original research. Liu et al. 2014a research was performed in vivo, conducted using animal exposure (mice).

Please, review these issues, because it is expected a list of research that has tested and shown nanotoxicity in mammal cells. I recommend restricting the cited works to the mentioned conditions (nanotoxicity on mammal cells based on new original research). Maybe, considering the main subject of the manuscript, it should only be based on human cell lines.

Subsection '3.2. Effects of physicochemical properties of nanomaterials on the environment' is out of context as the article, as mentioned in the title and previous sections, should focus on human health. There is no indication that nanotoxicity is related to human health in this part of the text; that humans have the same risks compared to other organisms; or even that the harmful effects of nanomaterial to other organisms could directly or indirectly jeopardize humans. As the purpose of this item is unclear, I recommend removing or making it clear and coherent with the article's subject. Inserting humans into an ecosystem and as part of a trophic web could explain this subitem, also associating this info with some exposure routes, such as gastrointestinal.

Table 2: There is no indication of why those nanomaterials were selected to be shown in the table. Were the most produced, used, or tested nanomaterials? Or the selection was purely random? As the table contains important data, the criteria for selection should be mentioned to support its objective.

☑ **4. Commonly used nanomaterial and their adverse health effects:**

The starting paragraph must be revised. In this part is mentioned that nanoparticles can 'naturally occur' from diesel exhaust (line 307). As it stands, it could be understood that the generation of nanoparticles by a combustion engine is a natural process. However, it would be more appropriate to indicate its origin as a by-product or a derived form. Perhaps it would be interesting to point to 'derived forms' and 'produced forms' of nanoparticles rather than 'naturally occurring' and 'artificial' forms. Please, review.

The last part of this introduction is fine, but it is not clear why only metal and carbon-based nanoparticles were preferred to be explored in the following subsections.

Subsection '4.1. Toxicity of metal-based nanoparticles' emphasizes silver, copper, and titanium nanomaterials. Among the metallic nanoparticles, these are the most studied, being already recognized by their toxicity. However, the authors insist to present data on adverse effects on other non-human species (lines 329-331). This is confusing as human health is the aim of the discussion. Please, review.

Subsection '4.2. Toxicity of Carbon-based nanoparticles' describes information regarding the toxicity of these nanomaterials. However, research conducted with non-human species is mentioned again (e.g. lines 351 and 358). Despite animals and other species could indicate harmful effects that also could happen to humans, this extrapolation must be carefully made. So, it would be better to restrict the text to research that has tested human cell lines exposed to carbon-based nanoparticles. Also, a major part of this subsection is based on a single review work (lines 358-370). Please, review.

☑ **5. Routes of exposure of the nanoparticle:**

In my perception, this entire section should be placed after item 3. For me, it is more logical to describe the physicochemical properties, the main routes of exposure, and then the toxicity induced by nanomaterials. I recommend the authors consider this suggestion to enhance the quality of the article.

This section brings the main conceptions of exposure routes related to nanoparticles. There are no issues.

☑ **6. Biodistribution:**

It could be renamed as 'Nanomaterial toxicokinetics'. If section 5 is moved, section 6 must follow. Info in this section is adequate and clear. However, the term 'pharmacokinetic' is often used for nanoparticles. I suggest the use of 'kinect' as nanoparticles are not always a pharmaceutical.

Figure 1: This figure is relevant considering the routes of exposure and the kinect of nanoparticles. Nevertheless, transformations in arrows line, size, and width must be indicated below the figure.

☑ **7. Mechanisms of toxicity of nanoparticles:**

This is the 'icing on the cake' section of the article. The only recommendation that I do is to change the order of subsections to 1) oxidative stress, 2) genotoxicity, 3) immunomodulation, and 4) inflammation.

Figure 2: The figure is only for illustration. Maybe it could be more related to the kinetics of nanoparticles and then to cellular toxicity by ROS generation.

☑ **8. Methods for assessing the toxicity of nanomaterials:**

This section is interesting to guide some researchers on how nanotoxicity can be evaluated. I have no further suggestions on this part.

☑ **9. Conclusion and Future Prospects:**

This section comprises and closes the article. However, I missed some suggestions regarding how stakeholders and decision-makers should address nanoparticle safety. For example, pesticides must be tested and evaluated regarding their toxicity on non-target animals, such as bees, before being sold. Should not nanoparticles pass through the same process? After all the presented information, I missed this author's statement.

An insight into impact of nanomaterials toxicity on human health

Wajhul Qamar[1], Shweta Gulia[2], Mohammad Athar[3,4], Razi Ahmad[5,a], Mohammad Tarique Imam[6], Prakash Chandra[2], Bhupendra Pratap Singh[7], Rizwanul Haque[8], Md. Imtaiyaz Hassan[9], Shakilur Rahman[10,b,*]

[1]*Department of Pharmacology and Toxicology and Central Laboratory, College of Pharmacy, King Saud University, Riyadh, KSA. Email: wqidris@ksu.edu.sa*

[2]*Department of Biotechnology, Delhi Technological University, New Delhi, India. Email: shwetagulia_2k20phdbt02@dtu.ac.in, prakashchandra@dtu.ac.in*

[3] *Science and Technology Unit, Umm Al-Qura University, Makkah, Saudi Arabia.*

[4] *Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia. Email: mabedar@uqu.edu.sa*

[5]*Department of Chemistry, Indian Institute of Technology Delhi, New Delhi, India. Email: razi.jmi@gmail.com*

[6]*Department of Clinical Pharmacy, College of Pharmacy, Prince Sattam Bin Abdul Aziz University, Al Kharj, Pin-11942, Saudi Arabia, m.imam@psau.edu.sa*

[7]*Department of Environmental Studies, Deshbandhu College, University of Delhi, New Delhi, India. Email: bpsingh0783@gmail.com*

[8]Department of *Biotechnology, Central University of South Bihar, Gaya, Bihar, India. Email: rhaque@cub.ac.in*

[9]*Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, Jamia Nagar, New Delhi 110025, India. Email: mihassan@jmi.ac.in*

[10]*Department of Medical Elementology and Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi, India. Email: rshakilur25@gmail.com*

Current address:

[a]*Quality and Research Department, Anantaa GSK Innovations Pvt Ltd., DLF Industrial Area, Faridabad 121003, India.*

[b]*Department of Anesthesiology and Perioperative Medicine, University of Alabama at Birmingham, Birmingham, Alabama*

Address for correspondence:

Dr. Shakilur Rahman, Department of Medical Elementology and Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi, India. E-mail: rshakilur25@gmail.com

ABSTRACT

In recent years, advances in nanotechnology have significantly influenced electronics manufacturing, industrial processes and medical research. Various industries have seen a surge in the use of nanomaterials. However, several researchers have raised the alarm about the toxicological nature of nanomaterials, which appear to be quite different from their crude forms. This altered nature can be attributed to their unique physicochemical profile. They can adversely affect human health and the environment. Nanomaterials that have been released into the environment tend to accumulate over time and can cause a significant impact on the ecosystem and organisms with adverse health effects. Increased use of nanoparticles has led to increased human exposure in their daily lives, making them more vulnerable to nanoparticle toxicity. Because of their small size, nanomaterials can readily cross biological membranes and enter cells, tissues, and organs. Therefore, the effect of nanomaterials on the human environment is of particular concern. The toxicological effects of nanomaterials and their mechanisms of action are being researched worldwide. Technological advances also support monitoring new nanomaterials marketed for industrial and household purposes. It is a challenging area because of the exceptional physiochemical properties of nanomaterials. In this updated review, we have discussed the diverse toxicological perspective of nanomaterials, including the use of different types of nanoparticles and their physiochemical properties responsible for toxicity, their potential routes of exposure in humans, their bio- distribution and mechanism of toxicity, and various *in vivo* and *in vitro* methods of assessing the toxicity of nanomaterials. Finally, this review will provide a detailed insight into nonmaterial's toxicological response, which can be beneficial in designing safe and effective nanoparticles.

Keywords: Nanotoxicology, Nanomaterials, Toxicology, Human health, Environmental health

As these keywords already appear on the title, I recommend changing them for others, which could enhance the paper's visibility.

1. Introduction

Toxicology is a multidimensional science branch involving the interaction of biological organisms, chemicals, and other agents to detect possible hazards (Krewski et al. 2020). The toxicity or toxic insult can be defined as any adverse biological effects of a product, irrespective of its origin. Many chemicals have been studied, and their toxicity and impact mechanisms have been published over several decades (Carlin et al. 2015; Genuis & Kyrillos 2017; Jaishankar et al. 2014; Lanphear 2017). Several studies have documented toxic events caused by nanomaterials over the past decade (Barabadi et al. 2019; Chen et al. 2020; Ferdous & Nemmar 2020). If we accept the declaration of the father of toxicology, Paracelsus (1493-1541), that "All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy", the nano-sized materials (Nanoparticles, nanomaterials) are not an exception to this statement. In the case of nanomaterials, the surface area of the nanoparticles, relative to their general counterparts, intensifies their effects even at lower doses (Ahmad et al. 2014a; Ahmad et al. 2015; Ahmad & Sardar 2015b; Khatoon et al. 2015; Sardar & Ahmad 2016; Sardar et al. 2014). Under the aegis of 'nanotoxicology,' the possible toxicological effects of nanomaterials are being examined. Nanomaterials possess unique catalytic, mechanical, and optical properties and electrical conductivity mainly due to their size in nanometers (Ahmad et al. 2023; Ahmad & Sardar 2015a; Ding & Chen 2009; Ghosh et al. 2021a; Ghosh et al. 2021b; Goel et al. 2023).

However, the branch of nanotoxicology may seem new, but it contains the same basic toxicological concepts. The instruments and techniques for the analysis can vary from the basic ones, depending on the nature of the substance (nanomaterials in this case). The associated health issues are also growing with the increasing demand for nanomaterials in different industries and household goods. Toxicological approaches play a key role in regulating numerous substances and products' impacts on human and environmental health (Klaine et al. 2008; Singh et al. 2021; Stark et al. 2015).

Nano-sized materials have shown their benefit over their crude forms and have their place in the formulations of products. There are now recorded formulations containing one or more nanomaterials in many cosmetic products, pharmaceuticals, paints, and pigments, etc. For example, nano-sized titanium dioxide (TiO_2) is used in biocatalytic processes and cosmetics

(Ahmad et al. 2019a; Ahmad et al. 2013), and silver nanoparticles are used as bactericides and make stain and odour-resistant clothing (Baker et al. 2005; Rajeshkumar et al. 2021).

Nanotoxicology can address all the health and environmental issues raised by nano-sized materials. The physicochemical causes, exposure paths, biodistribution, molecular determinants, genotoxicity, and regulatory aspects are incorporated into nanotoxicology (Arora et al. 2012; Goel et al. 2021). The toxicity of nanomaterials depends on the fundamental association of the materials' physicochemical assets with the molecular components of an organism's cellular pathways. Nanoscale materials can negatively affect human health despite having more innovative and distinctive physicochemical features than bulk materials. Nanomaterials are highly reactive and may be harmful when interacting with biological systems and the environment because of quantum size effects and enormous surface area to volume ratio (Ganguly et al. 2018; Sahu & Hayes 2017). Studies on animals and humans have revealed that NPs do not stay in one place after being inhaled or consumed; instead, they spread to several organs, including the liver, heart, spleen, brain, lungs, and gastrointestinal system, leading to adverse health effects (Bahadar et al. 2016; Takenaka et al. 2001).

Describe it when written for the first time.

This review aimed to get deeper insights into the toxicity profiling of nanomaterials and their consequential impacts on human health. It outlines the different classes of nanomaterials and their physiochemical properties responsible for toxicity. It discusses some commonly used nanoparticles and their associated adverse effects. It also discusses the possible routes of exposure to humans and animals (respiratory tract, skin, and gastrointestinal tract) and its biodistribution. Furthermore, it discusses the detailed toxicological perspective with genotoxicity, immunotoxicity, oxidative stress, and inflammation as a mechanism of cellular toxicity. The discussion desk also discusses an entire section on the methods for assessing the toxicity of nanomaterials. Importantly, the present review summarizes the overall concept behind the toxicology of nano-sized materials and includes the advancements in nanotoxicology research and methods during the past two decades.

The rationale of the study

Check the font size from line 120 to 133.

The growing usage of nano-sized materials in various sectors and applications drove this study on nanotoxicology and its effects on human health. Although nanoparticles have many advantages, there are

concerns about their possible negative impacts on human health and the environment. In order to ensure the safe and responsible use of these materials, it is necessary to recognize the potential hazards involved. By studying biodistribution, genotoxicity, molecular drivers, and regulatory factors, the present study seeks to offer more understanding of nanomaterials' toxicity profile. Researchers can comprehend the underlying processes of toxicity and possible detrimental impacts on human health and the environment by researching the physicochemical features of nanomaterials and their effects on cellular pathways. Nanotoxicology investigates the toxicity of nanomaterials and their interactions with biological systems to address these issues.

As nanotechnology develops, regulatory considerations are also crucial. Establishing suitable regulations and standards for the responsible management and application of nanomaterials is essential. This study can direct the safe and responsible development and implementation of nanotechnology by thoroughly evaluating the toxicity of nanomaterials and including regulatory issues. It is intended to fill the gaps in concepts and understanding of nanotoxicology for students and researchers and provides up-to-date information covering nanoparticles' toxicological aspects, which will eventually be beneficial to evaluating the hazards and risks of nanomaterials and developing sustainable and safe nanotechnology.

Again, check the font size.

Overall, this study advances the science of nanotoxicology by shedding important light on the possible health risks of nanomaterials. This study provides decision-making information and aids in risk mitigation by knowing their toxicity and taking regulatory considerations, offering the safe application of nanotechnology for the benefit of human health and the environment.

Search Methodology:

I believe that 'nanotoxicity' would be the proper expression, as nanotoxicology is the study area.

This study used a survey and search approach to look at the effects of nanotoxicology on human health. The subsequent actions were taken:

Review of the literature: Using particular keywords relating to nanotoxicology, nanomaterials, toxicity profiling, human health, and environmental consequences, relevant scientific literature was carefully examined from databases including PubMed, Scopus, and Web of Science. Articles were chosen based on predefined criteria, which included an emphasis on molecular components, cellular routes, physicochemical reasons, exposure pathways, biodistribution, genotoxicity, and regulatory issues.

Extraction of key data from chosen papers on the physicochemical characteristics of nanomaterials, experimental procedures, toxicity assessment methods, molecular determinants, and regulatory issues.

Data were thematically analyzed in order to spot patterns, trends, and knowledge gaps in nanotoxicology. A thorough grasp of the subject was provided by the synthesis of the findings.

Interpretation and Conclusion: The data were analyzed to reach relevant findings, respond to study-related questions, and obtain a better understanding of the toxicity of nanomaterials and their effects on human health. This study used a survey and search technique to acquire pertinent data, pinpoint knowledge gaps, and present a thorough analysis of nanotoxicology and its consequences for human health.

This information was already mentioned before, then I suggest the revision of this part, keeping it related mainly with the conclusion section.

2. Classes of Nanomaterials

For each subsection of classification, a review study could be cited. Then, if the reader desires to go deep into this knowledge, it could be consulted.

Nanomaterials can be classified based on size, morphology, state, and chemical composition depending on their practical application. Generally, NMs are ranked based on their dimensionality, morphology, state, and chemical content (Gleiter 2000; Saleh 2020). This classification is also based on their size, which varies between 1-100 nm in at least one dimension.

Describe it when written for the first time.

The above paragraph must be rewritten because the information presented is redundant.

2.1. Classification of nanomaterials based on Dimensions, morphology and state

There are numerous structural, dimensional, morphological and compositional measures based on which nanomaterials are classified, which provide unique properties to nanomaterials that affect their fate and toxicity in the environment and, ultimately, human health (Pokropivny & Skorokhod 2007; Saleh 2020).

Based on shape and dimensions, nanomaterials are further distributed into four classes. Zero-dimensional (0D) nanomaterials have all their dimensions <100 nm. These include nanomaterials shaped as cubes, spheres, polygons, nanorods, and hollow and nanomaterials existing as quantum dots (QDs). One-dimensional nanomaterials have only one dimension on the nanoscale. Examples of 1D nanomaterials are nanorods, nanotubes, nanofibers, nanowires, and metallics. Two-dimensional nanomaterials have two dimensions in the nanoscale, including thin films, nanoplates, and so on, which may be in single or double layers. 2D nanomaterials can exist in crystalline or amorphous forms. 3D nanomaterials have more than two dimensions. Nanotubes, fullerenes, honeycombs, and fibres are a few examples of 3D nanomaterials (Aversa et al. 2018; Pokropivny & Skorokhod 2007; Shiau et al. 2018). Morphologically, nanoparticles can be distributed based on their formation as flat spheres and the aspect ratio (High and low), including nano zigzags, nanopillars, nanospheres, nanopyramids, etc. Another class of nanomaterials is centered on the

states in which they can exist, such as suspension, colloid, or dispersed, e.g., magnetic nanoparticles. Elements of nanomaterials are an essential factor in categorizing them (Saleh 2020).

2.2. Classification of Nanomaterials-based on the chemical composition

The largest category of Nanomaterials is based on chemical composition and constitutes nanomaterials that are single constituents, composite, and inorganic and organic nanomaterials (Saleh 2020). This class comprises nanomaterials like composites, carbonaceous, metallic, metallic oxide, polymeric, etc., and nanomaterials (Pokropivny & Skorokhod 2007; Saleh 2020). This word is correct here? Please review the sentence.
Four types of nanomaterials categories are based on chemical composition: carbon-based nanomaterials, inorganic-based nanomaterials, organic-based nanomaterials, and composite-based nanomaterials (Majhi & Yadav 2021). Carbon is the core constituent of carbonaceous nanomaterials, while metallic nanomaterials are differentiated based on the metals they are made from. Carbon-based nanomaterials include graphene, fullerene, single-walled carbon nanotube, multi-walled carbon nanotube, carbon fiber, activated carbon, and carbon black. The metals forming metallic nanomaterials are usually Cu, Ag, Al, Zn, Fe, etc., thus having catalytic and adsorptive properties (Kim & Lee 2018; Li et al. 2019; Vijayakumar et al. 2022). Through certain processes like hydrothermal, doping or sol-gel reactions, metallic oxide nanoparticles (e.g., TiO_2, Fe_2O_3, and SiO_2) can be produced. This class of nanomaterials comes with additional applications, like sensors, semiconductors, etc. (Saleh & Fadillah 2019). The organic-based nanomaterials are formed from organic materials, excluding carbon materials, for instance, dendrimers, cyclodextrin, liposomes, and micelle. Next, Bimetallic nanomaterials are a combination of metals with different properties, such as Ag-Cu and Fe-Cu, which are further classified based on their structure (Hao et al. 2020; Lozhkomoev et al. 2019; Saleh & Fadillah 2019). Other nanomaterials based on composition are branched dendrimers, ceramic nanomaterials, nanogels, core-shell nanomaterials and polymeric nanomaterials (Saleh 2020). A class of nanomaterials is designed for drug delivery known as Lipid-based nanomaterials (García-Pinel et al. 2019). These nanomaterials could target certain hydrophilic and hydrophobic molecules in the human body and are relatively stable, less toxic, and specific, e.g., nanostructure lipid carriers, solid lipid nanoparticles, and liposomes. Liposomes are mainly formed of cholesterol and phospholipid compounds (García-Pinel et al. 2019; Zhong & Zhang 2019). Nanomaterials also occur as Quantum dots and can absorb UV light, and white light and reemitting them at certain

wavelengths. Hence, quantum dots come up with exclusive optical properties and electronic ones (Alavi et al. 2021).

3. Physico-chemical properties of nanomaterials responsible for toxicity

Studying nanomaterials from the physicochemical angle is essential for understanding the toxic effect. Properties such as particle shape, size, composition, stability, structural dimensions, concentration, nanoparticle morphology, and surface properties (area, roughness, energy, charge), including functional groups, are responsible for the interaction between the nanoparticle and the target molecule or cell (Chandra et al. 2013). These interactions also determine the entry of nanoparticles inside the cellular pathways, their translocation, exposure and further interactions with the molecules and entities inside the cell. Such interactions can be exemplified as hydrophobic, electrostatic, steric, solvent, and biological interactions. There is various mechanism of nanoparticles toxicity by catalytic activity and cellular dysfunction like the release of more reactive ionic form from nanoparticle surface, ROS generation, lipid peroxidation, protein denaturation, inflammation, endothelial dysfunction, mitochondrial perturbation, phagocytic function impairment and altered cell cycle regulation (Gupta & Xie 2018). In vivo Study conducted Please, fix. by Zhang et al. demonstrates size-dependent nanoparticle toxicity, indicating that 10 nm and 60 nm PEG-coated gold nanoparticles increased alanine transaminase and aspartate transaminase levels resulting in liver injury (Zhang et al. 2011). In an in vitro experiment conducted by Ng at al., MRC5 lung cells treated with ZnO NP-treated released a substantial amount of extracellular lactate dehydrogenase and had lower cell viability, indicating cellular damage and cytotoxicity (Ng et al. 2017).

3.1. Effects of physicochemical properties of nanomaterials on a cellular level

All the properties mentioned above are responsible for the toxicity displayed by nanomaterials in particular ways, like increased cellular uptake (endocytosis), receptor stimulation, oxidation, ionic species generation-related toxicity, mutagenesis, ROS generation, affected metabolic activity, and so on. Several research studies using different types of cells have been conducted to examine nanotoxicity generated due to the physicochemical properties of nanoparticles and engineered nanomaterials. One such study reported the toxic effect of graphene family nanomaterials on

ocular cells and the possible risk of applying graphene family nanomaterials in biomedical (Borandeh et al. 2021). Numerous studies currently state the possibility of the application of graphene family nanomaterials in ocular drugs, contact lenses, and ocular drug delivery due to their large π-conjugated aromatic structure and specific surface area. For example, graphene oxide and reduced graphene oxide are analogs of graphene family nanomaterial and affect cell viability (Borandeh et al. 2021; Ge et al. 2018; Oliveira et al. 2022). Graphene oxide nanoplates with 11 nm dimensions can enter the human mesenchymal stem cell nucleus more quickly and cause genotoxicity than a graphene oxide nanoplate with a 3 μ dimension. Also, nanoplates higher in concentration can cause more toxicity than those at low concentrations, as stated in studies (Borandeh et al. 2021; Ge et al. 2018; Oliveira et al. 2022). Reduced graphene oxide nanoplates of micron size were highly toxic at a high concentration of 100 μg/ml.

Please, fix: μm.

In contrast, the smaller-sized reactive graphene oxide nanoplate (11 nm), at a concentration as low as 0.1-1 μg/ml, caused genotoxicity on translocation to the nucleus (Akhavan et al. 2012). The shape of the nanomaterial is another nanotoxicity-determining factor in living cells. In another study about the effect and mechanism of nanotoxicity, it was concluded that there was growth inhibition and apoptosis in primary rat osteoblasts by hydroxyapatite nanoparticles which are needle-shaped and short. At the same time, this was less likely in the case of nanoparticles, having spherical, long rod shapes (Jain & Patel 2021; Xue et al. 2012). The chemical composition of nanomaterials is also a nanotoxicity causal factor. Particle dissolution, for example, can generate toxicity imparting ionic species. Nanotoxicity of nanoparticles like CuO, CdO and TiO_2 was found to cause DNA damage, which was high in the case of CuO but comparatively lower in TiO and CdO (Franklin et al. 2007; Zhu et al. 2013). **Table 1** depicts few examples of nanomaterials' size, shape, and concentration-dependent toxicity in mammalian cells.

Please, fix to O2.

3.2. Effects of physicochemical properties of nanomaterials on the environment

The properties of nanomaterials that are accountable for nanotoxicity among living cells are the source of nanotoxicity in the environment. These factors can affect microorganisms, plants, soil quality, water quality, quality of air, crop production, pollution levels, and aquatic life. To simplify this physical and chemical effect, it should be first understood that when nanomaterials encounter water, atmosphere or soil, a corona forms on their outer surface. Thus, the nanomaterials interact

with their corona and molecules present on the cell wall and membrane (Docter et al. 2015; Foroozandeh & Aziz 2015; Juárez-Maldonado et al. 2021; Nel et al. 2009). Consequently, conditional to the corona composition, nanomaterials with the same physical and chemical properties and the same corona composition but variable properties will unlikely affect a cell or microorganism. Table 2 presents a few examples of nanomaterials and their physicochemical properties affecting mammalian cells and their associated mechanism. Although it hasn't been understood how nanomaterials or nanoparticles produce toxicity in flora and microorganisms, reactive oxygen species (ROS) generation can stimulate specific defence mechanisms in cells or microorganisms that can lead to cell death (Smerkova et al. 2020; Zhao et al. 2020). However, ROS generation is not the only mechanism that can cause cytotoxicity, and antimicrobial molecule Reactive nitrogen species (RNS) generation enabled by nanomaterials can also cause extreme levels of cellular stress that can lead to toxicity (Balážová et al. 2020; Juárez-Maldonado et al. 2021; Wang et al. 2020b; Zhao et al. 2020). Several nanoparticles are known for inducing cytotoxicity and inhibition in soil microorganisms by acting as potent antimicrobial agents, and cytotoxicity depends on the concentration of nanomaterials to which the microbe is imperilled (Abdulla et al. 2021; Kumari et al. 2014). A higher concentration of nanomaterials has been reported to cause DNA damage, lipid peroxidation, ROS generation, ion release, ATP depletion and cell damage in soil microflora which is directly associated with reduced nitrification, growth inhibition, enzyme activity decrease and alterations in microbial structure. Toxicity of nanomaterials towards microorganisms also depends upon the type and nature of the nanomaterial, e.g., nanoparticles metallic in nature such as ZnO, CuO, Ag, CeO_2 or Fe_3O_4 nanoparticles could amend the composition of soil microflora (McKee & Filser 2016), but some nanoparticles like carbon nanoparticles are known to be more toxic to microbes in soil (Chen et al. 2019). Nanomaterials have a variable effect on distinct microorganisms depending on their concentration, size or parent material. In a research study based on the effect of a few engineered nanomaterials on two different organisms, it was deduced that the growth inhibition of both microbes varied when subjected to unrelated nanomaterials with different concentrations. Nanomaterials such as ZnO (4 mg L^{-1}), In_2O_3, γ-Al_2O_3, and TiO_2 inhibited the growth in Skeletonoma Costatumin up to 100% (with ZnO).

Please, pay attention to scientific name and follow the recommendation of use italic font.

In comparison, in the case of nanomaterials like α-Al_2O_3 and SnO_2 (1280 mg L^{-1}), inhibition did not occur fully, being 35% in the case of α-Al_2O_3 and 24% with SnO_2 (Wong et al. 2020). As a matter of size, shape, material, and coating, it has been described that increased concentration of nanomaterials to a certain level at which it becomes toxic and bio-stimulatory activity starts decreasing (Agathokleous et al. 2019). Similarly, nanomaterials bigger in size have less effect on cells (Ball 2017; Klaine et al. 2008).

4. Commonly used nanomaterial and their adverse health effects

Please, fix the redundancy.

Nanotechnology, the study of creating particles with sizes on the nanoscale scale, is a fast developing field of study. NPs (with a diameter of 100 nm or less) are found in the air and in some workplaces, exposing humans to them. Nanoparticles (NPs) can be either naturally occurring (like dust, diesel exhaust, and welding fumes) or artificially produced (like titanium dioxide, carbon black, carbon nanotubes, silver, zinc, and copper oxide) (Buzea et al. 2007). Engineered nanomaterials have exceptionally large surface areas and high percentages of their component atoms on the surface because they have at least one dimension smaller than 100 nm. Many nanoparticles acquire exceptional reactivity as a result, which opens up new avenues for their application in electronics, medicine, environmental cleanup, catalysis, and consumer goods. Concern regarding the possible hazardous effects of nanoparticles due to their usage or inadvertent release into the environment has grown in tandem with the growing interest in the advantages they may provide (Dreher 2004; Moore 2006; Nowack & Bucheli 2007).

This should be explained earlier. Please, revise.

Is this a natural source of NPs?

4.1. Toxicity of metal-based nanoparticles

The elements gold, silver, copper, nickel, cobalt, zinc, and titanium dioxide have all been used to create nanoparticles. A significant portion of the expanding nanotechnology business is made up of metal and metal oxide nanoparticles. The use of metallic nanoparticles is rising, and with that comes the risk that these particles will be released into the environment.

As well as being utilized as a bactericide, silver nanoparticles have found use in the production of stain- and odor-resistant textiles, sensors, inks, and catalysts (Baker et al. 2005). Antimicrobial

products using silver nanoparticles are becoming increasingly popular in the Western Hemisphere. More and more research points to silver nanoparticles' extreme toxicity to mammalian cells (Braydich-Stolle et al. 2005; Gopinath et al. 2008). Brain, liver, and stem cells are all vulnerable to the toxicity of silver nanoparticles (Braydich-Stolle et al. 2005; Panyala et al. 2008). Bactericidal treatments made with nano copper are increasingly popular (Cioffi et al. 2005). Coatings and sealants with these additives have better thermal and electrical conductivity and may be used to filter air and liquids and can be applied to integrated circuits and batteries. Several aquatic species, including zebrafish, have had it shown that copper and silver nanoparticles are extremely hazardous to them (Griffitt et al. 2008). TiO_2 nanoparticles are one type of manmade nanoparticle with widespread application in food colorings, sunscreens, and cosmetics. Because of their great stability, anti-corrosion, and photocatalytic capabilities, titanium dioxide nanoparticles (TiO_2-NPs) were mass-produced and employed extensively. It is one of the most extensively utilized nanomaterials and has lately found use in several agricultural industries. Emerging data suggest that TiO_2 nanoparticles can cause inflammatory and genotoxic reactions in various animal and human cell lines (Reeves et al. 2008; Xu et al. 2009). Damage to DNA is caused by the high levels of hydroxyl free radical produced by TiO_2 nanoparticles (Reeves et al. 2008; Zhu et al. 2008).

4.2. Toxicity of Carbon-based nanoparticles

Carbon is one of the components noticed earlier and is readily available. Diamond, graphite, amorphous carbon, carbon nanotubes (CNTs), graphene, and fullerenes compose the allotropes. Due to their special physicochemical and electro-mechanical properties and biological compatibility. This sentence is loose in the paragraph. But, they also possess harmful effects on biological processes and cellular compartments. Koike and Kobayashi, 2006 investigated carbon-based nanoparticles' chemical and biological oxidative effects (CBNP) and found that CBNPs with smaller sizes have greater oxidative potential than larger alveolar epithelial cells (Koike & Kobayashi 2006). In general, nanomaterials exist in various forms and structures such as particles, tubes, fibers, spheres, points, cubes, truncated triangles, wires, and films that influence nanoparticle (NP) kinetics and transportation in the environment (Gonzalez-Munoz et al. 2015; Madannejad et al. 2019; Walters et al. 2016). Srikanth et al., 2015 assayed the cytotoxicity of four types of carbon nanomaterial (carbon nanowire, carbon nanotube, graphene, and fullerene) on L929 mouse fibroblast cancerous cells and found that graphene was the most toxic substance with average toxicity of 52.24%

followed by CNTs, fullerene, and CNW, based on morphology, concentration, and duration of exposure. Srikanth et al., 2015, data indicate that toxicity variations are related to different structural arrangements and aspect ratios (Srikanth et al. 2015). In another study, observe the role of surface area in oxidative and DNA damage potential of carbon-based (CB) nanomaterial. They found that CB with smaller and larger surface areas demonstrate higher potencies for oxidative stress and DNA damage in rats (Almaimani 2021; Chuang et al. 2015). Carbon nanotubes (CNTs) have drawn much scientific interest and possible applications due to their specific physical, biological, electrical and mechanical properties. As the production and application of CNTs on a wide-scale increase, the general public is more likely to be directly or indirectly exposed to CNTs, prompting substantial attention to human health and safety concerns related to CNTs. Several significant factors, such as impurities, amorphous carbon, surface charge, form, volume, aggregation, and layer numbers, explain the differences in the experimental results of nontoxicity. The in vivo actions and fate of CNTs can also be affected by exposure paths, including inhalation, intravenous injection, or dermal or oral exposure. Oxidative stress, inflammatory reactions, malignant transformation, DNA damage and mutation (errors in chromosome number and mitotic spindle disruption), the development of granulomas, and interstitial fibrosis are the underlying mechanisms of CNT toxicity. These results provide valuable insights into the de novo nature and safe application and risk assessment of carbon nanotubes for human health (Liu et al. 2013).

5. Routes of exposure of the nanoparticle

The human body is a complex structure of several organs, such as the heart, liver, lung, kidney, etc. Any damage (cellular, biochemical, or molecular level) can disrupt the entire system and lead to various pathological conditions. A drug has to penetrate the body through certain routes to cause specific harm or general toxicity. These are referred to as exposure channels, which involve the skin (dermal exposure), gastrointestinal (oral exposure), and lung (inhalation exposure) exposure (De Matteis 2017). Oral exposure is the most prevalent natural route of exposure to a significant range of toxicants, including nanoparticles. However, any material with a lipophilic nature is more likely to penetrate the body or systemic circulation when it comes into contact with the skin. In this scenario, inhalation is the most sensitive route of exposure, and the skin is the least sensitive. Exposure to nanomaterials in food or the atmosphere via either of the above routes. In addition to these exposure pathways, numerous clinical pathways of administration for various therapies are

'Route' is a better term.

This sentence is loose in the paragraph.

383 also in operation (Romeyke & Stummer 2012). These include administration using a syringe
384 through an intravenous (IV), intraperitoneal (IP), subcutaneous (SC), intradermal (ID),
385 transdermal (TD), intramuscular (IM), etc. These pathways are primarily used for therapeutic
386 administration during treatment.

387 *5.1. Gastrointestinal tract*

388 Nanoparticles are gradually making their place in the formulations of consumer goods. Food, via
389 the gastrointestinal tract, is a significant source of nanoparticle exposure. Flavour enhancers, food
390 pigments and supplementations can be part of the range of nanomaterials. As a source of oral
391 exposure to nanomaterials, some non-edibles can also work. They can shed the nanomaterials used
392 in their development. These products may include toothbrushes, baby bottles, pacifiers, containers
393 for processed or unprocessed food items, and containers for processed or unprocessed food items
394 (Bergin & Witzmann 2013). Possible exposures to nanomaterials include consuming marine food
395 (fish or shellfish) with accumulated nanomaterials for toxic waste ingestion or absorption (Ahmad
396 et al. 2019b; Gaiser et al. 2012; Toussaint et al. 2019; Wang et al. 2020a). Researchers have
397 proposed that the absorption mechanism of nanoparticles is possibly endocytosis in the
398 gastrointestinal tract (Frohlich & Roblegg 2012). In contrast to larger counterparts, nanoparticles
399 of comparatively smaller sizes are more readily absorbed.

400 Silver nanoparticles, gold nanoparticles, titanium dioxide (TiO_2) nanoparticles, copper
401 nanoparticles, silicon dioxide (SiO_2) nanoparticles, quantum dots, carbon-based nanoparticles
402 (multi- or single-walled carbon nanotubes) and polymer/dendrimer nanoparticles (Bergin &
403 Witzmann 2013) are the materials recorded to exhibit their toxic effects after ingestion.

404 *5.2. Skin*

405 Skin serves as a physical barrier for all the xenobiotics and pathogenic microorganisms. It can
406 prevent the entry of microorganisms and hydrophilic substances. Skin remains in direct contact
407 with the environment but provides less interaction with the toxic components present in the
408 ambience. It works more as a physical barrier; it has a small surface area (1.73 m^2, an adult human's
409 average body surface area). It has less blood supply and an upper layer of dead cells as a physical
410 obstruction for most hydrophilic xenobiotics. Lipophilic substances (<600 Dalton [Da]) can easily

cross the skin passively and enter systemic circulation (Barry 2001; Coates et al. 2019). There are several reports which dealt with the skin penetration of the nanoparticles. Still, they show discrepancies in results likely related to differences in techniques and methods, laboratory conditions, and the absence of standardized evaluation protocols (Crosera et al. 2009; Ghasemiyeh & Mohammadi-Samani 2020).

In most cases, skin exposure to nanoparticles occurs through cosmetics. Various personal care products contain nano-sized components in the form of nano-emulsions and microscopic vesicles. The issue of dermal penetration is highlighted by applying TiO_2 and ZnO nanoparticles to cosmetic products. Sunscreens contain insoluble titanium dioxide (TiO_2) or zinc oxide (ZnO) nanoparticles, which are efficient filters of ultraviolet (UV) light (Geppert et al. 2020; Lu et al. 2015; Mohammed et al. 2019). Several in vivo toxicity studies, including in vivo intravenous studies, showed that TiO_2 and ZnO nanoparticles are non-toxic and have excellent skin tolerance (Nohynek & Dufour 2012). But, this may not be the case with other nanoparticles with potential applications in cosmetics. Nohynek and Dufour, in their review, concluded that the nanoparticles in skin care products or sunscreens pose no or negligible threats to humans in terms of toxicity. Factors that may affect the absorption of any agent include (i) skin integrity and regional variation, (ii) dimensions of orifices, aqueous pores, and lipidic fluid paths, and (iii) density of appendages (Baroli 2010).

5.3. Respiratory tract

Because of its importance for survival and exposure to a toxicant, inhalation deserves special attention. Chimney smoke, car fumes, cigarette smoke, forest fire smoke, and other harmful inhalants are inescapable in today's world. Inhalation exposure is more significant in environmental interactions. The lung and the skin both serve as interfaces between the body and its external environment, but their anatomical and physiological structures and functions are distinct. The physiology of the lungs is sensitive to even little variations in air composition. Toxic substances in the air are taken into the lungs along with the breath. Lung toxicities are very common due to the direct interaction of lung alveolar epithelium with inhaled toxicants. The sensitivity of the lung toward a variety of airborne toxic agents, including carcinogens, leads to a brisk inflammatory response (Wong et al. 2016).

The number and exposure of nanomaterials are rapidly increasing in the present environment. Most of them can cause damage to the lung. Airborne toxicants, including nanoparticles, are significant concerns regarding human health. Many reports are available regarding the toxicological effects of nanoparticles after inhalation (Bierkandt et al. 2018). More specifically, several effects of inhaled nanoparticles are attributed to their (i) direct effects on the central nervous system, (ii) their translocation from the lung into the bloodstream, and (iii) their capacity to invoke inflammatory responses in the lung with subsequent systemic effects (Borm & Kreyling 2004).

The nano-sized particles which can be inhaled and pose a serious threat to human health include suspended particulate matter (SPM), combustion-derived nanoparticles (CDNP), asbestos fibres and silicon nanoparticles (Borm et al. 2006). CDNPs are generated in some scenarios, including internal combustion engines, large-scale coal burning for power generation, and industrial processes where they often could be produced along with larger particles. CDNPs may include diesel exhaust particulates, welding fumes, nanoparticulated carbon black, and coal fly ash. These all cause oxidative stress and lung inflammation after inhalation (Donaldson et al. 2005). Asbestosis results from inhalation exposure to nano-sized asbestos fibers, resulting in mesothelioma (cancer of the pleural lining of the lung), lung cancer, and laryngeal cancer (Offermans et al. 2014; Ross 2014). Most lung ailments are associated with acute or chronic inflammation. The main reason behind the sensitivity of the lung lies in its anatomy, which is constituted by a large number of alveoli, increasing its surface area up to 75 m^2, and a large number of vasculature. High blood flow in the lungs makes it more vulnerable to inflammatory responses and the primary organ responsible for most nanoparticle-related toxicities. Chronic obstructive pulmonary disease (COPD), an accumulation of emphysema, bronchitis, and fibrosis, is primarily a chronic inflammatory disease. Other examples of lung inflammatory ailments include bronchiolitis, asthma, acute inflammation after toxic exposure, pneumonitis, etc. Most of the lung cancer cases are also associated with chronic lung inflammation-related episodes. It has been found that COPD and lung cancer positively correlate in most cases. Such an episode's exposure to any nano-sized particle may impose similar or more serious consequences. Several studies have indicated that nanoparticles react aggressively when exposed to biological systems due to their physic-chemical properties being different from their normal forms (Cheng et al. 2013).

6. Biodistribution

Due to their physicochemical properties, which are different from their crude counterparts, the nanomaterials may have a different distribution pattern in a biological system. Several pharmacokinetics studies provide a set of their distribution pattern, and the information is efficiently applied in the nanomaterials' medical diagnostics, pharmacology, and toxicology (Madru et al. 2013; Singh & Pai 2014). Studies indicate that the distribution of the nanoparticles initially depends on the particle shape and size (Kreyling et al. 2014; Toy et al. 2014). The dependence of the biodistribution of nanoparticles on their physical properties signifies the role of the physicochemical nature of the nanomaterials in toxicological effects and the different toxicological nature of the nanomaterials when compared with their crude forms (Duan & Li 2013). Reports also indicate the dependency of the biodistribution of nanoparticles on phagocytosis. In a rat model, Li et al.,(2014)showed that the distribution of polyethylene glycol-coated polyacrylamide nanoparticles across highly perfused organs depends on phagocytosis (Li et al. 2014). In their study, various target organs include the spleen, liver, bone marrow, lungs, heart, and kidneys. However, apart from phagocytosis, the nanoparticles also interact with the plasma proteins as soon as they enter the physiological environment. According to their specific functions, adsorbed proteins can be divided into opsonins and dysopsonins. Opsonins often induce the rapid blood clearance of nanoparticles, while dysopsonins benefit prolonged blood circulation (Gao & He 2014). Biodistribution can greatly be influenced by nanoparticle-protein interactions (Baimanov et al. 2019; Ovais et al. 2020). Though biological tools like phagocytosis and plasma proteins contribute to the ~~pharmaco~~kinetics of nanoparticles, the physicochemical properties of the nanoparticles themselves and the way how they are modified for delivery into the system also directly influence their distribution in the organ system (Ahmad et al. 2021; Patra et al. 2018). In certain cases, polyethylene glycol (PEG) density and lipids on the nanoparticle surface, such as Lipid-Calcium-Phosphate (LCP) nanoparticles, can influence the ~~pharmaco~~kinetics and biodistribution of the nanoparticle. Liu et al.,(2014,)reported that the delivery of LCP nanoparticles to hepatocytes depends on the concentration of PEG and the surface lipids (Liu et al. 2014b). LCP nanoparticles could be directed from hepatocytes to Kupffer cells by decreasing PEG concentration on the particle surface. This study signifies the role of the physical characteristics of the nanoparticles in their ~~pharmaco~~kinetics and biodistribution. It boils down to that the

biodistribution of nanoparticles is controlled by a complex array of interrelated, physicochemical and biological factors of the nanoparticles. These factors should be taken into consideration while planning a ~~pharmaco~~kinetics study of any nano-sized material. A schematic representation of the toxic fate of nanoparticles is given in **Fig. 1.**

7. Mechanisms of toxicity of nanoparticles

Nanoparticle causes adverse health effect by different means. Some possible toxicity mechanisms include oxidative stress, inflammation, genetic damage, and immune system dysfunction (Crisponi et al. 2017; Manke et al. 2013; Nel et al. 2006). Lysosomes often target NP toxicity because most pass into the cells by endocytosis. NPs produce lysosomal dysfunction by cytoskeleton disruption, alkalization of the lysosomal lumen, or NP overload (Crisponi et al. 2017; Stern et al. 2012).

7.1. Genotoxicity

Like any other general chemical forms, nanomaterials have the very same potential to modulate immune responses in an organism and the potential to alter the constitution of genetic material. Several researchers have proposed disturbances in nucleic acid sequences or simple damage to the DNA strands by nanoparticles (Akhtar et al. 2016; Ghosh et al. 2018; Ghosh et al. 2019; Jeevanandam et al. 2018; Kumar & Dhawan 2013). The main mechanism of genotoxicity by nanomaterials in in-vitro or in-vivo studies is oxidative damage, which is proposed to be the main mechanism of most nanomaterials for their toxic potential (Akhtar et al. 2016; Dobrzynska et al. 2014; Kumar & Dhawan 2013; Powell et al. 2010; Xia et al. 2009). Reactive oxygen species and other free radicals/oxidative species are very well known to cause DNA damage (Cooke et al. 2003; Kunwar & Priyadarsini 2011). Other mechanisms may include the direct interaction of nanomaterials with DNA/RNA and the release of toxic ions. A range of nanoparticles is reported or suggested to exhibit genotoxic potentials in in-vitro, in-vivo or in-silico studies (Kumar & Dhawan 2013). The reported nanoparticles for their genotoxic potential include, but are not limited to, nano-sized silver, titanium dioxide, nickel, copper oxide, carbon nanotubes etc.

7.2. Immunomodulation

Activation of neutrophils is considered the central event in acute inflammation episodes (Dwivedi et al. 2011; Rahman et al. 2011; Rahman et al. 2008). Gonçalves et al. 2010 reported the in-vitro activation of the human neutrophils by titanium dioxide (TiO_2) nanoparticles. The same study also demonstrated that TiO_2 markedly and rapidly induced tyrosine phosphorylation events, including phosphorylation of two key enzymes significantly. The nanoparticles exhibit their immune-modulatory properties. Because of their small size, nanoparticles can penetrate and accumulate much deeper cellular and even sub-cellular boundaries, which can cause many undesirable and uncharacterized effects. Nanoparticles may bind to serum proteins and act as haptens to induce an immunological response, including activation of the complement system and disturbance in the balance of the helper T cells. They may escape the phagocytic activity of macrophages. They may discompose the adaptive or innate immunity leading to a hyper-activated immune state or even a suppressed immune phenotype, p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases-1/2 (Erk-1/2) (Goncalves et al. 2010). However, this *in-vitro* study exhibits the influence of the TiO_2 nanoparticles on the molecular pathways involved in regulating inflammation and other immunological events. The probabilities of such incidences can be extrapolated to the *in-vivo* conditions. Different types of materials may have different effects on the immune system. For instance, cobalt and nickel nanoparticles have inflammatory effects, whereas hydroxyapatite crystals release TNF-α from macrophages, activating other phagocytes (Dwivedi et al. 2009). A hyperactive immune state or even a suppressed immunophenotype may result from nanoparticles that evade the phagocytic activity of macrophages, bind to serum proteins and act as haptens, activate complement cascades, upset the Th1/Th2 balance, cause hemolysis or thrombogenicity, or disrupt adaptive or innate immunity (Dwivedi et al. 2011). Certain NPs build up in local lymph nodes, where dendritic cells can take them up and process them. They then interact with self-proteins to change their antigenicity, which in turn causes altered immunological reactions, including autoimmunity (Di Gioacchino et al. 2011; Dwivedi et al. 2009). Studies conducted in vitro showed that NPs could direct the production of cytokines toward either Th1 (Pl, Pd, Ni, Co) or Th2 (Ti, mw, and sw Carbon) production patterns (Di Gioacchino et al. 2011). Delogu et al. (Delogu et al. 2012) investigated the impact of functionalized CNTs on immune cells and found strong activation of NK cells. Thus, considerable data suggest that NPs exert immunotoxicity by affecting different effector cells of the immune system.

7.3. Oxidative stress

Oxidative stress is caused either by an increase in the production of ~~reactive oxygen species (~~ROS) or depletion in the ability of cells to destroy ROS, or a combination of both. Several *in vivo* and *in vitro* studies have pointed out that ROS generation and oxidative stress play an essential role in NP-induced toxicity (Manke et al. 2013; Oberdorster et al. 2007; Shvedova et al. 2012). Federici et al., 2007, found increased ROS generation in rainbow trout by exposure to TiO_2 NPs in a concentration-dependent manner (Federici et al. 2007). Nanoparticles induce oxidative stress in several ways (Crisponi et al. 2017; Risom et al. 2005). It can be generated directly from the surface of nanoparticles (Buzea et al. 2007; Risom et al. 2005). Transition metal (iron, copper, chromium, vanadium, etc.) nanoparticles can generate reactive oxygen, acting as catalysts in Fenton-type reactions (Risom et al. 2005). Nanoparticles may enter mitochondria (Crisponi et al. 2017; Li et al. 2003) and alter their function, producing ROS. Activation of inflammatory cells, such as alveolar macrophages and neutrophils, induced by phagocytosis of nanoparticles, can lead to the generation of reactive oxygen species and reactive nitrogen species (Buzea et al. 2007; Risom et al. 2005). Oxidative stress corresponds with the physicochemical reactivity of NP. It involves mitochondrial dysfunction, depletion of antioxidant enzymes, lipid peroxidation of cellular membranes, protein modification, and DNA damage associated with cell and tissue injury (Buzea et al. 2007; Crisponi et al. 2017). NP-driven ROS generation also contributes to the activation of cell signaling pathways, inflammatory cytokine and chemokine expressions, and specific transcription factor activation, which may lead to pathological consequences (Crisponi et al. 2017; Manke et al. 2013; Medina et al. 2007). A schematic representation of the toxic effects of nanoparticles at the cellular level is given in **Fig. 2.**

Use the already mentioned abbreviation.

7.4. Inflammation

ROS and inflammation demonstrate an interdependent relationship in the case of exposure to NP (Manke et al. 2013). Inflammation is the typical response of the body to injury. When generated in moderation, inflammation helps fight against infection and eliminate foreign invaders; however, in excess and chronic conditions, it can lead to pathological conditions leading to the cause of many diseases (Buzea et al. 2007; Donaldson & Stone 2003). Various in vitro and in vivo experiments suggest that exposure to nanoparticles is associated with mild to severe forms of

inflammation, depending upon the size and composition of the nanoparticle (Buzea et al. 2007; Risom et al. 2005).

An intricate cascade of intracellular and extracellular processes regulates inflammation. Pro-inflammatory mediators, also known as cytokines, are secreted in response to oxidative stress and are largely responsible for the immune system's activation (Buzea et al. 2007; Long et al. 2004; Rahman et al. 2015). Inflammation has also been demonstrated to directly cause toxicity and increase cell death through the development of toxic by-products of inflammation such as ROS and complement proteins and through receptor-induced necrosis (Khanna et al. 2015; Wallach et al. 2014). ~~(Khanna et al. 2015; Wallach et al. 2014).~~ Increased production of pro-inflammatory proteins IL-6, IL-8, and MCP-1, as well as activation of the JNK and p53 pathways, have been linked to silica nanoparticle toxicity (Liu & Sun 2010). The PI3-K/Akt/mTOR signaling cascade is vital for cell survival and proliferation because it controls the cell cycle. PI3-K signaling was reported to promote the upregulation of Cox-2, iNOS, and pro-inflammatory cytokines (IL-6, IFN-γ, TNF-α, IL-17, and regulatory cytokine IL-10) in macrophages with exposure to zinc oxide nanoparticles (Khanna et al. 2015; Roy et al. 2014). ~~(Khanna et al. 2015; Roy et al. 2014)~~. As a result, there is evidence that NPs can have an inflammatory effect by activating many signaling pathways and producing reactive oxygen species.

8. Methods for assessing the toxicity of nanomaterials

To assure a responsible and sustainable growth of nanotechnology, the health, and safety associated issues of engineered nanomaterials and related products need to be addressed at a rate commensurate with nanotechnology's expansion. The toxicity assessment of any nano-sized material follows the same path as any other chemical. Both in-vitro and in-vivo approaches are considered while assessing the safety of a nano material-related product. The related safety concerns are also increasing with the emerging applications of nanotechnology in industries and household products. Human health concerns for nanomaterials are established historically by epidemiological and clinical studies on naturally occurring fibers and particles such as asbestos and silica (Ahmad et al. 2014b; Alam et al. 2018; Ghosh et al. 2019; Hillegass et al. 2010; Mohajerani et al. 2019). The nano-sized fibers of asbestos and silica enter the body through inhalation and cause a range of ailments, including lung fibrosis and cancer.

8.1. In vitro methods

In vitro model, which mainly includes cultured cells, provides a rapid and effective assessment of some toxicological endpoints associated with nanomaterial exposure (Eskes et al. 2017; Natoli et al. 2012). In-vitro assessments allow mechanism-based toxicity evaluations and provide precise information on how nanoparticles interact with biological systems in many ways (Gupta & Xie 2018; Savage et al. 2019). In-vitro assays include (i) cell viability/cytotoxicity assay, mechanistic estimations, (ii) microscopic analyses, (iii) gene expression evaluation and (iv) genotoxicity estimations etc.

Several assays have been mentioned to assess the effects of nanomaterials on cell viability or cytotoxicity. The review of the in-vitro studies by Hillegas et al., 2010 recommends collecting standard growth curve data to determine the baseline growth properties of selected cells (Hillegass et al. 2010). Various in-vitro estimations are performed to estimate a chemical or agent (Including nanomaterials). The assays include (i) trypan blue exclusion assay, (ii) microculture tetrazolium assay (MTA), (iii) clonogenic assay or colony-forming efficiency (CFE), (iv) lactate dehydrogenase (LDH) Assay, (v) TdTdUTP nick end labeling (TUNEL) and apostate assays. However, several other assays can also be employed to estimate cytotoxicity (Balouiri et al. 2016). Other in vitro nanotoxicity assays include the examination of lipid peroxidation to elucidate the role played by oxidative stress and methods to investigate apoptosis, including cytochrome c release from mitochondria and caspase activation (Hillegass et al. 2010).

In-vitro assays can also be exploited to estimate the nanomaterials' cytotoxicity and their probable effect on cell proliferation (Leudjo Taka et al. 2021). For cellular proliferation estimation, the assays which have been recommended include (i) DNA content estimation, (ii) Incorporation of Bromodeoxyuridine (BrdU), (iii) Ki-67 nuclear antigen estimation(iv)proliferating cell nuclear antigen (PCNA).

The genotoxicity of the nanomaterials can be evaluated in both in-vitro and in-vivo systems. Estimations in in-vitro are much simpler and easier when compared with the in-vivo systems. In-vitro systems are less time-consuming, and one can get the results in a short period (Barabadi et al. 2019; Elespuru et al. 2018). The in-vitro assays for genotoxicity include (i) Ames assay in

Salmonella typhimurium, and *Escherichia coli,* (Pan 2021) (ii) oxidized guanine bases estimation, (Cui et al. 2013) (iii) analysis of chromosomal aberration, (Clare 2012) (iv) micronuclei assay (Doherty 2012) (v) single cell gel electrophoresis (comet) assay (Karlsson 2010).

8.2. In-vivo methods

Over the last decade, nanotoxicology methods have mostly relied on in vitro cell-based estimations, which provide a good amount of information related to mechanistic approaches. Still, they do not account for the complexity of in vivo systems concerning biodistribution, metabolism, hematology, immunology, target organ, and neurological consequences. Compared to many reports published about nanoparticles, only 3% are devoted to studying their critical biological effects in-vivo (Greish et al. 2012; Hussain et al. 2020).

Various nanotoxicological studies incorporate the traditional in-vivo models for safety evaluation. The models include zebrafish, Xenopus embryos, mice, rats, and sometimes a primate (Macaque) (Yong et al. 2013).

Zebrafish (*Danio rerio*) is extensively used as an animal model for toxicological studies (Hill et al. 2005; Sipes et al. 2011). The Zebrafish genome project has placed zebrafish in an attractive position for use as a toxicological model. The zebrafish embryo is also a useful small model for investigating vertebrate development because of its transparency, low cost, transgenic and morpholino capabilities, conservation of cell signaling, and similarities with mammalian developmental phenotypes (Sipes et al. 2011). The zebrafish has also been proposed and used as an animal model in toxicological studies associated with nano-sized materials (Bohnsack et al. 2012; Fako & Furgeson 2009).

Mouse and rat models have been used in life science research for several decades. They are also used for toxicological evaluations of industrial and environmental chemicals. The main reason for the selection of mice as a model is its genomic similarity with humans, and it makes the primary base for the wide use of mice in the life sciences, pharmacological and toxicological research; the mouse as a mammalian model provides corresponding experimental conditions and analogous results to humans, though with certain limitations. The use of animal models sometimes raises certain ethical issues. However, it has been proved a successful animal model for preclinical

research. Nanotoxicological studies have also adopted the mouse as a model for estimating the toxic potential of nanomaterials (Bahadar et al. 2016; Ferreira et al. 2013; Lam et al. 2004). Rats are also used for experimental purposes similarly (Devoy et al. 2020; Ferreira et al. 2013; Konoeda et al. 2020; Mendonca et al. 2016; Warheit et al. 2004). Animal models are required to estimate the absorption of material into the biological system, its distribution in the body, its fate after metabolism and ultimately, modes of its elimination from the body (ADME studies). Absorption, distribution, metabolism, excretion, pharmacokinetics (ADME/PK) and carcinogenic and teratogenic studies are also required to evaluate nanomaterial toxicity (Greish et al. 2012; Raja et al. 2017; Zielinska et al. 2020). While studying the toxicological effects of nanoparticles, one should cautiously consider the physicochemical properties that include size, shape, surface area, surface charge, charge density, chemical composition, the density of structure, presence of pores and surface activating sites. These characteristics of nanomaterials may influence their toxicological effects.

9. Conclusion and Future Prospects

In conclusion, as the application of nanomaterials has expanded many times in recent years, there are increasing concerns regarding its potential adverse effect on human health and the environment. Therefore, regulatory guidelines should emphasize the safer design of nanomaterials. Also, extensive characterization of the physicochemical properties during the manufacturing of nanoparticles for therapeutic and diagnostic purposes must be emphasized to prevent adverse health effects. Proper storage, handling, and use of newly designed nanomaterials should be well-researched and investigated before marketing. This will minimize occupational exposure and environmental release of nanomaterials. Toxicologists are working to investigate the risks and hazards of nanomaterials. Developing advanced and sensitive toxicological methods contributes to a faster and more effective assessment of nanomaterials, toxicology in general, and safety pharmacology. The physicochemical characteristics of anthropogenic and artificial NMs, such as size, chemical content, crystal structure, surface morphology, surface charge and energy, and aggregation state, may be closely related to their toxicity. Therefore, more environmentally favorable and nontoxic NPs need to be developed. The circumstances of synthesis, processing, chemical makeup, and doses are additional elements that influence the levels of risk. Therefore, focused research on the interaction between physicochemical characteristics and the toxicity of

nanoparticles needs to be developed. Additionally, in-silico methods should be explored for nanotoxicology and systems biology approaches.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Data Availability: The data used to support the findings of this study are included in the article.

Acknowledgments: The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (23UQU4330890DSR002).

References

Abdulla NK, Siddiqui SI, Fatima B, Sultana R, Tara N, Hashmi AA, Ahmad R, Mohsin M, Nirala RK, and Linh NT. 2021. Silver-based hybrid nanocomposite: a novel antibacterial material for water cleansing. *Journal of Cleaner Production* 284:124746.

Agathokleous E, Feng Z, Iavicoli I, and Calabrese EJ. 2019. The two faces of nanomaterials: a quantification of hormesis in algae and plants. *Environment International* 131:105044.

Ahmad I, Ahmad A, Iftekhar S, Khalid S, Aftab A, and Raza SA. 2019a. Role of Nanoparticle in Cosmetics Industries. *Biological Synthesis of Nanoparticles and Their Applications*: CRC Press, 173-204.

Ahmad R, Fiodor A, Nain L, and Pranaw K. 2023. Microbially synthesized nanoparticles: implications and their applications in agriculture. In: Kumar V, and Singh M, eds. *Microbial Nanotechnology: Advances in Agriculture, Industry and Health Sectors*: De Gruyter, 25-52.

Ahmad R, Khatoon N, and Sardar M. 2013. Biosynthesis, characterization and application of TiO_2 nanoparticles in biocatalysis and protein folding. *Journal of Proteins & Proteomics* 4:115-121.

Ahmad R, Khatoon N, and Sardar M. 2014a. Antibacterial Effect of Green Synthesized TiO_2 Nanoparticles. *Advanced Science Letters* 20:1616-1620.

Ahmad R, Mishra A, and Sardar M. 2014b. Simultaneous Immobilization and Refolding of Heat Treated Enzymes on TiO_2 Nanoparticles. *Advanced Science, Engineering and Medicine* 6:1264-1268.

Ahmad R, Mohsin M, Ahmad T, and Sardar M. 2015. Alpha amylase assisted synthesis of TiO_2 nanoparticles: structural characterization and application as antibacterial agents. *Journal of hazardous materials* 283:171-177.

Ahmad R, Pranaw K, and Khare SK. 2019b. Effect of nanomaterials and their possible implication on the plants. *Plant Biotechnology: Progress in Genomic Era*: Springer, 213-229.

Ahmad R, and Sardar M. 2015a. Enzyme Immobilization: An Overview on Nanoparticles as Immobilization Matrix. *Biochemistry & Analytical Biochemistry* 4:2161-1009.1000178.

Ahmad R, and Sardar M. 2015b. Evaluation of Green Synthesis TiO_2 Nanoparticles as Antibacterial Agents. IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL: SPRINGER 233 SPRING ST, NEW YORK, NY 10013 USA. p S26-S26.

Ahmad R, Srivastava S, Ghosh S, and Khare SK. 2021. Phytochemical delivery through nanocarriers: a review. *Colloids and Surfaces B: Biointerfaces* 197:111389.

Akhavan O, Ghaderi E, and Akhavan A. 2012. Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. *Biomaterials* 33:8017-8025.

Akhtar MJ, Kumar S, Alhadlaq HA, Alrokayan SA, Abu-Salah KM, and Ahamed M. 2016. Dose-dependent genotoxicity of copper oxide nanoparticles stimulated by reactive oxygen species in human lung epithelial cells. *Toxicology and industrial health* 32:809-821.

Alam S, Ahmad R, Pranaw K, Mishra P, and Khare SK. 2018. Asparaginase conjugated magnetic nanoparticles used for reducing acrylamide formation in food model system. *Bioresource technology* 269:121-126.

Alavi M, Jabari E, and Jabbari E. 2021. Functionalized carbon-based nanomaterials and quantum dots with antibacterial activity: a review. *Expert Review of Anti-infective Therapy* 19:35-44.

Almaimani RA. 2021. The Association between oxidative stress marker Beta-2 microglobulin and type 2 diabetes mellitus in context of glycaemic index. *Journal of Umm Al-Qura University for Medical Sciences* 7:28-31.

Arora S, Rajwade JM, and Paknikar KM. 2012. Nanotoxicology and in vitro studies: the need of the hour. *Toxicology and applied pharmacology* 258:151-165.

Aversa R, Modarres MH, Cozzini S, Ciancio R, and Chiusole A. 2018. The first annotated set of scanning electron microscopy images for nanoscience. *Scientific data* 5:1-10.

Bahadar H, Maqbool F, Niaz K, and Abdollahi M. 2016. Toxicity of nanoparticles and an overview of current experimental models. *Iranian biomedical journal* 20:1.

Baimanov D, Cai R, and Chen C. 2019. Understanding the chemical nature of nanoparticle-protein interactions. *Bioconjugate Chemistry* 30:1923-1937.

Baker C, Pradhan A, Pakstis L, Pochan DJ, and Shah SI. 2005. Synthesis and antibacterial properties of silver nanoparticles. *Journal of nanoscience and nanotechnology* 5:244-249.

Balážová Ľ, Baláž M, and Babula P. 2020. Zinc oxide nanoparticles damage tobacco BY-2 cells by oxidative stress followed by processes of autophagy and programmed cell death. *Nanomaterials* 10:1066.

Ball P. 2017. Quantum materials: Where many paths meet. *MRS bulletin* 42:698-705.

Balouiri M, Sadiki M, and Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal* 6:71-79. 10.1016/j.jpha.2015.11.005

Barabadi H, Najafi M, Samadian H, Azarnezhad A, Vahidi H, Mahjoub MA, Koohiyan M, and Ahmadi A. 2019. A systematic review of the genotoxicity and antigenotoxicity of biologically synthesized metallic nanomaterials: are green nanoparticles safe enough for clinical marketing? *Medicina* 55:439.

Baroli B. 2010. Penetration of nanoparticles and nanomaterials in the skin: fiction or reality? *Journal of pharmaceutical sciences* 99:21-50.

Barry BW. 2001. Novel mechanisms and devices to enable successful transdermal drug delivery. *European journal of pharmaceutical sciences* 14:101-114.

Belyaeva EA, Sokolova TV, Emelyanova LV, and Zakharova IO. 2012. Mitochondrial electron transport chain in heavy metal-induced neurotoxicity: effects of cadmium, mercury, and copper. *The Scientific World Journal* 2012.

Bergin IL, and Witzmann FA. 2013. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. *International journal of biomedical nanoscience and nanotechnology* 3:163-210.

783 Bierkandt FS, Leibrock L, Wagener S, Laux P, and Luch A. 2018. The impact of nanomaterial
784 characteristics on inhalation toxicity. *Toxicology research* 7:321-346.
785 Bohnsack JP, Assemi S, Miller JD, and Furgeson DY. 2012. The primacy of physicochemical
786 characterization of nanomaterials for reliable toxicity assessment: a review of the zebrafish
787 nanotoxicology model. *Nanotoxicity*: Springer, 261-316.
788 Borandeh S, Alimardani V, Abolmaali SS, and Seppälä J. 2021. Graphene Family Nanomaterials
789 in Ocular Applications: Physicochemical Properties and Toxicity. *Chemical research in*
790 *toxicology*.
791 Borm PJA, and Kreyling W. 2004. Toxicological hazards of inhaled nanoparticles-potential
792 implications for drug delivery. *Journal of nanoscience and nanotechnology* 4:521-531.
793 Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V,
794 Kreyling W, and Lademann J. 2006. The potential risks of nanomaterials: a review carried
795 out for ECETOC. *Particle and fibre toxicology* 3:11.
796 Braydich-Stolle L, Hussain S, Schlager JJ, and Hofmann M-C. 2005. In vitro cytotoxicity of
797 nanoparticles in mammalian germline stem cells. *Toxicological sciences* 88:412-419.
798 Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, and Stark WJ.
799 2006. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the
800 effect of particle solubility. *Environmental science & technology* 40:4374-4381.
801 Buzea C, Pacheco II, and Robbie K. 2007. Nanomaterials and nanoparticles: sources and toxicity.
802 *Biointerphases* 2:MR17-MR71.
803 Carlin DJ, Larson TC, Pfau JC, Gavett SH, Shukla A, Miller A, and Hines R. 2015. Current
804 research and opportunities to address environmental asbestos exposures. *Environmental*
805 *health perspectives* 123:A194-A197.
806 Chandra P, Kim J, and Rhee SW. 2013. Quantitative analysis of growth of cells on
807 physicochemically modified surfaces. *Bulletin of the Korean Chemical Society* 34:524-
808 530.
809 Chen M, Sun Y, Liang J, Zeng G, Li Z, Tang L, Zhu Y, Jiang D, and Song B. 2019. Understanding
810 the influence of carbon nanomaterials on microbial communities. *Environment*
811 *international* 126:690-698.
812 Chen R-J, Chen Y-Y, Liao M-Y, Lee Y-H, Chen Z-Y, Yan S-J, Yeh Y-L, Yang L-X, Lee Y-L,
813 and Wu Y-H. 2020. The current understanding of autophagy in nanomaterial toxicity and
814 its implementation in safety assessment-related alternative testing strategies. *International*
815 *journal of molecular sciences* 21:2387.
816 Cheng L-C, Jiang X, Wang J, Chen C, and Liu R-S. 2013. Nano-bio effects: interaction of
817 nanomaterials with cells. *Nanoscale* 5:3547-3569.
818 Chuang H-C, Chen L-C, Lei Y-C, Wu K-Y, Feng P-H, and Cheng T-J. 2015. Surface area as a
819 dose metric for carbon black nanoparticles: A study of oxidative stress, DNA single-strand
820 breakage and inflammation in rats. *Atmospheric Environment* 106:329-334.
821 Cioffi N, Ditaranto N, Torsi L, Picca RA, De Giglio E, Sabbatini L, Novello L, Tantillo G, Bleve-
822 Zacheo T, and Zambonin PG. 2005. Synthesis, analytical characterization and bioactivity
823 of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. *Analytical and*
824 *bioanalytical chemistry* 382:1912-1918.
825 Clare G. 2012. The in vitro mammalian chromosome aberration test. *Genetic Toxicology*:
826 Springer, 69-91.
827 Coates M, Lee MJ, Norton D, and MacLeod AS. 2019. The Skin and Intestinal Microbiota and
828 Their Specific Innate Immune Systems. *Frontiers in Immunology* 10:2950.

Cooke MS, Evans MD, Dizdaroglu M, and Lunec J. 2003. Oxidative DNA damage: mechanisms, mutation, and disease. *The FASEB Journal* 17:1195-1214.

Crisponi G, Nurchi VM, Lachowicz JI, Peana M, Medici S, and Zoroddu MA. 2017. Toxicity of nanoparticles: etiology and mechanisms. *Antimicrobial Nanoarchitectonics*: Elsevier, 511-546.

Crosera M, Bovenzi M, Maina G, Adami G, Zanette C, Florio C, and Larese FF. 2009. Nanoparticle dermal absorption and toxicity: a review of the literature. *International archives of occupational and environmental health* 82:1043-1055.

Cui L, Ye W, Prestwich EG, Wishnok JS, Taghizadeh K, Dedon PC, and Tannenbaum SR. 2013. Comparative analysis of four oxidized guanine lesions from reactions of DNA with peroxynitrite, singlet oxygen, and γ-radiation. *Chemical research in toxicology* 26:195-202.

De Matteis V. 2017. Exposure to inorganic nanoparticles: routes of entry, immune response, biodistribution and in vitro/in vivo toxicity evaluation. *Toxics* 5:29.

Delogu LG, Venturelli E, Manetti R, Pinna GA, Carru C, Madeddu R, Murgia L, Sgarrella F, Dumortier H, and Bianco A. 2012. Ex vivo impact of functionalized carbon nanotubes on human immune cells. *Nanomedicine* 7:231-243.

Demir E. 2021. A review on nanotoxicity and nanogenotoxicity of different shapes of nanomaterials. *Journal of Applied Toxicology* 41:118-147.

Devoy J, Nunge H, Bonfanti E, Seidel C, Gate L, and Cosnier F. 2020. Quantitative measurement of carbon nanotubes in rat lung. *Nanotoxicology*:1-14.

Di Gioacchino M, Petrarca C, Lazzarin F, Di Giampaolo L, Sabbioni E, Boscolo P, Mariani-Costantini R, and Bernardini G. 2011. Immunotoxicity of nanoparticles. *International journal of immunopathology and pharmacology* 24:65S-71S.

Ding Y, and Chen M. 2009. Nanoporous metals for catalytic and optical applications. *MRS bulletin* 34:569-576.

Dobrzynska MM, Gajowik A, Radzikowska J, Lankoff A, DuÅ¡inskÃ¡ M, and Kruszewski M. 2014. Genotoxicity of silver and titanium dioxide nanoparticles in bone marrow cells of rats in vivo. *Toxicology* 315:86-91.

Docter D, Westmeier D, Markiewicz M, Stolte S, Knauer S, and Stauber R. 2015. The nanoparticle biomolecule corona: lessons learned–challenge accepted? *Chemical Society Reviews* 44:6094-6121.

Doherty AT. 2012. The in vitro micronucleus assay. *Genetic Toxicology*: Springer, 121-141.

Donaldson K, and Stone V. 2003. Current hypotheses on the mechanisms of toxicity of ultrafine particles. *Annali dell'Istituto superiore di sanitÃƒ* 39:405-410.

Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, Mills N, MacNee W, and Stone V. 2005. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Particle and fibre toxicology* 2:1-14.

Dreher KL. 2004. Health and environmental impact of nanotechnology: toxicological assessment of manufactured nanoparticles. *Toxicological sciences* 77:3-5.

Duan X, and Li Y. 2013. Physicochemical characteristics of nanoparticles affect circulation, biodistribution, cellular internalization, and trafficking. *Small* 9:1521-1532.

Dwivedi PD, Misra A, Shanker R, and Das M. 2009. Are nanomaterials a threat to the immune system? *Nanotoxicology* 3:19-26.

Dwivedi PD, Tripathi A, Ansari KM, Shanker R, and Das M. 2011. Impact of nanoparticles on the immune system. *Journal of Biomedical Nanotechnology* 7:193-194.

875 Elespuru R, Pfuhler S, Aardema MJ, Chen T, Doak SH, Doherty A, Farabaugh CS, Kenny J,
876 Manjanatha M, Mahadevan B, Moore MM, Ouédraogo G, Stankowski LF, Jr., and Tanir
877 JY. 2018. Genotoxicity Assessment of Nanomaterials: Recommendations on Best
878 Practices, Assays, and Methods. *Toxicol Sci* 164:391-416. 10.1093/toxsci/kfy100
879 Eskes C, Boström A-C, Bowe G, Coecke S, Hartung T, Hendriks G, Pamies D, Piton A, and
880 Rovida C. 2017. Good cell culture practices & in vitro toxicology. *Toxicology in Vitro*
881 45:272-277.
882 Fako VE, and Furgeson DY. 2009. Zebrafish as a correlative and predictive model for assessing
883 biomaterial nanotoxicity. *Advanced drug delivery reviews* 61:478-486.
884 Federici G, Shaw BJ, and Handy RD. 2007. Toxicity of titanium dioxide nanoparticles to rainbow
885 trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects.
886 *Aquatic toxicology* 84:415-430.
887 Ferdous Z, and Nemmar A. 2020. Health Impact of Silver Nanoparticles: A Review of the
888 Biodistribution and Toxicity Following Various Routes of Exposure. *International journal*
889 *of molecular sciences* 21:2375.
890 Ferreira AJ, Cemlyn-Jones J, and Cordeiro CR. 2013. Nanoparticles, nanotechnology and
891 pulmonary nanotoxicology. *Revista Portuguesa de Pneumologia (English Edition)* 19:28-
892 37.
893 Foroozandeh P, and Aziz AA. 2015. Merging worlds of nanomaterials and biological environment:
894 factors governing protein corona formation on nanoparticles and its biological
895 consequences. *Nanoscale research letters* 10:1-12.
896 Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, and Casey PS. 2007. Comparative
897 toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga
898 (Pseudokirchneriella subcapitata): the importance of particle solubility. *Environmental*
899 *science & technology* 41:8484-8490.
900 Frohlich E, and Roblegg E. 2012. Models for oral uptake of nanoparticles in consumer products.
901 *Toxicology* 291:10-17.
902 Gaiser BK, Fernandes TF, Jepson MA, Lead JR, Tyler CR, Baalousha M, Biswas A, Britton GJ,
903 Cole PA, and Johnston BD. 2012. Interspecies comparisons on the uptake and toxicity of
904 silver and cerium dioxide nanoparticles. *Environmental Toxicology and Chemistry* 31:144-
905 154.
906 Ganguly P, Breen A, and Pillai SC. 2018. Toxicity of nanomaterials: exposure, pathways,
907 assessment, and recent advances. *ACS Biomaterials Science & Engineering* 4:2237-2275.
908 Gao H, and He Q. 2014. The interaction of nanoparticles with plasma proteins and the consequent
909 influence on nanoparticles behavior. *Expert opinion on drug delivery* 11:409-420.
910 García-Pinel B, Porras-Alcalá C, Ortega-Rodríguez A, Sarabia F, Prados J, Melguizo C, and
911 López-Romero JM. 2019. Lipid-based nanoparticles: application and recent advances in
912 cancer treatment. *Nanomaterials* 9:638.
913 Gatti AM. 2004. Biocompatibility of micro-and nano-particles in the colon. Part II. *Biomaterials*
914 25:385-392.
915 Ge Z, Yang L, Xiao F, Wu Y, Yu T, Chen J, Lin J, and Zhang Y. 2018. Graphene family
916 nanomaterials: properties and potential applications in dentistry. *International journal of*
917 *biomaterials* 2018.
918 Genuis SJ, and Kyrillos E. 2017. The chemical disruption of human metabolism. *Toxicology*
919 *Mechanisms and Methods* 27:477-500.

Geppert M, Schwarz A, Stangassinger LM, Wenger S, Wienerroither LM, Ess S, Duschl A, and Himly M. 2020. Interactions of TiO_2 nanoparticles with ingredients from modern lifestyle products and their effects on human skin cells. *Chemical research in toxicology* 33:1215-1225.

Ghasemiyeh P, and Mohammadi-Samani S. 2020. Potential of Nanoparticles as Permeation Enhancers and Targeted Delivery Options for Skin: Advantages and Disadvantages. *Drug Design, Development and Therapy* 14:3271.

Ghosh S, Ahmad R, Banerjee K, AlAjmi MF, and Rahman S. 2021a. Mechanistic Aspects of Microbe-Mediated Nanoparticle Synthesis. *Frontiers in Microbiology* 12:638068.

Ghosh S, Ahmad R, and Khare SK. 2018. Immobilization of Cholesterol Oxidase: An Overview. *The Open Biotechnology Journal* 12:176-188.

Ghosh S, Ahmad R, and Khare SK. 2019. Refolding of thermally denatured cholesterol oxidases by magnetic nanoparticles. *International journal of biological macromolecules* 138:958-965.

Ghosh S, Ahmad R, Zeyaullah M, and Khare SK. 2021b. Microbial Nano-Factories: Synthesis and Biomedical Applications. *Frontiers in Chemistry* 9:626834.

Gleiter H. 2000. Nanostructured materials: basic concepts and microstructure. *Acta materialia* 48:1-29.

Goel N, Ahmad R, Fatima H, and Khare SK. 2021. New threatening of SARS-CoV-2 coinfection and strategies to fight the current pandemic. *Medicine in Drug Discovery* 10:100089.

Goel N, Hashmi Z, Khan N, Ahmad R, and Khan WH. 2023. Recent Strategies to Combat Multidrug Resistance. *Non-traditional Approaches to Combat Antimicrobial Drug Resistance*: Springer, 1-27.

Goncalves DM, Chiasson S, and Girard D. 2010. Activation of human neutrophils by titanium dioxide (TiO_2) nanoparticles. *Toxicology in Vitro* 24:1002-1008.

Gonzalez-Munoz M, Diez P, Gonzalez-Gonzalez M, Degano RM, Ibarrola N, Orfao A, and Fuentes M. 2015. Evaluation strategies of nanomaterials toxicity. *Nanomaterials-Toxicity and Risk Assessment InTech*:23-37.

Gopinath P, Gogoi SK, Chattopadhyay A, and Ghosh SS. 2008. Implications of silver nanoparticle induced cell apoptosis for in vitro gene therapy. *Nanotechnology* 19:075104.

Greish K, Thiagarajan G, and Ghandehari H. 2012. In vivo methods of nanotoxicology. *Nanotoxicity*: Springer, 235-253.

Griffitt RJ, Luo J, Gao J, Bonzongo JC, and Barber DS. 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environmental Toxicology and Chemistry: An International Journal* 27:1972-1978.

Gupta R, and Xie H. 2018. Nanoparticles in Daily Life: Applications, Toxicity and Regulations. *J Environ Pathol Toxicol Oncol* 37:209-230. 10.1615/JEnvironPatholToxicolOncol.2018026009

Hanley C, Thurber A, Hanna C, Punnoose A, Zhang J, and Wingett DG. 2009. The influences of cell type and ZnO nanoparticle size on immune cell cytotoxicity and cytokine induction. *Nanoscale research letters* 4:1409-1420.

Hao H, Li H, Wang S, Cheng Z, and Fang Y. 2020. Epitaxial growth of Ag-Cu bimetallic nanoparticles via thermal evaporation deposition. *Applied Surface Science* 505:143871.

Hill AJ, Teraoka H, Heideman W, and Peterson RE. 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological sciences* 86:6-19.

Hillegass JM, Shukla A, Lathrop SA, MacPherson MB, Fukagawa NK, and Mossman BT. 2010. Assessing nanotoxicity in cells in vitro. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 2:219-231.

Hu W, Peng C, Lv M, Li X, Zhang Y, Chen N, Fan C, and Huang Q. 2011. Protein corona-mediated mitigation of cytotoxicity of graphene oxide. *ACS nano* 5:3693-3700.

Huang J, Wang L, Lin R, Wang AY, Yang L, Kuang M, Qian W, and Mao H. 2013. Casein-coated iron oxide nanoparticles for high MRI contrast enhancement and efficient cell targeting. *ACS applied materials & interfaces* 5:4632-4639.

Huang L-H, Sun X-Y, and Ouyang J-M. 2019. Shape-dependent toxicity and mineralization of hydroxyapatite nanoparticles in A7R5 aortic smooth muscle cells. *Scientific reports* 9:1-18.

Hussain Z, Thu HE, Elsayed I, Abourehab MAS, Khan S, Sohail M, Sarfraz RM, and Farooq MA. 2020. Nano-scaled materials may induce severe neurotoxicity upon chronic exposure to brain tissues: A critical appraisal and recent updates on predisposing factors, underlying mechanism, and future prospects. *Journal of Controlled Release* 328:873-894.

Jain MI, and Patel MP. 2021. Effects and Mechanism of Nanotoxicity: An Overview. *Technology* 6.

Jain SK, Sahni Y, Rajput N, and Gautam V. 2011. Nanotoxicology: An Emerging Discipline. *Veterinary World* 4.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, and Beeregowda KN. 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary toxicology* 7:60.

Jeevanandam J, Barhoum A, Chan YS, Dufresne A, and Danquah MK. 2018. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein journal of nanotechnology* 9:1050-1074.

Ji Z, Wang X, Zhang H, Lin S, Meng H, Sun B, George S, Xia T, Nel AE, and Zink JI. 2012. Designed synthesis of CeO2 nanorods and nanowires for studying toxicological effects of high aspect ratio nanomaterials. *ACS nano* 6:5366-5380.

Jia Y-P, Ma B-Y, Wei X-W, and Qian Z-Y. 2017. The in vitro and in vivo toxicity of gold nanoparticles. *Chinese Chemical Letters* 28:691-702.

Jin C-Y, Zhu B-S, Wang X-F, and Lu Q-H. 2008. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. *Chemical research in toxicology* 21:1871-1877.

Juárez-Maldonado A, Tortella G, Rubilar O, Fincheira P, and Benavides-Mendoza A. 2021. Biostimulation and toxicity: The magnitude of the impact of nanomaterials in microorganisms and plants. *Journal of Advanced Research* 31:113.

Karlsson HL. 2010. The comet assay in nanotoxicology research. *Analytical and bioanalytical chemistry* 398:651-666.

Khanna P, Ong C, Bay BH, and Baeg GH. 2015. Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials* 5:1163-1180.

Khatoon N, Ahmad R, and Sardar M. 2015. Robust and fluorescent silver nanoparticles using *Artemisia annua*: Biosynthesis, characterization and antibacterial activity. *Biochemical Engineering Journal* 102:91-97.

Kim C, and Lee H. 2018. Light-assisted surface reactions on metal nanoparticles. *Catalysis Science & Technology* 8:3718-3727.

Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, and Lead JR. 2008. Nanomaterials in the environment: behavior, fate, bioavailability,

and effects. *Environmental Toxicology and Chemistry: An International Journal* 27:1825-1851.
Koike E, and Kobayashi T. 2006. Chemical and biological oxidative effects of carbon black nanoparticles. *Chemosphere* 65:946-951.
Konoeda H, Takizawa H, Gower A, Zhao M, Adeyi OA, and Liu M. 2020. Pharmacokinetics, tissue distribution and safety of gold nanoparticle/PKC Delta inhibitor peptide hybrid in rats. *Nanotoxicology* 14:341-354.
Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, Wambaugh JF, Jones D, Whelan M, and Thomas R. 2020. Toxicity testing in the 21st century: progress in the past decade and future perspectives. *Archives of toxicology* 94:1-58.
Kreyling WG, Hirn S, MoÌˆller W, Schleh C, Wenk A, Celik Gl, Lipka J, SchaÌˆffler M, Haberl N, and Johnston BD. 2014. Air-blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size. *ACS nano* 8:222-233.
Kumar A, and Dhawan A. 2013. Genotoxic and carcinogenic potential of engineered nanoparticles: an update. *Archives of toxicology* 87:1883-1900.
Kumari J, Kumar D, Mathur A, Naseer A, Kumar RR, Chandrasekaran PT, Chaudhuri G, Pulimi M, Raichur AM, and Babu S. 2014. Cytotoxicity of TiO2 nanoparticles towards freshwater sediment microorganisms at low exposure concentrations. *Environmental research* 135:333-345.
Kunwar A, and Priyadarsini KI. 2011. Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical & Allied Sciences* 1:53.
Lam C-W, James JT, McCluskey R, and Hunter RL. 2004. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicological sciences* 77:126-134.
Lanphear BP. 2017. Low-level toxicity of chemicals: No acceptable levels? *PLoS biology* 15:e2003066.
Lee E, Jeon H, Lee M, Ryu J, Kang C, Kim S, Jung J, and Kwon Y. 2019. Molecular origin of AuNPs-induced cytotoxicity and mechanistic study. *Scientific reports* 9:1-13.
Leudjo Taka A, Tata CM, Klink MJ, Mbianda XY, Mtunzi FM, and Naidoo EB. 2021. A review on conventional and advanced methods for nanotoxicology evaluation of engineered nanomaterials. *Molecules* 26:6536.
Li D, Johanson G, Emond C, Carlander U, Philbert M, and Jolliet O. 2014. Physiologically based pharmacokinetic modeling of polyethylene glycol-coated polyacrylamide nanoparticles in rats. *Nanotoxicology* 8:128-137.
Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, and Nel A. 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental health perspectives* 111:455-460.
Li Z, Wang L, Li Y, Feng Y, and Feng W. 2019. Carbon-based functional nanomaterials: Preparation, properties and applications. *Composites Science and Technology* 179:10-40.
Lin Y-S, and Haynes CL. 2010. Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *Journal of the American Chemical Society* 132:4834-4842.
Liu L, Sun M, Li Q, Zhang H, Alvarez PJ, Liu H, and Chen W. 2014a. Genotoxicity and cytotoxicity of cadmium sulfide nanomaterials to mice: comparison between nanorods and nanodots. *Environmental Engineering Science* 31:373-380.

Liu X, and Sun J. 2010. Endothelial cells dysfunction induced by silica nanoparticles through oxidative stress via JNK/P53 and NF-κB pathways. *Biomaterials* 31:8198-8209.

Liu Y, Hu Y, and Huang L. 2014b. Influence of polyethylene glycol density and surface lipid on pharmacokinetics and biodistribution of lipid-calcium-phosphate nanoparticles. *Biomaterials* 35:3027-3034.

Liu Y, Li W, Lao F, Liu Y, Wang L, Bai R, Zhao Y, and Chen C. 2011. Intracellular dynamics of cationic and anionic polystyrene nanoparticles without direct interaction with mitotic spindle and chromosomes. *Biomaterials* 32:8291-8303.

Liu Y, Zhao Y, Sun B, and Chen C. 2013. Understanding the toxicity of carbon nanotubes. *Accounts of chemical research* 46:702-713.

Long H, Shi T, Borm PJ, Maatta J, Husgafvel-Pursiainen K, Savolainen K, and Krombach F. 2004. ROS-mediated TNF-α and MIP-2 gene expression in alveolar macrophages exposed to pine dust. *Particle and fibre toxicology* 1:1-8.

Lozhkomoev A, Pervikov A, Chumaevsky A, Dvilis E, Paygin V, Khasanov O, and Lerner M. 2019. Fabrication of Fe-Cu composites from electroexplosive bimetallic nanoparticles by spark plasma sintering. *Vacuum* 170:108980.

Lu P-J, Huang S-C, Chen Y-P, Chiueh L-C, and Shih DY-C. 2015. Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. *Journal of food and drug analysis* 23:587-594.

Lucarelli M, Gatti AM, Savarino G, Quattroni P, Martinelli L, Monari E, and Boraschi D. 2004. Innate defence functions of macrophages can be biased by nano-sized ceramic and metallic particles. *European cytokine network* 15:339-346.

Madannejad R, Shoaie N, Jahanpeyma F, Darvishi MH, Azimzadeh M, and Javadi H. 2019. Toxicity of carbon-based nanomaterials: Reviewing recent reports in medical and biological systems. *Chemico-biological interactions* 307:206-222.

Madru R, Tran TA, Axelsson J, Ingvar C, Bibic A, Stahlberg F, Knutsson L, and Strand S-E. 2013. 68Ga-labeled superparamagnetic iron oxide nanoparticles (SPIONs) for multi-modality PET/MR/Cherenkov luminescence imaging of sentinel lymph nodes. *American journal of nuclear medicine and molecular imaging* 4:60.

Majhi KC, and Yadav M. 2021. Synthesis of inorganic nanomaterials using carbohydrates. *Green Sustainable Process for Chemical and Environmental Engineering and Science*: Elsevier, 109-135.

Manke A, Wang L, and Rojanasakul Y. 2013. Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed research international* 2013:942916.

Mao Z, Xu B, Ji X, Zhou K, Zhang X, Chen M, Han X, Tang Q, Wang X, and Xia Y. 2015. Titanium dioxide nanoparticles alter cellular morphology via disturbing the microtubule dynamics. *Nanoscale* 7:8466-8475.

McKee MS, and Filser J. 2016. Impacts of metal-based engineered nanomaterials on soil communities. *Environmental Science: Nano* 3:506-533.

Medina C, Santosâ€□Martinez MJ, Radomski A, Corrigan OI, and Radomski MW. 2007. Nanoparticles: pharmacological and toxicological significance. *British journal of pharmacology* 150:552-558.

Mendonca MCP, Soares ES, de Jesus MB, Ceragioli HJ, Irazusta SP, Batista AG, Vinolo MAR, Junior MRM, and da Cruz-Hofling MA. 2016. Reduced graphene oxide: nanotoxicological profile in rats. *Journal of nanobiotechnology* 14:53.

1099 Mohajerani A, Burnett L, Smith JV, Kurmus H, Milas J, Arulrajah A, Horpibulsuk S, and Abdul
1100 Kadir A. 2019. Nanoparticles in construction materials and other applications, and
1101 implications of nanoparticle use. *Materials* 12:3052.
1102 Mohammed YH, Holmes A, Haridass IN, Sanchez WY, Studier H, Grice JE, Benson HAE, and
1103 Roberts MS. 2019. Support for the safe use of zinc oxide nanoparticle sunscreens: lack of
1104 skin penetration or cellular toxicity after repeated application in volunteers. *Journal of*
1105 *Investigative Dermatology* 139:308-315.
1106 Moore MN. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic
1107 environment? *Environment international* 32:967-976.
1108 Natoli M, Leoni BD, D'Agnano I, Zucco F, and Felsani A. 2012. Good Caco-2 cell culture
1109 practices. *Toxicology in Vitro* 26:1243-1246.
1110 Nel A, Xia T, Madler L, and Li N. 2006. Toxic potential of materials at the nanolevel. *science*
1111 311:622-627.
1112 Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, and
1113 Thompson M. 2009. Understanding biophysicochemical interactions at the nano–bio
1114 interface. *Nature materials* 8:543-557.
1115 Ng CT, Yong LQ, Hande MP, Ong CN, Yu LE, Bay BH, and Baeg GH. 2017. Zinc oxide
1116 nanoparticles exhibit cytotoxicity and genotoxicity through oxidative stress responses in
1117 human lung fibroblasts and Drosophila melanogaster. *Int J Nanomedicine* 12:1621-1637.
1118 10.2147/ijn.S124403
1119 Nguyen KC, Rippstein P, Tayabali AF, and Willmore WG. 2015. Mitochondrial toxicity of
1120 cadmium telluride quantum dot nanoparticles in mammalian hepatocytes. *Toxicological*
1121 *sciences* 146:31-42.
1122 Nohynek GJ, and Dufour EK. 2012. Nano-sized cosmetic formulations or solid nanoparticles in
1123 sunscreens: a risk to human health? *Archives of toxicology* 86:1063-1075.
1124 Nowack B, and Bucheli TD. 2007. Occurrence, behavior and effects of nanoparticles in the
1125 environment. *Environmental pollution* 150:5-22.
1126 Oberdorster G, Stone V, and Donaldson K. 2007. Toxicology of nanoparticles: a historical
1127 perspective. *Nanotoxicology* 1:2-25.
1128 Offermans NSM, Vermeulen R, Burdorf A, Goldbohm RA, Kauppinen T, Kromhout H, and van
1129 den Brandt PA. 2014. Occupational asbestos exposure and risk of pleural mesothelioma,
1130 lung cancer, and laryngeal cancer in the prospective Netherlands cohort study. *Journal of*
1131 *occupational and environmental medicine* 56:6-19.
1132 Oliveira AML, Machado M, Silva GA, Bitoque DB, Tavares Ferreira J, Pinto LA, and Ferreira Q.
1133 2022. Graphene Oxide Thin Films with Drug Delivery Function. *Nanomaterials* 12:1149.
1134 Ovais M, Nethi SK, Ullah S, Ahmad I, Mukherjee S, and Chen C. 2020. Recent advances in the
1135 analysis of nanoparticle-protein coronas. *Nanomedicine* 15:1037-1061.
1136 Pan X. 2021. Mutagenicity Evaluation of Nanoparticles by the Ames Assay. *Environmental*
1137 *Toxicology and Toxicogenomics*: Springer, 275-285.
1138 Panyala NR, Pena-Mendez EM, and Havel J. 2008. Silver or silver nanoparticles: a hazardous
1139 threat to the environment and human health? *Journal of applied biomedicine* 6:117-119.
1140 Patra JK, Das G, Fraceto LF, Campos EVR, del Pilar Rodriguez-Torres M, Acosta-Torres LS,
1141 Diaz-Torres LA, Grillo R, Swamy MK, and Sharma S. 2018. Nano based drug delivery
1142 systems: recent developments and future prospects. *Journal of nanobiotechnology* 16:71.

Perez JE, Contreras MF, Vilanova E, Felix LP, Margineanu MB, Luongo G, Porter AE, Dunlop IE, Ravasi T, and Kosel J. 2016. Cytotoxicity and intracellular dissolution of nickel nanowires. *Nanotoxicology* 10:871-880.

Peters K, Unger RE, Kirkpatrick CJ, Gatti AM, and Monari E. 2004. Effects of nano-scaled particles on endothelial cell function in vitro: studies on viability, proliferation and inflammation. *Journal of Materials Science: Materials in Medicine* 15:321-325.

Pokropivny V, and Skorokhod V. 2007. Classification of nanostructures by dimensionality and concept of surface forms engineering in nanomaterial science. *Materials Science and Engineering: C* 27:990-993.

Powell JJ, Faria N, Thomas-McKay E, and Pele LC. 2010. Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. *Journal of autoimmunity* 34:J226-J233.

Puppi J, Mitry RR, Modo M, Dhawan A, Raja K, and Hughes RD. 2011. Use of a clinically approved iron oxide MRI contrast agent to label human hepatocytes. *Cell transplantation* 20:963-976.

Rahman S, Ansari RA, Rehman H, Parvez S, and Raisuddin S. 2011. Nordihydroguaiaretic acid from creosote bush (Larrea tridentata) mitigates 12-O-tetradecanoylphorbol-13-acetate-induced inflammatory and oxidative stress responses of tumor promotion cascade in mouse skin. *Evidence-Based Complementary and Alternative Medicine* 2011.

Rahman S, Bhatia K, Khan AQ, Kaur M, Ahmad F, Rashid H, Athar M, Islam F, and Raisuddin S. 2008. Topically applied vitamin E prevents massive cutaneous inflammatory and oxidative stress responses induced by double application of 12-O-tetradecanoylphorbol-13-acetate (TPA) in mice. *Chemico-Biological Interactions* 172:195-205.

Rahman S, Farooque A, Anjum S, Ansari RA, Adhikari JS, Dwarakanath BS, and Raisuddin S. 2015. Nordihydroguiaretic acid attenuates skin tumorigenesis in Swiss albino mice with the condition of topical co-administration of an immunosuppressant. *Chemico-Biological Interactions* 233:106-114.

Raja PMV, Lacroix G, Sergent J-A, Bois F, Barron AR, Monbelli E, and Elgrabli D. 2017. Nanotoxicology: role of physical and chemical characterization and related in vitro, in vivo, and in silico methods. *Metrology and Standardization of Nanotechnology: Protocols and Industrial Innovations*:363-380.

Rajeshkumar S, Tharani M, Rajeswari VD, Alharbi NS, Kadaikunnan S, Khaled JM, Gopinath K, Vijayakumar N, and Govindarajan M. 2021. Synthesis of greener silver nanoparticle-based chitosan nanocomposites and their potential antimicrobial activity against oral pathogens. *Green Processing and Synthesis* 10:658-665.

Reeves JF, Davies SJ, Dodd NJF, and Jha AN. 2008. Hydroxyl radicals (OH) are associated with titanium dioxide (TiO2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 640:113-122.

Risom L, Moller P, and Loft S. 2005. Oxidative stress-induced DNA damage by particulate air pollution. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 592:119-137.

Romeyke T, and Stummer H. 2012. Clinical pathways as instruments for risk and cost management in hospitals-a discussion paper. *Global journal of health science* 4:50.

Ross R. 2014. Asbestosis, not asbestos exposure, is the primary risk factor for lung cancer. *American journal of respiratory and critical care medicine* 189:114-115.

Roy R, Parashar V, Chauhan LKS, Shanker R, Das M, Tripathi A, and Dwivedi PD. 2014. Mechanism of uptake of ZnO nanoparticles and inflammatory responses in macrophages require PI3K mediated MAPKs signaling. *Toxicology in Vitro* 28:457-467.

Ruenraroengsak P, Novak P, Berhanu D, Thorley AJ, Valsami-Jones E, Gorelik J, Korchev YE, and Tetley TD. 2012. Respiratory epithelial cytotoxicity and membrane damage (holes) caused by amine-modified nanoparticles. *Nanotoxicology* 6:94-108.

Sadaf A, Ahmad R, Ghorbal A, Elfalleh W, and Khare SK. 2020. Synthesis of cost-effective magnetic nano-biocomposites mimicking peroxidase activity for remediation of dyes. *Environmental Science and Pollution Research* 27:27211-27220.

Sahu SC, and Hayes AW. 2017. Toxicity of nanomaterials found in human environment: a literature review. *Toxicology Research and Application* 1:2397847317726352.

Saleh TA. 2020. Nanomaterials: Classification, properties, and environmental toxicities. *Environmental Technology & Innovation*:101067.

Saleh TA, and Fadillah G. 2019. Recent trends in the design of chemical sensors based on graphene–metal oxide nanocomposites for the analysis of toxic species and biomolecules. *TrAC Trends in Analytical Chemistry* 120:115660.

Sardar M, and Ahmad R. 2016. Green Synthesized Metal Nanoparticles: Applications in Biology and Medicine. IN VITRO CELLULAR & DEVELOPMENTAL BIOLOGY-ANIMAL: SPRINGER 233 SPRING ST, NEW YORK, NY 10013 USA. p S43-S43.

Sardar M, Mishra A, and Ahmad R. 2014. *Biosynthesis of metal nanoparticles and their applications*. USA: Scrivener Publishing.

Savage DT, Hilt JZ, and Dziubla TD. 2019. In Vitro Methods for Assessing Nanoparticle Toxicity. *Methods Mol Biol* 1894:1-29. 10.1007/978-1-4939-8916-4_1

Schmid G. 2008. The relevance of shape and size of Au 55 clusters. *Chemical Society Reviews* 37:1909-1930.

Schmid G, Reuter T, Simon U, Noyong M, Blech K, Santhanam V, Jäger D, Slomka H, Lüth H, and Lepsa MI. 2008. Generation and electrical contacting of gold quantum dots. *Colloid and Polymer Science* 286:1029-1037.

Seabra AB, Paula AJ, de Lima R, Alves OL, and Durán N. 2014. Nanotoxicity of graphene and graphene oxide. *Chemical research in toxicology* 27:159-168.

Shiau B-W, Lin C-H, Liao Y-Y, Lee Y-R, Liu S-H, Ding W-C, and Lee J-R. 2018. The characteristics and mechanisms of Au nanoparticles processed by functional centrifugal procedures. *Journal of Physics and Chemistry of Solids* 116:161-167.

Shvedova AA, Pietroiusti A, Fadeel B, and Kagan VE. 2012. Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicology and applied pharmacology* 261:121-133.

Singh BP, Kumar K, and Jain VK. 2021. Source identification and health risk assessment associated with particulate-and gaseous-phase PAHs at residential sites in Delhi, India. *Air Quality, Atmosphere & Health* 14:1505-1521.

Singh G, and Pai RS. 2014. Pharmacokinetics and in vivo biodistribution of optimized PLGA nanoparticulate drug delivery system for controlled release of emtricitabine. *Drug delivery* 21:627-635.

Singh N, Jenkins GJ, Asadi R, and Doak SH. 2010. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). *Nano reviews* 1:5358.

1232 Sipes NS, Padilla S, and Knudsen TB. 2011. Zebrafish-As an integrative model for twenty-first
1233 century toxicity testing. *Birth Defects Research Part C: Embryo Today: Reviews* 93:256-
1234 267.
1235 Smerkova K, Dolezelikova K, Bozdechova L, Heger Z, Zurek L, and Adam V. 2020.
1236 Nanomaterials with active targeting as advanced antimicrobials. *Wiley Interdisciplinary*
1237 *Reviews: Nanomedicine and Nanobiotechnology* 12:e1636.
1238 Soenen SJ, Parak WJ, Rejman J, and Manshian B. 2015. (Intra) cellular stability of inorganic
1239 nanoparticles: effects on cytotoxicity, particle functionality, and biomedical applications.
1240 *Chemical reviews* 115:2109-2135.
1241 Soto K, Carrasco A, Powell T, Garza K, and Murr L. 2005. Comparative in vitro cytotoxicity
1242 assessment of some manufacturednanoparticulate materials characterized by
1243 transmissionelectron microscopy. *Journal of Nanoparticle Research* 7:145-169.
1244 Srikanth M, Misak H, Yang S-Y, and Asmatulu R. 2015. Effects of morphology, concentration
1245 and contact duration of carbon-based nanoparticles on cytotoxicity of l929 cells. ASME
1246 International Mechanical Engineering Congress and Exposition: American Society of
1247 Mechanical Engineers. p V014T011A027.
1248 Stark WJ, Stoessel PR, Wohlleben W, and Hafner A. 2015. Industrial applications of nanoparticles.
1249 *Chemical Society Reviews* 44:5793-5805.
1250 Stern ST, Adiseshaiah PP, and Crist RM. 2012. Autophagy and lysosomal dysfunction as emerging
1251 mechanisms of nanomaterial toxicity. *Particle and fibre toxicology* 9:20.
1252 Sudhakar C, Poonkothai M, Selvankmuar T, Selvam K, Rajivgandhi G, Siddiqi MZ, Alharbi NS,
1253 Kadaikunnan S, and Vijayakumar N. 2021. Biomimetic synthesis of iron oxide
1254 nanoparticles using Canthium coromandelicum leaf extract and its antibacterial and
1255 catalytic degradation of Janus green. *Inorganic Chemistry Communications* 133:108977.
1256 Sukhanova A, Bozrova S, Sokolov P, Berestovoy M, Karaulov A, and Nabiev I. 2018. Dependence
1257 of nanoparticle toxicity on their physical and chemical properties. *Nanoscale research*
1258 *letters* 13:1-21.
1259 Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, and Heyder J.
1260 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats.
1261 *Environmental health perspectives* 109:547-551.
1262 Tkach AV, Yanamala N, Stanley S, Shurin MR, Shurin GV, Kisin ER, Murray AR, Pareso S,
1263 Khaliullin T, and Kotchey GP. 2013. Graphene oxide, but not fullerenes, targets
1264 immunoproteasomes and suppresses antigen presentation by dendritic cells. *Small* 9:1686-
1265 1690.
1266 Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, Rio-Echevarria
1267 IM, and Van den Eede G. 2019. Review of micro-and nanoplastic contamination in the
1268 food chain. *Food Additives & Contaminants: Part A* 36:639-673.
1269 Toy R, Peiris PM, Ghaghada KB, and Karathanasis E. 2014. Shaping cancer nanomedicine: the
1270 effect of particle shape on the in vivo journey of nanoparticles. *Nanomedicine* 9:121-134.
1271 Vijayakumar N, Bhuvaneshwari VK, Ayyadurai GK, Jayaprakash R, Gopinath K, Nicoletti M,
1272 Alarifi S, and Govindarajan M. 2022. Green synthesis of zinc oxide nanoparticles using
1273 Anoectochilus elatus, and their biomedical applications. *Saudi Journal of Biological*
1274 *Sciences* 29:2270-2279.
1275 Wallach D, Kang T-B, and Kovalenko A. 2014. Concepts of tissue injury and cell death in
1276 inflammation: a historical perspective. *Nature Reviews Immunology* 14:51-59.

1277 Walters C, Pool E, and Somerset V. 2016. Nanotoxicology: a review. *Toxicology-new aspects to*
1278 *this scientific conundrum InTech*:45-63.
1279 Wang Y-L, Lee Y-H, Chiu IJ, Lin Y-F, and Chiu H-W. 2020a. Potent impact of plastic
1280 nanomaterials and micromaterials on the food chain and human health. *International*
1281 *journal of molecular sciences* 21:1727.
1282 Wang Y, Yang Y, Shi Y, Song H, and Yu C. 2020b. Antibiotic-free antibacterial strategies enabled
1283 by nanomaterials: progress and perspectives. *Advanced Materials* 32:1904106.
1284 Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, and Webb TR. 2004.
1285 Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats.
1286 *Toxicological sciences* 77:117-125.
1287 Wong J, Magun BE, and Wood LJ. 2016. Lung inflammation caused by inhaled toxicants: a
1288 review. *International journal of chronic obstructive pulmonary disease* 11:1391.
1289 Wong SW, Zhou G-J, Kwok KW, Djurišić AB, Han J, Lee J-S, and Leung KM. 2020. In vivo
1290 toxicities of nine engineered nano metal oxides to the marine diatom Skeletonema costatum
1291 and rotifer Brachionus koreanus. *Marine pollution bulletin* 153:110973.
1292 Wu X, Tan Y, Mao H, and Zhang M. 2010. Toxic effects of iron oxide nanoparticles on human
1293 umbilical vein endothelial cells. *International journal of nanomedicine* 5:385.
1294 Xia T, Li N, and Nel AE. 2009. Potential health impact of nanoparticles. *Annual review of public*
1295 *health* 30:137-150.
1296 Xu A, Chai Y, Nohmi T, and Hei TK. 2009. Genotoxic responses to titanium dioxide nanoparticles
1297 and fullerene in gpt delta transgenic MEF cells. *Particle and fibre toxicology* 6:1-13.
1298 Xue Y, Wu J, and Sun J. 2012. Four types of inorganic nanoparticles stimulate the inflammatory
1299 reaction in brain microglia and damage neurons in vitro. *Toxicology letters* 214:91-98.
1300 Yong K-T, Law W-C, Hu R, Ye L, Liu L, Swihart MT, and Prasad PN. 2013. Nanotoxicity
1301 assessment of quantum dots: from cellular to primate studies. *Chemical Society Reviews*
1302 42:1236-1250.
1303 Zhang XD, Wu D, Shen X, Liu PX, Yang N, Zhao B, Zhang H, Sun YM, Zhang LA, and Fan FY.
1304 2011. Size-dependent in vivo toxicity of PEG-coated gold nanoparticles. *Int J*
1305 *Nanomedicine* 6:2071-2081. 10.2147/ijn.S21657
1306 Zhao L, Lu L, Wang A, Zhang H, Huang M, Wu H, Xing B, Wang Z, and Ji R. 2020. Nano-
1307 biotechnology in agriculture: use of nanomaterials to promote plant growth and stress
1308 tolerance. *Journal of agricultural and food chemistry* 68:1935-1947.
1309 Zhao Y, Sun X, Zhang G, Trewyn BG, Slowing II, and Lin VSY. 2011. Interaction of mesoporous
1310 silica nanoparticles with human red blood cell membranes: size and surface effects. *ACS*
1311 *nano* 5:1366-1375.
1312 Zhong Q, and Zhang L. 2019. Nanoparticles fabricated from bulk solid lipids: Preparation,
1313 properties, and potential food applications. *Advances in colloid and interface science*
1314 273:102033.
1315 Zhu R, Wang S, Chao J, Sun X, and Yao S. 2008. Oxidative and binding effect of nano-TiO2 on
1316 plasmid DNA and pepsin. 2008 2nd International Conference on Bioinformatics and
1317 Biomedical Engineering: IEEE. p 1013-1016.
1318 Zhu X, Hondroulis E, Liu W, and Li Cz. 2013. Biosensing approaches for rapid genotoxicity and
1319 cytotoxicity assays upon nanomaterial exposure. *Small* 9:1821-1830.
1320 Zhu X, Li N, Huang C, Li Z, and Fan J. 2020. Membrane Perturbation and Lipid Flip-Flop
1321 Mediated by Graphene Nanosheet. *The Journal of Physical Chemistry B* 124:10632-10640.

Zielinska A, Costa B, Ferreira MV, Migueis D, Louros J, Durazzo A, Lucarini M, Eder P, Chaud MV, and Morsink M. 2020. Nanotoxicology and nanosafety: Safety-by-design and testing at a glance. *International Journal of Environmental Research and Public Health* 17:4657.

Legend to Figure:

Fig. 1. Toxic interactions of nanoparticles in human and animal bodies.

Fig. 2. Toxicity of nanoparticles at the cellular level. A human is exposed to these nanoparticles by different means, which enters into the cells by lysosomes mediated endocytosis, which results in lysosome dysfunction and ROS generation. These ROS inside the cells causes lipid peroxidation of the membrane, protein modification, mitochondrial dysfunction, and DNA damage

Figure 1

Toxic interactions of nanoparticles in human and animal bodies

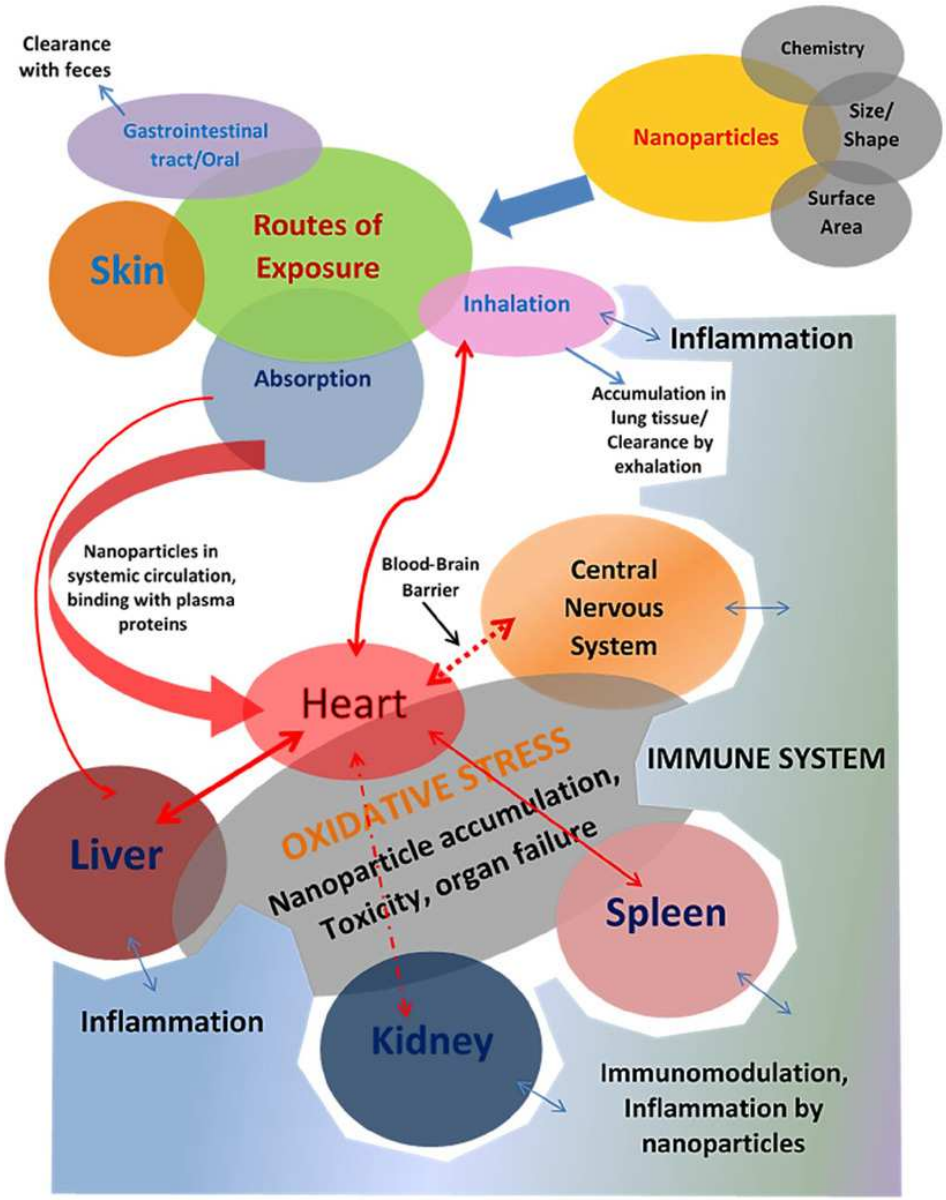
Clearance with feces
Gastrointestinal tract/Oral
Chemistry
Size/ Shape
Surface Area
Nanoparticles
Routes of Exposure
Skin
Inhalation
Inflammation
Absorption
Accumulation in lung tissue/ Clearance by exhalation
Nanoparticles in systemic circulation, binding with plasma proteins
Blood-Brain Barrier
Central Nervous System
Heart
IMMUNE SYSTEM
OXIDATIVE STRESS
Nanoparticle accumulation,
Toxicity, organ failure
Liver
Spleen
Inflammation
Kidney
Immunomodulation, Inflammation by nanoparticles

Fig. 1.

Figure 2

Toxicity of nanoparticles at the cellular level

Toxicity of nanoparticles at the cellular level. A human is exposed to these nanoparticles by different means, which enters into the cells by lysosomes mediated endocytosis, which results in lysosome dysfunction and ROS generation. These ROS inside the cells causes lipid peroxidation of the membrane, protein modification, mitochondrial dysfunction, and DNA damage.

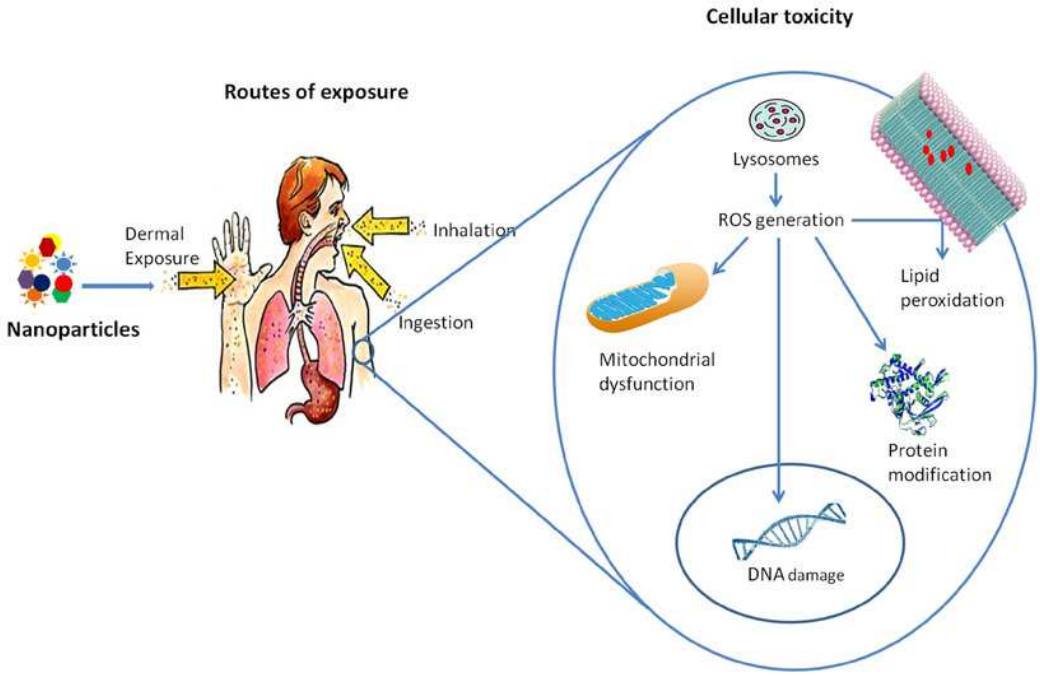

Fig. 2.

1

Table 1(on next page)

Nanomaterials and their toxicity on mammalian cells

Nanomaterials and their toxicity on mammalian cells

1 **Table 1. Nanomaterials and their toxicity on mammalian cells**

S. No.	Nanoparticle Type/Nanomaterial	Size/shape/concentration of Nanomaterial	Effect on mammalian cells	References
1.	Silver	20 μm	Cytotoxic to murine macrophage cell line has been found in the colon and blood of patients with colon cancer and blood cancer, respectively	(Gatti 2004; Jain et al. 2011; Soto et al. 2005; Takenaka et al. 2001)
2.	Superparamagnetic Iron oxide	Concentration >100 μg/ml	Reportedly, impairs the functions of DNA, nucleus and mitochondria and causes inflammation	(Brunner et al. 2006; Nel et al. 2006; Oberdorster et al. 2007; Singh et al. 2010; Sudhakar et al. 2021)
3.	Zirconium dioxide	11 nm particle size	Increases viral receptor expressions	(Lucarelli et al. 2004)
4.	Cadmium nanorod	30-50 nm diameter & 50-1100 nm length, concentration 1000-10000 mg/kg.	Genotoxicity, oxidative stress and DNA damage in Kunming mice	(Demir 2021; Liu et al. 2014a)

5.	Cerium oxide Nanorods	Length >200 nm	Progressive pro-inflammatory effect and cytotoxicity in Human myeloid cell line	(Ji et al. 2012)
6.	Hydroxyapatite (nano-HAP)	Crystals, H-rod, H-needle, H-sphere, H-plate	Decreased cell viability and consequent necrosis in rat aortic smooth muscle cells	(Huang et al. 2019)
7.	Citrate capped gold nanoparticle	5 nm	Toxicity, increased Cytokine production in mouse fibroblast	(Jia et al. 2017)
8.	Nickel Nanowire	33 nm diameter, 5.4 µm length, 5 µg/ml	Cytotoxicity and decreased cell viability in human colorectal carcinoma HCT 116 cells	(Perez et al. 2016)
9.	Zinc oxide Nanoparticle	4-20 nm diameter	Low viability, ROS production, cytotoxicity in human immune cells (e.g., monocyte)	(Hanley et al. 2009)
10.	Titanium oxide	70 nm diameter, 50 µg/ml	Inflammation, elevated IL-8 in human microvascular endothelial cells	(Peters et al. 2004)
		3 and 600µg/ml	Shrinking of cells, lower metabolic activity, releasing of LDH,ROS production in mouse fibroblastL929 Please, fix and add a space.	(Jin et al. 2008)

11.	Graphene oxide	Upton 25µg/ml	Effected antigen inhibition linked to downregulated intracellular levels of immune proteasome	(Seabra et al. 2014; Tkach et al. 2013)
12.	Mesoporous Silica nanoparticle	100 nm	Membrane deformities and hemolysis in human red blood cells	(Lin & Haynes 2010)

2

Table 2(on next page)

Physico-chemical properties of nanomaterial responsible for its cytotoxicity and associated mechanism

Physico-chemical properties of nanomaterial responsible for its cytotoxicity and associated mechanism

1 **Table 2: Physico-chemical properties of nanomaterial responsible for its cytotoxicity and associated mechanism.**

S. No.	Type of Nanoparticle	Source of nanomaterial	Physico-chemical property responsible for cytotoxicity	Mechanisms for Cytotoxic effect	References
1.	Mesoporous silica nanoparticles (MSNs)	Tetra ethyl orthosilicate	Size (~ 600 nm) dependent cytotoxicity	severe local membrane distortion resulting in RBC spherocytosis, internalization of the particles, and ultimately hemolysis.	(Zhao et al. 2011)
2.	Graphene oxide (GO) nanosheets	Oxidation of graphite oxide	concentration-dependent cytotoxicity	Direct interactions between the cell membrane and GO nanosheets that cause physical harm to the cell membrane cause cytotoxicity due to high absorption	(Hu et al. 2011; Zhu et al. 2020)
3.	Titanium dioxide (TiO_2) nanoparticles	Titanium dioxide	Concentration (100 $\mu g\ ml^{-1}$) dependent neurotoxicity	Tau-TiO_2 NP interaction is observed when neuroblastoma cells are exposed to the nanoparticles. Microtubules may become unstable as a result of interactions between TiO_2 and tau proteins, tubule heterodimers, and microtubules, which increases the neurotoxicity of TiO_2 NPs	(Mao et al. 2015; Sukhanova et al. 2018)
4.	Iron oxide nanoparticles	Iron oxide	Citrate and Dextran coating on the	Highly reactive hydroxyl radicals are produced by the Haber-Weiss	(Puppi et al. 2011; Sadaf et

	(IONPs)		surface of NP.	reaction in cells (e.g., HUVECs). At larger intracellular quantities of iron salts, the Fenton reaction is detectable. The free radicals have the potential to harm DNA, cell membranes, the cytoskeleton, and ECM. They can also directly or indirectly mediate signal transduction pathways through bioactive mediators	al. 2020; Sukhanova et al. 2018; Wu et al. 2010)
5.	Au55 clusters	Reduction of (triphenylphosphine)gold chloride with diborane followed by incorporation into SBA-15 silica mesopores	cuboctahedral structure and 1.4nm size	Cytotoxicity(necrosis) brought on by the remarkably strong interaction of the 1.4 nm particles with the main grooves of DNA	(Schmid 2008; Schmid et al. 2008)
6.	Cadmium telluride quantum dot NPs	1-thioglycerol, L-cysteine, thioglycolic acid and Cd^{2+}	Chemical compostion-presence of cadmium and tellurium	CdTe-QDs alter the potential of the mitochondrial membrane via increasing Ca^{2+} levels. The cadmium component of the NPs causes CdTe-QDs' impact on Ca2+. Increased intracellular Ca2+ has been linked to cadmium-induced apoptosis	(Belyaeva et al. 2012; Nguyen et al. 2015)
7.	Polystyrene	n-pentanol, sodium	Surface charge	Positively charged nanoparticles	(Liu et al. 2011;

	NPs	dodecyl sulphate, ammonium persulfate,		(NPs) more efficiently cross the membrane, but also because they are more tightly linked to the negatively charged DNA, damaging it and lengthening the G0/G1 phase of the cell cycle as a result	Sukhanova et al. 2018)
8.	Casein-coated iron oxide nanoparticles	Casein, iron chloride tetrahydrate, and iron chloride hexahydrate	NP Shell	Shell enhances durability and guards NP against desalination and oxidative or photolytic deterioration. As a result, the toxicity of NP is reduced. Due to the wide range of modifiers, altering the NP characteristics as well as their particular transport and accumulation is possible that can increase or decrease the toxicity	(Huang et al. 2013)
9.	Zinc oxide NP	Zinc salts	Zn^{2+} ions	ZnO NPs generate an increase in cytoplasmic and mitochondrial Zn^{2+} levels, which can ultimately result in mitochondrial damage and apoptosis in cells	(Soenen et al. 2015)
10.	Gold NP	Au^{+1} or Au^{3+}	Plasmonic properties	The cytotoxicity is caused by the ionic interactions of the Au-NPs with the plasma membrane. Genes including those connected to the cell cycle, particularly those engaged in the G1 phase and those involved in	(Lee et al. 2019)

				the nucleic metabolic process, are down-regulated	
11.	Polystyrene latex NP	Styrene, sodium hydrogen carbonate and potassium persulfate	Surface modification and particle size (50 nm and 100 nm)	On cell membranes, there have been reports of significant damage and holes that have not been seen with other kinds of NPs. Along with enhanced CXCL8 production, this also induces apoptotic (caspase-3/7 and caspase-9) cell death (IL-8) and cell detachment	(Ruenraroengsak et al. 2012)