Too small to matter? Physicochemical transformation and toxicity of engineered $nTiO_2$, $nSiO_2$, nZnO, carbon nanotubes, and nAg

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Too small to matter? Physicochemical transformation and toxicity of engineered $nTiO_2$, $nSiO_2$, nZnO, carbon nanotubes, and nAg.

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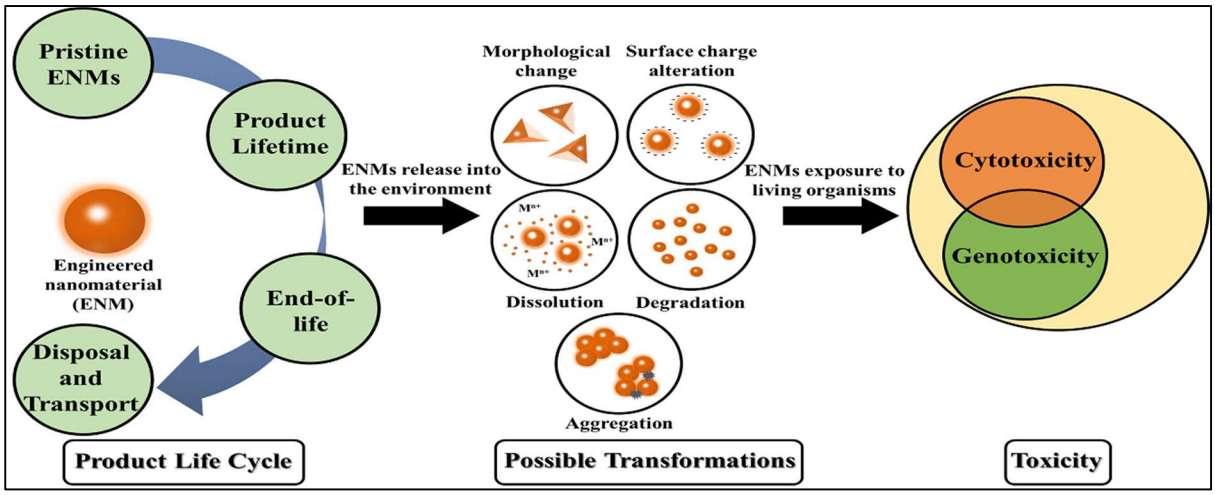
Abstract

Engineered nanomaterials (ENMs) refer to a relatively novel class of materials that are increasingly prevalent in various consumer products and industrial applications – most notably for their superlative physicochemical properties when compared with conventional materials. However, consumer products inevitably degrade over the course of their lifetime, releasing ENMs into the environment. These ENMs undergo physicochemical transformations and subsequently accumulate in the environment, possibly leading to various toxic effects. As a result, a significant number of studies have focused on identifying the possible transformations and environmental risks of ENMs, with the objective of ensuring a safe and responsible application of ENMs in consumer products. This review aims to consolidate the results from previous studies

related to each stage of the pathway of ENMs from being embodied in a product to disintegration/transformation in the environment. The scope of this work was defined to include the five most prevalent ENMs based on recent projected production market data, namely: $nTiO_2$, $nSiO_2$, nZnO, carbon nanotubes, and nAg. The review focuses on: (i) models developed to estimate environmental concentrations of ENMs; (ii) the possible physicochemical transformations; (iii) cytotoxicity and genotoxicity effects specific to each ENM selected; and (iv) a discussion to identify potential gaps in the studies conducted and recommend areas where further investigation is warranted.



Keywords: nanomaterials; nanoparticles; nanotoxicity; transformation; cytotoxicity; genotoxicity.

Contents

4. Physicochemical Transformation of ENMs
   4.1. $nTiO_2$
   4.2. $nSiO_2$
   4.3. nZnO
   4.4. CNTs
   4.5. nAg
5. Toxicity of ENMs
   5.1. Cytotoxicity of ENMs
   5.1.1. $nTiO_2$
   5.1.2. $nSiO_2$
   5.1.3. nZnO
   5.1.4. CNTs
   5.1.5. nAg
   5.2. Genotoxicity of ENMs
   5.2.1. $nTiO_2$
   5.2.2. $nSiO_2$
   5.2.3. nZnO
   5.2.4. CNTs
   5.2.5. nAg
6. Conclusions and Future prospects
   6.1. Development of comprehensive and unified inventory database
   6.2. Development of sophisticated computational approaches
   6.3. Establishment of scientific correlations and development of cause-pathway-influence-effect relationship
   6.4. Implementation of systematised standard protocols and harmonized reporting

List of Abbreviations	
CAGR	Compound annual growth rate
cit-nAg	citrate-capped Ag nanomaterial(s)
CNM	Carbon nanomaterial
CNT	Carbon nanotube
DLVO	Derjaguin-Landau-Verwey-Overbeek
ENM	Engineered nanomaterial
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GD	Guidance document

HA	Humic acid
ISO	International organization for Standards
L-cys	L-cysteine
LOEC	Lowest observed effect concentration
MDA	Malondialdehyde
MOA	Mode of action
MWCNT	Multi-walled CNT
NAL-cys	N-acetyl L-cysteine
nAg	Ag nanomaterial(s)
$nCeO_2$	CeO_2 nanomaterial(s)
$nSiO_2$	SiO_2 nanomaterial(s)
$nTiO_2$	TiO_2 nanomaterial(s)
nZnO	ZnO nanomaterial(s)
NOM	Natural organic matter
O-MWCNT	Oxidised MWCNT
OECD	Organisation for Economic Co-operation and Development
OHT	OECD harmonised template
PEI	Polyethylenimine
PVP	Polyvinylpyrrolidone
ROS	Reactive oxygen species
SWCNT	Single-walled CNT
SAS	Synthetic amorphous silica
TEM	Transmission electron microscope
TG	Testing guideline
TLR	Toll-like receptors
US	United States of America
UV	Ultraviolet
WHO	World Health Organization

WWTP	Wastewater treatment plants

1. Introduction

Engineered nanomaterials (ENMs) are manufactured materials with any external dimension or internal structure or surface structure in the nanoscale (ca. 1 to 100 nm) that are designed for a specific purpose or function (ISO/TS, 80004-1:2015). The invention of ENMs has allowed us to imbue revolutionary functions into otherwise ordinary materials. By engineering ordinary chemicals or materials at nanoscale, the resulting products are enhanced with additional properties: ultraviolet (UV) protection, antimicrobial effects, increased strength, flexibility and conductivity (Mitrano et al., 2015). The potential applications of ENMs into both consumer and industrial products are expected to be ubiquitous in the near future (Aitken et al., 2006). According to the Organisation for Economic Co-operation and Development (OECD) (2016), the global market for nanomaterials was estimated at 11 million tonnes with market value of 20 billion Euros in 2012. Grillo et al. (2018) reported an estimated production output of up to 270,000 tonnes/year for SiO_2, TiO_2, FeO_x, AlO_x, ZnO and CeO_2 ENMs combined. As more ENM-containing products enter the market, significant volumes of ENMs would be released gradually into the natural environment due to degradation over their lifetime, posing unknown toxic effects on the organisms they encounter. It was estimated that 189,200, 51,600, 8,100 and 69,200 tonnes/year of the studied ten ENMs being released into landfill, soil, air and water, respectively (Keller and Lazareva, 2014). In order to quantify the toxic effects of ENM release, it is essential to (i) estimate the concentration of ENMs in various environmental reservoirs (Keller and Lazareva,

2014), (ii) understand the transformations of ENMs prior to accumulation in the environment, and (iii) assess the different mechanisms and modes of toxicity of ENMs at relevant environmental concentrations (Gottschalk et al., 2013). Hence, it would be beneficial if the increasing prevalence of ENMs is accompanied by rigorous studies that verify whether ENMs pose any harm towards the environment and various communities of organisms they host (Ren et al., 2016).

Numerous studies have been reported in the three areas of research (i-iii) mentioned above. (Mitrano et al., 2015) provided a comprehensive review of the transformation of ENMs with respect to environmental parameters. Various models were proposed to simulate ENM flow and accumulation in the environment (Gottschalk et al., 2013; Sun et al., 2014b). However, considering the ENM's impact on the ecosystems, previous studies indicate that any consensus would be inconclusive regarding the effects of environmental parameters on the exposure rate, physicochemical transformation, and toxicity effects of ENMs (Ren et al., 2016). Furthermore, testing for toxicity of ENMs on biological systems has proven to be challenging due to the difficulty in observing their concomitant effects at a cellular level. While *in vitro* and *in vivo* studies have attempted to identify and explain the toxicity mechanisms of ENMs, these studies sometimes arrive at contrasting conclusions. Nevertheless, research should progress in all three areas to ensure safe and sustainable use of ENMs on a global scale.

Giusti et al. (2019) reported on 20 grouping strategies for ENMs by international organizations and national agencies based on various criteria such as physicochemical properties, reactivity, human health risks, hazards, exposure, toxicity, and other tiered

approaches. However, it was also highlighted that none of the approaches have been formally validated and the applicability of these grouping approaches is yet to be demonstrated via case studies. Hence, this study reviewed the ENMs based on individual aspects rather than under a specific grouping approach due to the high uncertainties associated with the different types of ENMs. Grouping of the types of ENMs would be an inconsiderate generalization to the enormous variations in the effects exhibited by the individual ENMs. Instead, each of these ENMs should further be explored as a distinct group or umbrella covering the variations within these individual ENMs including coated, enriched, and modified forms. Importantly, an integrated review of the prevalent ENMs covering their market projections, environmental flow and transport models, physicochemical transformations, cytotoxicities, and genotoxicities has been sparsely reported. This review aims at consolidating recent information on the flow models of ENMs in environmental media, physicochemical transformations of ENMs, and cytotoxicity and genotoxicity of ENMs from a wide range of studies (Figure 1). Specifically, this review focuses on the state of knowledge of the five most prevalent ENMs in potential applications during this decade, based on the current trajectories of production levels and market share. Large-scale production implies increased demand and application that would lead to increased release and exposure to the natural environment. These ENMs are focused as priorities as the implications of releasing these materials present greater importance. We categorically review the scientifically reported physicochemical transformations of the five ENMs and present the cytotoxic and genotoxic implications caused by these ENMs

on the various organisms. Further, interactions of ENMs with materials of biological nature, including biomacromolecules, organisms, individual cells, and enzymes, leading to biotransformations of the inorganic ENMs are excluded in this review. The study would aid the readers to understand the implications of the prevalent ENMs in the environment, understand the current state of research and development, highlight the gaps, and progress towards future research.

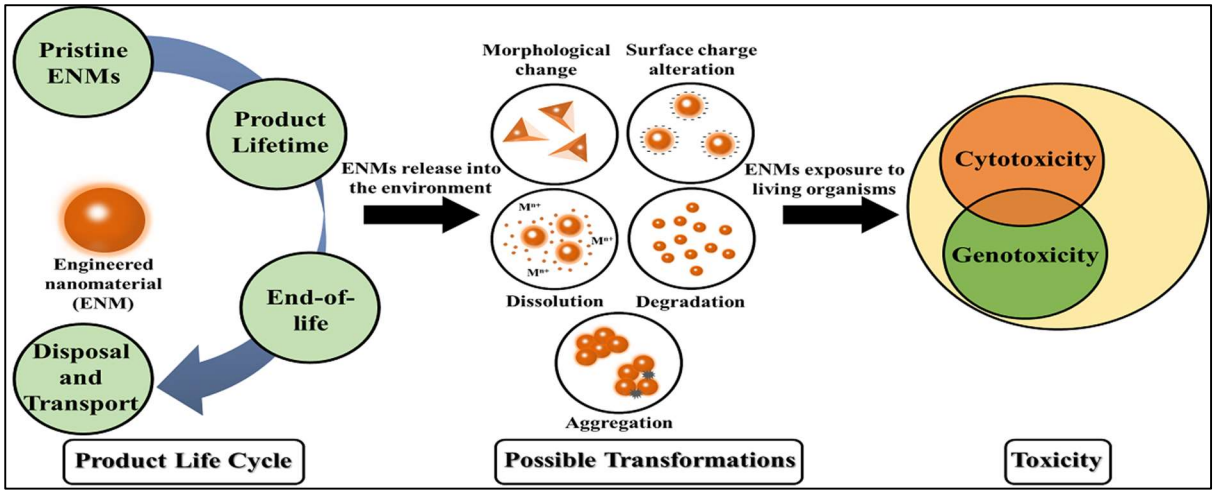


Figure 1. Life cycle, possible transformations, and toxicity of engineered nanomaterials

2. Trajectory of ENMs production and applications

Although concealed, ENMs are present in many consumer products, such as nAg in textiles, $nTiO_2$ in sunscreens and paints, and carbon nanotubes (CNTs) in wiring and plastic composites (Aitken et al., 2006). As there are numerous ENM-related materials currently incorporated in products, the top five ENMs with the largest production volumes in the near future are identified and the scope is limited to the studies that

investigated these five ENMs. A similar approach was implemented by (Sun et al., 2014b) and (Keller et al., 2013).

The novelty of ENMs makes the market data for global ENM production limited, thereby increasing the uncertainty in the scale of production of several ENMs. Table 1 shows the current scale of production/market share of several ENMs. While numerous types of ENMs exist, the figure only displays those with relatively high volumes of production or market share.

Table 1. Scale of production (tonnes/year) of various prevalent ENMs within the last decade.

Source	**Average value or range of production (t/y)**					**Remarks**
	$nTiO_2$	**$nSiO_2$**	**nZnO**	**CNT**	**nAg**	
Hendren et al., 2011	(7,800-38,000)	-	-	(55-1,101)	(2.8-20)	Within USA
Farré et al., 2011	-	-	-	1,000	-	Global
Piccinno et al., 2012	3,000 (550-5,500)	5500 (55-55,000)	550 (55-550)	300 (55-550)	55 (5.5-550)	Global
Piccinno et al., 2012	550 (55-3,000)	5500 (55-55,000)	55 (5.5-28,000)	550 (180-550)	5.5 (0.6-5.5)	Within EU
(Lazareva and Keller, 2014	(83,500-88,000)	(82,500-95,000)	(31,500-34,000)	(2,916-3,200)	(360-450)	Global
(Sun et al., 2014b)	10,000	-	1,600	380	30	Within EU

Wang et al., 2016a	-	(32,448-245,549)	-	-	-	Within EU
Sun et al., 2016	39,000	-	7,300	730	50	Within EU
Part et al., 2018	10,000,000	1,500,000	>1,000	500-600	22	Global
Suzuki et al., 2018	(3841-3914)	(106-465)	-	-	-	Within Japan (Construction sector)

Based on the information shown in Table 1, the following five ENMs were selected as focus materials for this review in descending order: $nTiO_2$, $nSiO_2$, nZnO, CNTs and nAg. Despite a lower production volume, nAg was preferred because of its significance as an antimicrobial agent, which could pose significant impacts on ecosystems by interfering with bacterial communities and associated ecosystem processes (McGillicuddy et al., 2017). The following sections briefly summarise the applications associated with each ENM as well as their production, distribution and market projection.

2.1. $nTiO_2$

The $nTiO_2$ is one of the most widely produced ENMs amongst various industries, with its principal applications in cosmetics, paints and coatings. At least half the annual production of $nTiO_2$ in the European Union (EU) is used in cosmetics, and approximately 10-30% was found in paints or coatings as a white pigment or as a protective shield against UV irradiation due to its photocatalytic nature (Mitrano et al., 2015; Piccinno et al., 2012). The $nTiO_2$ is mainly found bounded in an inert matrix of

varying chemical compositions in their pristine form. Gradual degradation of this matrix will lead to the eventual release of ENMs into the surroundings, which would influence the environmental safety of ENMs' usage (Mueller and Nowack, 2008). Robichaud et al. (2009) predicted that the global $nTiO_2$ production levels would exceed 2.5 million tonnes by 2025. Likewise, recent market data suggest a similar growth in global production with a market share approximating US$ 15.76 billion in 2018 and a compound annual growth rate (CAGR) of 8.7% from 2019-2025 (Grand View Research, 2019).

2.2. $nSiO_2$

The $nSiO_2$ predominantly serves as a filler for concrete, paints and other materials (Nguyen et al., 2012). Another application investigated was its suitability as a substitute for cement in concrete in order to reduce the production cost and carbon emissions of concrete production (Quercia and Brouwers, 2010). Similar to the $nTiO_2$, degradation of the host matrix could release ENMs, exposing biological systems to the $nSiO_2$. The production of $nSiO_2$ within the EU reached nearly 6000 tonnes with a majority of its applications found in paints, cosmetics and plastics (Mitrano et al., 2015). In the near future, the global $nSiO_2$ market size is projected to reach approximately US$ 5.14 billion by 2025, with a CAGR of 7.6% from 2016-2025 (Grand View Research, 2017).

2.3. nZnO

The major applications of the nZnO are similar to that of the $nTiO_2$ as both ENMs exhibit similar photocatalytic properties and UV light scattering ability (Ng et al., 2017).

Like the $nTiO_2$, the nZnO is often found bounded within a host matrix. However, the difference between these two ENMs is the superlative light screening effects of the nZnO, thereby reducing the rate of photodegradation of the host matrix (Mitrano et al., 2015). Furthermore, the nZnO is water-soluble unlike the $nTiO_2$; hence no chalking ensues unlike the $nTiO_2$-containing paints. Therefore, the nZnO is appropriate for products that are regularly subject to exposure and removal via water, such as cosmetics and sunscreens (Mitrano et al., 2015). Recently, another possible characteristic of the nZnO as an antimicrobial agent was explored (Wang et al., 2017a), suggesting potential applications in the food industry and for biomedical purposes (Ng et al., 2017). It is estimated that approximately 1600 tonnes/year of the nZnO are produced within the EU, with almost 80% utilized in cosmetics, and the rest mainly applied in plastics and paints (Mitrano et al., 2015; Sun et al., 2014b). In 2015, the global nZnO market was valued at US$ 2.10 billion and is expected to reach US$ 7.68 billion by 2022, registering a CAGR of 20.4% during the forecast period 2016 – 2022 (Sahu, 2016).

2.4. Carbon nanotubes (CNTs)

The pristine form of CNTs is “seamless cylinders of one or more layers of graphene, with open or closed ends” (De Volder et al., 2013). The CNTs can be single-walled (SWCNTs) or multi-walled CNTs (MWCNTs), with one or more functional surface groups depending on the desired properties of the material. In polymer nanocomposites, the CNTs are embedded within the polymer matrix to provide unique functionalities and properties, thereby making nanocomposite material production an emerging industry with increasing demand (De Volder et al., 2013). The utilization of CNTs has

recently extended into many product sectors because of their excellent electrical conductivity, mechanical integrity, and versatility. For example, CNTs have often been incorporated as a filler in polymeric media to enhance the mechanical and electrical properties of thermoplastic polyurethane (TPU) nanocomposites (Wohlleben et al., 2013). Most CNTs (ca 70%) produced in the EU are used in manufacturing plastics/polymers (Mitrano et al., 2015) and the potential future applications of CNTs may focus on microelectronics, energy storage, biotechnology and coatings (De Volder et al., 2013). The global CNTs market was valued at US$ 1.03 billion and is expected to reach US$ 3.81 billion by 2022, registering a CAGR of 20.6% during the forecast period 2016 – 2022 (Sahu and Prasad, 2016).

2.5. nAg

Due to their antimicrobial properties, nAg is currently used in a range of products such as textiles, food, electronics and coatings (Foldbjerg et al., 2015). The nAg in products can be engineered to release at a controlled rate, increasing the lifespan of the product's antimicrobial properties (Mitrano et al., 2015). Compared to the other ENMs, nAg is produced in relatively small quantities as shown in Table 1. However, to benefit from the antimicrobial properties of nAg, more products containing nAg are anticipated in the near future. Sun et al. (2014b) claimed that approximately 30 tonnes of nAg were produced annually in the EU and another survey proposed a global consumption of 800 tonnes in 2012 (Avalon Global Research, 2012). The global nAg market was valued at US$ 433 million in 2015, and is expected to reach US$ 1.61 billion by 2022, manifesting a CAGR of 20.5% (Doshi, 2016).

Hence, all the five ENMs foresee a CAGR between 7 - 20% until 2025. The high CAGR emphasizes the importance and prerequisite for an established ENM characterization procedure, development of ENM data repository, potential risk assessment, life cycle - fate, exposure, and toxicity evaluation in the natural environment.

3. Models that study the fate and transport of ENMs in the environment

Robust models that accurately analyse and parametrize the distribution of ENMs between various media and environmental compartments are required in order to predict the environmental impacts of ENMs, including the toxicity potentials. Modelling approaches provide an indispensable role in understanding and predicting ENM concentrations in the environment that are difficult to achieve by analytical measurements (Sun et al., 2017). Abbas et al. (2020) elaborately reviewed the fate and environmental behaviour of ENMs including environmental compartment wise evaluation. They concluded that the most important entry routes of ENMs in the environment are via discharge of treated/untreated wastewater and solid waste. Further, they recommended that various dynamic transformations, which could alter the behaviour, fate, and toxicity of ENMs, need to be considered in future studies. For example, Garner et al. (2017) reported orders of magnitude increase in ZnO sediment concentration by a dynamic multimedia simulation model (nanoFate model) predictions when the dissolution effect was disregarded. As presented in Table 2, many approaches hitherto have focused on modelling the flow of $nTiO_2$ and nAg, possibly due to their prevalence and antimicrobial properties, respectively. Generally, these models are

categorized under one of the two types: deterministic or stochastic. Both types of models are briefly summarised in the following sub-sections.

3.1.Deterministic models

The deterministic approach entails the assumption of the various parameters in the flow of ENMs between environmental compartments. These parameters are often evaluated based on the current/projected ENM production, consumption and emission rates within a geographical region. For example, Blaser et al. (2008) proposed three emission scenarios of nAg based on 25 member countries in the EU. Their model assumed nAg in wastewater solution undergoes one of three potential pathways – direct discharge into the environment, treatment in sewage wastewater treatment plants (WWTPs) or release as effluents from WWTPs. The source of nAg was restricted to plastics and textiles; data of nAg product usages and productions were extrapolated from 15 countries in the EU to demographics of 25 countries in the EU in 2010.

Mueller and Nowack (2008) studied the life cycle emissions of various ENMs in Switzerland. The authors assumed two scenarios for each outflow - realistic exposure and high exposure. In their model, the predicted environmental concentration was calculated from substance flow analysis while assuming a steady-state system where a proportion of ENMs at each node would flow to its next environmental compartment. To parametrize production and flow rates, the authors used two factors: 1) a weighing factor “article” that describes the distribution of total ENM production to different

product categories; and 2) a weighing factor “weight” (weight of ENM in each product category, estimated from data on various products).

Often, the nature of these models assumes a steady-state in the flow of ENMs among environmental compartments. As ENM production and consumption are expected to ramp up in the near future, these models may not necessarily reflect the current ENM flow unless the models are constantly updated by recalculating their flow parameters.

3.2. Stochastic models

Subsequent models by Gottschalk et al. (2010) relaxes the assumption of a steady-state through a stochastic approach. In the model by Gottschalk et al. (2010), the authors described each flow rate and environmental concentration of $nTiO_2$ in the form of a probability distribution curve to account for the variability and uncertainty of ENM flows. Subsequently, Markov Chain Monte Carlo algorithms were used to obtain steady-state solutions to the system and reiterated repeatedly. Furthermore, the authors applied sensitivity analysis to determine the impact of each parameter on the overall output concentration. Moreover, Meesters et al. (2016) expanded their methodology to model other ENMs, such as nZnO and $nCeO_2$ while focusing on predicting the most important sources of uncertainty and variability in environmental concentrations. Song et al. (2017) introduced a stochastic dynamic model to estimate the life cycle release with a multilayer time lag between ENMs production and release. Specifically, the authors proposed that the during-lifetime and end-of-life release of ENMs are stochastic and modelled by probability distributions. From their established model, the authors

suggested that previous models tend to overestimate ENM emissions into the environment due to the lack of consideration of ENM stocks that remain within products during their lifetime.

Many studies employ Monte Carlo methods to simulate the flow of ENMs within the system accustomed to a considerable set of varying parameters. Upon release into the environment, the ENMs will undergo a series of dynamic transformation processes that complicate the understanding and increases the difficulties in modelling (Baun et al., 2017). While stochastic models may offer greater precision when compared with deterministic approaches, the reliance on accurate data of ENM production and consumption rates (to precisely describe the flow parameters) are unavoidable. Such data will remain indispensable for characterising and verifying the safety of ENM flow into the environment. For example, Suzuki et al. (2018) studied the environmental fate of ENMs from Japanese construction sector in 2016 via dynamic flow model with environmental sinks including all the environmental compartments. The input data for the study were acquired by interviewing various relevant Japanese Associations in the field. They concluded that about 95% of the ENMs remained in the buildings and roads, while about 5% were released into the waste streams and the environment.

Table 2. Types of models used to study ENM flow in the environment based on previous works

Source	**ENM**	**Environmental Compartment**	**Assessment model**	**Transformations included in the study**

Kaegi et al., 2013	nAg	WWTP (Wastewater)	Experimental study	Heteroaggregation, Sulfidation
Hendren et al., 2013	nAg, coated nAg	WWTP (Wastewater)	Monte Carlo simulation	Heteroaggregation
Lazareva and Keller, 2014	$nTiO_2$, nSiO2, nZnO, CNTs, nAg	Wastewater, solid waste and all the environmental compartments	Life cycle release model	No
Van Koetsem et al., 2015	nAg	Surface water and sediments	Experimental study	No
Quik et al., 2015	nAg	River (Surface water)	Spatially explicit hydrological model (NanoDUFLOW)	Homoaggregation, Heteroaggregation, Dissolution, Degradation
Sun et al., 2016	$nTiO_2$, nZnO, CNTs, nAg	All environmental compartments	Dynamic probabilistic model	No
Garner et al., 2017	$nTiO_2$, nZnO	All environmental compartments	Dynamic multimedia simulation model (nanoFate)	Heteroaggregation, Dissolution, Oxidation, Sulfidation, Interaction with NOM, Degradation, Attachment to aerosols, soils, and sediments, Transformation to other ENMs/compounds,
Sun et al., 2017	$nTiO_2$, nZnO, CNTs, nAg	All environmental compartments	Dynamic probabilistic model	Sulfidation, Combustion

Adam and Nowack, 2017	$nTiO_2$, nZnO, CNTs, nAg	Landfilling, Incineration and Recycling	Dynamic probabilistic model	No
Suzuki et al., 2018	$nTiO_2$, $nSiO_2$	Wastewater, solid waste and all the environmental compartments	Dynamic probabilistic model	No
Avant et al., 2019	CNTs	Surface water and sediments	Dynamic spatial simulation model (Water Quality Analysis Simulation Program 8)	Heteroaggregaion, Phototransformation
Saharia et al., 2019	$nTiO_2$	Combined sewer overflow in an urban river (Surface water)	Combination of Urban hydrologic model and 3D river hydrodynamic model	Heteroaggregation
Rajkovic et al., 2020	$nTiO_2$, nZnO, CNTs, nAg	Wastewater, solid waste and all the environmental compartments	Dynamic probabilistic model	Sulfidation, Combustion

Table 2 summarizes the types of models used to study ENM flow in the environment. Recently, Garner et al. (2017) developed a dynamic multimedia simulation model (nanoFate) to predict the time-dependent accumulation of metallic ENMs across environmental media. They simulated ten years of the release of CeO_2, CuO, TiO_2 and ZnO ENMs in San Francisco Bay area. The model predicted that eventually the highest concentration and mass fractions of ENMs will be found in agricultural soils, freshwater

and marine sediments, while aerosol and suspended sediment concentrations are high but with smaller mass fraction. The simulation incorporated most of the ENM transformations and compartmental transfers within the nanoFate model. However, more mesocosmic experimental studies would aid in replicating the simulated models on a smaller scale and validate the proposed models in the natural environment (Rawat et al., 2018).

Nonetheless, Cornelis et al. (2014), Dale et al. (2015), Baalousha et al. (2016), Park et al. (2016), Nowack (2017), Avilov et al. (2017), Baun et al. (2017), Part et al. (2018), Williams et al. (2019), and Abbas et al. (2020) provided comprehensive reviews on the environmental fate, transport, and exposure models for ENMs in various environmental compartments including end-of-life waste streams. The readers are referred to these reviews for further information on the developments, gaps, and challenges in the ENM flow models.

4. Physicochemical Transformation of ENMs

ENMs in the environment are subjected to dynamic physicochemical changes depending on the transport medium that will drive the materials towards a largely unknown endpoint and products from their pristine or manufactured forms (Lowry et al., 2012; Nguyen et al., 2011). The triggering environmental factors are light, temperature, oxidants, reductants, pH change (acidity/alkalinity), ionic strength, ROS species, minerals, NOM, physical and mechanical stress such as abrasion, and intrinsic composition of the media (Sigmund et al., 2018). ENMs are released from the body of

the product and enter the environment via different modes of transport over the course of its lifetime. The consequence of the gradual release process is that the pristine form of ENMs fails to adequately represent the state of ENM stocks in the environment (Lowry et al., 2012; Zhang et al., 2018). Hence, the toxic effects of transformed ENMs may vary significantly from that of pristine ENMs (Ren et al., 2016; Zhang et al., 2018). Therefore, to appropriately quantify the toxicity of ENMs, identification of the possible physicochemical transformations with respect to relevant environmental parameters is essential. The following sections aim to analyse the studies that attempted to describe the physicochemical transformations of the five studied ENMs. The physicochemical transformations include changes in particle size, porosity, aggregation, surface reactions, dissolution, photolysis, sulfidation, redox reactions, and interactions with NOM and other particles (Abbas et al., 2020; Lowry et al., 2012; Sigmund et al., 2018; Zhang et al., 2018).

4.1. $nTiO_2$

Sun et al. (2014a) reported the effects of increasing UV irradiation time of $nTiO_2$ in water with respect to the principal application of $nTiO_2$ in photoprotection. The $nTiO_2$ aggregation accelerated over time, leading to a clear separation between the solid and liquid phases of the suspensions. Even after re-dispersing irradiated $nTiO_2$ via ultrasonication, the ENMs reaggregated spontaneously. Further measurements of the zeta potential and the isoelectric point also revealed that the UV irradiation led to reduction in the charges on the particle surface. Combined with the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory - with a decrease in the electrostatic barrier, van der

Waal forces between ENMs predominate, forcing them to aggregate (Sun et al., 2014a). However, the trends in aggregation size were more evident than that of the energy barrier, suggesting another force that accelerates aggregation. A possible reason could be the bridging effect between acidic bridging hydroxyls and basic terminal hydroxyl groups among adjacent $nTiO_2$ (Soria et al., 2010). In natural systems, humic acid (HA) may coat onto ENMs that interfere with the charge interactions and reduce the agglomeration of ENMs (Dasari and Hwang, 2013).

Sun et al. (2014a) further investigated how aggregation impacts the generation of reactive oxygen species (ROS). In aggregated ENMs, the ROS generation (·OH) rate increased until 15h of exposure but decreased subsequently. However, in aggregated-then-redispersed ENMs, the ·OH generation rate remained stable for the entire duration. Photoluminescence decay measurements of $nTiO_2$ suspensions exhibited a similar trend involving the charge transfer efficiency of the samples. According to Folli et al. (2010), the reason behind the aforementioned phenomenon was hypothesised as a result of the reduction in the specific surface area in large aggregates. Thus, the possible transformations of $nTiO_2$ are aggregation, ROS generation via UV irradiation, change in surface charge, and bridging between functional groups.

4.2. $nSiO_2$

In the case of the $nSiO_2$, previous studies have shown insignificant increase in $nSiO_2$ release due to mechanical stress relative to conventional product use (Koponen et al., 2009). For example, most $nSiO_2$ contained in paints or food products remained

undissolved in experiments mimicking natural weather conditions or human gastrointestinal tract environment (Al-Kattan et al., 2015; Dekkers et al., 2013). It was also noted that most $nSiO_2$ were embedded with parts of the host matrix instead of suspended single particles (Al-Kattan et al., 2015). Hence, such materials would produce particles within a wide size distribution when applied with destructive mechanical forces.

Specially, when $nSiO_2$ is used as nanofiller, the degradation rate of matrix material also has influence on the fate of embedded $nSiO_2$. For example, Nguyen et al. (2012) focused on the surface morphological changes and the release of $nSiO_2$ during UV irradiation of epoxy/$nSiO_2$ composites. During UV irradiation, there was an increasing mass loss of epoxy matrix due to photodegradation, which potentially led to the accumulation and subsequent release of the nanofillers on the surface, though the $nSiO_2$ filler used neither catalyse/stabilise the photodegradation of the epoxy nor the nanocomposite.

Recently, Sharma et al. (2020) studied aggregation, dissolution, and photodegradation behaviours of surface-modified $nSiO_2$ under the influence of ionic strength, pH, and visible light irradiation. It was reported that $nSiO_2$ possessed good photostability but showed susceptibility to salts and pH variation in the form of aggregation or dissolution, possibly due to the particle-particle electrostatic interactions.

In summary, the $nSiO_2$, as nanofiller, exhibited low levels of dissolution or release from commercialized products due to mechanical stress but showed increasing release with the significant degradation of host material within nanocomposites. In addition, the

stability of surface-modified $nSiO_2$ would be affected by environmental factors like ionic strength and pH.

4.3.nZnO

While nZnO is primarily applied in sunscreens and cosmetics, with photochemistry and aqueous conditions similar to $nTiO_2$, only nZnO is prone to dissolve via acid wash or rainwater (Mitrano et al., 2015). Various studies have examined the transformations of nZnO under broad-spectrum conditions. Wang et al. (2017a) reported that as alkalization time of nZnO in ultrapure water increased, the physical morphology of nZnO changed from distinct, cylindrical, rod-like particles to showing signs of dissolution and agglomeration, coupled by an increase in hydrodynamic size of the ENMs.

Wang et al. (2014) studied the aggregation of nZnO under a range of pH conditions and in the presence of HA. The authors reported that the 'z-average' hydrodynamic diameter of nZnO varied non-linearly solely with respect to pH. When HA concentration was increased at different pH ranges relative to the pH of zero zeta potential (pH_{PZC}), negatively charged HA molecules were rapidly adsorbed onto the positively charged nZnO. This reduced the zeta potential of nZnO until charge inversion occurred and repulsion of surface charge ensued. In general, the particle size decreased as the concentration of HA increased (with the exception during $pH < pH_{PZC}$), indicating that the HA molecules interfere with the charge interactions between nZnO. The authors noted an assortment of (dis)aggregation states of nZnO with respect to the

environmental pH and presence of natural organic matter (NOM) when a combination of different pH and HA concentrations was applied, as shown in Figure 2.

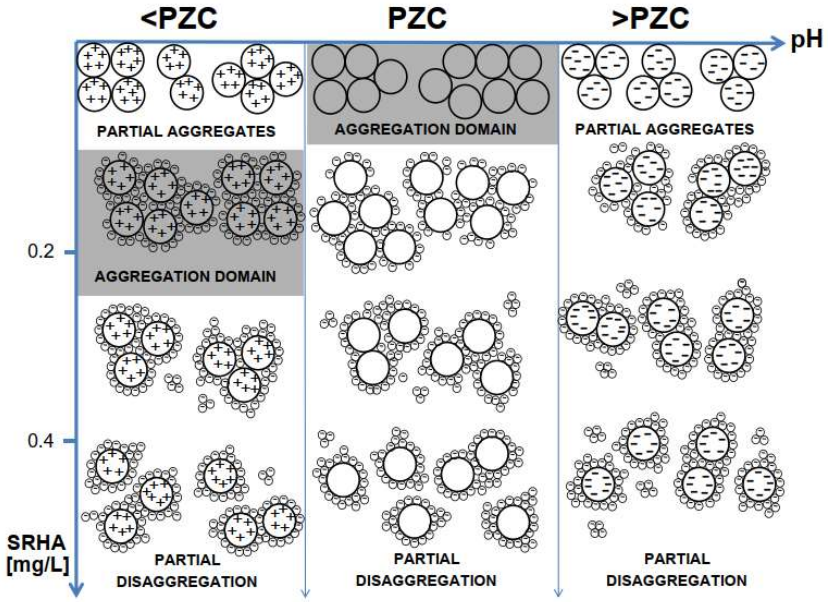


Figure 2. Assortment of (dis)aggregation of nZnO with respect to different pH and humic acid concentration (Omar et al., 2014).

Another study by Lv et al. (2012) reported the dissolution and transformation effects of nZnO from the natural phosphate contamination in the environment. Phosphate ions either form an amorphous layer on the solid phase surface (adsorption) or complexes with dissolved Zn^{2+} in solution to form precipitate (sequestration). Pristine nZnO may exist as amorphous $Zn(OH)_2$ via hydrolysis in aqueous solution, which slightly dissolves to release Zn^{2+}. Results demonstrated that dissolved Zn^{2+} experienced sharp decrease when the P/Zn ratio increase from 0 to 0.5, owing to the rapid sequestration reaction with aqueous phosphate. Subsequently, slight increase of Zn^{2+} ions was observed when P/Zn ratio further increased from 0.7 to 4.0, as the precipitation that consumed free Zn^{2+} ions would stimulate continuous release of Zn^{2+} until reaching a

new equilibrium. The authors also reported that at low P/Zn ratios, sequestration was preferred between Zn^{2+} and PO_4^{3-} while at high P/Zn ratios, adsorption of PO_4^{3-} was preferred instead. However, the layer of Zn phosphate formed during adsorption is insufficient to fully protect nZnO from dissolution (because of the small size of nZnO), resulting in a continuous discharge of Zn^{2+} ions.

Ma et al. (2013) reported a concentration dependant sulfidation of the nZnO to nZnS (<5nm) by dissolution and re-precipitation mechanisms. Sulfidation promoted aggregation, decreased the surface charge, and exhibited greater stability of the Zn form in a reduced environment, while the small size (<5nm) suggested high reactivity of the nZnS. Further, the ZnO core influences the subsequent solubility in the case of partially sulfidised nZnO. Therefore, influenced by various environmental factors such as different pH, sulfides, NOM or phosphate contamination, nZnO may undergo transformations of surface charge alteration, aggregation, dissolution and morphological change.

4.4.CNTs

As CNTs are widely used as nanofillers due to their favourable properties, their release and transformation behaviours were also investigated by previous studies under various environmental factors and mechanical stress (Nguyen et al., 2011; Wohlleben et al., 2013). For example, Wohlleben et al. (2013) studied the fate of CNTs embedded in TPU nanocomposites under the influence of three exposure scenarios – sanding, taber abrasion and UV irradiation. No free CNTs from TPU debris were observed above

detection limit after mechanical treatments, similar with the results obtained when detecting abraded paints and filament coatings that contain CNTs (Anas et al., 2019). In terms of UV irradiation on CNT-polymer nanocomposites, several studies confirmed that although the polymer matrix (such as TPU, epoxy) may undergo photodegradation, the embedded CNTs would remain and form dense and entangled network with no sign of environmental release. Also, it was proposed that the dense superficial network of CNTs protected the polymer matrix underneath, reducing the rate of photodegradation and subsequent release of nanofillers (Nguyen et al., 2011; Nguyen et al., 2017; Wohlleben et al., 2013).

Bitter et al. (2014) investigated the transformation of oxidised MWCNTs (O-MWCNTs) exposed to UVC radiation (254nm). Photoreduction dominated on the surfaces that were oxidised prior to UVC exposure. Transformations of O-MWCNTs observed in the study included a rapid increase in particle size (>400 nm), aggregation, and clear phase separation in the MWCNTs suspension. Surface chemistry analysis revealed that exposure to UVC radiation led to an increase in -C-OH and -C=O groups, and a decrease in -COOH groups. The loss of negatively charged deprotonated carboxylic groups is responsible for the loss of colloidal stability and subsequent aggregation, supporting the observation that solutions of low ionic strength and high pH provided strong aggregation resistance. The authors noted that irradiated O-MWCNTs experienced minor structural transformation and mineralisation throughout the experiment. Generally, CNTs exhibited aggregation, UV induced photodegradation, surface charge transformation as well as bridging effects in functionalised forms.

4.5. nAg

For shape transformation of nAg to occur, oxidative dissolution and redox agents are required for the photoconversion process of nAg spheres into prisms and then into larger agglomerates (Zou et al., 2015). Components of NOM such as fulvic acid and HA found naturally in the soil are potential electron donors/acceptors required for Ag redox cycles. Specifically, Zou et al. (2015) found that at low NOM concentrations, NOMs exert a bridging effect that helps chain multiple nanomaterials together. However, at high concentrations, electrostatic repulsion and steric hindrance dominate, causing most nAg to exist as individual particles. The authors continued to investigate the coupled impacts of NOM presence and UV irradiation on the chemistry of citrate-capped nAg (cit-nAg), then proposed a schematic for the mechanism during the redox process, as shown in Figure 3.

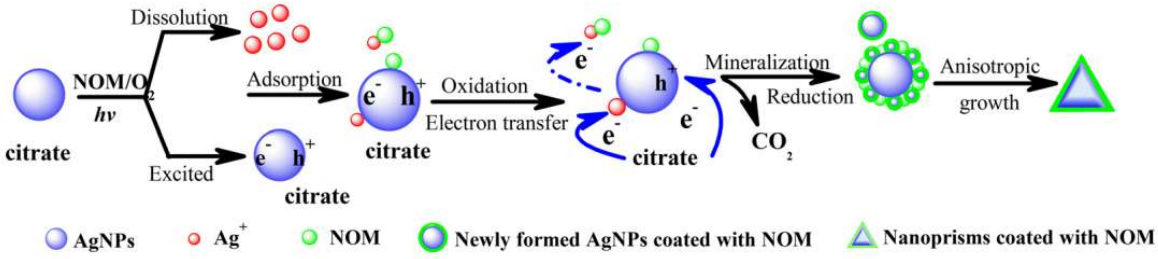


Figure 3. Proposed mechanism for the redox-induced transformation of citrate-capped nAg and their interactions with NOMs (Zou et al., 2015).

A study by Afshinnia et al. (2018) to investigate the effect of different natural organic ligands on the colloidal stability of cit-nAg by comparing the effects of L-cysteine (L-cys) against that of N-acetyl L-cysteine (NAL-cys). Both molecules contain the thiol

group (S), which adsorbs onto the surface of cit-nAg, forming a layer of AgS that prevents oxidation. At neutral conditions, the bulky acetyl group in NAL-cys shielded the molecule from the positively charged cit-nAg, leading to reduced charge interactions and aggregation rate. In the mixture of fulvic acid and L-cys, fulvic acid increased nAg stability, supporting the findings by Zou et al. (2015); however, the presence of L-cys reduced this effect. Overall, the presence of NOM generally lowered the rate of aggregation of nAg. However, other functional groups in NOM molecules should be taken into account as well.

Sulfidation is an important transformation process that influences the metal containing ENMs, which readily dissolve in aqueous solutions. nAg entering the WWTP is mostly sulfidised (Kaegi et al., 2011; Kim et al., 2010). The Ag being a class B soft metal preferentially binds to inorganic sulphides and sulphur containing molecules and resulting in low solubility of Ag_2S (Andren and Bober, 2002). Fletcher et al. (2019) studied the chemical transformation of cit-nAg (5-10 nm) in sulfide-rich conditions in WWTPs. The sulfidation process was (i) direct and immediate, (ii) the product was found to be stable including partial sulfidation scenarios, and (iii) the release of Ag^+ ion was prevented by the process, which was verified over a period of two months. Further, sulfidation rate increased with decreasing cit-nAg size (20-200 nm) and increasing HA concentration (Thalmann et al., 2016). The sulfidation half-life of the 20 nm cit-nAg decreased from 12 to 1 min with an increase in HA concentration from 0 to 1000 mg/L (Thalmann et al., 2016). Similarly, chloride ions are predominant in the natural waters. The kinetics of dissolution of the PVP coated nAg in chloride containing system was

strongly dependent on the Cl/Ag ratio and the thermodynamically expected speciation of Ag in the presence of chloride was observed (Levard et al., 2013b).

Further, (Zou et al., 2017) investigated the effect of dissolved oxygen on the stability of mono-dispersed nAg in natural and synthetic freshwater. In synthetic freshwater, nAg aggregated due to the greater ionic strength of the solution, according to the DLVO theory. The addition of NOM dispersed the aggregated particles, possibly due to the formation of Ag_2S complexes and adsorption of NOMs onto moderately charged particles, supporting the findings in the abovementioned studies. Dissolved oxygen serves as an oxidising agent alongside H^+ ions to oxidise nAg into Ag^+ ions, resulting in more rapid dissolution of nAg under oxic conditions. However, NOMs blocked oxidising sites on nAg or directly reduced Ag^+ to Ag, mitigating the oxidising effect of dissolved oxygen. To summarise the complex interactions between dissolved oxygen, NOM, and nAg, Zou et al., (2017) provided a schematic of the mechanisms explained, as shown in Figure 4. Besides, it was reported that heteroaggregation and/or deposition also affect the fate and transport of the nAg in urban wastewater systems due to the enormous dilution effect in the wastewater or the activated sludge (Kaegi et al., 2013). Hence, nAg demonstrated transformations such as morphology change, aggregation, dissolution, change in surface charge, sulfidation, chlorination, and interactions with NOM.

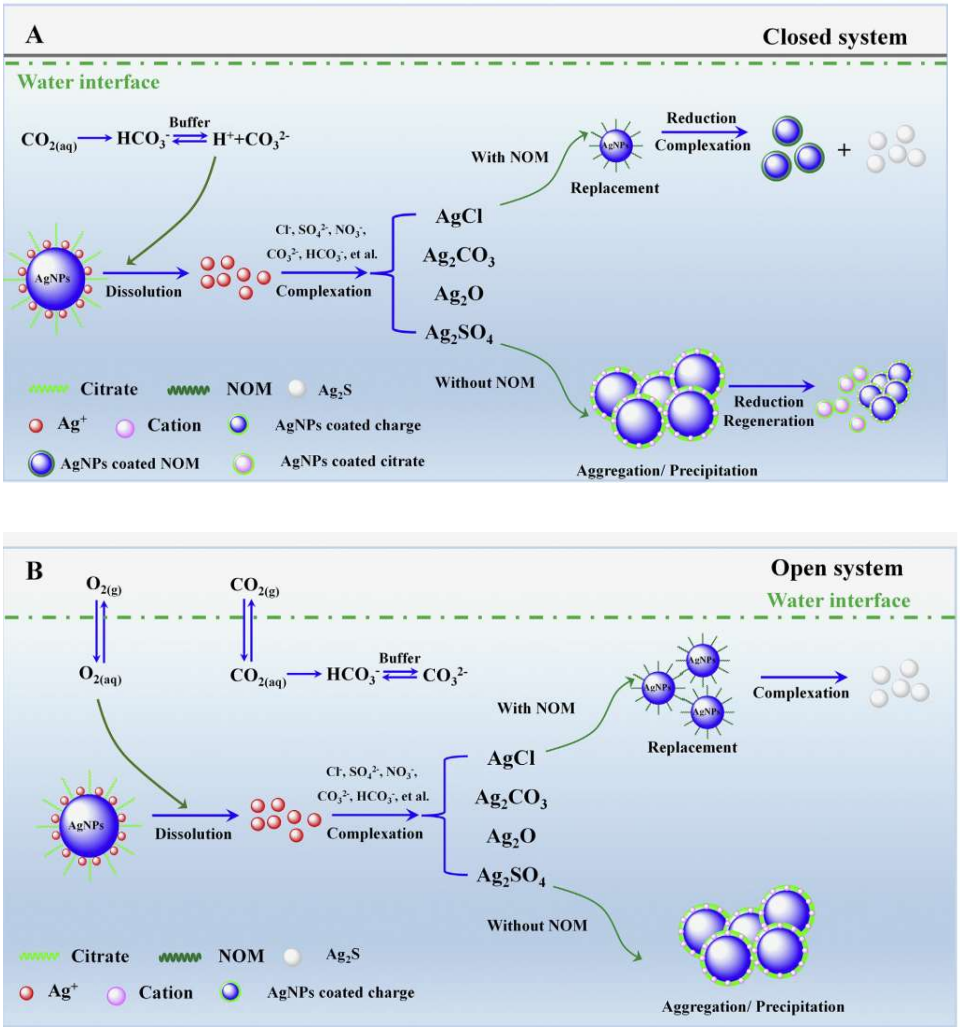


Figure 4. Schematic of the redox reactions of nAg in anoxic (A) and oxic (B) conditions (Zou et al., 2017).

5. Toxicity of ENMs

The ENMs might pose several toxicity risks due to their small size during the course of their transport and transformations from the original products to the environment. This permits the ENMs to infiltrate organisms and interfere with critical biochemical processes at cellular level. Cytotoxicity and genotoxicity are two of the eminent components of the toxicity that draws review. Both components are covered in the

following sections, specific to chemical properties and post-transformation states of the ENMs.

5.1. Cytotoxicity of ENMs

Cytotoxicity of ENMs at a cellular level encompasses cell viability, growth rates, and damage to cell organelles. Subsequently, these effects may be extrapolated to macro-organisms, depending on tissue distribution, bioaccumulation levels and removal mechanisms possessed by the organism. Each of the five ENMs in this review presents different chemical properties, physicochemical transformations, and environmental concentrations. Hence, the following sub-sections attempt to review the cytotoxicity effects of each ENM reported by various studies, under *in vitro* and *in vivo* conditions.

5.1.1. $nTiO_2$

The $nTiO_2$ containing products are frequently exposed to aqueous environments (such as rain, river and seawater), causing a significant volume of $nTiO_2$ to enter water bodies. The photocatalytic nature of $nTiO_2$ would act as a significant redox agent in the environment that might damage biological systems. Kim et al. (2011a) examined the $nTiO_2$ and nAg inhibition on the growth of an algae *Lemna paucicostata*. Growth inhibition occurred at the lowest observed effect concentration (LOEC) of 250ppm and the growth rate was inhibited by a factor of 0.5 at approximately 500ppm. Other studies reported similar LOEC in willow trees (Seeger et al., 2009), *Allium cepa*, and *Nicotiana tabacum* (Ghosh et al., 2010). When the goldfish *Carassius auratus* was considered as the test subject, it was noted that the $nTiO_2$ primarily accumulated in the intestines, gills

and liver. By further using malondialdehyde (MDA) as a marker for lipid peroxidation and oxidative stress, the presence of increased MDA in the livers of the high-exposure fish groups as compared to that of the control group was observed. The study demonstrated the potential of the introduced $nTiO_2$ to exert oxidative stress onto biological systems, even if the stress was eventually non-lethal (Ates et al., 2013).

In terms of microorganisms, it was confirmed that sunlight irradiation, HA treatment and their dynamic interactions all contribute to the significant cytotoxic effects of $nTiO_2$ on bacterial assemblages (Dasari and Hwang, 2013). Specifically, $nTiO_2$ could further inhibit cell viability of bacterial assemblages under light condition compared with dark condition due to their photosensitive nature. Also, as a main type of NOM, HA is capable of reducing agglomeration of ENMs via adsorption and binding (Lin et al., 2012) as well as exerting light attenuation effect caused by its dark coloration, leading to the reduction of $nTiO_2$ cytotoxicity and increase of bacterial viability (Dasari and Hwang, 2013).

Similarly, Simon-Deckers et al. (2009) studied the toxicity of various dimensions of $nTiO_2$ on *Escherichia coli* MG1655 and *Cupriavidus metallidurans* CH34, considering the post-transformation physical state of $nTiO_2$. The cytotoxic effect was discovered to be related with smaller particle size (<100nm), positive surface charge, concentration and shape, while the crystalline structure (anatase and rutile) failed to have significant difference.

Recently, Zhao et al. (2019) reported that $nTiO_2$ induced mitochondrial damage and dysfunction in HT22 cells, which led to the accumulation of undesired ROS and the reduction in mitochondrial membrane potential. Meanwhile, the consequences may further accelerate mitochondrial fragmentation. The above interaction may be one of the possible mechanisms of cell apoptosis under $nTiO_2$ exposure.

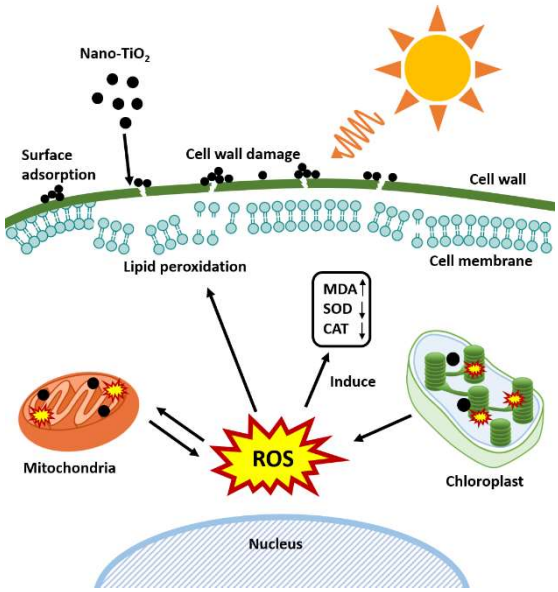


Figure 5. Scheme of possible cytotoxicity induced by $nTiO_2$ on algal species.

Toxicity impact of $nTiO_2$ has also been widely studied on algae, a classical biological model for aquatic toxicity study. Evidence showed the adsorption and aggregation of $nTiO_2$ on the algal surface, causing slight photosynthesis inhibition due to the shading effect (Hu et al., 2018; Navarro et al., 2008) and cell wall damage via physical contact (Wang et al., 2016b). While the major cause of cytotoxicity is the increased intracellular ROS induced by photoactive $nTiO_2$ (Ozkaleli and Erdem, 2018; Xia et al., 2015). It was proved that the overproduction of ROS destroys the balance between pro-

oxidation/anti-oxidation system, resulting in the inhibition of antioxidant enzyme activity such as superoxide dismutase and catalase (Xia et al., 2015). Besides, increase of MDA content was also observed, indicating the lipid peroxidation and loss of membrane integrity. Moreover, it was confirmed that chloroplast is the site where ROS is produced in *K. brevis* cells (Li et al., 2015), which may be the reason of thylakoids lamellar structure damage observed on another algal species *Chlorella pyrenoidosa* (Middepogu et al., 2018). Figure 5 demonstrates the possible cytotoxicity induced by $nTiO_2$ on algal species. Upon the $nTiO_2$ exposure in algae, excess ROS generated from the electron transfer chain of the chloroplast eventually led to lipid peroxidation, mitochondrial damage, and algal growth inhibition.

Furthermore, Zhang et al. (2016b) examined the transformation of various ENMs in surface waters under several water quality parameters and reported the algal cytotoxicity of ENMs towards *C. pyrenoidosa*. In both seawater and freshwater samples, the $nTiO_2$ exhibited minimal cytotoxicity at low concentrations. While at the highest concentration, it was reported the most toxic amongst the four tested ENMs ($nTiO_2$, nZnO, nAg, and CNTs). As ENM-cell heteroagglomeration was only observed in seawater samples, the study deduced that physical interaction failed to explain the observed $nTiO_2$ cytotoxicity in the freshwater samples. Instead, joint impacts of complicated water quality parameters may eventually lead to the cytotoxic effect of $nTiO_2$.

5.1.2. $nSiO_2$

Fruijtier-Pölloth (2012) provided a comprehensive review of the toxicity of synthetic amorphous silica (SAS) in biological systems. Although, SAS tends to supersaturate and dissolve fully in biological systems, its bioaccumulation occurs minimally due to the lack of lipophilicity that prevents SAS from adhering to lipid-based biological membranes.

Table 3. List of results from past studies investigating toxicities of $nSiO_2$ *in vitro* and *in vivo*.

Source	**Cell type/Organism**	**Toxic effect**
In vitro		
Jiang et al., 2009	*Bacillus subtiles* (bacteria) *Escherichia coli* (bacteria) *Pseudomonas fluorescens* (bacteria)	Partial cell death
García-Saucedo et al., 2011	*Saccharomyces cerevisiae* (yeast)	Small amounts of membrane damage
Kim et al., 2015	A549 cells; NIH/3T3 fibroblasts; HepG2 cells	Reduction in viability; membrane disruption; oxidative stress
Katsumiti et al., 2016	Mussel hemocytes and gill cells	Promotion of inflammatory processes
Decan et al., 2016	Mouse lung epithelial (FE1) cells	Decreased cell viability
Bermejo-Nogales et al., 2017	*Poeciliopsis lucida* liver cell line (PLHC-1); *Oncorhynchus mykiss* fibroblast-like gonadal cell line (RTG-2)	Negligible cytotoxicity

Ahamed et al., 2019	HepG2 cells; HT1080 cells	$nSiO_2$: no cytotoxicity Co-exposure of $nSiO_2$ and As: augmentation of oxidative stress and mitochondria-mediated apoptosis
Book et al., 2019	*Oncorhynchus mykiss* gill cell line (RTgill-W1)	Size- and concentration-dependent inhibition of cell viability
Sharma et al., 2020	*Staphylococcus aureus; Escherichia coli* (bacteria)	Elevated oxidative stress
In vivo		
Fujiwara et al., 2008	*Chlorella kessleri* (algae)	Size-dependent growth inhibition; low toxicity
Cho et al., 2009	BALB/c mice	Significant but transient inflammatory cell foci
Wei et al., 2010	*Scenedesmus obliquus* (algae)	Reduced chlorophyll content
Li et al., 2014	Adult zebrafish	Potentially Parkinson's disease-like behaviour
Hassankhani et al., 2015	Male Wistar rats	Cellular necrosis
Karunakaran et al., 2015	*Porphyridium aerugineum Getitler* (algae)	Nil
Schiavo et al., 2016	Dunaliella tertiolecta (algae)	Oxidative stress
Vicentini et al., 2017	*Daphnia magna* (water flea)	Decreased longevity, reproduction and growth
Vidya and Chitra, 2017)	*Oreochromis mossambicus* (fish)	Concentration-dependent fish mortality
Yang et al., 2017	Male Wistar rats	Pulmonary lesions
Du et al., 2019	Male Wistar rats	Cardiovascular injury
Eom and Choi, 2019	*Caenorhabditis elegans* (nematode)	Reduced reproductive ability
Rekulapally et al., 2019	*Artemia* (brine shrimp)	Oxidative stress

Vranic et al., 2019	*Danio rerio* (Zebrafish embryo)	Nil
Athif et al., 2020	*Oreochromis mossambicus* (fish)	Reduced hepatic protein content
Lozano et al., 2020	*ex vivo* rat hearts	Reduction of relaxation
Shariati et al., 2020	*Daphnia magna* (water flea)	Dose-dependent daphnia mortality
Sharma et al., 2020	*Phytoplankton* (algae)	Reduced total chlorophyll content; cell growth restriction
Zhang et al., 2020	*Caenorhabditis elegans* (nematode)	Enhanced germ cell apoptosis

The toxicity of $nSiO_2$ differs significantly across organism types. Table 3 shows a list of toxic effects observed in organisms from exposure assays across several studies. Varying degrees of cytotoxicity were observed amongst the reported studies. The study by Fruijtier-Pölloth (2012) continued to elaborate on the possible mode of actions (MOAs) that lead to potential cytotoxicity of $nSiO_2$ in organisms. Some of these MOAs include adsorption onto biological cells and membrane damage via the denaturation of proteins through the proton-donating Si-O-H groups on the ENMs (Pandurangi et al., 1990). Additionally, $nSiO_2$ at high concentrations could exert a shading effect on the cells that inhibit photosynthetic capacity of the green algae (Wei et al., 2010). Internalisation of ENMs could occur through endocytosis of specifically silica-containing phagosomes fusing with endosomes, thus damaging internal membranes and leaking out cellular contents. However, $nSiO_2$ could also interrupt the lysosome exocytosis pathway, preventing the discharge of internalised ENMs, and possibly exacerbating damage to cells (Decan et al., 2016). Another MOA is through oxidative

stress wherein the cells may produce ROS that overloads their natural, in-built antioxidation mechanism (Lin et al., 2006; Ye et al., 2010). Interestingly, as shown in Figure 6, co-exposure of $nSiO_2$ and Arsenic (As) significantly aggravated oxidative damage of HepG2 and HT1090 cells compared with single As treatment, while $nSiO_2$ alone exhibited no toxicity (Ahamed et al., 2019).

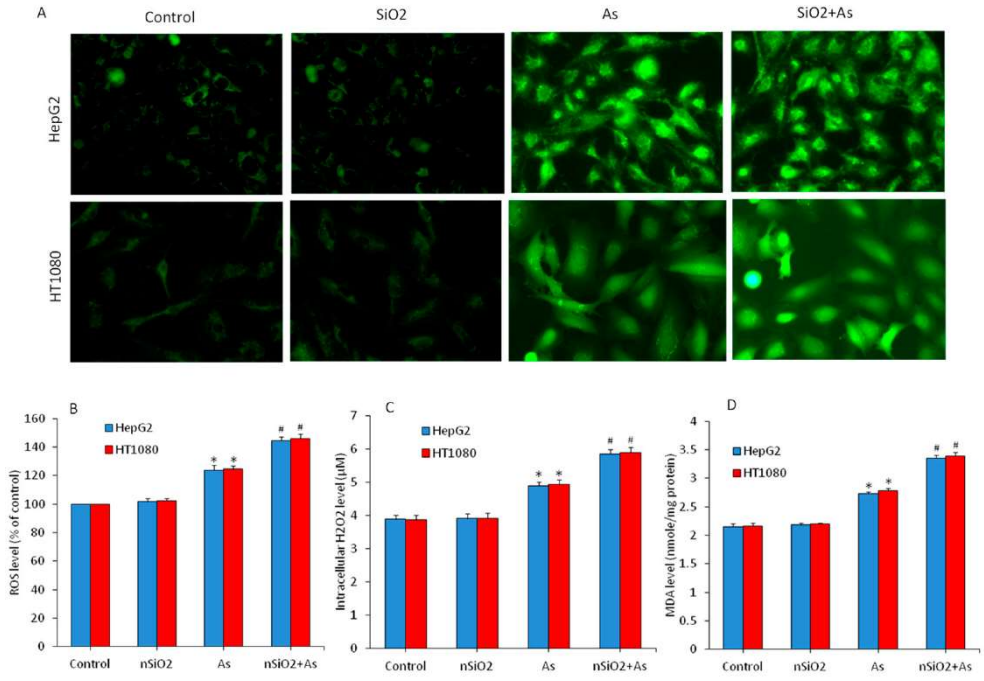


Figure 6. Pro-oxidant levels in HepG2 and HT1080 cells exposed for 24h to $nSiO_2$ (10 μg/mL), As (1μg/mL) or $nSiO_2$ + As (10μg/mL + 1μg/mL) for 24h (Ahamed et al., 2019).

Furthermore, cellular uptake of ENMs was significantly correlated with size, smaller $nSiO_2$ (12 nm) may pose greater cytotoxicity due to their ease of internalisation and interference with the cellular clearance (Decan et al., 2016). (Slowing et al., 2011),

however, proposed a contrasting result that bioaccumulation in mammalian cells was generally unobserved as cells were able to utilise functional exocytosis to remove internalised ENMs. The above was supported by the animal experiments conducted by (Cho et al., 2009), which discovered that mice were able to clear their internalised ENMs via urine and bile. More systematic case studies are required to obtain comprehensive and comparable cytotoxicity mechanisms of $nSiO_2$ toward various organisms.

5.1.3. nZnO

As mentioned in section 5.1.1, Zhang et al. (2016b) assessed algal toxicity of four types of ENMs towards *C. pyrenoidosa*, among which nZnO were the most toxic at low concentrations; and they were slightly less toxic compared to $nTiO_2$ at high concentrations. Due to the ease of nZnO dissolution and the diverse toxicities of ENMs tested, the study concluded that the release of Zn^{2+} would contribute extensively to the toxicity of nZnO. The interaction between nZnO and algae was also shown in the TEM image (Figure 7), demonstrating that transmembrane nZnO increased intracellular total Zn, which led to microalgae growth inhibition (Zhang et al., 2016a). Apart from dissolved Zn^{2+}, it was speculated that large aggregates of nZnO observed in the solution also contribute to cytotoxicity in green algae by encircling and immobilizing the algal cells (Ji et al., 2011). Conversely, it was observed that larger nZnO are less toxic when using mammalian cells in the experiments. The agglomeration degree of nZnO gradually increased with the elongated alkalisation time in pure water environment, leading to increased hydrodynamic size of the ENMs and reduction of cytotoxicity

(Wang et al., 2017a). Hence, pristine and transformed nZnO are likely to exert altered cytotoxicity across various organisms and more evidence is needed for identifying detailed nZnO toxicity.

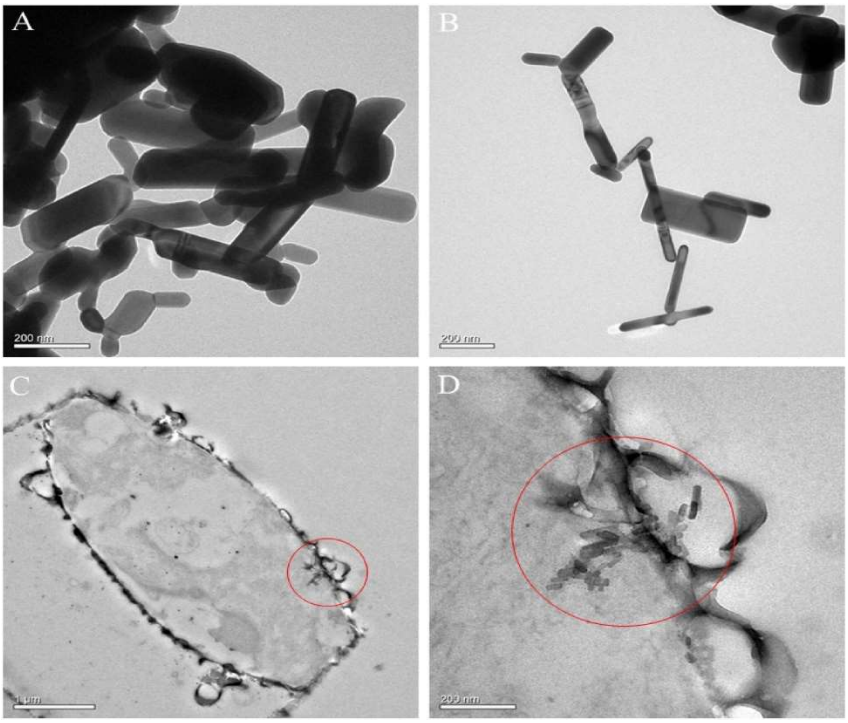


Figure 7. TEM image of nZnO (A and B) and interaction between nZnO and microalgae *Skeletonema costatum* (C and D) (Zhang et al., 2016a).

Additionally, nZnO with diverse morphological structures caused different toxic effects. Peng et al. (2011) tested the toxicity of spherical and rod-shaped nZnO on marine diatoms. They reported that the latter induced relatively higher cytotoxicity on one of the marine algae, indicating the possible shape effect of nZnO should be considered when assessing their cytotoxicity. However, recently Falfushynska et al. (2019) reported that nZnO particles activated stronger inflammatory response and higher cell

apoptosis when compared with nZnO rods, where blue mussels were used as test organisms. These divergent results suggest that nZnO also exhibit varying cytotoxic effects across different organisms.

Furthermore, Dasari and Hwang (2013) examined the effect of HA and sunlight on the cytotoxicity of nZnO to riverine bacterial assemblages. Although lower cell viability of bacterial assemblages was observed in the nZnO treatment group under light condition, the ROS generation showed insignificant difference compared with the group treated in dark condition, indicating the existence of other mechanisms that account for nZnO cytotoxicity instead of its photocatalytic property alone. The study proposed that the toxicity of nZnO could be due to adsorption onto cell membranes, which induced membrane damage and partial cell lysis. Such a hypothesis was supported by electron microscopy imagery showing nZnO attached to the cell walls in the target cells and the presence of HA inhibited ENM-cell physical contact, thus correlated with increased cell viability. nZnO presented higher sensitivity and bioavailability to *Eisenia fetida* (earthworm) than the sulfidised form (nZnS) (Bao et al., 2020). However, neither exhibited acute toxicity to the organism. Significant decrease in MDA level of *E. fetida* was observed on exposure to nZnS indicating mitigating role in facing oxidative stress (Bao et al., 2020). nZnS exhibited a more pronounced effect on the root growth of *Lepidium sativum* (plant) than the nZnO in sewage sludge-amended soil (Oleszczuk et al., 2019). In the same study, higher dosage (>250 mg/kg) of nZnO/nZnS induced mortality and inhibited reproduction in *Folsomia candida* (arthropod), while lower

doses had insignificant influence. Contrarily, *Vibrio fischeri* (bacterium) exhibited better reduction in toxicity for nZnO than nZnS (Oleszczuk et al., 2019).

In summary, the cytotoxicity of nZnO may be attributed to metal ion release, agglomeration, physical adsorption to cell membranes, photocatalytic property, sulfidation as well as morphological structure of the material.

5.1.4. CNTs

CNTs modified with functional groups usually possess altered physicochemical properties which may affect their toxicity toward different organisms. For example, polyethylenimine (PEI) modified MWCNTs exhibited significantly higher positive zeta potential and greater toxicity on *Daphnia magna* when compared to acid-treated MWCNTs (Petersen et al., 2011). The authors deduced that the polymer size had a significant role in determining cytotoxicity, while the surface charge may not be the primary cause, considering only minor differences in the uptake rate by *D. magna* amongst the different modified MWCNTs were observed. Additionally, proper functionalization of MWCNTs can reduce the toxicity of pristine MWCNTs. For example, MWCNTs modified with carboxy and amino groups demonstrated higher negative zeta potential and better solubility in culture media, leading to decreased cytotoxicity on HEK293 cells when compared with primary MWCNTs (Chowdhry et al., 2019). However, the *in vivo* experiments showed that zebrafish was resistant to both types of MWCNTs, which may be due to the limited effective exposure concentration and immune defence. Besides, algal feeding was essential in achieving high elimination

rates in water flea species like *Ceriodaphnia dubia* and *D. magna* (Kennedy et al., 2008; Petersen et al., 2011), which can be a practical approach to eliminate CNTs within water flea species or even other aquatic organisms. In general, the cytotoxic effects of MWCNTs were not only related with surface charge, functionalization and polymer size, but also vary between *in vivo* and *in vitro* results, indicating the importance of evaluating the CNT cytotoxicity at multiple biological levels.

When examining the toxicity of raw- (with Fe impurities) and purified-MWCNTs towards *E. coli* MG1655 and *C. metallidurans CH34*, it was reported that MWCNTs were bactericidal to *E. coli* MG1655 regardless of purity (Simon-Deckers et al., 2009). The absence of the Fe group's impact from the raw-MWCNTs may be attributed to the metal groups' location, which was situated inside the tubes and therefore avoided direct contact with bacteria. Besides, it was deduced that the resistance mechanisms within *C. metallidurans CH34* could promote cell membrane integrity or overexpress protective components, which were relatively weak within *E. coli.* Generally, the toxicity of MWCNTs is not correlated with purity but may rely on the sensitivity and response of bacterial species to the nanomaterial.

In seawater samples, CNTs showed lower algal toxicity ascribed to their significantly enhanced homoaggregation behaviour, which could limit physical contact between CNTs and cells (Zhang et al., 2016b). A study by Long et al. (2012) proposed that CNT toxicity is jointly due to oxidative stress, physical interactions, and the shading effect. Recently, (Peng et al., 2020) also reported that oxidative stress is the main toxicity

mechanism of carbon nanomaterials including CNTs, graphene and graphene oxide (Figure 8).

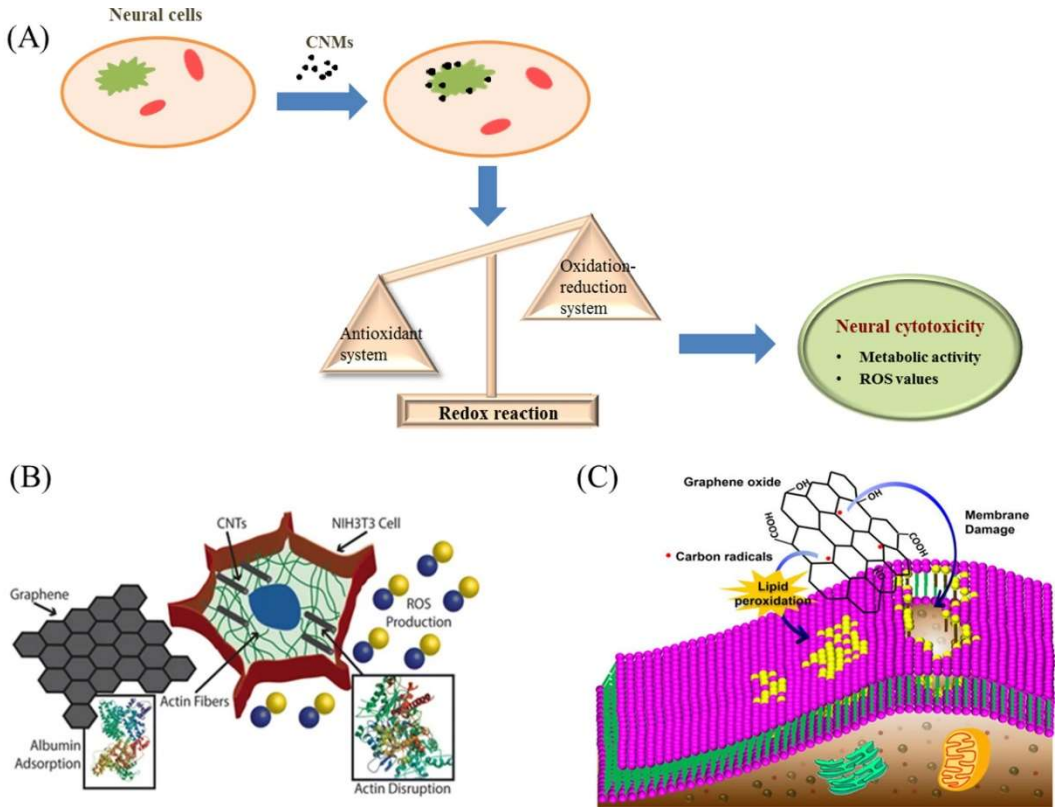


Figure 8. Toxicity mechanism of carbon nanomaterials (CNMs) to cells (A) The equilibrium state of the ROS in the cells is broken by the addition of CNMs, resulting in the production of large amounts of oxidants and affecting cellular metabolic activity. (B) Toxic effects of CNMs on NIH3T3 cells: agonistic protein destruction and ROS production. (C) Toxic effects of graphene oxide on macrophages: lipid peroxidation and membrane damage. (Peng et al., 2020).

5.1.5. nAg

As shown in Figure 9, previous studies reported the possible cellular uptake methods of nAg (Foldbjerg et al., 2015; Stern et al., 2012), which rely on the physiochemical properties of ENMs and cell type tested (Brkić Ahmed et al., 2017; Zhang et al., 2015). In addition, the localization of nAg in endosome/lysosome, mitochondria, endoplasmic reticulum and nucleus has also been discovered (Foldbjerg et al., 2015). The existence of nAg and released Ag^+ ions may interrupt protein function, cause oxidative stress and interfere with cell signalling cascade, leading to inhibition of cell proliferation (Abramenko et al., 2018; Asharani et al., 2009; Du et al., 2018).

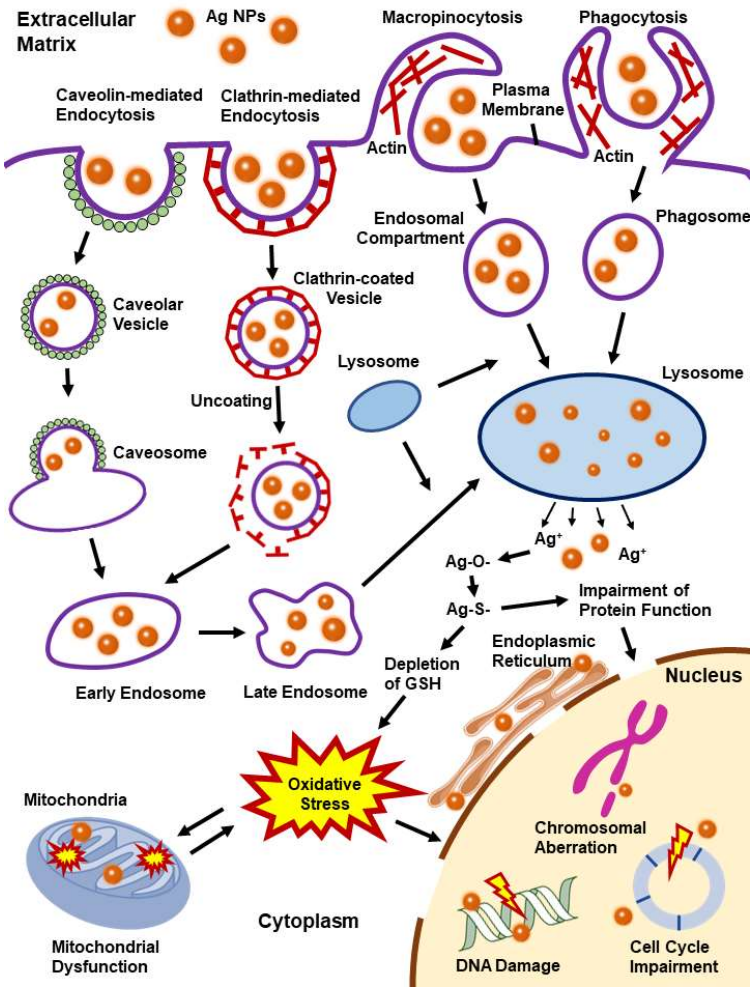


Figure 9. Schemes of possible pathways that lead to nAg induced cytotoxicity and genotoxicity

As mentioned in section 5.1.1, (Kim et al., 2011a) examined the $nTiO_2$ and nAg inhibition effect on the growth of an algae *L. paucicostata*. Unlike $nTiO_2$, nAg exhibited higher toxicity towards the algae, with the LOEC as low as 1ppm, and complete growth inhibition above 100ppm. By comparing the results to the uptake limit of terrestrial plants *Brassica juncea* and *Medicago sativa* (> 1000ppm) reported by Harris and Bali (2008), the authors suggested that aquatic plants could be more sensitive than terrestrial plants to the toxic effects of nAg.

Conversely, no toxicity was observed when examining citrate and polyvinylpyrrolidone (PVP) -coated nAg in two marine organisms *Ampelisca abdita* and *Americamysis bahia* (cellular survival rate > 90%) (Wang et al., 2014). Seawater medium possessing high ionic strength would promote aggregation coupled with the tendency of nAg to precipitate as AgCl or Ag_2S, leading to reduced bioavailability of nAg. While there were equally significant levels of bioaccumulation of citrate/PVP-coated ENMs in the organisms with accompanying tissue weight loss, the direct correlation of the results with the concentration of ENMs remains unclear.

Sulfidation of PVP coated nAg resulted in reduced toxicity in *E. coli* (Reinsch et al., 2012), *Danio rerio* (zebrafish) (Devi et al., 2015; Levard et al., 2013a), *Caenorhabditis elegans* (nematode) (Levard et al., 2013a; Starnes et al., 2015), *Fundulus heteroclitus* (killifish) (Levard et al., 2013a), and *Lemna minuta* (duckweed) (Levard et al., 2013a) due to the reduced Ag^+ concentration or bioavailability and loss of surface area owing

to the aggregation of Ag_2S. Hence, sulfidation of metallic ENMs is considered as one of the detoxification mechanism (Reinsch et al., 2012). The effect of various chloride concentrations on the PVP-coated nAg toxicity in *E. coli* growth inhibition was governed by the amount of dissolved $AgCl_x^{(x-1)-}$ species suggesting an ion effect rather than a nanoparticle effect (Levard et al., 2013b). nAg (10 mg/L) in high saline media demonstrated significant increase in the toxicity on hatching rate of Japanese medaka eggs (Kataoka et al., 2015). Similarly, nAg and $AgCl_x^{(x-1)-}$ species exhibited higher bioavailability and medaka embryo toxicity when compared to $AgNO_3$ in saline conditions (Kataoka et al., 2015).

Moreover, nAg is capable of exerting toxicity on the ecosystem via food chain accumulation (Luo et al., 2016). By applying a food chain model from *E. coli* to *C. elegans*, the authors observed that the nAg accumulated in *E. coli* were transferred to *C. elegans*, exhibiting a toxicity effect at a higher trophic level. Furthermore, smaller nAg (25nm) were able to accumulate at a greater concentration in *C. elegans* and display stronger toxicity by causing germ cell death, interfering with reproductive integrity, and shortening life span.

Similar to nZnO, the release of Ag^+ ions played a vital role in determining toxicity. It was reported that Ag^+ ions showed higher toxicity than Zn^{2+} ions and comparable or greater toxicity than nAg towards algal species, though the rate and extent of dissolution were determined to be dependent on multiple water chemistry parameters simultaneously (Miao et al., 2009; Zhang et al., 2016a).

5.2.Genotoxicity of ENMs

Genotoxicity of ENMs encompasses oxidative DNA damage, chromosomal aberrations, strand breaks, gene mutation, and micronucleus formation, as the potential effects. Some of these effects are linked to physicochemical transformations of ENMs (such as ROS generation), which may also exhibit cytotoxicity simultaneously. This sub-section summarises the genotoxic effects of each ENM reported by various studies, *in vitro* and *in vivo*.

5.2.1. $nTiO_2$

$nTiO_2$ could induce a concentration-dependent increase in oxidative DNA damage and micronucleus formation on human HepG2 liver cells, which was correlated with the increase in the uptake of ENMs, ROS generation, and oxidative stress (Shukla et al., 2013). Similar findings regarding apoptosis were identified in other cell types, such as human lung epithelial cells (Park et al., 2008) and rat neuronal cells (Liu et al., 2010). Besides, $nTiO_2$ induced ROS production is possibly controlled by nuclear factor-E2-related factor-2 signalling pathway (Song et al., 2016), while other underlying mechanisms remain to be further explored (Figure 10). Though numerous studies reported ROS generation as one of the main pathways for genotoxic effects of $nTiO_2$, a deeper understanding of the underlying mechanism is required with further research studies.

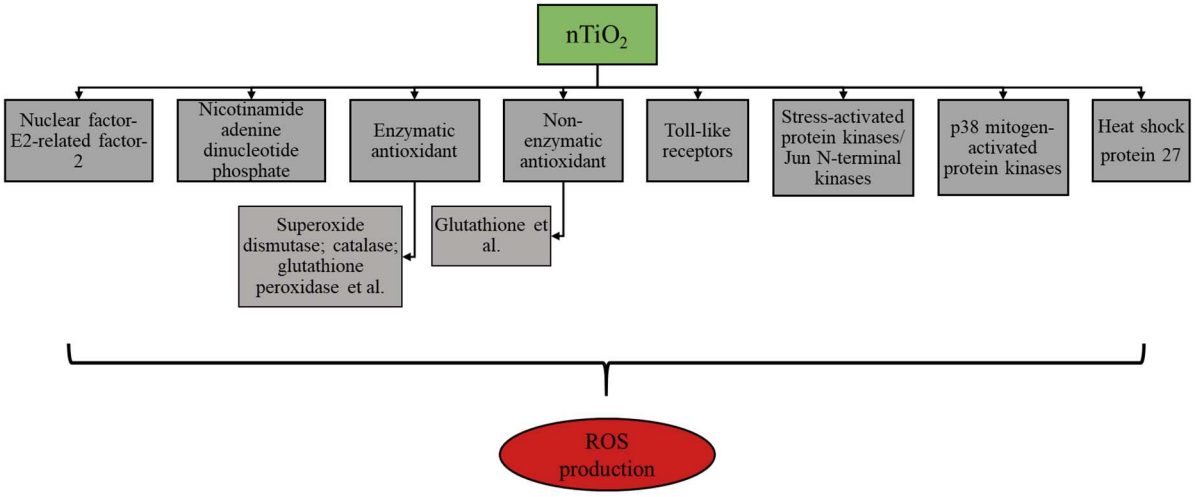


Figure 10. Simple illustration of mechanisms underlying $nTiO_2$-induced reactive oxygen species (ROS) production (Dhupal et al., 2018; Hanot-Roy et al., 2016; Song et al., 2016).

$nTiO_2$ with two crystalline forms (anatase and rutile) induced intracellular ROS generation and DNA strand break in HepG2 cells (Petković et al., 2011; Shukla et al., 2013; Simon-Deckers et al., 2009). However, both forms inflicted DNA damage to a different extent, i.e. anatase stimulated severe DNA strand break, while rutile induced mRNA expression of p21, gadd45a, and mdm2 genes that are associated with the DNA repair mechanism (Petković et al., 2011). Nonetheless, the presence of increased expression of the aforementioned genes provided strong evidence for the genotoxic potential of $nTiO_2$ in both crystalline forms.

Furthermore, $nTiO_2$ with different morphologies (tube- and anatase-type) were reported to exhibit epigenomic toxicity on human respiratory cells (16HBE and A549), resulting in reduced global DNA methylation and changed the expression level of methylation-

related genes and proteins (Ma et al., 2017). Moreover, anatase-type $nTiO_2$ exerted significantly higher genotoxicity than the tube-type $nTiO_2$ on the 16HBE cell line, indicating the cell-specific sensitivity to the nanomaterial.

While there is a general agreement that $nTiO_2$ induce genotoxicity, it is difficult to establish a clear, singular trend of the extent of toxicity across all organisms. For example, Andreoli et al. (2018) reported a significant increase in DNA strand breaks in human peripheral blood cells exposed to anatase and rutile concentrations of 50 and 100 μg/ml, respectively. Monocytes exhibited a considerable increase in ENM uptake and ROS generation when compared to lymphocytes in the study. However, Rocco et al. (2015) reported that DNA damage was detected in erythrocytes of *D. rerio* at the highest concentration (10μg/L) tested after 14 days. Such variations in the reported results suggest that the mechanisms involving $nTiO_2$ induced genotoxicity may be highly complicated and specific to each type of organism/cell, making it challenging to develop a common toxicological paradigm regarding genotoxicity across all biological systems.

Additionally, the potential of $nTiO_2$ as carriers for heavy metals in aquatic systems has been broadly reported. Previous studies demonstrated that $nTiO_2$ could enhance heavy metal bioavailability and bioaccumulation in various biological systems (such as mammalian cells, zebrafish and carb) or even exert synergistic genotoxicity effect with heavy metals (Liu et al., 2015; Sun et al., 2007; Wang et al., 2017b). This phenomenon demonstrates the indirect genotoxic effects of $nTiO_2$ that may occur in biological systems, which require further studies to ensure the safe utilization of ENMs.

5.2.2. $nSiO_2$

The genotoxic responses of amorphous $nSiO_2$ with different sizes were investigated in pulmonary cells of Wistar rats under both *in vivo* and *in vitro* conditions (Maser et al., 2015). The *in vitro* studies showed that larger ENMs induced more severe DNA damage at a lower concentration when compared to smaller ENMs. Nevertheless, no consistent genotoxicity response was detected under *in vivo* conditions, possibly due to a lower dose of ENMs reaching the target cells *in vivo*. However, contrary size-dependent genotoxicity of $nSiO_2$ *in vitro* was reported in another study, where smaller ENMs (12 nm) induced lysosomal dysfunction and more significant genotoxicity (micronuclei formation) as a result of higher ENMs internalization and alteration of gene expressions associated with lysosomal functions (Decan et al., 2016). Besides, chromosomal aberrations were observed in *A. cepa* root tip cells treated with surface-modified $nSiO_2$, indicating *in vitro* genotoxic effect (Sharma et al., 2020). Different from the abovementioned *in vitro* genotoxicity, only minimal or no *in vitro* genotoxic effects were observed in organisms exposed to $nSiO_2$ (Johnston et al., 2000; Kwon et al., 2014; Maser et al., 2015; Sayes et al., 2010). The research studies reporting the genotoxicity of $nSiO_2$ are scant, hence, more studies are needed to further investigate the possible genotoxicity of $nSiO_2$ for safer future applications.

5.2.3. nZnO

Particle size was reported to have an influence on the genotoxic effects of ZnO particles, while Zn^{2+} ions release may not be the primary factor. For example, when investigating

the genotoxic effects of ZnO particles (nano- and micro-sized) on Madin-Darby canine kidney cells, a significant increase in DNA damage and micronucleus formation was observed specific to nZnO (Kononenko et al., 2017) and ROS generation may serve as the key explanation of the genotoxicity (Ng et al., 2017). As genotoxic effects were unobserved when an equimolar concentration of ionic zinc was applied, it may be surmised that the magnitude of Zn^{2+} ion release was insufficient to cause measurable genotoxicity (Kononenko et al., 2017).

Despite using similar doses of nZnO, the disparity between the observed genotoxic effects in cell cultures *in vitro*, and the lack of genotoxic effects *in vivo* still remains to be elucidated. For example, de Melo Reis et al. (2015) tested the genotoxicity and mutagenic potential of nZnO on the Chinese Hamster V79 cells *in vitro* and the organism *Drosophila melanogaster in vivo*, respectively. In the former test, the authors reported a statistically significant increase in micronucleus in the cells at the highest concentration tested (120µM). For the *in vivo* test, only elevated nZnO concentration (12.5mM) exhibited mutagenic effects on *D. melanogaster* in the form of a significantly increased number of total spots. However, the mutagenic potential was found to be concentration-independent, indicating that other factors such as particle structure may play a critical role. Furthermore, it was reported that nZnO entered primary human epidermal keratinocytes (skin cells) and induced DNA damage when mimicking the application of nZnO in sunscreens applied to human skin (Sharma et al., 2011). Conversely, no evidence of genotoxic effect was observed in mice orally administered with nZnO and micro-ZnO suspensions when mimicking the uptake of ZnO through

food products (Li et al., 2012). Hence, more efforts are needed to further explain the different ENMs toxicity observed between *in vivo* and *in vitro* experiments.

5.2.4. CNTs

Berlo et al. (2012) summarised an extensive list of studies documenting the genotoxicity of CNTs *in vitro* and *in vivo*. Based on the reported studies, the authors reported a significantly higher number of studies demonstrating the genotoxicity of CNTs *in vitro* than studies that focused on *in vivo*. However, most of the *in vitro* studies typically discounted the possible physicochemical transformations of CNTs. Szendi and Varga (2008) claimed that they failed to find any urinary mutagenicity in rats orally administrated with CNTs. Similarly, Ema et al. (2013) tested the genotoxicity of SWCNTs using various assays on bacteria and mammalian erythrocytes and reported negative findings, therefore dismissing the genotoxic risks of SWCNTs. Outside mammalian cells, no genotoxicity was observed in the erythrocytes of the amphibian *Xenopus laevis* larvae exposed to double-walled CNTs in water (Mouchet et al., 2008).

As CNTs encompass a diverse range of materials of varying structural and chemical modifications, their genotoxic potentials are expected to vary. DNA methylation was observed *in vitro* with dosages of 10 and 20 μg/mL of MWCNTs in BEAS-2B human bronchial epithelial cells in 2-4 weeks after exposure, indicating MWCNTs concentration and exposure duration affected the hypomethylation in 755 CpG sites (Sierra et al., 2017). *In vivo* experiments on mice treated with 25μg MWCNTs suspension also confirmed the existence of global DNA hypomethylation in the lung

tissue and white blood cells, which eventually resulted in lung inflammation (Brown et al., 2016). Another study reported that straight MWCNTs possessed superior genotoxicity to tangled MWCNTs (Catalán et al., 2016). In *in vitro* studies, straight MWCNTs at low concentrations induced significant DNA strand breaks while tangled MWCNTs only inflicted DNA damage at the highest concentration tested. Moreover, in *in vivo* experiments, straight MWCNTs induced both DNA damage and micronuclei formation in mouse lung cells with a highly significant dose-dependent response, while such effect was undetected for tangled MWCNTs.

Recently, the considerable variation in CNT toxicity experienced across similar organisms was further exemplified. When different strains of Wisteria mice were orally administrated with MWCNTs over an extended period, it was reported that most of the statistically significant differences occurred in interspecies genotoxicity rather than intraspecies genotoxicity (Gerencser et al., 2016). In addition, no cytotoxicity, oxidative stress-related DNA damage or DNA methylation effects were observed in A549 and HEK293T cells on exposure with 10-50 μg/mL MWCNTs (Chen et al., 2017). Therefore, coupled with significant discrepancies in describing the genotoxic potential of CNTs mentioned above, the mechanisms involving CNT genotoxicity could be highly complex and variable across different biological systems, which require further investigation.

5.2.5. nAg

Foldbjerg et al. (2015) and McShan et al. (2014) provided comprehensive reviews of the previous studies examining the genotoxicity of nAg. The predominant mechanism for genotoxicity in nAg involves ROS generation, in turn leading to DNA strand breaks, micronuclei formation, cell cycle impairment and chromosomal aberrations (Figure 9). By applying ROS scavenger superoxide dismutase on BEAS 2B cells treated with nAg, a significant decrease in genotoxic effects was observed, confirming nAg induced ROS generation serves as a critical indicator of genotoxicity (Kim et al., 2011b).

In addition, Ag^+ ions released from endosome/lysosome also contributed to genotoxicity to a certain extent (Foldbjerg et al., 2015). However, despite numerous reports on the toxicity of nAg/Ag^+ being published, the underlying mechanism and the dominant toxicity cause are still under debate. Studies that test for the genotoxic potential of nAg have reported mixed results, depending on the target organism. Within a single study, different assays may not necessarily indicate a consistent genotoxic effect. For example, PVP-coated nAg were reported to exhibit dose-dependent DNA damage effect in the BEAS 2B cells, while chromosomal aberrations and micronuclei formation were undetected (Nymark et al., 2013). Conversely, in another study, nAg induced dose-dependent DNA damage as well as micronuclei formation in the same type of cells (Kim et al., 2011b). Furthermore, several studies reported that nAg induced insignificant genotoxic effects on organisms like Sprague-Dawley rats (Kim et al., 2011c), mice and human testicular cell lines (Asare et al., 2012).

As mentioned in 5.1.5, both 25 and 75nm nAg evinced toxicity through food chain transportation (Luo et al., 2016).The authors conducted further experiments to

investigate the genotoxicity of nAg on *C. elegans*. The results showed that the damage caused to the germ cells could be transferred to subsequent generations. However, only 25nm nAg were found in the F1 generation and the toxicity effect was pronounced in the F2 and F3 generations but reduced in the F4 generation, indicating the potential size-dependent genotoxicity of nAg.

nAg with different sizes (10 and 100nm) could induce dose-dependent DNA damage in the CHO-K1 and CHO-XRS5 cell lines (Souza et al., 2016). Among the CHO-XRS5 cell line, considerable increase in micronuclei and nucleoplasmic bridges was observed when exposed to the 100nm nAg, validating that larger ENM size generally inflicted greater genotoxic effects, which is similar to the trend in $nSiO_2$ (Maser et al., 2015). This effect was significantly less pronounced in the CHO-K1 cell line, suggesting CHO-K1 cells are tolerant to genotoxic effects from nAg. A gradual decrease in DNA damage was observed in the roots and shoots of *A. cepa* and *N. tabacum* exposed to nAg, which may be due to the predominant ENM-ENM interactions at higher concentration, limiting the chance of nAg to interact with the biological systems (Ghosh et al., 2012). Further, distinct transcriptomic responses were observed from *C. elegans* exposed to pristine and sulfidised forms of nAg corresponding to different toxicity mechanisms (Starnes et al., 2016). Overall, the correlation between genotoxicity impacts of nAg and the dosage of ENMs across most organisms still remain inconclusive.

6. Conclusions and Future prospects

The environmental flow models, physicochemical transformations, cytotoxicities and genotoxicities of $nTiO_2$, $nSiO_2$, nZnO, CNTs, and nAg have been reviewed and discussed. From the reported studies, it is evident that extensive work has been performed to model ENM levels in the environment, and to investigate the physicochemical transformations, and the subsequent toxicities of ENMs. In light of the growing applications of ENMs, focussed research on their end-of-life management and toxicity are paramount. The stochastic models on fate, transport, and exposure are progressively becoming advanced and comprehensive. The availability of accurate production and release data would further improve the precision of the models from current predictions. However, experimental validations would aid to minimize the uncertainties associated with the proposed models. Subsequently, large-scale ENM fate models exploring transformations beyond simple first-order descriptions of ENM dissolution are essential (Dale et al., 2015). The physicochemical transformations occurring in the ENMs are well recorded. On the other hand, scattered approaches in cytotoxicity and genotoxicity assessments and evaluation methods complicate the systematic understanding and validation of the studies. Despite the increased attention and funding for the nanomaterial safety research and development, there is no consensus on the safety and toxicity of the ENMs (Maynard and Aitken, 2016; Valsami-Jones and Lynch, 2015). The published research by different groups on the same ENM fail to arrive at a consensus on the conclusions in some instances and even contradicting at times thereby increasing the uncertainties of the findings, and becoming increasingly

challenging to make evidence-based decisions. Key areas are highlighted that would require future research efforts to improve the toxicity evaluation.

6.1. Development of comprehensive and unified inventory database

Firstly, insufficient ENM production and distribution data to precisely evaluate ENM concentrations in the environment makes it difficult to further assess the toxicity of ENMs. As mentioned previously, while there are models available to estimate ENM levels in the environment, these models rely on accurate data to provide useful projections of ENM concentrations in environments as the ENM consumption increases manifold in the near future. For example, Piccinno et al. (2012) reported a high variance of $nTiO_2$ and $nSiO_2$ production amounts as shown in Table 1, which would affect the estimation of environmental concentration via distribution models. Hence, we assert the need to improve current data collection for ENM production and consumption, possibly through establishing a shared database among companies involved in the trading of ENM-containing products. Thenceforward, suitable modelling of ENM concentrations in the environment can be designed for future studies by basing their experiments around environmentally relevant concentrations. Furthermore, toxicity studies are largely reliant on environmental concentration data, as nanotoxicity may be immensely influenced by ENM concentration in the environmental medium. For instance, nanotoxicity may not always be dose-dependent, as reported in the findings by Ghosh et al. (2012) and Wang et al. (2017b). Hence, an increased flow of ENMs into the environment may not pose significant cytotoxicity due to increased aggregation, thus lowering bioavailability (Wang et al., 2014). Development of extensive toxicity

databases by a combination of interdisciplinary scientific fields including chemistry, physics, biology, mathematics, statistics and informatics would further aid in the resolution of the present issues (Pikula et al., 2020).

6.2. Development of sophisticated computational approaches

There are enormous amount of literature published with respect to ENMs in the past decade. Human comprehension of all of the available information and arriving at a consensus is a challenging feat primarily due to the variations and uncertainties between studies. Hence, in this aspect, the assistance of data mining and analysis technologies would enable to sort, and comprehend the available research information. Further, artificial intelligence technology could be used for simulations and achieve more accurate models for environmental concentrations. For instance, Quantitative Structure Activity Relationship computational approach uses the molecular descriptors and physicochemical properties to predict the biological functioning of a material. However, there is a need for a more sophisticated prediction of fate, transformations, biological interactions, and toxicities by *in silico* approaches including nanoinformatics, statistical analysis, and machine learning platforms. These approaches have demonstrated the ability for reliable prediction and evaluation of ENMs' behavior and their toxic endpoints (Furxhi et al., 2020; Furxhi et al., 2019; Pikula et al., 2020; Sheehan et al., 2018). Moreover, a thorough evaluation of the interactions of the ENMs in a representative environmental matrix factoring in numerous permutations and combinations between the ENMs and the environmental system is possible via the artificial intelligence technology.

6.3. Establishment of scientific correlations and development of cause-pathway-influence-effect relationship

Only few studies analysed herein reached a consensus regarding the toxic effects of ENMs. Further, no predictable pattern or correlations have been observed. This may be attributed to their failure in accounting for the effects of environmental parameters on the exposure rate and the physicochemical transformations of ENMs – an important point of consideration as demonstrated by Wang et al. (2014) and Zhang et al. (2016b). The toxicity of ENMs may be mediated by multiple mechanisms caused by various factors, including ENM characteristics, tested organism properties, and environmental parameters. The dynamic and stochastic nature of the environmental systems imposes significant physicochemical changes to the ENMs including incidental coatings and subsequent reactions that further complicate the understanding of the risks associated with the environmental release (Lowry et al., 2012). ENMs exposed to the environment may possess different physicochemical properties during various phases of its life cycle and the corresponding toxicity varies. Even amongst studies that have considered environmental parameters, these parameters may interact simultaneously (Omar et al., 2014), complicating the eventual toxic effect (Dasari and Hwang, 2013). Hence, future studies should consider the dynamic interactions between multiple environmental factors in determining the final physicochemical state of ENMs to evaluate their nanotoxicity. Additionally, when exploring the toxic effect of a specific type of nanomaterial, most studies focus solely on the material itself. However, in the real environment, previously released nanomaterials and various environmental pollutants

co-exist and are likely to interact with the ENMs under evaluation. Hence, co-existing ENMs and contaminants need to be considered when assessing nanomaterial toxicity. Moreover, existing studies mainly focus on pristine nanomaterials, while only limited data on the end-of-life stage of spent nanoproducts is available (Miseljic and Olsen, 2014; Zhang et al., 2018). The spent ENMs are most likely to be released into the environment and exposed to organisms. The effects of particle size, agglomeration, dissolution and other transformations on the toxicity of organisms are poorly understood (McGillicuddy et al., 2017). Therefore, it is also necessary to evaluate the toxic potency of the spent/transformed nanomaterials. Further, the data of possible toxicity mechanisms is limited and remains to be developed. It is recommended that future works expend more resources for establishing the mechanisms of toxicity instead of the effects themselves, as observed in studies by Foldbjerg et al. (2015) and Kim et al. (2011b). The observed mechanisms are reported directly, however, an in-depth cause-pathway-influence-effect relationship needs to be established. This would facilitate the prediction of the effects of ENMs across a broader spectrum of organisms and improve our understanding of the environmental impacts of ENM consumption at an ecosystem level.

6.4. Implementation of systematised standard protocols and harmonized reporting

There is a significant contrast between toxic effects observed in *in vitro* and *in vivo* across several studies, such as in Maser et al. (2015) and de Melo Reis et al. (2015). The toxic effects of ENMs reported by diverse literature are usually inconsistent across

various organisms. Furthermore, even within the same species, the toxicity observed under similar exposure scenarios may be different, as demonstrated by Gerencser et al. (2016). This could be due to bioremoval (Slowing et al., 2011), protective mechanisms (Simon-Deckers et al., 2009), natural sensitivity of the species, etc. One possible approach to further understand the differences between the observations of *in vitro* and *in vivo* is via toxicity studies that target the same organism, followed by sharing the data and comparing differences in their experimental setup and toxicity evaluation methods. OECD established an OECD harmonised template (OHT) system that is a standard data format for reporting information and results aimed at developing a database. Currently, the ENM specific OHT addresses the physicochemical properties. Such a systemised strategy may help in explaining the variations observed in experimental setups and choice of assays that influence the final toxicity observed and ultimately improve future experimental design in nanotoxicity assessments. Concurrently, it is crucial to establish a comprehensive and standardized evaluation system for researchers to effectively assess the toxicity of ENMs in the environment. On the other hand, the accurate identification of toxicity biomarkers that detect potential ENM-induced damage to living organisms needs to be further advanced (Yu et al., 2020).

Recent trends suggest that there are numerous published research, which study various aspects of the ENMs' fate, transport, exposure, and toxicities. Each of these researches studies a specific material and parameter. However, an organized research could facilitate in the development of an invaluable data repository and aid in validation.

There are efforts to promote international cooperation between research groups, develop internationally harmonized standards, develop data repository, and integrated testing strategies by international organizations including OECD, ISO, FAO, and WHO (Takeuchi et al., 2014). ISO has published standard nanomaterial characterization procedures and OECD has published a dossier on the safety of ENMs. A BILAT USA 4.0 (2019) meeting at Harvard University on EU-US priorities in nanosafety was focussed on EU-US cooperation in research and innovation. Seven research priorities were identified in the meeting including environment and human hazard, emerging ENMs and potential risks, social and natural science research, nanoinformatics, exposure assessment at both environment and human population levels, standard methodologies, reference materials and harmonization, life cycle and transformation.

Further, OECD testing guidelines program ensured the development and updating of several testing guidelines (TGs) and guidance documents (GDs) for nanomaterials under the OECD working party for manufactured nanomaterials (WPMN). TGs and GDs aid in transparent and traceable physicochemical characterization, hazard, fate and risk assessments of ENMs facilitating the internationally recognized ENM grouping and data collection via OHT (Rasmussen et al., 2019). For example, there are few latest TGs addressing specific hazards such as TG412 (subacute inhalation toxicity), TG413 (subchronic inhalation toxicity) and TG318 (dispersion stability on nanomaterials in simulated environmental media) developed in 2017 and 2018 (OECD, 2017; OECD, 2018a; OECD, 2018b). Further, new and updated TGs are under development based on the proposals from various WPMN recommendations.

Hence, it is essential to establish standardised reference materials and experimental methodologies, sample characterization based on international standards, development of data repository, and characteristic and representative test organisms and cell types. Further, other relevant controllable factors should be predefined based on international consensus. This would aid to minimize the uncertainties associated with the experimental research and facilitate reproducible outcomes.

On the other hand, despite the reported toxicity concerns, the largescale production and subsequent release of the ENMs into the environment are imminent primarily due to the superior benefits offered by the nanotechnology to humankind. However, the toxicity can be minimised by approaching the technology with environmentally sensitive product designs with predefined end-of-life treatment options, improving the recyclability of the products thereby facilitating circularity of the material flow, and shifting towards the usage of renewable, biodegradable, and biocompatible materials.

CRediT authorship contribution statement

Ashiq Ahamed: Conceptualization, Methodology, Literature review, Investigation, Validation, Writing - Original Draft. **Lili Liang**: Literature review, Investigation, Validation, Writing - Review & Editing. **Ming Yang Lee**: Literature review, Investigation, Writing - Original Draft. **Johan Bobacka**: Supervision, Writing - Review & Editing. **Grzegorz Lisak**: Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing - Review & Editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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