# Modelling the interactions of advanced micro- and nanoparticles with novel entities

Zhang, F.

# Chapter 1

# General Introduction

## 1.1 Novel entities

In September 2021 Claire Asher wrote an essay entitled: Novel chemical entities: Are we sleepwalking through a planetary boundary? This title gives the exact expression for humans' low state of consciousness while performing activities with increasing pollution which result in affecting the Earth system in a variety of ways. These "new substances with potential geophysical and/or biological effects" are referred to as "novel entities" (Persson et al., 2022). Scientists generally recognize that novel entities become the focus of global environmental attention when they exhibit the potential for lasting impact, large-scale distribution, and influence on important Earth system processes (De Souza Machado et al., 2019). The majority of man-made chemical "novel entities" have been reported to enter the environment (L. Chen et al., 2023; Kuznetsova et al., 2023; Nunes et al., 2023; Zhang et al., 2023), including engineered nanoparticles (ENPs), microplastics (MPs), pesticides, per- and polyfluoroalkyl substances, flame retardants, antibiotics even novel living organisms.

The Planetary Boundaries framework created by the international scientific team of the Stockholm Resilience Centre is trying to get a handle on the myriad of environmental impacts of this chemical onslaught (Steffen et al., 2015). Because novel entities are so pluriform, just recently quantification of the extent and magnitude of the pollution was estimated (Diamond et al., 2015; MacLeod et al., 2014; Persson et al., 2022, 2013; Steffen et al., 2015) with a lot of focus and help of visible plastic pollution (Arp et al., 2021).

The "planetary boundary" is the boundary value used to define the global "safe operating space" (Rockström et al., 2009), which relates

to e.g., biosphere integrity, climate change, and novel entities (Steffen et al., 2015). "Planetary boundaries" are proposed to maintain the normal functioning of the Earth system and human society by defining variables that humans need to control, setting thresholds, and reducing the risk of human activities exceeding Earth system thresholds (MacLeod et al., 2014). However, these "novel entities" are being created at such a rapid pace that they are far outpacing the risk assessments conducted by governments and organizations. This makes that the majority of the novel entities are still existing in the natural environment, and production and associated contamination are likely to rise further (Persson et al., 2022).

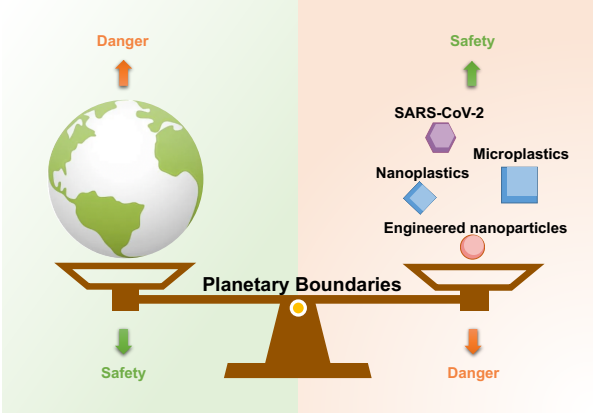


***Figure 1.1.*** *Potential control of the quality of the Earth's ecosystem by novel entities.*

At present, it is still difficult to control the impacts on the Earth's ecosystem from these "novel entities". To maintain the balance of planetary health in relation to the continued increase of novel entities (Figure 1.1), there is an urgent need to investigate the environmental behavior and toxicological effects of novel entities. Toxicological testing has confirmed the ecotoxicity of typical novel entities such as

ENPs and MPs (Minetto et al., 2016; Rai et al., 2021). Most notably, novel entities often enter the environment as mixtures (Kar and Leszczynski, 2019; Martinez et al., 2022; Trinh and Kim, 2021). Simultaneous exposure to multiple novel entities requires consideration of the possible interactions between these novel entities and their effects on organisms.

### 1.1.1 Engineered nanoparticles

Nanotechnology has emerged as the most promising technology of the 21st century (Sharma et al., 2023). Nanomaterials are widely used in many fields (Singh et al., 2023), and most of them are engineered and gradually integrated into people's lives and used in thousands of products (Pérez-Hernández et al., 2021), such as cosmetics, sunscreens, fabrics, pharmaceuticals, and sports equipment. Along with the large-scale industrial production, transportation, and disposal processes of nanomaterials, especially during the wash-off process of personal care products doped with nanomaterials (e.g., cosmetics, sunscreens, textiles, etc.) and during the use and decomposition of industrial products, ENPs are released or flow into and seep into the atmosphere, water bodies, and soil environment, becoming "environmental nano-pollutants" (Abbas et al., 2020; Ahmed et al., 2018).

ENPs have been detected in surface water (Azimzada et al., 2021; Sanchís et al., 2020; Wu et al., 2020) and sediments (Tou et al., 2021), and nanoparticles containing titanium, copper, zinc, and silver have been detected in aquatic organisms for instance marine mollusks (e.g., oysters, mussels, scallops, and clams) (Xu et al., 2020). When a particle is small and approaches the nanoscale, its physicochemical

properties are greatly altered and it has the potential to freely cross cell membranes and enter the cells of organisms (Verma et al., 2008). Numerous studies have confirmed that ENPs have a significant impact on ecology and human health (Das et al., 2016; Jogaiah et al., 2021; Tiede et al., 2016). In fact, environmental nano-pollutants have become one of the most dominant and important objects of environmental science research and environmental protection technologies.

### 1.1.2 Microplastics and nanoplastics

In terms of time span change, humankind has taken a big step into the "plastic era" (Lee et al., 2022). However, with the growing awareness of environmental protection, it has been recognized that "microplastics" (MPs, < 5 mm) pollution has taken over the world (Jung et al., 2022). MP pollution not only affects the development of human society, but also negatively affects the function of marine organisms, biomes, and the entire global ecosystem, and is a potential global boundary threat (Allen et al., 2021; González-Pleiter et al., 2021; Romera-Castillo et al., 2023). MP pollution has posed an unprecedented challenge to geoscience research and has become a global challenge to address. MPs include micron- to millimeter-scale plastics (> 1000 nm to 5 mm) as differentiated from nanoplastics (NPs, 1 to 1000 nm) (Junaid et al., 2023). NPs are characterized by large fugitive quantities, high adsorption efficiency, and high trans-biofilm capacity, and may exhibit more intense ecological and toxic effects compared to MPs (Z. Chen et al., 2023; Qi et al., 2023).

MPs and NPs can cause damage to biological organisms through oxidative stress (Rodrigues et al., 2023), cytotoxicity (Shi et al., 2022),

inflammatory responses (Wang et al., 2023), metabolic alterations (Zhao et al., 2023), and neurotoxicity (J.-L. Xu et al., 2022). MPs and NPs are frequently found in the environment and are easily ingested by organisms and can enter the food chain by virtue of their small size (Kim et al., 2022; Zhu et al., 2021), ultimately affecting human health (Dong et al., 2023; W. Wang et al., 2022). *In vitro* studies have also confirmed that MPs and NPs have toxic effects on the human digestive, respiratory, immune, and reproductive systems (Bastyans et al., 2022; J.-L. Xu et al., 2022). Due to the strong specific surface area, MPs and NPs may have more serious negative effects on organisms when they act as "Trojan horses" carrying other contaminants through biofilms or across tissues (Katsumiti et al., 2021; Roje et al., 2019; Sun et al., 2023). Therefore, the phenomenon of complex contamination caused by the interaction of MPs and NPs with harmful "novel entities" coexisting in the environment cannot be ignored.

### 1.1.3 Viral particles

Different from engineered chemical "novel entities", biological "novel entities" include amongst others viruses (like, among others the coronavirus). Viruses can exist in sizes ranging in between 1–100 nm, with novel coronaviruses typically having a diameter of 60–140 nm (Gunathilake et al., 2022).

These biological entities distribute globally. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spreads mostly through direct contact and respiratory droplets (Jennings and Perez, 2020; Ragab et al., 2020), although pathways via water, air, and soil may also be important vectors of coronavirus transmission (Sanchez-Galan et al.,

2021). Coronavirus-containing waste or wastewater can enter the aquatic environment, especially in urban water environments, by several routes (Saingam et al., 2023). Wastewater contains a large number of viruses, bacteria, fungi and chemical reagents, which are mixed together and are highly infectious. This allows them to easily cause various human diseases and complications, posing a serious public health risk. At the same time, the aquatic environment contains a large number of viruses and other pathogens that are extremely destructive to other ecological species. A more comprehensive understanding of the environmental behavior of viruses or viral ribonucleic acid (RNA) in environmental media is not only beneficial to the development of more scientific-based control and prevention strategies, but also allows for a deeper understanding and scientific cognition of the relevance of ecological changes to viruses.

## 1.2 Interactions of micro- and nanoparticles with other novel entities

Small particles have a relative large surface area (e.g., ENPs, MPs, NPs, and virus) and inherently have good adsorption capacity owing to their special physicochemical properties, including small particle size, huge specific surface area, and high hydrophobicity at nanostructured surfaces. Once these micro- and nanoparticles (MNPs) enter the environment, they are likely to sorb, enrich and carry other pollutants in the environment. This will not only alter the transport and transformation behavior of other pollutants in the environment, but these pollutants will also change the dissolution, agglomeration, precipitation, bioconcentration and migration, microbial degradation

and other behavioral properties of MNPs themselves in the environment (Figure 1.2). In addition, when MNPs act simultaneously with other novel entities on organisms, they may induce toxic responses that are different from the effects of a single toxicant (Figure 1.2). Thus, it can be seen that the carrier effect is the key by which MNPs influence the environmental behavior and toxic effects of other novel entities.

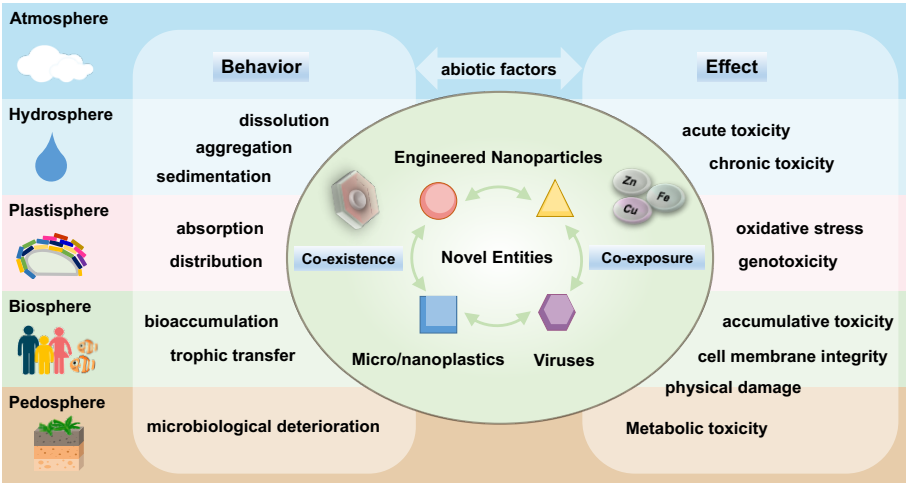


***Figure 1.2.*** *Interactions between novel entities and their potential environmental behavior and toxicological effects.*

### 1.2.1 Interaction of ENPs with metals

ENPs readily adsorb metals/metal ions and their oxides from the environment (Deshwal et al., 2023; Rosenfeldt et al., 2016). This adsorption behavior can affect not only the physicochemical properties of ENPs such as particle size and surface charge, but also the environmental transport and transformation of the adsorbed substances. When the composite system belongs to the category of colloids (particle size between 1 nm and 10 μm), the particles have

well-defined physicochemical properties such as surface charge and kinetic diameter. Meanwhile, the classical colloid dispersion theory — the Derjaguine-Landaue-Verwaye-Overbeek (DLVO) theory — can provide a solid theoretical basis for the analysis of nanoparticle composite systems (Nur et al., 2015).

### 1.2.2 Interaction of ENPs with organic pollutants

ENPs have large chemical activity and surface energy, which make them have strong interaction with organic pollutants and allows them to adsorb a large amount of organic pollutants (Pan and Xing, 2008; Qi et al., 2014; Xin et al., 2023; Yang and Xing, 2010). In recent years, scientists have conducted various studies on the ENPs' capacity to bind organic contaminants and demonstrated that the interaction of ENPs with organic pollutants is mainly related to the hydrophilicity and polarity of organic pollutants (Chen et al., 2007). The mechanisms of interaction between ENPs and hydrophobic organic pollutants are mainly based on hydrophobic and π-π interactions, while the adsorption of hydrophilic organic pollutants is mainly through partitioning, electrostatic force, and hydrogen bonding (Pan and Xing, 2008).

### 1.2.3 Interaction of ENPs with microorganisms

The interactions between ENPs and microorganisms are complex and diverse. For instance, once ENPs are released into the environment, a large fraction of the nanoparticles flow into wastewater treatment plants and come into contact with microorganisms present in the wastewater treatment (Saravanan et al., 2022; Zhou et al., 2023). These microorganisms can be present in biological wastewater

treatment systems in many forms. ENPs discharged from wastewater plants or directly into the environment can also come into contact with environmental microorganisms such as bacteria and algae (Gong et al., 2023). Therefore, understanding the interaction between ENPs and microorganisms is important to recognize the migration, toxicity, and elimination of ENPs in the microbial environment.

Recently, scientists have identified novel coronaviruses in wastewater in several countries (Conde-Cid et al., 2021). Previous studies have shown that both hepatitis viruses (Brisebois et al., 2018) and enteroviruses (Upfold et al., 2021) are transmitted to humans through wastewater. Therefore, wastewater containing novel coronaviruses can pose a serious threat to the population. After going through the entire standard wastewater treatment process, it may be sufficient to remove or destroy the virus. However, if there is still virus remaining in the effluent of wastewater treatment plants or in the effluent of treatment plants with poor wastewater treatment, the virus may still enter surface waters (Kolarević et al., 2022) with the effluent discharge (e.g., lakes, rivers, and various recreational sites) and may also become a source of infection. In addition, virus-laden effluent that seeps into natural waterways can cause infection through airborne aerosols (Q. Wang et al., 2022). Furthermore, the consumption of fruits and vegetables irrigated with wastewater that has not been properly disinfected may also be an indirect route of infection (Fernandes et al., 2023). Studies have reported that novel coronavirus RNA fragments have been detected in wastewater samples (Langeveld et al., 2023). The residence of novel coronaviruses and their RNA fragments in different water bodies and aerosols provides an opportunity for their coexistence with ENPs in

the environment.

The interaction between ENPs and microorganisms involves three main processes: physical, chemical, and biological. Both the agglomeration of nanoparticles and their deposition on microbial surfaces involve common physical interaction forces, as described by the traditional DLVO theory, hydrophobic interactions, spatial site resistance, and multimer bridging. ENPs also undergo a number of chemical transformation processes in the microbial environment, including redox transformations, photochemical degradation processes, surface adsorption, precipitation, complexation and cation bridging. In addition to physicochemical processes, some biologically relevant processes also occur between ENPs and microorganisms, including endocytosis, mitochondrial effects and biodegradation.

### 1.2.4 Interaction of MPs and NPs with microorganisms

MPs and NPs provide substrates and ecological niches for microorganisms to attach and selectively enrich environmental microorganisms to form biofilms, a new artificial ecosystem called the "plastisphere" (Zettler et al., 2013). Harmful microorganisms in the environment are adsorbed by MPs or NPs and transported over long distances in the environmental media. In these media they propagate and spread, facilitating the spread of antibiotic resistance genes and thus causing potential harm to the ecosystem. Numerous studies have focused on the enrichment and succession of microbial communities (algae, bacteria, archaea, fungi, viruses, protozoa, etc.) in the plastisphere, exploring the interactions within microbial communities, metabolic capabilities, and how microbial communities affect the surrounding environment (Barros and Seena, 2021; Junaid et al.,

2022; Wang et al., 2022). Thus, the impact of MPs and NPs is not only associated with themselves, but the microbial impact on their surface may be even more profound.

Possibly harmful effects on biota being amplified by interactions between SARS-CoV-2 and plastic pollutants in the aquatic environment is another growing subject. For example, recent studies have shown that particles of the SARS-CoV-2 virus can adhere to MPs' surfaces (Belišová et al., 2022), raising concerns about potential increased infectivity and virus transmission in humans, as well as the possibility of ecotoxicological risk that such interactions could present to organisms that are not the targets. For example, MPs can bind SARS-CoV-2 pseudoviruses on their surface and enhance infection of human cells *in vitro*, suggesting that plastic particles present in the environment or in the respiratory or gastrointestinal tracts of humans (target organism) have the potential to interact with SARS-CoV-2 and increase the risk of viral infection (Zhang et al., 2022). In addition, airborne MPs from waste could also serve as a transmission vector for SARS-CoV-2 (Liu and Schauer, 2021), and knowledge on this interaction can help to understand the transmission process.

### 1.2.5 Main factors affecting the interaction between MNPs and other novel entities

The adsorption of novel entities to MNPs can be described by considering that:

1) The ability of MNPs to adsorb other novel entities is closely related to the inherent properties of MNPs. It is both size and surface

related. Additionally, the physicochemical properties of MNPs are essentially determined by their composition, morphology, and internal structure.

2) The ability of MNPs to adsorb other novel entities is also related to the physicochemical properties of the other novel entities with which it has the interaction, such as the molecular size, structure and polarity of novel entities.

3) The ability of MNPs to adsorb other novel entities is also influenced by external field conditions such as pH, ionic strength and temperature.

## 1.3 Combined toxic effects of MNPs and other novel entities

MNPs can affect the biotoxicity and bioefficacy of other novel entities through adsorption, enrichment, and carrier effects, while altering their environmental behavior and fate. However, the current ecological risk assessment of MNPs typically relies on toxicity data regarding the toxicity of single constituents, and the potential risks caused by combined adverse effects are not fully taken into account. Therefore, the study of the mixture toxicity of MNPs has important theoretical value and practical significance, which is conducive to improving the scientific and rational nature of ecological risk evaluation.

To address the ecological risks caused by MNPs and their mixtures, studies on the combined toxicity of MNPs with other novel entities are gradually emerging. Current studies have focused on the mixture toxicity of MNPs with metals and organic pollutants, while studies on

the mixture toxicity of MNPs with other novel entities in the particulate state (other ENPs, M/NPs) have lagged behind. Up-to-date 1st generation metal oxide ENPs were tested most frequently on these test systems (Das et al., 2022; Mansouri et al., 2016; Yu et al., 2016a).

The mechanisms of interaction of MNPs and other novel entities in the particulate state and the combined toxicity of the mixture of these two entitites to ecological species are relatively complex, and the mechanisms of toxicity due to particle-particle interactions may also differ from those of particle-organic substance and particle-metal ion interactions. In addition, a non-homogeneous mixture composed of multiple particulate components has very different properties as compared to the properties of a single particulate component. This makes the combined toxicity of MNPs and other novel entities in the particulate state being affected by a combination of factors, including the composition, structure, and properties of each particulate component, the agglomeration and stability of particles, and the composition of exposure medium.

There are currently two approaches to determine the mixture toxicity of novel entities, namely a bottom-up approach (i.e., component-based or reductionist approach) and a top-down approach (i.e., whole mixture-based or holistic approach). Mixture toxicity studies depend on the methodology chosen. In the bottom-up approach, the challenge may be to relate experimental results of carefully controlled and well-defined mixtures to real-world situations. To respond to this challenge, mixtures consisting of chemicals with actual scenario exposure information or with common toxicity indicators can be prioritized for evaluation in studies of

mixture toxicity (Rider and Simmons, 2018). In the top-down approach, on the other hand, whole mixtures are often treated like single chemicals. However, such method is needed to determine the similarity between different mixtures. It is also essential to determine the interacting effects between components in a mixture.

## 1.4 Computational simulation methods for interactions between MNPs and other novel entities

Computational simulation models can be used to assist in solving complex problems and in data-rich situations. Along with the innovation of computer technology and the explosive development of big data, machine learning (ML) and artificial intelligence, as well as computational simulation (or *in silico*) methods have been rapidly applied to investigate the environmental behavior of novel entities (Gastaldi et al., 2023). Currently, the use of computational simulation methods in the field of nanotoxicology has increased significantly. Computational simulation methods can not only reduce the time and money spent on identifying the impacts of novel entities for *in vivo* and *in vitro* experiments, but also improve the understanding of the toxicity of novel entities.

Four major categories of computational simulation methods are commonly used: molecular simulations, physiologically based pharmacokinetics (PBPKs), quantitative structure-activity relationship (QSAR) models, and ML. Among them, molecular simulation methods are frequently used to study the structure-effect relationship of nanoparticle-novel entity interactions (Brinkmann et al., 2022; Dowlatabadi et al., 2019; Geitner et al., 2017). Molecular simulation uses computers to simulate the structure and behavior of

molecular models at the microscopic (atomic), nanoscopic, and mesoscopic scales, and then obtain various physical and chemical properties of molecular systems (Figure 1.3).

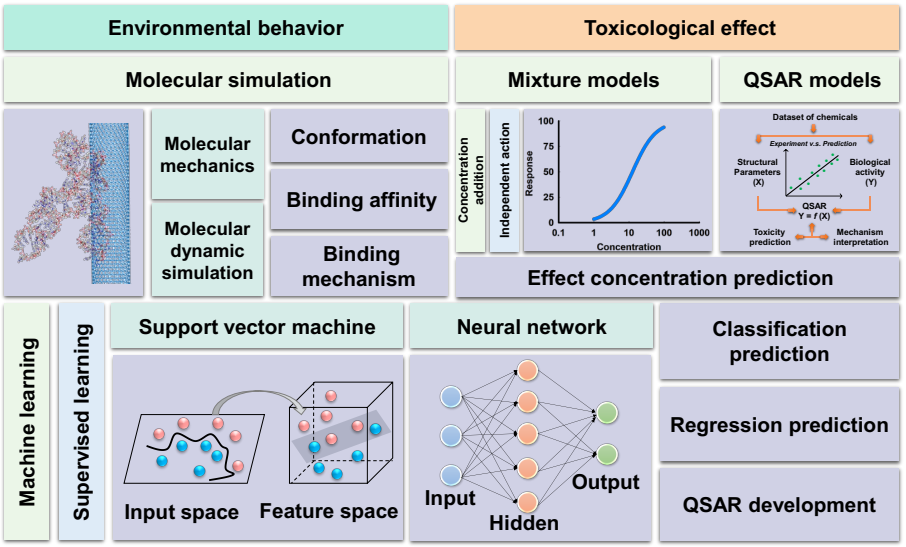


***Figure 1.3.*** *Computational simulation methods for environmental behavior and toxicological effects of mixtures of novel entity.*

It constructs a set of models and algorithms based on physical laws with basic principles to calculate reasonable molecular structures and molecular behaviors. Molecular simulation can simulate not only the static structure of molecules, but also the dynamics of molecular systems. The typical methods of molecular simulation are: quantum mechanics (QM), molecular mechanics (MM), molecular dynamics (MD) simulation, molecular docking, Monte Carlo simulation.

MD simulation can simulate the flexible binding processes of multi-molecular systems and the dynamic conformations of complexes, providing more comprehensive information on the interaction mechanisms. Unlike other molecular simulation methods

(e.g., molecular docking, Monte Carlo simulation) that can deal with the static binding properties of multi-molecular systems, a MD method is capable of simulating both static and dynamic processes in multi-molecular systems. In addition, the MD method is more time efficient in dealing with multi-molecular systems than a QM method that takes the electron motion into account. Moreover, the MD method can also increase the accuracy. Therefore, the method has an irreplaceable advantage in simulating multi-molecular systems at the microscopic scale.

A QSAR is a statistical model that relates a set of structural parameters that describe a chemical compound to its biological activity (Buglak et al., 2019; Chen et al., 2017), as depicted in Figure 1.3. These parameters, which are called descriptors, are typically related to the steric and electronic properties of the compound, and they can be computed or measured in experiments. Biological activity, however, is determined through biological assays involving organisms of different trophic levels like algae, daphnids, and fish. Currently, the development of QSAR can offer a new way to rapidly screen chemicals alone or in mixtures and prioritize testing (Chatterjee and Roy, 2023; Reddy et al., 2023).

Recently, ML algorithms and deep learning (DL) algorithms have been developed for the study of multi-component interactions. ML modeling mainly uses publicly available structural information and *in chemico*, *in vivo*, and *in vitro* bioactivity data to construct QSAR models built on ML algorithms to further deepen the analysis of contaminant-biomolecule interactions in complex biological contexts (Figure 1.3). Therefore, ML modeling becomes a new computational technique that is completely different from molecular simulation.

Commonly used ML algorithms include support vector machines, neural networks, and random forests. These algorithms are capable of uncovering the intrinsic connections between numerous molecular features and predicted endpoints. Each algorithm has its own strengths, and the ability to make good use of such strengths is related to a variety of factors (e.g., the structure of the data). However, there is a key problem with ML modeling — poor interpretability. Previous statistical methods with excellent predictive power for linear data, such as linear-based multiple linear regression and partial least squares regression, are no longer able to cope with the explosive growth of biological "big data". The huge volume of data and the complexity of biological processes have led to a more advanced nonlinear class of data analysis methods. ML algorithms as applied to QSAR models with decision boundaries and hyperparametric dependence prediction problems that allow ML modeling with both high prediction accuracy and low interpretability. Combining multiple computational toxicology techniques to develop an integrated workflow for analysis of the underlying mechanisms can compensate for their respective shortcomings and allows to obtain optimal results. However, how to add computational flux screening while maintaining mechanistic analysis is still a critical issue to be addressed in the future.

## 1.5 Mixtures modeling

In ecotoxicological studies, screening of contaminant compound risks is mainly achieved by qualitatively assessing the mode of action of combined toxicity and quantitatively predicting the magnitude of combined toxicity. Two pharmacological concepts can be used for

data description and data interpretation for mixture toxicity, namely: "concentration addition" (CA) is used to predict the toxicity of mixed systems composed of compounds with similar modes of toxic action (Loewe and Muischneck, 1926); whilst "independent action" (IA) is used to predict the toxicity of mixed systems composed of compounds with different modes of toxic action (Bliss, 1939).

The CA and IA models have been applied to the assessment and prediction of nanoparticle mixture toxicity (Baek et al., 2020; Lai et al., 2022; Liu et al., 2016; Martín-de-Lucía et al., 2019). For example, the CA and IA models were used to effectively predict the combined toxicity of Cu and ZnO nanoparticles to *Lactuca sativa* L., and it was found that the fit of the IA model to experimental data on the combined toxicity of the two ENPs was better than the fit in case of the CA model (Liu et al., 2016). However, it is worth pointing out that the CA and IA models also require toxicity tests to determine the concentration-response relationships for single components. This increases the resources, money and time invested in the experiments on the one hand. On the other hand, it reduces the efficiency of the risk assessment of mixtures.

## 1.6 Extrapolation methods for assessing and predicting the combined toxicity of MNPs with other novel entities

Testing the toxicity of all chemicals and their mixtures is impractical, so there is an increasing need to rely on expert systems and computational methods (e.g., PBPKs, QSAR models) in future risk assessments. QSAR models can compensate for the low predictive efficiency of CA and IA models for mixture toxicity. As mentioned

above, QSAR models are mathematical relationships between toxicity indicators (e.g., lethality) and descriptors (e.g., physicochemical properties of chemicals) (Chen et al., 2017). In particular, QSAR model inputs do not require information on the concentration-response relationships of all single components in a mixture.

In previous studies, many breakthroughs have been achieved in the application of QSAR approaches to predict single toxicity studies of ENPs (Ehret et al., 2014; Epa et al., 2012; Huang et al., 2020; Puzyn et al., 2011), and hence QSAR model has become one of the most effective approaches for predicting the toxicity of single ENPs. However, QSAR studies on quantitative prediction of the toxicity of nanoparticles in combination with other novel entities are still in their infancy. The primary problem could be a lack of sufficient experimental data and standardized toxicity endpoints for developing predictive models. In addition to toxicity indicators, descriptors are also important for the development of QSAR models. Descriptors for nanoparticles can be obtained based on their properties at different multiple scales (Wang et al., 2018), which include quantum chemical properties of nanoclusters (e.g., total energy, orbital energy, and thermodynamic parameters), intrinsic structural properties of nanoparticles (e.g., chemical components, primary size, surface charge, specific surface area, and solubility), and colloidal properties of nanoparticles at mesoscopic scales (e.g., zeta potential and hydrodynamic diameter). Since nanoparticle mixtures contain both nanoparticle and mixture components, it is necessary to screen or calculate the features that can describe the hybrid system. Currently, some pioneering studies have developed QSAR models for predicting the biotoxicity of n$TiO_2$-based nanoblends (Mikolajczyk et al., 2019;

Trinh et al., 2022). The aforementioned $nTiO_2$-based nano-mixtures are mainly concerned with mixtures composed of metals and organics (Mikolajczyk et al., 2019; Trinh et al., 2022). However, there are still limited studies on the application of QSAR models for toxicity prediction of mixtures between nanoparticles and other novel entities in granular form.

## 1.7 Aims, research questions and objectives

The Earth will be under constant threat when the pressure from new types of entities persists over time. The coexistence of multiple novel entities in the environment is bound to exacerbate this threat. Exploring the interactions between different novel entities and clarifying the effects of these interactions on the environmental behavior, exposure pathways, toxic effects, and toxicity mechanisms of novel entities is a key part to understand and to enable mitigation actions. Thus, in this thesis, three novel entities of interest, namely ENPs, MPs, and SARS-CoV-2, were selected for study.

This thesis aimed to (scheme as shown in Figure 1.4):

1) Assess and quantify the interactions of carbon nanoparticles (CNPs) of different types and dimensions with the key fragment of the SARS-CoV-2 RNA.

2) Characterize the interactions of MPs with the non-enveloped structural materials of SARS-CoV-2 including a nucleocapsid protein and a SARS-CoV-2 RNA fragment in the water phase and in vacuum.

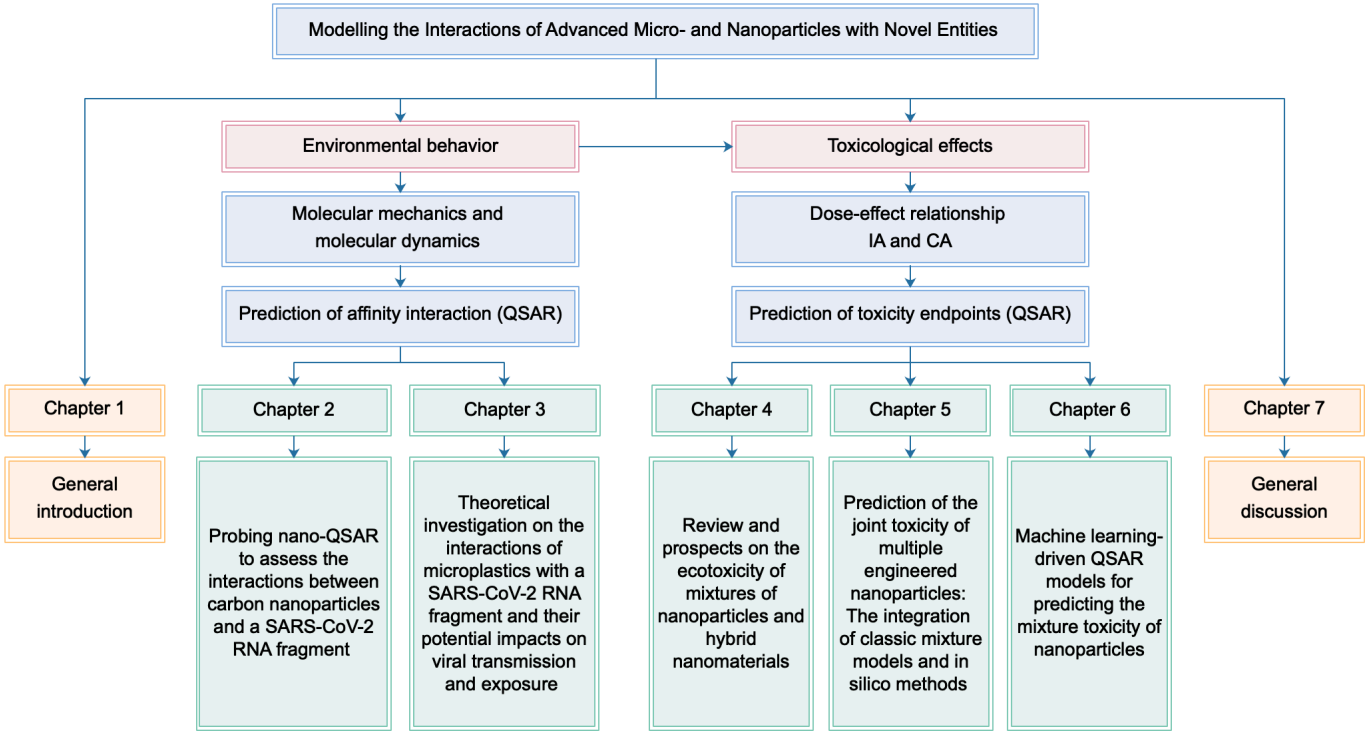


***Figure 1.4.*** *Scheme of main research contents in this thesis.*

3) Collate information on the mixture toxicity of ENPs spanning trophic levels as well as aquatic and terrestrial environments available in the literature.

4) Propose a smart strategy for forecasting the toxicity of a mixture of ENPs.

5) Rebuild existing nano-QSAR models by incorporating ML methods to describe the toxicity of a mixture of ENPs.

According to the aims, the following research questions were addressed:

1) What is the mechanism of interaction between CNPs and the SARS-CoV-2 RNA fragment? How can the interactions be predicted? How do these interactions affect the human exposure and transmission of novel coronavirus? (**Chapter 2**)

2) What is the mechanism of interaction between MPs and the SARS-CoV-2 RNA fragment? What are the factors affecting this interaction? How does the interaction affect exposure and transmission of novel coronavirus? (**Chapter 3**)

3) What joint interactions have been reported after exposure of a range of aquatic and terrestrial test species to multiple ENPs? Which factors determine the toxicity of a mixture of multiple ENPs? Is there a difference between the environmental behavior and fate of multiple ENPs compared to single ENPs and do such differences subsequently affect the induced ecotoxicological effects? Which important knowledge gaps and further research needs have been identified in assessing mixture-nanoecotoxicology by experimentalists, computational modelers, risk assessors, and regulators? (**Chapter 4**)

4) How to quantitatively predict the joint toxicity of emerging or untested/unknown mixtures of multiple ENPs? How to establish reliable prediction models for mixed toxicity of multicomponent ENPs based on ML methods? Which ML models have better predictive performance and efficacy based on study samples? How do ML methods differ from traditional hybrid models in terms of predictive ability? (**Chapters 5 and 6**)

Focusing on the above scientific issues, theoretical models and computational simulations were used to assess and predict the interaction of MNPs with other novel entities and their combined toxicity. To accomplish this overall purpose the following objectives were achieved:

1) Probe the molecular interactions between CNPs and a SARS-CoV-2 RNA fragment using molecular mechanics simulations; Develop QSAR models to describe the interactions of 17 different types of CNPs from three dimensions with the SARS-CoV-2 RNA fragment. (**Chapter 2**)

2) Explore the molecular interactions between five MPs and a SARS-CoV-2 RNA fragment at temperatures ranging from 223 to 310 K in vacuum and in water using MD simulations. (**Chapter 3**)

3) Assess the toxicity of mixtures of ENPs to a variety of different species, covering algae, bacteria, daphnia, fish, fungi, insects, and plants using data mining methods. (**Chapter 4**)

4) Develop nano-QSAR models by ML techniques to predict the joint toxicity of seven metallic ENPs for *Escherichia coli* at different mixing ratios; Compare the differences in the ability to predict the

joint toxicity by means of ML-based nano-QSAR models and component-based mixture models. (**Chapters 5 and 6**)

## 1.8 Thesis outline

**Chapter 1** A general introduction is given to the definition of novel entities and to the types of micron or nano scaled novel entities. Moreover, the interactions between MNPs and other novel entities and the impacts of these interactions on the behavior and toxicity of novel entities are illustrated. Meanwhile, the theoretical models and *in silico* methods for the studies of the interactions between MNPs and other novel entities and their joint toxicity are provided. Furthermore, the objectives and research questions of this thesis are proposed in this chapter.

**Chapter 2** The molecular interactions between CNPs and a SARS-CoV-2 RNA fragment were investigated using molecular mechanics simulations. The mechanism of molecular interactions between the CNPs and the SARS-CoV-2 RNA fragment was elucidated. A predictive model was developed that quantifies the relationship between the structural properties of CNPs and these interactions.

**Chapter 3** The molecular interactions between five model MPs and a SARS-CoV-2 RNA fragment at temperatures ranging from 223 to 310 K in vacuum and in water were investigated using MD simulations. The mechanism of the molecular interactions between the MPs and the SARS-CoV-2 RNA fragment was elucidated. A correlation was established between the interaction affinity and molecular parameters of MP monomers.

**Chapter 4** The toxicity of mixtures of individual ENPs and of hybrid nanomaterials to a variety of different species, covering algae, bacteria, daphnia, fish, fungi, insects, and plants was reviewed. The strength of the joint interactions of multiple nanoparticles and the main factors influencing the joint response of the mixtures were identified. The knowledge of building a computational approach that is able to reduce the experimental costs of ecotoxicity testing of mixtures of nanoparticles of varying composition and to include both nanohybrids and mixtures of different ENPs was concluded.

**Chapter 5** The classical mixture toxicology models and advanced computational toxicology models for toxicity prediction of chemical mixtures are summarized and demonstrated. The key strategy to quantitatively predict the joint toxicity of an emerging or untested/unknown mixtures of multiple ENPs is proposed.

**Chapter 6** Nano-QSAR models by incorporating ML methods to predict the cytotoxicity of a mixture of ENPs were developed. The differences in the ability to predict the combined toxicity by means of the ML-based methods and two component-based mixture models (IA and CA) are compared.

**Chapter 7** The research questions and main findings of the thesis are discussed. This includes discussion of the interactions between ENPs and SARS-CoV-2 genetic materials, mechanisms and influencing factors, and the impacts of these interactions on exposure and transmission of novel coronavirus; modes of action, influencing factors and assessment methods for the toxicity of ENP mixtures to ecological species; computational simulation methods for interaction and joint toxicity prediction between nanoparticles and other novel

entities; research perspectives on experimental and computational simulations for the study of environmental behavior, toxic effects and mechanisms of mixtures of novel entities; and future risk and hazard assessment of novel entities and their mixtures.