# The toxicity of nanoplastics to marine organisms

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## ABSTRACT

The abundance of nanoplastics in the marine environment is expected to increase due to the increasing use of engineered nanoplastics in various applications (primary nanoplastics), as well as due to the continuous degradation of macro- and microplastics at sea (giving rise to so called secondary nanoplastics).

Generally, the ability of (engineered) nanomaterials to interfere with cellular metabolic processes through the generation of reactive oxygen/nitrogen species (ROS, RNS), induction of oxidative stress and subsequent oxidative damage in exposed cells is widely considered to play a central role in their ecotoxicity and is thus an accepted means for testing and comparing the toxicity of various nanomaterials. What is more, the toxicity of a contaminant is directly affected by the chemical, physical and biological transformations it is subject in the environment, which in turn determine its "speciation" and fate, including its distribution, transport as well as partitioning into environmental compartments and lastly its bioavailability to living organisms.

The present document provides a brief synopsis of the (potential) mechanisms underlying the toxicity of synthetic, polymer (plastic) nanoparticles, i.e. nanoplastics, towards marine organisms. General hypotheses concerning material properties and their ROS generating capacities are roughly outlined, most of which remain to be verified for nanoplastics, and the concept of bioavailability is presented as a crucial concept for understanding and assessing toxicity, which allows to link chemical and physical speciation with possible biological effects.

Keywords: environmental nanotoxicology, bioavailability, mechanisms of toxicity, paradigm of oxidative stress

## 1. INTRODUCTION

The present document provides a brief synopsis of the mechanisms underlying the toxicity of synthetic polymer (plastic) nanoparticles, i.e. nanoplastics (NPs), towards marine organisms. Engineered nanomaterials[1] (ENMs) have unique, size-dependent physicochemical properties

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[1] The European Commission has recently recommended the following definition of nanomaterials (European Commission 2011): "'*Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%.*"

(mechanical, optical, catalytic, electronic and magnetic material features) that arise at the nano-scale and significantly differ from their bulk equivalents. Advances in nanotechnology enable mankind to harness these very properties, making ENMs the next generation materials for various commercial, industrial and research applications (Bhatt and Tripathi, 2011; Seal and Karn, 2014).

These new material properties, in combination with expected elevated levels of ENMS and their likely persistence in the environment (especially metal and metal oxide ENMs) have raised concern with respect to their environmental impact (see also contributions by F. Galgani and others in this volume). Ecotoxicological ENM hazard assessment has been complicated by the fact that ENMs differ in two crucial ways from conventional pollutants such as trace metals or organic pollutants (Scown *et al.*, 2010):

- ENMs enter aquatic environments as colloids where they undergo physical and chemical transformation leading to a yet unknown "speciation" (by analogy to conventional pollutants such as metals) characterized by an inhomogeneous mix of homo- and hetero-agglomerates, free/complexed and partially dissolved/chemically transformed ENMs uncertain environmental fate and bioavailable fraction.
- ENMs are expected to have high surface reactivity due to their extremely high surface-to-weight/volume ratio (specific surface area) and potentially, electrically charged surfaces, which may also entail high redox activity enormous variety with uncertain reactivity.

A main ecotoxicological concern is that ENMs have the potential to physically interact with vital cellular and sub-cellular organelles, which in combination with their (potentially) very high reactivity could cause considerable harm to exposed organisms. It is assumed that the behavior and interactions of ENMs with living organisms, even those composed of low-toxic materials (in bulk) such as polystyrene or the metal oxide $TiO_2$, could distinctly differ from conventional (bulk) pollutants and cause harm due to 1) their small size, possibly facilitating access to subcellular compartments previously inaccessible to direct interactions with (conventional "bulk") contaminants, 2) their extraordinary high surface area and potentially enhanced reactivity and/or 3) other unique, physicochemical nano-scale properties (Scown *et al.*, 2010; Ward and Kach, 2009).

The abundance of NPs in the marine environment is expected to become higher due to the increasing use of engineered NPs in various applications (so called primary nanoplastics), such as polystyrene (Brijmohan *et al.*, 2005), and to the continuous degradation of macro- and microplastics at sea (giving rise to so called secondary nanoplastics) (Wright *et al.*, 2013). Synthetic polymers such as polyethylene (Karlsson and Albertsson, 1998; Zhao *et al.*, 2005) and polystyrene (Snell and Hicks, 2009) for example, are generally considered chemically and biologically inert (Andrady, 2011; Cole *et al.*, 2011; Karlsson and Albertsson, 1998; Welch, 1990), unless they possess reactive surface functional groups. Generally, adverse effects of ENMs to marine habitats can theoretically arise from either indirect or direct interactions. Possible indirect effects of NPs include the interference with and disruption of vital ecosystem services, such as for example biogeochemical cycles, abiotic processes (Bergmann and Klages, 2012) or changes in community structure and abundance (Thiel and Gutow, 2005b). Possible direct effects could result from direct physical interactions between NPs and biological entities (e.g. organisms, cells, organelles, proteins and molecules) via ad- and desorption, which may cause physical (e.g. clogging, obstruction, perforation, etc.) or chemical harm (e.g. generation of reactive oxygen/nitrogen species, denaturation of proteins, etc. ) that can lead to toxic effects. There are two possible chemical effects distinctive of NPs. These include the leaching of constitutive toxic (monomer) additives (e.g. by polyvinylchloride, polystyrene, polycarbonate, polyethylene, polyester and polyurethane (Bhattacharya *et al.,* 2010)), of which some are known for their low-dose, endocrine disruptive mode of action in invertebrates, vertebrates, mammals and humans, e.g. Bisphenol A (Vom Saal and Hughes, 2005). The second important chemical effect involves their role as vectors of transport for persistent (often bioaccumulative and toxic) organic pollutants (e.g. polychlorinated biphenyls, dichlorodiphenyl, trichloroethane, nonylphenol) and other xenobiotics, endotoxins (e.g. lipopolysaccharides (Jovanović and Palić, 2012)) or even biofilms and microbial communities (Zettler *et al.,* 2013), which readily adsorb and concentrate on their surface due to their high surface area and high sorption capacity for hydrophobic organic contaminants (Bergmann and Klages, 2012; Cole *et al.,* 2011; Koelmans *et al.,* 2014a; Teuten *et al.,* 2007).

The toxicity of a contaminant is directly linked to the chemical, physical and biological transformations it undergoes in the environment, which in turn determine its "speciation" and fate, including its distribution, transport, partitioning into environmental compartments and lastly bioavailability to living organisms. The concept of bioavailability provides an important means for linking chemical and physical speciation with possible biological effects and is a crucial concept for understanding toxicity.

## 2. BIOAVAILABILITY

Bioavailability is defined as "*the extent to which a contaminant in a source is free for uptake by an organism and to which it can cause an effect at the site of action.*" (Newman, 2010; Nordberg *et al.,* 2010). A fundamental premise of toxicology is that interactions, whether direct or indirect, between a contaminant and a target organism, are a prerequisite for the contaminant to elicit toxic effects and that these interactions are decisively influenced by three interfaces, viz. i) the physicochemical properties of the contaminant, ii) the abiotic and biotic properties of the ambient medium as well as iii) the biology of the target organism (Kashiwada, 2006; von Moos *et al.,* 2014; Ward and Kach, 2009). Table 1 provides examples of how these three interfaces may affect the bioavailability and toxicity of NPs specifically.

Table 1. Examples illustrating how the three interfaces affect the bioavailability and toxicity of nanoplastics.

| **Material properties** | **Exposure conditions** | **Biology of species** |
|---|---|---|
| **- NP size-dependent uptake**<br>Size dependent adsorption and accumulation of latex NPs by Medaka eggs (Kashiwada, 2006). | **- Salinity dependent effects**<br>Lethal effects of latex NPs increased dramatically with increasing salinity and simultaneously, aggregation of NPs occurred and their adsorption as well as accumulation by fertilized Medaka eggs decreased (Kashiwada, 2006). | **- different species uptake rates**<br>Capture efficiencies for particle mass, shape and size vary between species of the same taxon (Ward and Kach, 2009).<br>Medaka adsorbed 494 nm PS beads much more readily than larger (42 000, 18 600, 932 nm) or smaller beads (39 nm) (Kashiwada, 2006). Rotifers absorbed 37-nm particles much more readily than particles larger than 83 nm (Snell and Hicks, 2009). |
| **- NP size-dependent effects**<br>Rotifers ingested polystyrene NPs ca. 30 – 3000 nm in size. Larger particles remained confined in the gut and caused no reduction in population growth rate. The smallest particles penetrated the gut wall, entered tissues and reduced population growth rate significantly (Snell and Hicks, 2009). | **- Marine aggregates facilitate ingestion**<br>Mussels and oysters more efficiently capture and ingest NPs that are incorporated into aggregates compared to those freely suspended (Ward and Kach, 2009). | **- differential species sensitivities** |
| **- NP surface charge dependent adsorption onto microalgal cells**<br>Positively charged polystyrene (amidine-latex) beads more readily adsorbed onto microalgal cells due to the electrostatic attraction to the negatively charged cellulosic cell wall (Bhattacharya *et al.,* 2010). | **- Microalgae facilitate ingestion.**<br>The uptake of PS NPs and the production of pseudofeces by the blue mussels were higher in the presence of algae, which probably formed large aggregates with the NPs that were more bioavailable (Wegner *et al.,* 2012). | |

For an interaction to take place, a contaminant must first translate from the bulk solution to the immediate vicinity of a cell/organism via diffusion or mass transport, during which physical, chemical and biological transformations can occur as a function of material properties and the abiotic factors of the ambient medium (Fig. 1). Possible chemical transformations include dissolution, sulfidation, redox surface reactions, (de-)protonation, ligand exchange, photodegradation or complexation, for example. Physical transformations may involve homo- and heteroaggregation with inorganic (e.g. metal) and organic (natural organic matter, polysaccharides, proteins, etc.) colloids. Direct interactions with biological surfaces occur via adsorption and desorption, either as released ions, transformed single nanoparticles, agglomerates or complexes. Cells can modify these interactions by their specific cell surface properties (e.g. by the presence of extracellular polymeric substances, etc.) or by the release of small molecules/proteins. Following adsorption, engineered nanomaterials may, depending on their material properties and the biological target organism, actively or passively be internalized by cells and induce biological responses, which however can also be independent of direct interactions and internalization (von Moos *et al.,* 2014). Hence, interactions occur between an heterogeneous assembly of differently

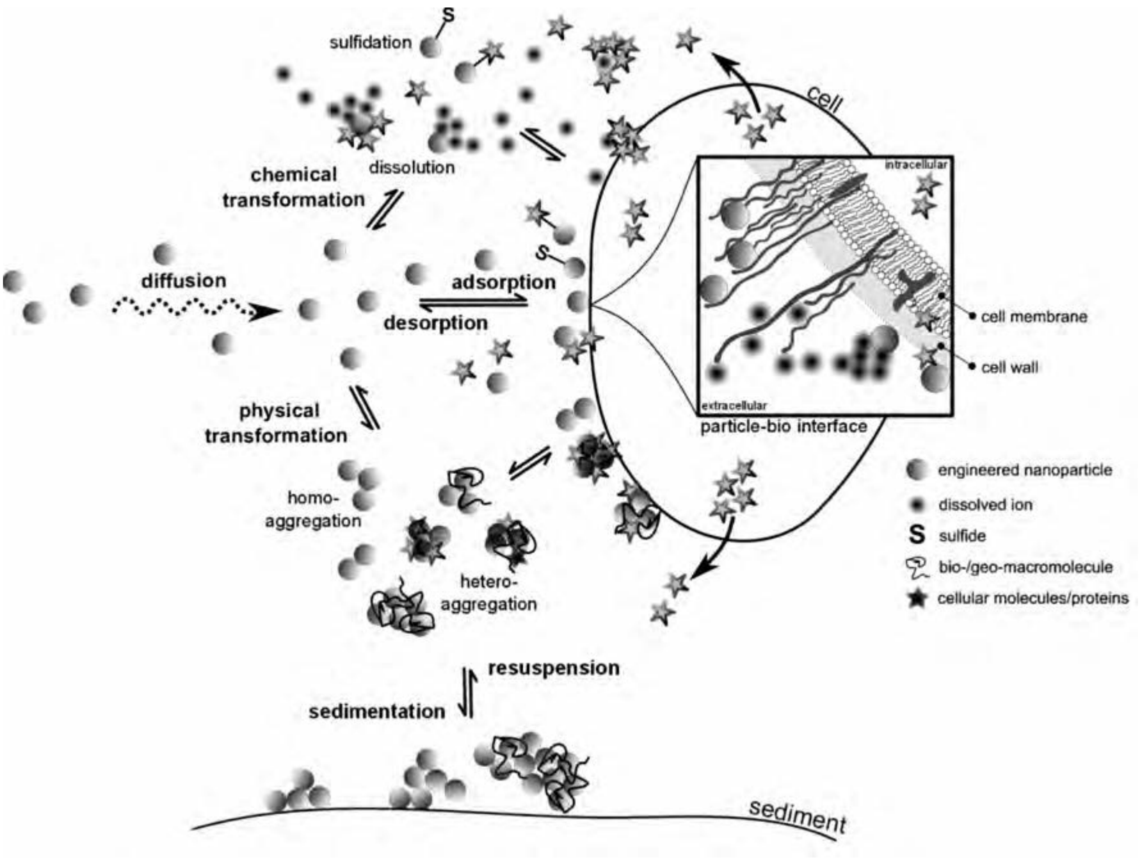

Figure 1. Processes at the medium–bio-interface underlying the bioavailability of engineered nanomaterials to aquatic microorganisms. All processes are highly dynamic. Adapted from von Moos *et al.* (2014), not to scale.

transformed nanomaterials and living organisms. The bioavailability and toxicity of NPs is ultimately shaped by the sum of the above processes at the particle, medium and biological interfaces (Fig. 1) occurring successively or simultaneously and subject to the spatial and temporal dynamics of natural water bodies (von Moos *et al.,* 2014).

The potential environmental transformations of NPs have major implications in terms of exposure of aquatic organisms and communities (Scown *et al.,* 2010). On the basis of laboratory studies, mainly performed on metal ENMs, and on some field studies on microplastics, we can infer the following behavior of NPs, which however, remains to be confirmed by conclusive experimental evidence and field work.

Low-density NPs such as polypropylene and polyethylene can predominantly be expected in the sea-surface microlayer where they are more likely to interact with pelagic organisms. However, their position in the water column can vary due to the accumulation of microbial biofilms, which propagate surface fouling and colonization, and lead to increases in density. Further, wind stress can significantly enhance the vertical mixing of buoyant micro-plastic in the water column (Bergmann and Klages, 2012). High-density NPs (e.g. polyvinylchloride, polyester and polyamide) can predominantly be expected in the benthos in which case sediment-dwelling and benthic organisms become the primary targets of exposure (Scown *et al.,* 2010), with (temporary) remobilization events possibly triggered by turbulence (e.g. storms) or physical perturbation by organisms (Montes *et al.,* 2012). Nonetheless, they could also remain suspended in estuaries due to tidal fronts, high-flow rates or sufficient momentum (Cole *et al.,* 2011). Generally however, high ionic strength favors the agglomeration of ENMs (at least in absence of counteracting surface coatings) and thus cation-rich marine and estuarine environments probably favor sedimentation

(Canesi *et al.,* 2012; Scown *et al.,* 2010). However, aggregation modifying interactions are very complex and hence are still difficult to generalize. It has for example been documented that water stirring and bubbling of exposure tanks during *in vivo* laboratory exposure actually lead to the disaggregation of previously aggregated metal oxide NPs and at the same time also to an increase of organic compounds released by the test species (e.g. mucus, fecal pellets, gametes), which in turn favor the formation of larger agglomerates (Canesi *et al.,* 2012). The relative bioavailability of single *vs* agglomerated NPs will strongly depend on the target organism. Free or embedded aggregated NPs have higher fluxes to the benthos and are generally more available to suspension-feeding bivalves whose particle capture efficiencies decrease with size (Ward and Kach, 2009). Agglomerates are captured and then broken down by the action of cilica on the gills and labial palps and the constituent particles ingested. It has been shown that NPs had longer gut retention times than microplastics (Canesi *et al.,* 2012; Ward and Kach, 2009). By capturing NPs from the water column and depositing pseudofeces on the benthos, mussels also represent an important pathway rendering suspended NPs bioavailable to the benthos (Montes *et al.,* 2012).

For a detailed description of colloidal stability and aggregation, I refer readers to Handy *et al.* (2008, pp. 298). With respect to NPs in oceans, the following two points seem to be key knowledge gaps:

- Development of standardized sampling and detection methods to measure environmental concentrations of NPs.
- Investigate their chemical and physical surface transformations, fate and behavior at sea.
- Adapt laboratory toxicity studies to insights gained from field monitoring.

For a more detailed discussion on technical limitations of ENM detection in organisms and environmental matrices, I direct readers to Powers *et al.* (2006), Bhatt and Triphathi (2011), section "9. Ecotoxicity test strategies and biological hazard assessment" as well as to Scown *et al.* (2010), last section "The future". Table 2 hereafter provides a more detailed listing of knowledge gaps concerning the environmental fate and behavior of NPs specifically.

Table 2. Knowledge gaps concerning environmental transformations, adsorption and uptake of nanoplastics.

| **Transformations and partitioning of NPs** | |
|---|---|
| **Known** | **Unknown** |
| • Increasing input and continuous fragmentation of already present microplastics. | • Effective concentrations of nanoplastics in oceans (Wegner *et al.,* 2012) → exposure concentrations?<br>• Predominant shapes and size distributions, chemical and physical transformations affecting bioavailability and toxicity. |
| • Trend: low-density plastics are buoyant, or neutrally buoyant. High-density plastics settle (Wegner *et al.,* 2012). | • Persistence, fate and partitioning/distribution (sinking rates) of nanoplastics in marine habitats and compartments.<br>→ main target organisms/communities according to plastic types? |
| • Algae can facilitate aggregation and possibly settling of nano-PS (Wegner *et al.,* 2012). | • General effects of biotic (biofilms, microbial communities, exopolymeric substances) and abiotic (salinity, ionic strength, pH, natural organic matter) factors on agglomeration, sedimentation and lastly on bioavailability and toxicity. |
| • Aggregation increases bioavailability to benthic organisms and suspension-feeding bivalves (Ward and Kach, 2009). | • Effect of aggregation on bioavailability.<br>• How particle properties affect partitioning and bioavailability. |
| • Sediments predominantly contain fibrous NPs (Browne *et al.,* 2011; Claessens *et al.,* 2011). | • Bioavailability and toxicity of fibrous NPs? |
| • Concentration of POPs on the surface of nanoplastics.<br>• Transfer of pollutants and additives from NPs to tissue is possible (Browne *et al.,* 2013; Koelmans *et al.,* 2013). | • Very little data on transfer of POPs from NPs to biota and across trophic levels.<br>• Effects of aggregation on release kinetics.<br>• Relative contributions of particle and POP toxicities? |

**Adsorption onto organisms/cells**

| Known | Unknown |
|---|---|
| • Positively charged NPs adsorb onto negatively charged microorganisms and cause negative effects (Bhattacharya *et al.,* 2010). | • Uptake of secondary nanoplastics? Uptake routes? Retention, egestion and toxicity?<br>• Effects on organism/cellular (behavior, feeding) and subcellular level (stress and damage?). |

**Ingestion and internalization**

| Known | Unknown |
|---|---|
| • Ingestion of microplastics and metal(oxide) NPs occurs across different feeding guilds. Ingestion of nanoplastics has been shown for several organisms (Canesi *et al.,* 2012; Cole *et al.,* 2011; Matranga and Corsi, 2012; Ward and Kach, 2009; Wegner *et al.,* 2012; Wright *et al.,* 2013).<br>• Trophic transfer probable (Matranga and Corsi, 2012). | • Uptake routes<br>• Retention, egestion & bioaccumulation<br>• Trophic transfer |
| • Mussels and oysters more easily ingest nanoplastic aggregates (Ward and Kach, 2009). | • Effect of agglomeration on bioaccumulation and toxicity? |
| • Nanoplastics translocate to the circulatory system/blood and penetrate tissue (Kashiwada, 2006; Snell and Hicks, 2009).<br>• Nanoplastics are internalized by cells and single-cell organisms (e.g. ciliates) (Jovanović and Palić, 2012; Pace, 1987). | • Mechanisms of internalization?<br>• Intracellular localization?<br>• Effects of protein corona on toxicity?<br>• Accumulation in tissue and organs?<br>• Toxic effects of accumulation? |
| • Size ranges of 30 – 50 nm are the optimum for endocytic pathways. Hence, they are theoretically accessible to nanoplastics (von Moos *et al.,* 2014). | • Effective uptake routes. |

Adsorption is a prerequisite for particle uptake and often also toxicity. Thus, particle charge crucially affects direct interactions with biological surfaces (e.g. membranes and organelles). Generally, the adsorption of ENMs onto surfaces is governed by van der Waals and hydrophobic forces, electrostatic attraction, hydrogen bonding and receptor-ligand interactions. Most inorganic ENPs are negatively charged in physiological media and natural pH values (Gregory, 2006), and most microorganisms, particularly bacteria and microalgae (Neal, 2008), and biological surfaces also exhibit a net negative surface charge (Hu *et al.,* 2009) due to the dissociation of carboxylic, phosphate and other acidic functional groups of the membrane. The plasma membrane (i.e. phospholipids), intracellular milieu and DNA are negatively charged, which favours interactions with cationic ENMs but does not preclude the uptake of anionic ENMs (Singh *et al.,* 2009). Natural waters are oxidizing environments, while carbon-rich sediments, groundwater (Lowry *et al.,* 2012), and physiological, intracellular conditions are reducing environments (Boxall *et al.,* 2007). Redox reactions highly depend on a compound's redox potential, the presence of redox agents, pH and on the ENM surface properties including the presence of adsorbed substances (Nowack *et al.*, 2012). Sunlight and saltwater are conducive to oxidation and may introduce oxygen containing functional groups on a particle's surface, hence introducing surface charge and enabling electrostatic interactions with biological surfaces. However, while electrostatic attraction is often considered a main driving force for adsorption/adhesion and for direct physical interaction, it does not account for all observed direct interactions between metal ENMs and aquatic microorganisms (von Moos *et al.,* 2014). Currently, literature is still very limited, especially with respect to secondary NPs. For more details on the chemistry and biology of surface modifying processes, readers are directed to contributions by Dussud and Ghighlione, Fotopoulou *et al.* and Raddadi *et al.* in this volume.

The internalization of metal and metal oxide ENMs by bacteria and microalgae has been shown by numerous studies, indicating that cell walls can be penetrated without prior damage. In most cases however, the exact uptake pathways remain unknown (von Moos *et al.,* 2014). Once the first barrier – the cell wall – is overcome, uptake must occur through the semi-permeable phospholipid bilayer, either by non-invasive passive diffusion (i.e. for very small, non-polar particles), membrane fusion or transport proteins. However, endocytic pathways (i.e. clathrin- and cavelolae-mediated) with optimal uptake sizes of 30 – 50 nm are generally considered the most likely internalization routes for ENMs into the cytoplasm (von Moos *et al.,* 2014). This, however, remains to be shown.

## 3. Mechanisms of toxicity of plastic nanoparticles

The field of nanotoxicology began with research on the toxicity of air-borne NPs (originally ultrafines) towards mammals and mammalian cell lines and more recently developed into what is now known as the field of nanoecotoxicology, dedicated to the ecotoxicity of ENMs towards aquatic freshwater and marine organisms/communities, primarily focusing on metal and metal oxide nanomaterials, which currently also represent the major manufactured class of ENMs. Though the proportion of manufactured NPs is certainly small, they are likely to gain in importance as a secondary byproduct emerging from the ongoing degradation of increasing amounts of macro- and microplastics in marine habitats. Very few studies, in fact I have only found a handful (summarized in Table 4), have specifically investigated the toxicity of NPs towards aquatic marine organisms (Ward and Kach, 2009). Early research on the toxicity of ultrafines lead to the insight that the ability of particles to generate reactive oxygen and nitrogen radical species (ROS and RNS, respectively) at or near their surfaces is a central mechanism by which ENMs engender toxicity (Donaldson *et al.,* 1996; Oberdorster *et al.,* 2005; Oberdorster *et al.,* 2007; Unfried *et al.*, 2007). This "oxidative stress hypothesis" then became one of the best-accepted paradigms for the assessment and comparison of ENM toxicity and was also successfully applied to nanoecotoxicological hazard assessment, and has been demonstrated for various (micro-) pollutants such as synthetic organic substances and trace metals.

The hierarchical oxidative stress hypothesis defines three levels of oxidative stress according to the predominating active biological processes and cellular responses with distinct enzymes and signaling pathways. The lowest level is associated with the induction of the protective antioxidant system to restore the cellular redox equilibrium. In the intermediate level, the activated cellular antioxidant system is overwhelmed and pro-inflammatory responses induce inflammation and cytotoxicity. During inflammation responses the signaling pathways mitogen activated protein kinase (MAPK) and the nuclear factor (NF)-κB are activated and trigger defense reactions that involve the release of pro-inflammatory cytokines (interleukins (IL), tumor necrosis factor (TNFs)) and additional ROS, which creates a negative ROS feedback loop. In the third and highest tier toxic oxidative stress, characterized by increased cytotoxicity and apoptosis, sets in and culminates in oxidative damage (Nel *et al.,* 2006; Singh *et al.,* 2009; Xia *et al.,* 2009; Zhang *et al.,* 2012). A non-exhaustive list of toxic effects of nanomaterials on different levels of biological organization is provided in Table 3.

Table 3. Toxic effects of engineered nanomaterials on different levels of biological organization (non-exhaustive). (Adapted from Jovanović and Palić, 2012; Matranga and Corsi, 2012; Scown *et al.*, 2010)

| **Subcellular level** | **Cellular/tissue level** | **Organism level** |
|---|---|---|
| • Increase/Decrease in Na+,K+-ATPase activity<br>• Generation of ROS<br>• Oxidative stress<br>• Lipid peroxidation<br>- Thiobarbituric acid reactive substances ↑<br>- Malondialdehyde ↑<br>• Activation of antioxidant enzymes (e.g. superoxide dismutase, catalase)<br>• Depletion of glutathione (GSH)<br>• Genotoxicity<br>- hydroxylation of guanine<br>- DNA strand breaks<br>- Changes in gene expression patterns<br>• Immunotoxicity<br>- Immnomodulation<br>- Immune deficiency<br>- Inflammatory responses<br>- Frustrated phagocytosis<br>- Impaired neutrophil function<br>- Altered Immune gene transcription<br>- Lysosomal destabilization<br>• Endocrine disruption | • physical obstruction and damage of organs (pores, gills, digestive tract)<br>• Deformations (e.g. notochord in fish)<br>• Changed haematological parameters<br>• Bradycardia (fish)<br>• Cardiac arrhythmia<br>• Slowed blood flow<br>• Proliferation/hypertrophy (e.g. epithelial cells in fish)<br>• Apoptosis<br>• Oedema (fish)<br>• Increased mucus secretion (fish)<br>• Altered mucocytes (fish)<br>• Hyperplasia in gills<br>• thickening of the gill lamellae | • Alterations in behavior<br>• Immobilization<br>• Changes in morphology<br>• Decreased reproduction<br>• Growth inhibition<br>• Increased mortality/ decreased survival<br>• Developmental abnormalities<br>• Delays in embryonic & larval development<br>• Delay in hatching of eggs<br>• Changes in (embryo) morphology |

To date, the ability of ENMs to interfere with cellular metabolic processes through the generation of ROS/RNS, induction of oxidative stress and subsequent oxidative damage in exposed cells is widely considered to play a central role in their ecotoxicity. The present body of evidence seems to support this notion for the toxicity of carbon, metal and metal (oxide) ENMs to aquatic, marine and freshwater model invertebrates (e.g. microalgae, bacteria, crustaceans, etc.) and vertebrates (e.g. fish) (Bhatt and Tripathi, 2011; Matranga and Corsi, 2012; Scown *et al.,* 2010; von Moos and Slaveykova, 2013). Out of the four studies I have found explicitly examining the toxicity of NPs towards marine organisms (Table 4), two reported ROS generation and oxidative stress as observed biological effects of exposure, indicating that the paradigm of oxidative stress may also apply to the effects of NPs in marine organisms.

Table 4. Toxicity of nanoplastics to marine organisms.

| **Model organism** | **nanoparticle** | **particle size & zeta potential** | **Exposure concentration, time & medium** | **Biological effects** | **Mechanism of toxicity** | **Reference** |
|---|---|---|---|---|---|---|
| blue mussel<br>*Mytilus edulis* | Polystyrene beads + *Pavlova lutheri* | 1° size: 30 nm<br>2° size: 1000 nm | 0, 0.1, 0.2, and 0.3 g $L^{-1}$, 8h in seawater | Reduced filtering activity, production of pseudofeces. | NA | Wegner *et al.,* 2012 |
| freshwater, single-celled microalga *Chlorella*<br>freshwater/marine, multicelled microalga *Scenedesmus* | Charged polystyrene nanospheres/beads:<br>- amidine latex (positive)<br>- carboxyl latex (negative) | 1° size: 20 nm<br>Zeta: 106 & -40 mV | 1.8 - 6.5 mg $L^{-1}$,<br>3 - 60 h in algal growth medium | Adsorption to cell walls, reduction of photosynthesis, increased **ROS** production | reduced photosynthesis<br>lead to increased respiration and increased $O_2$ activity. | Bhattacharya *et al.,* 2010 |
| marine rotifer<br>*Brachionus manjavacas* | polystyrene | 37, 83, 217, 546 and 2980 nm | 5.74, 2.87, 1.43, 0.96, 0.72, and 0 µg $mL^{-1}$,<br>2h and 48h in artificial sea water | Ingestion.Smallest NP reduced population growth rate. | Possibly suppressed ingestion or direct interference with metabolism. Oxidative stress. | Snell and Hicks, 2009 |
| Medaka/Japanese rice fish, eggs, larvae & adult fish<br>*Oryzias latipes* | Fluorescent solid latex (polystyrene) water solutions | 39 – 42000 nm | 1mg $L^{-1}$, 24 h - 7 d in embryo rearing medium | Size-dependent adsorption of NPs onto chorion and accumulation of NPs in oil droplets of eggs.<br>Uptake through gills and intestine by adult fish and entry into various organs via gill-blood route. Elimination via feces.<br>Penetration of blood-brain barrier. | NA | Kashiwada, 2006 |

There are two main theoretical mechanisms, by which ENMs in general can generate ROS, namely i) via chemical reactivity or ii) via direct physical interactions with subcellular compartments, especially electron transport chains (von Moos and Slaveykova, 2013). In the case of NPs, the second mechanism seems more likely, since plastics, and probably NPs too, are generally considered bio-inert, at least in their bulk form. Hence, it seems plausible that NPs are capable of appropriating previously inaccessible (to their equivalent bulk formulations) access ways to cells (e.g. endocytosis, pores, channels, etc.), which may enable direct, contact-mediated interactions that lead to the generation of ROS. This is supported by findings from the exposure of rotifera to different sized polystyrene beads, where the authors Snell and Hicks (2009) observed the following: "*The 2-day reproductive bioassays for 100-, 200-, 500-,and 3000-nm diameter particles, for exposures up to 1.2 µg $mL^{-1}$, had no statistically significant effect on population growth rate. Since all of these particles are composed of the same material, which lack chemical toxicity at the exposure concentrations, the adverse effects of the 37-nm nanoparticles must be due to the size of the particle; not its chemical composition.*" and concluded "*Larger particles remain confined in the intestinal tract (Fig. 1) and were eventually defecated away. Rotifers therefore can eliminate larger particles from their guts through defecation without absorption, but nanoparticles of about 37 nm are strongly absorbed. Because all of the nanoparticles tested were of identical chemical composition, this result indicates that below a threshold diameter (37–83 nm), nanoscale particles are able to pass through the gut wall and enter the tissues, perhaps via membrane pores or uptake by epithelial phagocytosis.*". It remains to be shown if this observation can be considered a more general mode of NP action concerning the generation of ROS or if in other cases, there may in addition actually also be nano-scale effects affecting the reactivity of NPs in general (e.g. due to increased surface area), especially with respect to the leaching of constituents and the sorption and desorption of xenobiotics.

Current efforts are directed to linking (measurable) material properties to ROS/RNS inducing abilities to enable more rapid and efficient hazard assessment and prediction of the overwhelming variability of emerging materials and exposure scenarios. These principally include properties such as chemical composition, purity and solubility, size/aggregation, surface area and shape, primary and secondary surface coatings and surface functionalization, surface chemistry and charge, photochemistry and band gap energy (Scown *et al.,* 2010; von Moos and Slaveykova, 2013). Detailed considerations on how these factors affect toxicity can, for example be found in Scown *et al.* (2010) and von Moos and Slaveykova (2013). General hypotheses concerning material properties and their ROS generating capacities are roughly outlined in Table 5, some of which remain to be verified for NPs specifically and in general. In the case of metal (oxide) ENMs, contradicting findings have been reported for practically every one of these hypotheses. This is thought to be mainly due to the extremely high material diversity as well as the high sensitivity of the exposure conditions to abiotic factors and treatment (exposure media, ENM preparation and handling, etc.).

Table 5. General hypotheses concerning ROS generating capacities.

| **Property** | **Hypothesis** |
|---|---|
| **Size & surface area** | • Smaller particles are more toxic than larger particles.<br>• Size-dependent generation of ROS (larger surface area). |
| **Surface chemistry** | • Bulk = non-toxic<br>• Nano = toxic |
| **Surface functionalization** | • Increases or decreases ROS generation |
| **Acquired surface coatings** | • Increases or decreases ROS generation |
| **Charge & Aggregation** | • Opposite charge can favor electrostatic attraction and increase ROS generation.<br>• Same charge decreases ROS generation due to increased repulsion.<br>• Aggregation decreases ROS generation (decreased surface area). |
| **Shape** | • Rod shaped NPs are more toxic than spherical (frustrated phagocytosis). |

## 4. CONCLUSION

The vast majority of studies have investigated the effects of microplastics or metal and metal oxide nanoparticles towards marine organisms. Some general concepts may also apply to nanoplastics, but there is a need to fill this research deficit to better understand their toxic potential. Most research has also focused on effects on single cells and organisms and their overall impact remains elusive. Studies investigating effects of chronic exposure to low, more realistic environmental exposure concentrations of nanoplastics are completely lacking.