Article details

Brun N. R., Peijnenburg W.J.G.M., Pomeren M. van & Vijver M.G. (2018), Implementing the current knowledge of uptake and effects of nanoparticles in an adverse outcome pathway (AOP) framework.. In: Hauser-Davis R.A., Parente T.E. (Eds.) Ecotoxicology - Perspectives on Key Issues. Baton Rouge, USA: CRC Press. 145-169.
Doi: 10.1201/b21896-8

8

Implementing the Current Knowledge of Uptake and Effects of Nanoparticles in an Adverse Outcome Pathway (AOP) Framework

Nadja Rebecca Brun,[1,*] *Willie J.G.M. Peijnenburg*,[2] *Marinda van Pomeren*[1] and *Martina G. Vijver*[1]

Introduction

Hazard identification of nanoparticles to organisms

Unlike most other environmental pollution issues, safety assessments of nanoparticles (NPs) were meant to be the prime example of how to foresee and tackle predicted environmental concerns. For once, research efforts were ahead of mass production and potential release into environments (Nowack and Bucheli 2007, Handy et al. 2008). Anyhow, these small particles with their inherent reactivity arose as a real challenge in fate and response assessments. Consequently, safety research is still mostly performed under controlled laboratory conditions, focusing on the central question of whether the unique properties of NPs cause fundamentally different effects as compared to their larger counterparts. Subsequently, it is asked: if so, what are the nano-specific induced responses we can expect? Ever since the appearance of

[1] CML - Institute of Environmental Sciences, Department of Conservation Biology, Leiden University, Einsteinweg 2, 2333 CC Leiden, The Netherlands.
E-mail: m.van.pomeren@cml.leidenuniv.nl
E-mail: vijver@cml.leidenuniv.nl
[2] Centre for Safety of Substances and Products, RIVM - National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands.
E-mail: willie.peijnenburg@rivm.nl
* Corresponding author: n.r.brun@cml.leidenuniv.nl

NPs and early safety and risk assessments, nanotechnology industry has expanded rapidly due to widespread use of NPs in commercially available products. The global production of engineered NPs was estimated to increase from 10,000 tons in 2011 to 60,000 tons in 2020, reaching a market value of $ 3 trillion by 2020 (Łojkowski et al. 2015, Piccinno et al. 2012). Although concentrations of environmental nanoparticles are currently forecasted to be low (Gottschalk et al. 2013), the continuous use and accumulation of non-degradable or slowly degradable NPs will inevitably impose increasing pressure on our natural environment.

The ecotoxicological profiling of particles in the nano range is specifically challenging due to their various features. NPs are defined as "particles, in an unbound state or as an aggregate or as an agglomerate where 50% or more of the particles in the number size distribution, in one or more external dimensions, are in the size range 1 nm–100 nm" (EU 2011). Nanosized particles find manifold applications in electronics, medicine, cosmetics and consumer products such as wall paint or sport clothing, only to name a few. NPs are not only manmade, but can come from natural sources such as products from combustion (e.g., forest fires), simple erosions or volcanic dust. In industrial and consumer products, their small particle size brings various desirable properties: NPs have a very large surface area compared to larger materials (from now one referred to as bulk materials), offering a larger surface area for chemical reactions and adsorption capacity (Auffan et al. 2008). The number of atoms located at the surface exponentially increases with the decrease of NP size. Although NPs are routinely defined as having a dimension between 1 and 100 nm, Auffan et al. (2009) point out that many particles undergo dramatic changes in crystalline structure at a size of 30 nm or less, suggesting to focus on this smaller set of NPs when conducting toxicity studies.

Experimental results indicate that metal-based NPs do not necessarily react in the same way as their bulk counterparts, nor as dissolved metals or metal ions (Gomes et al. 2011, Muller et al. 2015). In response to this, ecotoxicological assessments of NPs require input from the disciplines of chemical toxicology as well as colloidal chemistry. The main difficulty in assessing toxicity profiles of NPs is that their sizes and shapes are as manifold as their applications. Every modification in core, size, shape or coating of the NP can affect its fate and response, thus hampering general predictability. In addition, the physicochemical behavior of NPs changes with almost every new medium and is highly dynamic over time, making repeatability and translation between laboratories or test organisms difficult to control. Lastly, the mechanistic pathways of responses remain unclear. Detected biological effects are often related to the nanoparticle's inherent elements, such as metals masking the nanoparticle's effect and thus adding to the challenges in nanoparticle research. Nanospecific modes of actions are to a large extent unknown, but their endocytic uptake mechanism (Zhu et al. 2013) and reactive surface suggest different intracellular effects than soluble compounds.

NPs in the environment

Sediments, either from terrestrial or aquatic environments, are particularly at risk for NP contamination, because they act as sink for many contaminants discharged,

including NPs. This chapter will focus on the aquatic environment since 30 times more studies focus on this environment than terrestrial environments (Chen et al. 2015, Selck et al. 2016). Nanoparticles enter the aquatic systems indirectly from wastewater treatment plant (WWTP) runoffs or directly as surface runoffs and by deposition. WWTPs mainly collect NPs originating from medical uses, cosmetics and household products (e.g., TiO_2, ZnO, Ag), whereas surface runoffs mainly transport NPs from fuels (CeO_2), wall paints, sunscreens (TiO_2), anti-microbial coatings (Ag), leachates from landfills and accidentally released NPs at production sites. Unless NPs are released accidentally from the point of manufacturing, they will reach the environment mostly as degraded particles released from consumer goods, medical or industrial applications. Once the NPs have entered the aquatic environment, further transformation processes (e.g., agglomeration, aggregation, dissolution, sulfidation, see Fig. 1) affect the state of the particles. These processes may vary depending on salinity, pH, temperature and content of dissolved organic matter (DOM) of the receiving aquatic environments and finally determine the toxic potential of NPs. Regardless of the transformation processes that occur, NPs show a strong tendency to settle from the water column and are ultimately concentrated in the sediments where benthic organisms, sediment dwellers and microorganisms are at risk. Modeled concentrations of NPs in European lake sediments range from 0.1 to 10,000 µg kg^{-1} (Gottschalk et al. 2013). These complex interactions exemplify the need for NP characterization in exposure media to understand their ecotoxicological potential. Yet, few studies are available regarding how NPs will

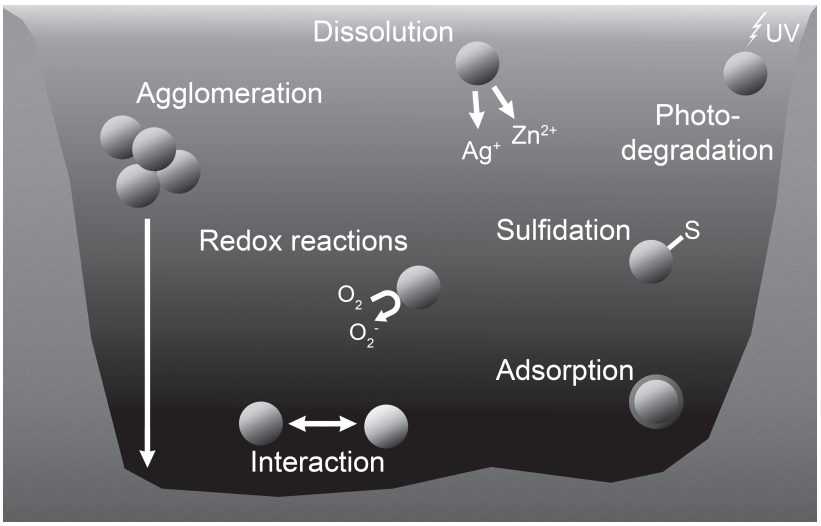

Fig. 1. Schematic overview of dynamic processes in surface waters. NPs can agglomerate into larger particles, which may settle out of the water column. Dissolution processes result in free ions and smaller particles. In the presence of Sulfur (S), NPs (e.g., AgNP or CuNP) are likely to dissolve and are sulfidized, which then decreases dissolution. Redox reactions can affect surface stability, affect dissolution and sulfidation rates. Adsorption of other compounds (e.g., humic acid) and photodegradation of coatings lead to surface modification. Particles may also interact with other particles and biota. (Figure drawn by N.R. Brun, modified from Lowry et al. 2012.)

behave in natural environments. It is challenging to measure NP behavior in a complex system where the presence of a multitude of particles mask the measurement of the target particle.

Uptake routes and effects

When it comes to NP effects on organisms, there are two features which get special attention: size and subsequent reactivity. NPs may be taken up by organisms through common uptake routes such as ingestion, skin lesions or gills in fish and then, due to their small size, be translocated through cell membranes. This could increase internal concentrations of core elements as one particle contains many densely packed elements. Metal-based NPs, for example, can deliver high amounts of free metal ions into the cell (Gilbert et al. 2012). NPs can cross the cell membrane *via* endocytic processes. In some cases, NPs are also reported to interact with membrane proteins such as toll-like receptors (Hu et al. 2016). Once entering the intracellular space, NPs can be translocated or stored in cell organelles which differ from their bulk or ionic counterpart. Due to the enlarged surface-volume ratio, the reactivity of a NP is enormous and hence influences the dissolution behavior of metal-based NPs. Their inherent reactivity is commonly related to generation of reactive oxygen species (ROS) at the target site (Nel et al. 2006). In addition, the reactive surface preferably forms ligands with proteins, which can lead to accelerated protein degradation or denaturation, ultimately leading to disrupted enzyme function. The protein binding can provoke macrophage uptake and complement activation, resulting in the release of inflammatory cytokines (Deng et al. 2011), which is a nonspecific effect ascribed to NPs. Both ROS and inflammatory reactions can lead to adverse effects in exposed organisms. Moreover, dissolution of metal-based NPs, either on the outer epithelial layer or in the intracellular space, results in a mixture of colloids and toxic metal ions, which can potentially lead to synergistic effects. However, how organisms deal with NPs over time as well as threshold values for the environment has been poorly investigated so far.

Adverse outcome pathways

Currently, the various possible biological effects of a large number of NPs are being assessed in laboratories all over the world. Screening every existing and newly designed particle for its potential ecotoxicological outcomes presents a huge challenge. Thus, common mechanistic pathways must be identified and based upon these pathways, high-throughput *in vitro* assays are to be developed. This fits in neatly with the Adverse Outcome Pathway (AOP) conceptual framework, linking a perturbation at molecular level of a biological system with an adverse (apical) outcome at higher levels of biological organization which are of regulatory relevance (e.g., impact on growth, reproduction, or survival; Fig. 2). This approach includes the description of key events (KEs) of responses at molecular, cellular, organ or sub-organismal levels which are measurable and necessary for an adverse outcome to occur. The first KE represents the molecular initiating event (MIE), whereas the last KE represents the adverse outcome (AO). The MIE is the direct site of interaction

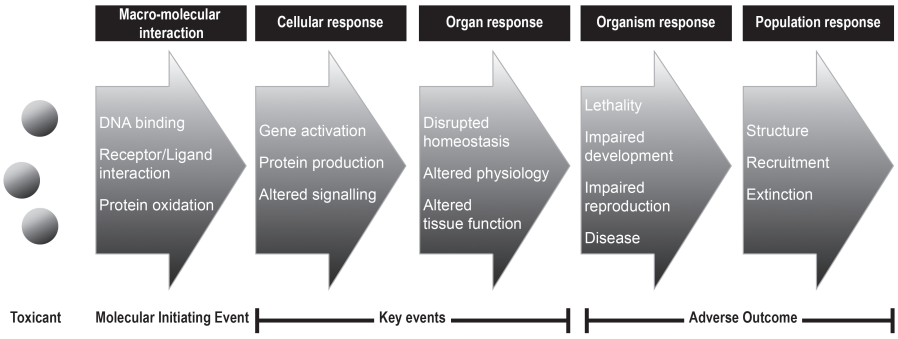

Fig. 2. Schematic overview of the key features of an Adverse Outcome Pathway across biological levels. (Figure adapted from the AOP knowledge base; http://www.aopkb.org/.)

between a toxicant and its molecular target within an organism. This interaction can be either highly specific, such as binding to a specific receptor, or non-specific, such as a reactive chemical that can covalently modify a wide range of proteins. The latter is likely to be the case for NPs, since NP recognition by receptors is rarely observed. The AO should be relevant to regulatory decision-making and thus will most often be an outcome of demographic significance.

In 2012, the OECD together with the EU-Commission's Joint Research Centre, US-EPA and US Army Engineer Research and Development Center, launched a new program to share and discuss the development of AOPs related knowledge. This so called AOP knowledge base brings together four different platforms (AOP-Wiki, Effectopedia, Intermediate Effects DB, and AOP Xplorer), facilitating the sharing of AOP knowledge between the scientific community and stakeholders.

Given the pace of NP development, advancements in understanding ecological effects of NPs are urgently needed. This chapter gives an overview of the present understanding of NP toxicity in aquatic organism. Briefly, state-of-the-art techniques to detect NPs in tissues are summarized and the present understanding of cellular and organismal NP uptake routes is given. The location of NPs in tissues bears several challenges but is the first step in identifying target organs or cells and, thus, is important in the search for mechanisms of action. The evaluation of our current knowledge of cellular and organismal responses when exposed to NPs, ultimately, allows for the identification of key knowledge gaps and foresees research directions and needs to develop Adverse Outcome Pathways for NPs.

Methods to Determine Uptake and Internalization of NPs

Several methods are available to determine uptake and cellular internalization in organisms. While some methods enable an overview of the spatial distribution (e.g., organs) of a contaminant in whole organisms, other methods determine accumulation in different subcellular compartments. In the following table, a summary of state-of-the-art techniques to localize NPs in organs or cells is given (Table 1). Many of the techniques rely on fluorescently labelled NPs, which is a superb technique

Table 1. Pros and cons of a variety of methods currently in use to study the distribution of nanoparticles.

Technique	Detected specific NPs; where?	Pro	Con
Fluorescence microscopy	Fluorescent NP; localization in tissue and whole organism	Can be used in many applications	No single particle detection due to limited resolution of 200 to 500 nm
Autoradiography microscopy	Radioisotopes of selected NP; distribution in cross sections of organs or whole body	High contrast in fine structures	Highly equipped lab needed, laborious sample preparation, strict health safety rules, resolution restricted to grain-size used
Light sheet microscopy (LSM)	Fluorescent and Au NP; live imaging technique, detected in whole organism and organs	Imaging in real time in a non-invasive manner	none
(cryo) Transmission electron microscopy (TEM)	Any NP; intracellular localization	Detailed information on subcellular structures up to 0,2 nm, good penetration depth, low photo-bleaching	Single slices, laborious sample preparation
Scanning electron microscopy (SEM)	Any NP; intracellular localization	Detailed information on subcellular structures with good field of depth	Single slices, laborious sample preparation, samples often need to be coated in conductive material
TEM or SEM in combination with Energy-dispersive X-ray spectroscopy (EDX)	Any NP; intracellular localization, useful for very small NPs	Identification of elemental composition, mapping of additional elements (P, Ca, Fe) may allow conclusions about toxic effects	Single slices, time consuming
Confocal scanning laser microscopy (CSLM)	Fluorescent and Au NP; live imaging technique, detected in whole organism, organs or subcellular fractions	Layer by layer imaging of thick samples, cellular details incl. circulating blood cells	High sensitivity is obtained by strong excitation light only, causing tissue photodamage and dye bleaching
Multifocal multi-photon microscopy (MPM)	Any NP; live imaging technique	Larger imaging depth than CSLM, high temporal resolution	Only 4 cell layers thick, lower spatial resolution than CSLM

Table 1 contd. ...

...Table 1 contd.

Technique	**Detected specific NPs; where?**	**Pro**	**Con**
Coherent anti-Stokes Raman scattering (CARS)	Any NP; live imaging technique	Detection of intrinsic and specific chemical structures with vibrational spectroscopy, increased depth penetration, low phototoxicity	Difficult to detect low concentrations
Induced coupled plasma mass spectroscopy (ICP-MS) and atomic absorption spectroscopy (AAS)	Metal-based NP; quantifying elements in extracted organs or subcellular fractions	Quantification of the metal present	Dissolved metals not distinct from metal-based NP
Time-resolved ICP-MS	Metal-based NP; detecting NPs in extracted organs or subcellular fractions	Distinction between NPs and dissoluted metals possible	If NPs contain elements with high natural abundance, separation of background difficult, recently developed method (under development)
Laser ablation ICP-MS	Metal-based NP; spatial distribution of elements in fixed tissues and organs	High sensitivity up to ppb with low detection limits	Dissolved metals not distinct from metal-based NP
Scanning micro X-ray fluorescence (μXRF) spectroscopy	Metal-based NP; spatial distribution of elements in fixed tissues and organs	Mapping of additional elements (P, Ca, Fe) may allow conclusions about toxic effects	Low spatial resolution
Flow cytometry	Fluorescent NP; intracellular localization	Analysis of thousands of cells in seconds, measurement of particle size distribution directly in biological fluids	Cannot distinguish between externally attached and fully internalized NP

to study biodistribution. However, fluorescence labeling of NPs always bears the risk of changing NPs bioreactivity. Other disadvantages are their time-dependent photobleaching and potential fluorophore leakage from the NP (Salvati et al. 2011). The use of metal NPs often possesses the risk of free metal dissolution and, thus, a final confirmation of NPs present in tissues is usually not given (Brun et al. 2014). To study subcellular distribution, various microscopic techniques are available. Such techniques can give detailed information on subcellular structure and localization. However, as they rely on single slices with sections of an organ or tissue, only a

small volume of 1 to 10 μm^3 is analyzed (Ostrowski et al. 2015). Therefore, the overall picture of the NP distribution might be biased as it is "seeking a needle in a hay stack".

Adsorption versus absorption

In practice, bioaccumulation is a summation of the amount of compound adsorbed and absorbed, the relative proportions usually not being quantified. However, not all accumulated NP burden is necessarily absorbed into the body (see Fig. 4). A proportion may remain in association with the external surface, or be bound to extracellular compounds by physicochemical forces (Handy et al. 2008). Adsorption of NPs to the membrane is therefore defined as an extracellular process, and to make it visible, it can even be washed off (Nowack and Bucheli 2007, Van Pomeren et al. 2017). To distinguish between accumulation of particles in the gut and absorption to the gut epithelium, a depuration time after exposure is usually included (Skjolding et al. 2014). Multiple ways to eliminate adsorbed chemicals and particles are reported in literature, many challenges are there and interpretation of data on the quantitative distinction of the two processes is needed.

Uptake Routes

An aquatic environment loaded with man-made NPs undoubtedly leads to exposure of the organisms living in this environmental compartment. Whether the particles are actually incorporated into cells or not is still a topic of scientific debate. NPs must overcome several challenges such as phospholipid membranes, harsh intracellular conditions and, finally, clearing mechanisms which hamper their uptake. Due to their distinct physicochemical properties, NPs interact with a cell in a different way than their soluble counterparts. This affects uptake mechanism, intracellular fate and target organs (and ultimately toxic effects). The current knowledge on cellular uptake mechanisms in aquatic organisms is given in the following section.

A NP reaching an organism will either interact with the outer or with the inner epithelial layer after being ingested. Upon contact with a biological surface, a NP can be adsorbed to the surface or actually be absorbed into the cell (Lesniak et al. 2013). Although an adsorbed NP is of lesser concern, it may act as point source for metal dissolution or for other pollutants adsorbed to the particle. Furthermore, particles adsorbed to the body surface may cause moving hindrance. This is described for particles adsorbed to the antennae and filtering screens of *Daphnia magna*, inhibiting movement and thus increasing mortality (Lewinski et al. 2010).

In general, the outer body surface is not the main route of uptake for NPs. Nevertheless, there are susceptible organs for uptake on the outside such as the gills and the eye. The gills represent a fish specific organ vulnerable to NPs, where aggregates accumulate on the surface of the gill epithelium and potentially increase uptake (Johnston et al. 2010, Scown et al. 2010). However, the fact that NPs aggregate to a large extent on the epithelial surface of the gill, renders the NPs less likely to

diffuse across cellular membranes. Recently, it has been shown that NPs can cross the retina of zebrafish embryos (Kim et al. 2013, Van Pomeren et al. 2017). It remains speculative whether the particles are stored in the eye or are further translocated into the brain for example. The predominant uptake route of NPs is ingestion (Skjolding et al. 2017, Fig. 3). In the gut, the NPs can encounter a variation in pH and ionic strength in comparison to the aquatic environment they come from. It is well known that aggregation and disaggregation are pH- and ionic strength-driven (Keller et al. 2010) and, thus, uptake behavior of NPs may change in the gut fluids. As a result, absorption may be favored under gut conditions. Indeed, most studies (Van Pomeren et al. 2017) describing accumulation of NPs in inner organs first observed the particles in the intestine of vertebrates and invertebrate organisms. Similar to food, translocation of NPs from the digestive tract to other organs occurs by uptake into the intestinal epithelium and distribution in the body *via* the bloodstream and lymphatic system in vertebrates or hemolymph in invertebrates. Since the blood vessels can only be reached after bypassing several cell layers, migration of macromolecules and nanoparticles into the bloodstream is not easy and depends on several factors.

Cellular uptake is determined by four main factors: size, shape, charge, and protein corona (Savolainen et al. 2013, Fig. 4). The most important driving factor for NP uptake, whether on the outer epithelium or on the inner, is again its size. Size not only determines whether a particle is taken up or not, but moreover how. Only the smallest NPs, with up to 6 nm and with fitted surface properties, are allowed by the cell to pass the plasma membrane by passive diffusion (Verma et al. 2008). Larger particles require an active transport system through the cell membrane by endocytosis, which is a vesicular transport (Xia et al. 2008). Depending on the particle size, different endocytic uptake mechanisms such as phagocytosis (> 1 μm), macropinocytosis (> 1 μm), clathrin-mediated endocytosis (~ 120 nm), and caveolin-mediated endocytosis (~ 60 nm), as well as clathrin- and caveolin-independent pathways (~ 90 nm) take care of the transport, each having its own dynamics and size rules (Zhu et al. 2013). Some of these uptake mechanisms are more efficient than others: NPs with a diameter of 50 nm are internalized by cells to a higher extent than smaller

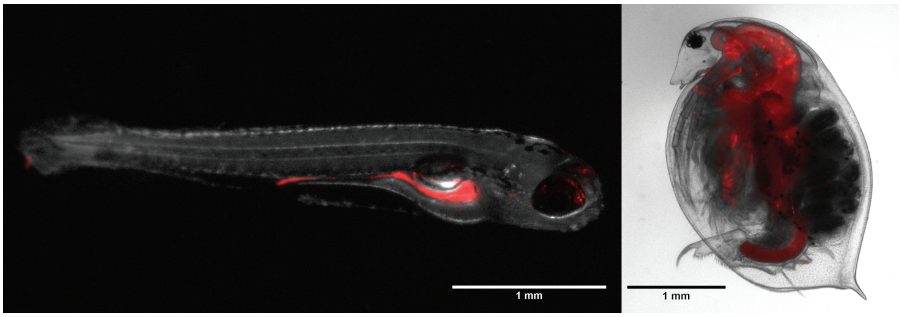

Fig. 3. Ingestion as the major route of uptake for NPs. Red fluorescent polystyrene NPs accumulate in the gut of 5 days old *Danio rerio* embryos (left) and adult *Daphnia magna* (right). (Pictures taken by M. van Pomeren and N.R. Brun.)

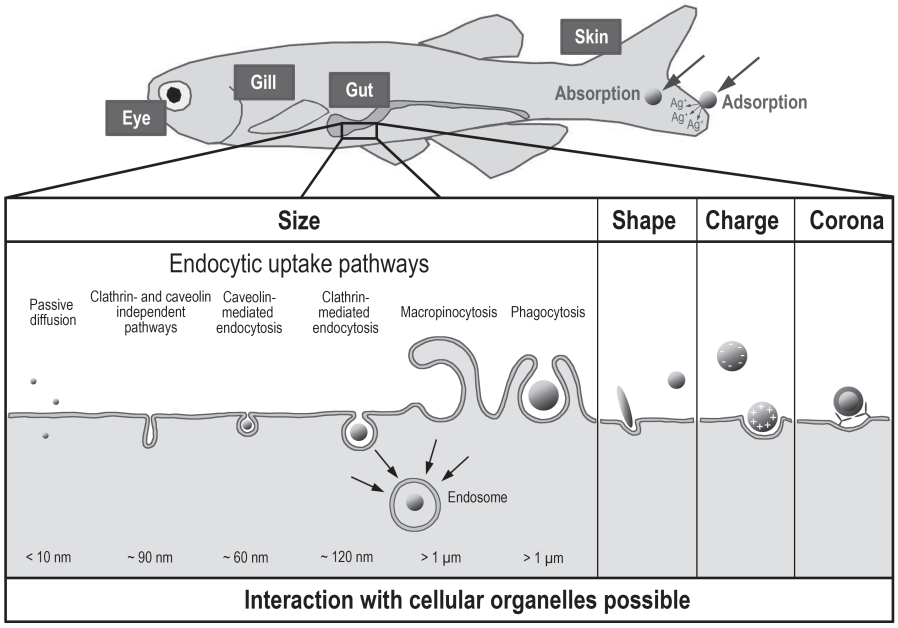

Fig. 4. Uptake routes and factors determining cellular uptake (absorption). Ingestion is likely to be the most important route of uptake. Passage through the cellular membrane is dictated by NP characteristics: NPs are mostly internalized through endocytic uptake mechanism, which are size restrictive; NPs with a higher aspect ratio can exhibit an increased internalization rate compared to spherical shaped NPs; positively charged NPs can be attracted by the negatively charged cell membrane; a protein corona is playing a vital role in defining surface charge and recognition by membrane receptors. (Figure drawn by N.R. Brun, adapted from Monopoli et al. 2012, Thurn et al. 2007, Zhu et al. 2013.)

or larger particles (Chithrani et al. 2006, Lu et al. 2009). In *Daphnia* for instance, nanowires of 40 nm and ZnO NPs of 10–30 nm are more frequently internalized into midgut cells than larger NPs of the same core (Mattsson et al. 2016, Santo et al. 2014). Not solely size, but also shape and aspect ratio determine uptake. Rod-shaped particles experience a facilitated uptake in comparison to spheres. Moreover, the length of the rod has an effect, with an intermediate length (aspect ratio of 2.1–2.5) internalized the fastest (Gratton et al. 2008, Meng et al. 2011, Zhu et al. 2013). Factors affecting NP excretion from the cells are lacking attention so far, but excretion was shown to be a size dependent exocytic event as well (Chithrani and Chan 2007, Fröhlich 2016). Furthermore, exocytosis is less effective than endocytosis, with reported release rates of 15 to 30% (Fang et al. 2011).

In addition to physical properties (size and shape), extrinsic factors that dictate cellular uptake also exist. When NPs enter a biological system, biomolecules adsorb to their surface, leading to the formation of a protein corona (Cedervall et al. 2007, Lundqvist et al. 2008). With this coating, surface properties such as surface charge (tendency to aggregate) and hydrodynamic diameter are altered and the presence of surface proteins usually increases cellular recognition (e.g., by receptors); thus,

uptake is likely to be enhanced. The composition of the protein corona varies over time (Cedervall et al. 2007), is dependent on the NPs surface properties (Esmaeili et al. 2008), size (Zhang et al. 2011), shape (Carnovale et al. 2016) and medium (e.g., blood or body fluid; Zhang et al. 2011) around it. Furthermore, the surface charge of NPs, which can be altered by the corona, can be decisive for increased uptake. Due to the slightly negative charged cell membrane, positively charged NPs are suggested to favor adhesion (Arvizo et al. 2010). In coherence with that, positively charged Au NPs induce more ROS in daphnid guts than negatively charged Au NPs (Dominguez et al. 2015). These factors shaping the NP's identity are highly variable and hard to control or determine.

While after vesicular internalization, the NP is enclosed in an endosome and thus coated by a membrane, it is uncoated after diffusion through the membrane. The latter process allows it to directly bind to plasma proteins and other molecules in the cell, nano-specific interactions can thus be expected. In contrast, NPs trapped in endosomes are subjected to acidification due to a drop in pH with increasing stage of the endosome (Zhu et al. 2013). This can be especially fatal in case the NP is metallic. Even though NPs are very small in size, they obviously contain considerably more atoms than their ionic counterpart. For example, a ZnO NPs of 50 nm contains up to 8 million zinc atoms. If diluted in a typical cell volume of approximately 500 femtoliters, it results in a concentration of up to 25 umol L^{-1} zinc, which is already in the cytotoxic range (Krug and Wick 2011). This cytotoxic effect of small amounts of ZnO NPs bypassing the cell membrane has been observed by several authors (George et al. 2010, Xia et al. 2008).

Once in the cell, the NP can be translocated (in their endosomes) to various regions and organelles. While NPs less than 100 nm in diameter have been shown to enter cells, a size of less than 40 nm allows it to enter the cell nucleus, and less than 35 nm to cross the blood-brain barrier (Dawson et al. 2009). Gold nanorod particles remain in their vesicular system and are finally stored in lysosomes (Zhang et al. 2013). Ag NPs were located in the perinuclear region but not in the cell nucleus, endoplasmic reticulum (ER) or Golgi complex (Greulich et al. 2011). Polystyrene, Ti NPs and Si NPs were transported to the nucleus as well as the mitochondria, strengthening the concept of direct interactions with cellular organelles and thus interfering with cellular functions (Hemmerich and von Mikecz 2013, Sun et al. 2011, Xia et al. 2008). When comparing ZnO NPs with soluble zinc, the particles were predominantly found in organelles and cytosol, whereas the metal ions were detected in the cell membrane (Li et al. 2011). NPs that are engulfed by endosomes in the cytosol during mitosis can be inherited by daughter cells (Rees et al. 2011). To this end, it can be concluded that depending on the NP properties, different cellular compartments are targeted and thus toxicological interactions and effects might vary.

In invertebrates, the organ specialized for vesicular uptake is the hepatopancreas, whereas in fish, endocytic transport is of particular importance in the intestinal epithelium (Moore 2006). NPs that were able to adsorb through the gut epithelium are potentially released into the blood stream from where they are distributed in the organism body, accumulate at target sites or are excreted. Due to the large concentration of tissue resident phagocytic macrophages, NPs are often cleared from the blood circulation into the liver and spleen.

Excretion through the kidney is highly dependent on size, as glomerular filtration eliminates NPs with a hydrodynamic diameter of less than 5.5 nm only (Choi et al. 2007). However, classical metabolism is not directly applicable for NPs. Other processes act on NPs such as dissolution, de-agglomeration and chemical degradation, leading to particle degradation. Degradation of Si NPs for example, leads to the formation of soluble silicic acid, which is excreted *via* feces and urine (Park et al. 2009). In contrast, metal oxides are bound and transformed by metallothioneins, which are abundantly expressed in liver and kidney.

Among aquatic organisms, fish is investigated the most regarding target organs of NPs. Indeed, biodistribution of NPs (Au, Ag, CNT, polystyrene) is described and accumulation of NPs is mainly found in liver, but also in the blood, brain, gill, eye, and heart (Kashiwada 2006, Kwok et al. 2012, Scown et al. 2010, Skjolding et al. 2017, Smith et al. 2007). However, the fate of NPs indicates that invertebrates populating the sediment are especially at risk. In most cases, detection of internalization in invertebrates failed, such as for ZnO and Cu NPs in *Daphnia magna* (Adam et al. 2014) as well as for fullerene NPs in the sediment-dwelling larvae *Chironomus riparius* (Waissi-Leinonen et al. 2012). However, recently it was demonstrated that nanowires can be translocated through the gut epithelium in *Daphnia magna* (Mattsson et al. 2016).

Response at Cellular Level

NPs trespassing the phospholipid membrane may interact with subcellular structures and proteins (Colvin 2003, Service 2004). If engulfed by endosomes, NPs are less prone to intracellular interactions and may manifest as overload of the endosomal or lysosomal system. Such accumulated NPs are often stored in fish liver and hepatopancreas or midgut gland of arthropods, molluscs and fish. If escaping from lysosomes, NPs can damage organelles or DNA (Nel et al. 2009, He et al. 2014). *In vitro* studies show that depending on the target organelle, different mechanistic pathways may be affected (Unfried et al. 2007). There is increasing evidence that NPs disrupt mitochondrial and ER functioning (Xia et al. 2006, Chen et al. 2014, Christen et al. 2014). The former activates the oxidative stress-mediated signaling cascade and the latter interferes with protein folding and maturation as well as mitochondrial perturbation and thus oxidative stress (Xia et al. 2006). A persisting state of oxidative stress induces the production of inflammatory cytokines and if all rescue attempts fail, programmed cell death is initiated (Khanna et al. 2015, Chen et al. 2016). Also, persistent ER stress can promote production of pro-inflammatory cytokines and lead to apoptosis (Chen et al. 2014). Moreover, the protein binding capacity of NPs can result in accelerated protein denaturation or degradation, leading to functional changes (e.g., enzyme function; Gao et al. 2016). Lastly, NPs which are translocated into the nucleus may damage the genetic material (Hemmerich and von Mikecz 2013). These described cellular responses are summarized in Fig. 5. To date, suggested NP-related cellular effects are based on known toxicological pathways. In the near future, high-throughput sequencing could enable the discovery of new NP specific effects.

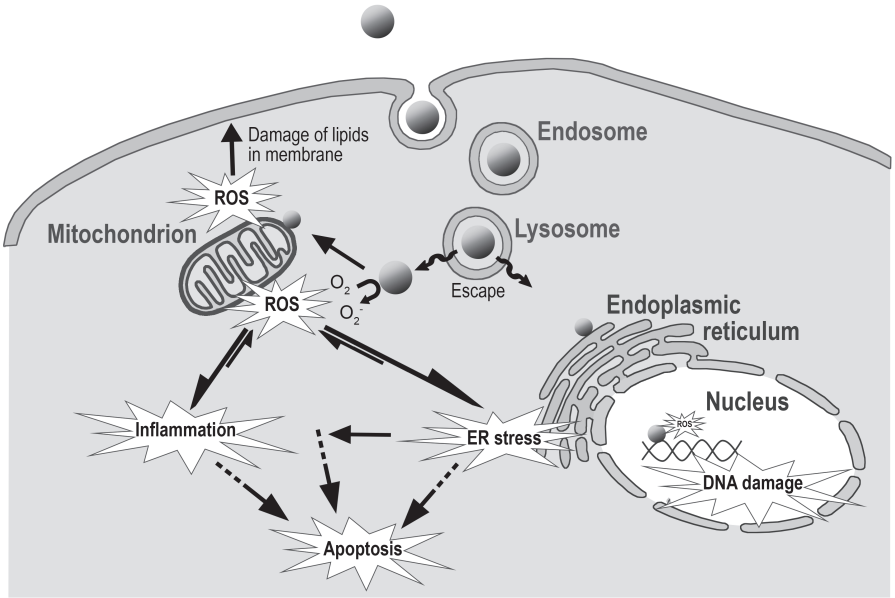

Fig. 5. Intracellular behavior of NPs, its potential translocation to the endoplasmic reticulum (ER), mitochondrion or nucleus and associated responses. (Figure drawn by N.R. Brun, adapted from Shang et al. 2014.)

Reactive Oxygen Species (ROS)

NPs, which are able to penetrate the cell, can induce ROS production and inflict oxidative stress (Xia et al. 2006). The generation of ROS is the key mechanism of NP toxicity and currently the best understood paradigm for NP toxicity. ROS formation can have different causes: (1) redox active surfaces of NPs interact with molecular dioxygen (O_2) and the capture of an electron leads to the formation of superoxide anion (O_2^-) which is a precursor of more reactive ROS such as H_2O_2 and $OH^\cdot$ (Foucaud et al. 2007), (2) the dissolution of transition metal ions acting as catalysts for ROS formation as they react with H_2O_2 to yield $OH^\cdot$ and an oxidized metal ion (Limbach et al. 2007), (3) NPs are translocated to the mitochondria where they disturb the balance in the respiratory chain and thereby increase mitochondrial ROS generation (Xia et al. 2006). In addition, organs which are not directly exposed to NPs can still show oxidative stress due to rapid distribution of ROS with blood circulation (Federici et al. 2007).

Under normal conditions, ROS are produced and neutralized in the mitochondrion. ROS at low concentrations play vital roles of controlling cellular processes such as gene expression, apoptosis and as a second messenger in signal transduction pathways (Blokhina et al. 2003, Valavanidis et al. 2006). In excess, ROS can cause severe damage to proteins, lipids and DNA, possibly resulting in deleterious effects on the cell such as apoptosis, lipid peroxidation and cancer initiating processes. Thus, in the presence of excessive ROS, cells activate their

antioxidant defense mechanism to restore the redox equilibrium. Upon NP exposure, the cells respond by activating an enzymatic antioxidant system. O_2^- is converted to oxygen or H_2O_2 by the enzyme superoxide dismutase (SOD), and catalase (CAT) catalyzes the transformation of H_2O_2 to water and oxygen (Valavanidis et al. 2006). Activation of these phase I antioxidant enzymes is frequently detected in aquatic organisms exposed to NPs, such as zebrafish embryos, carp, daphnids and mussels, and is an established method to detect NP damage (Brun et al. 2014, Gomes et al. 2011, Hao et al. 2009, Kim et al. 2010).

When the first defense against ROS, antioxidant enzymes, fails to restore the balance, cellular damage proceeds. The presence of free radicals can cause oxidative degradation of lipids in cell membranes, commonly called lipid peroxidation. Malondialdehyde (MDA), an indicator of lipid peroxidation, is recognized as molecular marker for evaluating progressive oxidative stress induced by nanoparticles (Ma et al. 2010). Increased MDA levels were measured in ZnO NPs exposed zebrafish embryos (Zhao et al. 2013), in liver of adult zebrafish where Ag NPs accumulated (Choi et al. 2010) and in the digestive gland of the mussel *Mytilus edulis* where Au NPs accumulated after exposure (Tedesco et al. 2010).

Inflammation

At higher levels of oxidative stress, the antioxidant response is overtaken by inflammation and mitochondrial-mediated apoptosis. Cellular inflammation is a response to tissue damage and/or infection and can, if unresolved, cause chronic conditions. The immune system responds by activating signaling cascades (MAPK and NF-κB) leading to the release of pro-inflammatory cytokines, such as TNFα or interleukins, activating kinases and inhibition of phosphatases. Moreover, there is a feedback loop to increase ROS production, as inflammatory phagocytes (e.g., neutrophils and macrophages) induce oxidative burst. When neutrophils are recruited to the site of injury, the assembly of NADPH oxidase is stimulated. Its activation leads to reduction of O_2 to form superoxide anions (O_2^-) and other ROS. The rapid release of these radicals is important for successful defense against invading bacteria and fungi and is termed oxidative burst. Although ROS production plays an important role in killing microorganisms and degrading particles, it can end in a vicious cycle for the production of free radicals and cause destruction of surrounding tissue (Machlin and Bendich 1987).

Whereas mammalian studies confirm the inflammation reactions and immune responses to NP exposure (Nel et al. 2006, Oh et al. 2010), such reports on aquatic organisms are scarce. Global gene expression analyses in zebrafish embryos exposed to waterborne TiO_2 NPs and Au NPs highlight genes involved in immune response and endocytosis (Park and Yeo 2013, Truong et al. 2013), and nanostructured graphene oxide induced an immune response in adult zebrafish spleen (Chen et al. 2016). Furthermore, when using primary kidney goldfish neutrophils as a model, several metal-oxide NPs increased neutrophil respiratory bursts and mRNA of pro-inflammatory genes (Ortega et al. 2015). Also in mussel (*Mytilus galloprovincialis*) hemocytes, rapid activation of MAPKs was measured after exposure to nanosized carbon black (Canesi et al. 2008). These few studies indicate that the inflammatory

processes may be a common response mechanism to NPs among human cells and cells of aquatic invertebrates and vertebrates.

Endoplasmic reticulum (ER) stress

Recent findings indicate inhibition of protein translation by NP aggregation in the ER (Chen et al. 2014, Gao et al. 2016, Han et al. 2014). The ER is the cellular organelle responsible for protein folding and maturation, synthesis of lipids and storage of free calcium. Failure of the ER's function results in accumulation of unfolded proteins and release of calcium and consequently in activation of the unfolded protein response (UPR), which is a protective mechanism to counteract the stress situation. Prolonged ER stress interferes with inflammatory pathways, oxidative stress or apoptosis (Hotamisligil 2010).

This injury pathway is triggered in zebrafish embryos when exposed to Ag NPs, including down-stream activation of inflammatory and apoptotic pathways (Christen et al. 2013). It is suggested that Ag NPs enter the ER of zebrafish embryos, thereby blocking protein synthesis and increase mortality at a later stage of development (Gao et al. 2016). In addition, in human cell lines exposed to ZnO NPs, Au NPs and SiO_2 NPs, ER stress was the predominant response and links to oxidative stress, inflammatory response and apoptosis were demonstrated (Chen et al. 2014, Christen et al. 2014, Noël et al. 2016, Tsai et al. 2011). The interaction of NPs with the ER is certainly underexplored, especially in aquatic organisms. However, this is a promising early marker for nanotoxicology.

If the stress situation is severe or persists for a longer period, the cell can initiate multiple signaling cascades of apoptosis. NPs are likely to activate the mitochondria-dependent caspase cascades and there are three major situations by which it can be triggered: (1) an extreme overload with ROS will result in mitochondrial membrane damage, leading to release of pro-apoptotic factors and ultimately cell death, (2) NPs can also take a short cut by directly targeting the mitochondria and thus trigger mitochondrial perturbation, (3) ER stress leads to calcium release from the ER and this calcium enters the mitochondria where it depolarizes the inner membrane and activates the caspase cascade.

Genotoxicity

Sustained oxidative stress can result in DNA damage through free-radical attack and ultimately abnormal cell growth. Furthermore, especially the smaller NPs may reach the nucleus *via* transportation through the nuclear pore and then directly interact with the DNA. Thus, genotoxicity can represent a particle-specific mechanism.

Genotoxic effects triggered by NPs may manifest as either damage to the genome or some adaptive changes in gene expression or both. Small sized Ag NPs (5 nm to 20 nm) induce high levels of γ-H2AX—a marker for double DNA strand breaks—in the liver of adult zebrafish (Choi et al. 2010). Moreover, an increased level of hepatic oxidative damage shows the role of oxidative damage as a precursor of genetic damage for NP toxicity in fish (Choi et al. 2010). A global transcriptomic analysis in *Daphnia magna* revealed particle specific gene expression profiles for Ag

NPs, including disruption of protein metabolism and DNA damage (Poynton et al. 2012). Biota exposed to genotoxic agents consequently may show long-lasting and profound adverse changes at cellular and organismal level. However, controversial results are found in literature suggesting no genotoxic activity of at least Si NPs (Barnes et al. 2008, Kwon et al. 2014).

In addition to the above described mechanistic pathways where NPs can interact, other forms of injury, such as membrane damage and the formation of foreign body granulomas are possible. It is also possible that NPs can lead to novel mechanisms of toxicity. Most of the effects described herein at the cellular level may not lead to a specific adverse outcome such as impaired reproduction but may generally reduce fitness of target organisms and thus weaken its health and capability of responding to other stressors. For AOP development, more knowledge is needed on the long term effects of these mechanistic pathways in ecotoxicological relevant species.

Response at Organismal Level

The adverse sub-lethal effects of NPs on aquatic organisms have been the main subject of research in nano-ecotoxicology. Sub-lethal responses assessed for NPs are diverse and thus allow evaluation of NP-related physiological and morphological effects. Of particular interest are adverse effects, which may cause impacts at community and ecosystem levels. The general dose-response model is commonly applied for NPs aiming at defining the threshold for a particular response. These threshold values are often in the mg L^{-1} range for NPs (Adam et al. 2015). Thus, even though environmental concentrations are largely unknown, effective concentrations can be expected to be rather high. However, species sensitivity between laboratory model organisms and free-living organisms can vary substantially and effects on the most sensitive species in an ecosystem may change the community already (Song et al. 2015). Sediment organisms are especially at risk to be exposed to elevated NP levels as they continuously settle out of the water column. Moreover, laboratory assessments often reveal acute effects, whereas chronic effects remain largely unexplored. NPs have the potential of being persistent in the environment (Savolainen et al. 2013) and continuous gradual input may lead to population decline. Whether organisms are able to acclimate to an increasing NP load remains an unanswered question.

Physiological and morphological responses

Morphological changes in response to NP exposure are mainly assessed as acute effects. These give a good indication of which organ or physiological process is targeted by the NPs. However, the vast majority of cases cannot be directly translated to environmental scenarios, as exposure concentrations at laboratory scale are beyond expected environmental concentrations. Increasingly, studies attempt to underpin the morphological response with molecular modes of actions. However, often the adverse outcomes at organism level can originate from several molecular mechanisms and connections are not yet established.

Fish embryos are the best studied organisms in terms of morphological response to NPs. The eye development is targeted by Ag and Au NPs, resulting in decreased

width (Asharani et al. 2011, Bar-Ilan et al. 2009, Kim et al. 2013, Lee et al. 2007, Wu et al. 2010). The occurrence of edema is frequently observed in embryos treated with Ag, Au, TiO_2, and ZnO NPs and is an indicator of a defective cardiovascular system (Hao et al. 2009, Wu et al. 2010, Zhu et al. 2009). Slow blood flow or decreased heart rate is likely to be a precursor of edema and is also observed in fish embryos exposed to Ag, Au, and Pt NPs (Kim et al. 2013, Park et al. 2013, Wu et al. 2010). Hatching interference is often observed with metal NPs. The underlying mechanism is the interference of metal ions with the hatching enzyme. However, NPs tend to accumulate on the chorion, resulting in more metal ions released into the perivitelline space compared to ionic exposures (Muller et al. 2015).

In invertebrates, a decrease in growth and reproduction can be measured after exposure to various NP. However, this is a more general response to stress occurring often with chemical exposure as metabolic rates increase under toxic stress while energy resources of organisms are limited.

Disruption of the microbiome

Uptake of NPs occurs mainly *via* ingestion and they are accumulated in the gut. The gastrointestinal tract is a site of complex, symbiotic interactions between host cells and the resident microbiome. There is increasing evidence that NPs change the populations of intestinal microbiota and modulate gut-associated immune response, but it is yet an unexplored field (Bergin and Witzmann 2013, Williams et al. 2014). In adult zebrafish, Cu NPs suppressed beneficial bacterial strains to non-detectable levels (Merrifield et al. 2013). In addition, Ag NPs depleted the gut microbiome in Nile tilapia (Sarkar et al. 2015). The effects of NPs on the microbiome of invertebrates are not assessed yet. However, it is known that the well being of *Daphnia magna* in respect to their growth, survival and fecundity is strongly dependent on their microbiota (Sison-Mangus et al. 2014). It is not far off to expect that NPs with antimicrobial properties (e.g., Ag NPs) may disrupt the microbial community of filter feeders in particular and subsequently affect its health.

Behavioral responses

Behavioral changes represent an important mechanism of environmental stress response. They appear to be among the most sensitive indicators for toxicity and impairments will reveal effects at the community and ecosystem level. Changes can be triggered internally by biochemical processes (neurotoxicity, hormones, energy metabolism) or externally by avoidance. Furthermore, behavior can be assessed in individuals (e.g., locomotion) and in communities (e.g., predator-prey and social interactions). Up to the present, NPs have been mainly assessed for their effects on individual behavior. There are indications that biochemical processes underlie the behavioral response, but more insights are needed.

Swimming responses of larval zebrafish are affected after exposure to Au, Ag, CuO and TiO_2 NPs (Chen et al. 2011, Kim et al. 2013, Powers et al. 2011, Sun et al. 2016). Interestingly, differences in NP coating and size were observed. Polyvinylpryrrolidone (PVP) coated Ag NPs caused hypoactivity in small sizes and

hyperactivity in larger dimension. A size dependency in behavioral response was also found in adult zebrafish exposed to SiO_2 NPs (Li et al. 2014). Smaller particles (15 nm) decreased locomotive activity and disrupted advanced learning and memory cognitive behaviors to a greater extent than their bigger (50 nm) counterparts. The feeding (and shoaling) behavior of fish can be affected by polystyrene NPs, increasing the time to consume their food significantly which might be related to the disrupted lipid metabolism, indicating reduced energy reserves in exposed fish (Cedervall et al. 2012, Mattsson et al. 2014). Also, invertebrates show behavioral alterations when exposed to NPs. For example, Cu NPs reduce feeding activity of the shredder *Allogamus ligonifer* (Pradhan et al. 2015).

Trophic transfer

Transfer of NPs through the aquatic food chain requires attention, since ingestion is a major route of NP uptake. Through trophic transfer, organisms can be exposed to higher concentrations than from waterborne exposure. For example, *Daphnia magna* accumulate ZnO NPs in their gut and then fish is served a concentrated form of NPs. In this manner, ZnO NPs reached more than tenfold higher levels in fish than through aqueous exposure (Skjolding et al. 2014). Also, the amphipod *Leptocheirus plumulosus* accumulates quantum dots to a greater extent when exposed through algal food than in water (Jackson et al. 2012). In trophic transfer, NPs adsorbed to the organism to be eaten may play a significant role, whereas absorption is of primary importance for toxicity and biodistribution.

Outlook Towards an AOP Development for NPs

Nanomaterials and nanotechnology are a scientific breakthrough in industry and consumer products. The production of NPs accelerates and therefore environmental concentrations will increase over time. However, impact of NPs on community and population levels in ecosystems is not assessed. In this chapter, the current understanding of NP effects on different biological levels was reviewed in order to evaluate opportunities and challenges to develop an AOP for NPs. In an AOP, a pollutant effect cascades from one biological level to the next. Biochemical interactions are the basic level and related to the functionality of a tissue or an organ. At higher biological levels, it is then evaluated whether such effects change the performance of the organism and whether this altered performance can affect the ecosystem unction. The development of AOPs for NPs can expedite the significance of the various events measured at cellular level. Moreover, AOP development is a regulatory driven plea.

In order to understand where molecular events are initiated, uptake routes need to be determined and fate of tissues and cells assessed. There are a number of techniques available to assess uptake and biodistribution of NPs, each with certain limitations. For NPs that can be made visible with fluorescent laser, fluorescent, confocal and light sheet microscopy are advised methods to track NPs in biota, the latter being the most promising technique. For non-fluorescent NPs, Raman spectroscopy is the technique of choice for bio-imaging. An important site of uptake is the intestinal

tract. Thus, as initial step of an AOP development, molecular mechanisms involved in cellular uptake as well as binding and processing of NPs should be identified in the intestinal tract. A lion's share of the particles might be accumulated in the gut and not cross the epithelium membrane. There are indications that NPs disrupt the gut microbiome which can have adverse effects on organism's health. This pathway is yet unexplored for NPs but might evolve into a future AOP with an altered microbiome as initiating event and subsequent health impacts as key events.

In this chapter, it becomes obvious that NPs crossing the membrane can trigger several cellular responses (Fig. 6), depending on the NP's intrinsic and extrinsic properties. Thus, different AOPs may be developed for different NPs. Oxidative stress and inflammation have been identified as major pathways affected. With this knowledge, the foundation is laid to develop AOPs for NPs. However, there is currently a knowledge gap connecting the cellular response with observed adverse outcomes at individual or population level such as decreased growth and survival as well as different behavioral alterations. Due to this missing link, the predictive potential of *in vitro* to *in vivo* is still in its infancy. In view of the limited availability of such data, future research should fill this knowledge gap.

In addition, we identified four research directions which need more attention when developing AOPs for NPs: (1) long term NP exposures are needed as they are likely to be more important for population decline (McKee and Filser 2016), (2) threshold values for the environment are needed for risk assessments, (3) more data on biodegradation of NPs and excretion pathways need to be added, and (4) benthic organisms need to be included in the assessments to understand whether these target organisms are at risk or well adapted, as they are living in a world of natural colloids. Ecotoxicology and environmental fate research communities will have to work together to identify cascading key events. Once established, adverse outcomes should be verified in ecologically relevant scenarios and at environmentally relevant concentrations, as NP behavior and fate in different environments plays a pivotal role for potential toxic effects. A developed AOP allows the bulk of screening analysis

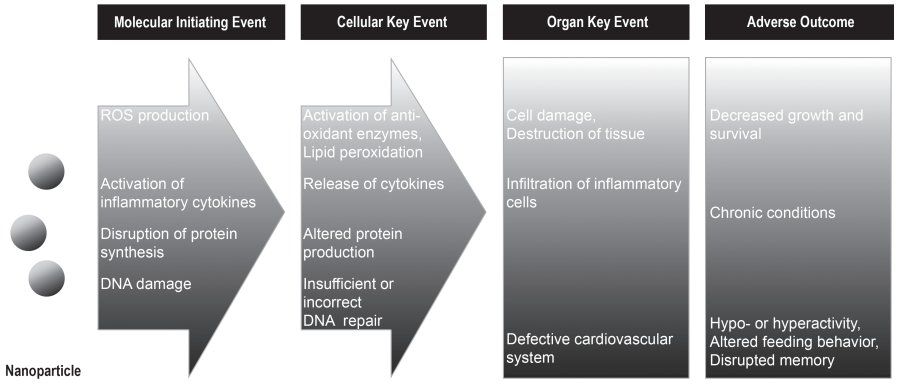

Fig. 6. Scheme summarizing the key event across biological levels and adverse outcomes described for NPs. Molecular initiating events are not defined yet, but may cover a broad spectrum including disruption of protein synthesis. The connections between measured cellular effects and observed adverse outcomes are not established yet. (Figure adapted from the AOP knowledge base; http://www.aopkb.org/.)

to be conducted *in vitro* in a high-throughput manner. Risk reduction for aquatic environments can then be carried out by limiting or avoiding exposures that trigger these toxicological responses.

References

Adam, N., F. Leroux, D. Knapen, S. Bals and R. Blust. 2014. The uptake of ZnO and CuO nanoparticles in the water-flea *Daphnia magna* under acute exposure scenarios. Environ. Pollut. 194: 130–137.

Adam, N., C. Schmitt, L. De Bruyn, D. Knapen and R. Blust. 2015. Aquatic acute species sensitivity distributions of ZnO and CuO nanoparticles. Sci. Total Environ. 526: 233–242.

Arvizo, R.R., O.R. Miranda, M.A. Thompson, C.M. Pabelick, R. Bhattacharya, J. David Robertson et al. 2010. Effect of nanoparticle surface charge at the plasma membrane and beyond. Nano Lett. 10: 2543–2548.

Asharani, P.V., Y. Lianwu, Z. Gong and S. Valiyaveettil. 2011. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. Nanotoxicology. 5: 43–54.

Auffan, M., J. Rose, O. Proux, D. Borschneck, A. Masion, P. Chaurand et al. 2008. Enhanced adsorption of arsenic onto maghemites nanoparticles: As(III) as a probe of the surface structure and heterogeneity. Langmuir. 24: 3215–22.

Auffan, M., J. Rose, J.-Y. Bottero, G.V. Lowry, J.-P. Jolivet and M.R. Wiesner. 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat. Nanotechnol. 4: 634–41.

Bar-Ilan, O., R.M. Albrecht, V.E. Fako and D.Y. Furgeson. 2009. Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. Small. 5: 1897–1910.

Barnes, C.A., A. Elsaesser, J. Arkusz, A. Smok, J. Palus, A. Leśniak et al. 2008. Reproducible comet assay of amorphous silica nanoparticles detects no genotoxicity. Nano Lett. 8: 3069–3074.

Bergin, I.L. and F.A. Witzmann. 2013. Nanoparticle toxicity by the gastrointestinal route: evidence and knowlege gaps. Int. J. Biomed. Nanosci. Nanotechnol. 3: 1–2.

Blokhina, O., E. Virolainen and K.V. Fagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot. 91: 179–194.

Brun, N.R., M. Lenz, B. Wehrli and K. Fent. 2014. Comparative effects of zinc oxide nanoparticles and dissolved zinc on zebrafish embryos and eleuthero-embryos: Importance of zinc ions. Sci. Total Environ. 476-477: 657–666.

Canesi, L., C. Ciacci, M. Betti, R. Fabbri, B. Canonico, Fantinati et al. 2008. Immunotoxicity of carbon black nanoparticles to blue mussel hemocytes. Environ. Int. 34: 1114–1119.

Carnovale, C., G. Bryant, R. Shukla and V. Bansal. 2016. Size, shape and surface chemistry of nano-gold dictate its cellular interactions, uptake and toxicity. Prog. Mater. Sci. 83: 152–190.

Cedervall, T., I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson et al. 2007. Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proc. Natl. Acad. Sci. USA. 104: 2050–2055.

Cedervall, T., L.A. Hansson, M. Lard, B. Frohm and S. Linse. 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One. 7: 1–6.

Chen, G., M.G. Vijver and W.J.G.M. Peijnenburg. 2015. Summary and analysis of the currently existing literature data on metal-based nanoparticles published for selected aquatic organisms: Applicability for toxicity prediction by (Q)SARs. ATLA Altern. to Lab. Anim. 43: 221–240.

Chen, M., J. Yin, Y. Liang, S. Yuan, F. Wang, M. Song et al. 2016. Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish. Aquat. Toxicol. 174: 54–60.

Chen, R., L. Huo, X. Shi, R. Bai, Z. Zhang, Y. Zhao et al. 2014. Endoplasmic reticulum stress induced by zinc oxide nanoparticles is an earlier biomarker for nanotoxicological evaluation. ACS Nano. 8: 2562–2574.

Chen, T., C. Lin and M. Tseng. 2011. Behavioral effects of titanium dioxide nanoparticles on larval zebrafish (*Danio rerio*). Mar. Pollut. Bull. 63: 303–308.

Chithrani, B.D., A.A. Ghazani and W.C.W. Chan. 2006. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett. 6: 662–668.

Chithrani, B.D. and W.C.W. Chan. 2007. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Lett. 7: 1542–1550.

Choi, H.S., W. Liu, P. Misra, E. Tanaka, J.P. Zimmer, B. Itty Ipe et al. 2007. Renal clearance of nanoparticles. Nat. Biotechnol. 25: 1165–1170.

Choi, J.E., S. Kim, J.H. Ahn, P. Youn, J.S. Kang, K. Park et al. 2010. Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. Aquat. Toxicol. 100: 151–9.

Christen, V., M. Capelle and K. Fent. 2013. Silver nanoparticles induce endoplasmatic reticulum stress response in zebrafish. Toxicol. Appl. Pharmacol. 272: 519–528.

Christen, V., M. Camenzind and K. Fent. 2014. Silica nanoparticles induce endoplasmic reticulum stress response, oxidative stress and activate the mitogen-activated protein kinase (MAPK) signaling pathway. Toxicol. Reports. 1: 1143–1151.

Colvin, V.L. 2003. The potential environmental impact of engineered nanomaterials. Nat. Biotechnol. 21: 1166–1170.

Dawson, K.A., A. Salvati and I. Lynch. 2009. Nanotoxicology: nanoparticles reconstruct lipids. Nat. Nanotechnol. 4: 84–85.

Deng, Z.J., M. Liang, M. Monteiro, I. Toth and R.F. Minchin. 2011. Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat. Nanotechnol. 6: 39–44.

Dominguez, G.A., S.E. Lohse, M.D. Torelli, C.J. Murphy, R.J. Hamers, G. Orr et al. 2015. Effects of charge and surface ligand properties of nanoparticles on oxidative stress and gene expression within the gut of *Daphnia magna*. Aquat. Toxicol. 162: 1–9.

Esmaeili, F., M.H. Ghahremani, B. Esmaeili, M.R. Khoshayand, F. Atyabi and R. Dinarvand. 2008. PLGA nanoparticles of different surface properties: Preparation and evaluation of their body distribution. Int. J. Pharm. 349: 249–255.

EU. 2011. Commission recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Off. Journal L. 38–40.

Fang, C.Y., V. Vaijayanthimala, C.A. Cheng, S.H. Yeh, C.F. Chang, C.L. Li et al. 2011. The exocytosis of fluorescent nanodiamond and its use as a long-term cell tracker. Small. 7: 3363–3370.

Federici, G., B.J. Shaw and R.D. Handy. 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): gill injury, oxidative stress, and other physiological effects. Aquat. Toxicol. 84: 415–30.

Foucaud, L., M.R. Wilson, D.M. Brown and V. Stone. 2007. Measurement of reactive species production by nanoparticles prepared in biologically relevant media. Toxicol. Lett. 174: 1–9.

Fröhlich, E. 2016. Cellular elimination of nanoparticles. Environ. Toxicol. Pharmacol. 46: 90–94.

Gao, J., C.T. Mahapatra, C.D. Mapes, M. Khlebnikova, A. Wei and M.S. Sepúlveda. 2016. Vascular toxicity of silver nanoparticles to developing zebrafish (*Danio rerio*). Nanotoxicology. 5390: 1–10.

George, S., S. Pokhrel, T. Xia, B. Gilbert, Z. Ji, M. Schowalter et al. 2010. Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. ACS Nano. 4: 15–29.

Gilbert, B., S.C. Fakra, T. Xia, S. Pokhrel, L. Mädler and A.E. Nel. 2012. The fate of ZnO nanoparticles administered to human bronchial epithelial cells. ACS Nano. 6: 4921–4930.

Gomes, T., J.P. Pinheiro, I. Cancio, C.G. Pereira, C. Cardoso and M.J. Bebianno. 2011. Effects of copper nanoparticles exposure in the mussel *Mytilus galloprovincialis*. Environ. Sci. Technology. 45: 9356–9362.

Gottschalk, F., T. Sun and B. Nowack. 2013. Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. Environ. Pollut. 181: 287–300.

Gratton, S.E.A., P.A. Ropp, P.D. Pohlhaus, J.C. Luft, V.J. Madden, M.E. Napier et al. 2008. The effect of particle design on cellular internalization pathways. Proc. Natl. Acad. Sci. USA. 105: 11613–11618.

Greulich, C., J. Diendorf, T. Simon, G. Eggeler, M. Epple and M. Köller. 2011. Uptake and intracellular distribution of silver nanoparticles in human mesenchymal stem cells. Acta Biomater. 7: 347–354.

Han, Y., S. Li, X. Cao, L. Yuan, Y. Wang, Y. Yin et al. 2014. Different inhibitory effect and mechanism of hydroxyapatite nanoparticles on normal cells and cancer cells *in vitro* and *in vivo*. Sci. Rep. 4: 7134.

Handy, R.D., F. von der Kammer, J.R. Lead, M. Hassellöv, R. Owen and M. Crane. 2008. The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology. 17: 287–314.

Hao, L., Z. Wang and B. Xing. 2009. Effect of sub-acute exposure to TiO_2 nanoparticles on oxidative stress and histopathological changes in juvenile Carp (*Cyprinus carpio*). J. Environ. Sci. 21: 1459–1466.

He, X., W.G. Aker and H.-M. Hwang. 2014. An *in vivo* study on the photo-enhanced toxicities of S-doped TiO_2 nanoparticles to zebrafish embryos (*Danio rerio*) in terms of malformation, mortality, rheotaxis dysfunction, and DNA damage. Nanotoxicology. 8: 185–195.

Hemmerich, P.H. and A.H. von Mikecz. 2013. Defining the subcellular interface of nanoparticles by live-cell imaging. PLoS One. 8.

Hotamisligil, G.S. 2010. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell. 140: 900–917.

Hu, H., Q. Li, L. Jiang, Y. Zou, J. Duan and Z. Sun. 2016. Genome-wide transcriptional analysis of silica nanoparticle-induced toxicity in zebrafish embryos. Toxicol. Res. 5: 609–620.

Jackson, B.P., D. Bugge, J.F. Ranville and C.Y. Chen. 2012. Bioavailability, toxicity, and bioaccumulation of quantum dot nanoparticles to the amphipod Leptocheirus plumulosus. Environ. Sci. Technol. 46: 5550–6.

Johnston, B.D., T.M. Scown, J. Moger, S.A. Cumberland, M. Baalousha, K. Linge et al. 2010. Bioavailability of nanoscale metal oxides TiO_2, CeO_2, and ZnO to fish. Environ. Sci. Technol. 44: 1144–51.

Kashiwada, S. 2006. Distribution of nanoparticles in the dee-through Medaka (*Oryzias latipes*). Environ. Health Perspect. 114: 1697–1702.

Keller, A.A., H. Wang, D. Zhou, H.S. Lenihan, G. Cherr, B.J. Cardinale et al. 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ. Sci. Technol. 44: 1962–1967.

Kanna, P., C. Ong, B.H. Bay and G.H. Baeg. 2015. Nanotoxicity: An interplay of oxidative stress, inflammation and cell death. Nanomaterials. 5: 1163–1180.

Kim, K.T., S.J. Klaine, J. Cho, S.H. Kim and S.D. Kim. 2010. Oxidative stress responses of *Daphnia magna* exposed to TiO_2 nanoparticles according to size fraction. Sci. Total Environ. 408: 2268–2272.

Kim, K.T., T. Zaikova, J.E. Hutchison and R.L. Tanguay. 2013. Gold nanoparticles disrupt zebrafish eye development and pigmentation. Toxicol. Sci. 133: 275–288.

Krug, H.F. and P. Wick. 2011. Nanotoxicology: An interdisciplinary challenge. Angew. Chemie—Int. Ed. 50: 1260–1278.

Kwok, K.W.H., M. Auffan, A.R. Badireddy, C.M. Nelson, M.R. Wiesner, A. Chilkoti et al. 2012. Uptake of silver nanoparticles and toxicity to early life stages of Japanese medaka (*Oryzias latipes*): Effect of coating materials. Aquat. Toxicol. 120-121: 59–66.

Kwon, J.Y., H.L. Kim, J.Y. Lee, Y.H. Ju, J.S. Kim, S.H. Kang et al. 2014. Undetactable levels of genotoxicity of SiO_2 nanoparticles in *in vitro* and *in vivo* tests. Int. J. Nanomedicine. 9: 173–181.

Lee, K.J., P.D. Nallathamby, L.M. Browning, C.J. Osgood and X.-H.N. Xu. 2007. *In vivo* imaging of transport and biocompatibility of single silver nanoparticles in early development of zebrafish embryos. ACS Nano. 1: 133–143.

Lesniak, A., A. Salvati, M.J. Santos-Martinez, M.W. Radomski, K.A. Dawson and C. Åberg. 2013. Nanoparticle adhesion to the cell membrane and its effect on nanoparticle uptake efficiency. J. Am. Chem. Soc. 135: 1438–1444.

Lewinski, N.A., H. Zhu, H.-J. Jo, D. Pham, R.R. Kamath, C.R. Ouyang et al. 2010. Quantification of water solubilized CdSe/ZnS quantum dots in *Daphnia magna*. Environ. Sci. Technol. 44: 1841–6.

Li, L.-Z., D.-M. Zhou, W.J.G.M. Peijnenburg, C.A.M. van Gestel, S.-Y. Jin, Y.-J. Wang et al. 2011. Toxicity of zinc oxide nanoparticles in the earthworm, *Eisenia fetida* and subcellular fractionation of Zn. Environ. Int. 37: 1098–104.

Li, X., B. Liu, X.-L. Li, Y.-X. Li, M.-Z. Sun et al. 2014. SiO_2 nanoparticles change colour preference and cause Parkinson's-like behaviour in zebrafish. Sci. Rep. 4: 3810.

Limbach, L.K., P. Wick, P. Manser, R.N. Grass, A. Bruinink and W.J. Stark. 2007. Exposure of engineered nanoparticles to human lung epithelial cells: Influence of chemical composition and catalytic activity on oxidative stress. Environ. Sci. Technol. 41: 4158–4163.

Łojkowski, W., H.-J. Fecht and A. Świderka-Środa. 2015. Quo Vadis Nanotechnology? pp. 79–94. *In*: Van de Voorde, M., M. Werner and H.-J. Fecht. (eds.). The Nano-Micro Interface: Bridging the Micro and Nano Worlds. Wiley-VCH, Weinheim, Germany.

Lowry, G.V., K.B. Gregory, S.C. Apte and J.R. Lead. 2012. Transformations of nanomaterials in the environment. Environ. Sci. Technology. 46: 6893–6899.

Lu, F., S.H. Wu, Y. Hung and C.Y. Mou. 2009. Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles. Small. 5: 1408–1413.

Lundqvist, M., J. Stigler, G. Elia, I. Lynch, T. Cedervall and K.A. Dawson. 2008. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc. Natl. Acad. Sci. USA. 105: 14265–14270.

Ma, L., J. Liu, N. Li, J. Wang, Y. Duan, J. Yan et al. 2010. Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. Biomaterials. 31: 99–105.

Machlin, L.J. and A. Bendich. 1987. Free radical tissue damage: protecitve role of antioxidant nutrients. FASEB J. 1: 441–445.

Mattsson, K., M.T. Ekvall, L.A. Hansson, S. Linse, A. Malmendal and T. Cedervall. 2014. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Env. Sci. Technol. 49: 553–561.

Mattsson, K., K. Adolfsson, M.T. Ekvall, M.T. Borgström, S. Linse, L.-A. Hansson et al. 2016. Translocation of 40 nm diameter nanowires through the intestinal epithelium of *Daphnia magna*. Nanotoxicology. 10: 1160–1167.

McKee, M.S. and J. Filser. 2016. Impacts of metal-based engineered nanomaterials on soil communities. Environ. Sci. Nano. 3: 506–533.

Meng, H., S. Yang, Z. Li, T. Xia, J. Chen, Z. Ji et al. 2011. Aspect ratio determines the quantity of mesoporous silica nanoparticle uptake by a small gtpase-dependent macropinocytosis mechanism. ACS Nano. 5: 4434–4447.

Merrifield, D.L., B.J. Shaw, G.M. Harper, I.P. Saoud, S.J. Davies, R.D. Handy et al. 2013. Ingestion of metal-nanoparticle contaminated food disrupts endogenous microbiota in zebrafish (*Danio rerio*). Environ. Pollut. 174: 157–163.

Monopoli, M.P., A. Salvati, C. Åberg, K.A. Dawson, C. Åberg, A. Salvati et al. 2012. Biomolecular coronas provide the biological identity of nanosized materials. Nat. Nanotechnol. 7: 779–786.

Moore, M.N. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ. Int. 32: 967–976.

Muller, E.B., S. Lin and R.M. Nisbet. 2015. Quantitative adverse outcome pathway analysis of hatching in zebrafish with CuO nanoparticles. Environ. Sci. Technol. 49: 11817–11824.

Nel, A., T. Xia, L. Mädler and N. Li. 2006. Toxic potential of materials at the nanolevel. Science. 311: 622–627.

Nel, A., L. Mädler, D. Velegol, T. Xia, E.M.V. Hoek, P. Somasundaran et al. 2009. Understanding biophysicochemical interactions at the nano-bio interface. Nat. Mater. 8: 543–557.

Noël, C., J.-C. Simard and D. Girard. 2016. Gold nanoparticles induce apoptosis, endoplasmic reticulum stress events and cleavage of cytoskeletal proteins in human neutrophils. Toxicol. *In Vitro*. 31: 12–22.

Nowack, B. and T.D. Bucheli. 2007. Occurrence, behavior and effects of nanoparticles in the environment. Environ. Pollut. 150: 5–22.

Oh, W.-K., S. Kim, M. Choi, C. Kim, Y.S. Jeong, B.-R. Cho et al. 2010. Cellular uptake, cytotoxicity, and innate immune response of silica-titania hollow nanoparticles based on size and surface functionality. ACS Nano. 4: 5301–5313.

Ortega, V.a., B.a. Katzenback, J.L. Stafford, M. Belosevic and G.G. Goss. 2015. Effects of polymer-coated metal oxide nanoparticles on goldfish (*Carassius auratus* L.) neutrophil viability and function. Nanotoxicology. 9: 23–33.

Ostrowski, A., D. Nordmeyer, A. Borenham, C. Holzhausen, L. Mundhenk, C. Graf et al. 2015. Overview about localization of nanoparticles in tissue and cellular context by different imaging techniques. Beilstein J. Nanotechnol. 6: 263–280.

Park, H.-G. and M.-K. Yeo. 2013. Comparison of gene expression changes induced by exposure to Ag, Cu-TiO_2, and TiO_2 nanoparticles in zebrafish embryos. Mol. Cell. Toxicol. 9: 129–139.

Park, J.-H., L. Gu, G. von Maltzahn, E. Ruoslahti, S.N. Bhatia and M.J. Sailor. 2009. Biodegradable luminescent porous silicon nanoparticles for *in vivo* applications. Nat. Mater. 8: 331–6.

Park, K., G. Tuttle, F. Sinche and S.L. Harper. 2013. Stability of citrate-capped silver nanoparticles in exposure media and their effects on the development of embryonic zebrafish (*Danio rerio*). Arch. Pharm. Res. 36: 125–133.

Piccinno, F., F. Gottschalk, S. Seeger and B. Nowack. 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J. Nanopart. Res. 14: 1109.

Powers, C.M., T.A. Slotkin, F.J. Seidler, A.R. Badireddy and S. Padilla. 2011. Silver nanoparticles alter zebrafish development and larval behavior: Distinct roles for particle size, coating and composition. Neurotoxicol. Teratol. 33: 708–714.

Poynton, H.C., J.M. Lazorchak, C.A. Impellitteri, B.J. Blalock, K. Rogers, H.J. Allen et al. 2012. Toxicogenomic responses of nanotoxicity in *Daphnia magna* exposed to silver nitrate and coated silver nanoparticles. Environ. Sci. Technol. 46: 6288–6296.

Pradhan, A., P. Geraldes, S. Seena, C. Pascoal and F. Cássio. 2015. Natural organic matter alters size-dependent effects of nanoCuO on the feeding behaviour of freshwater invertebrate shredders. Sci. Total Environ. 535: 94–101.

Rees, P., M.R. Brown, H.D. Summers, M.D. Holton, R.J. Errington, S.C. Chappell et al. 2011. A transfer function approach to measuring cell inheritance. BMC Syst. Biol. 5: 31.

Salvati, A., C. Aberg, T. dos Santos, J. Varela, P. Pinto, I. Lynch et al. 2011. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. Nanomedicine. 7: 818–826.

Santo, N., U. Fascio, F. Torres, N. Guazzoni, P. Tremolada, R. Bettinetti et al. 2014. Toxic effects and ultrastructural damages to *Daphnia magna* of two differently sized ZnO nanoparticles: Does size matter? Water Res. 53: 339–350.

Sarkar, B., M. Jaisai, A. Mahanty, P. Panda, M. Sadique, B.B. Nayak et al. 2015. Optimization of the sublethal dose of silver nanoparticle through evaluating its effect on intestinal physiology of Nile tilapia (*Oreochromis niloticus* L.). J. Environ. Sci. Heal. Part A. 50: 814–823.

Savolainen, K., U. Backman, D. Brouwer, B. Fadeel, T. Fernandes, T. Kuhlbusch et al. 2013. Nanosafety in Europe 2015–2025: Towards safe and sustainable nanomaterials and nanotechnology innovations. Finnish Institute of Occupational Health.

Scown, T.M., E.M. Santos, B.D. Johnston, B. Gaiser, M. Baalousha, S. Mitov et al. 2010. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicol. Sci. 115: 521–534.

Selck, H., R.D. Handy, T.F. Fernandes, S.J. Klaine and E.J. Petersen. 2016. Nanomaterials in the aquatic environment: A European Union-United States perspective on the status of ecotoxicity testing, research priorities, and challenges ahead. Environ. Toxicol. Chem. 35: 1055–1067.

Service, R.F. 2004. Nanotechnology grows up. Science. 304: 1732–1734.

Shang, L., K. Nienhaus and G.U. Nienhaus. 2014. Engineered nanoparticles interacting with cells: Size matters. J. Nanobiotechnology. 12: 5.

Sison-Mangus, M.P., A.A. Mushegian and D. Ebert. 2014. Water fleas require microbiota for survival, growth and reproduction. ISME J. 9: 59–67.

Skjolding, L.M., M. Winther-Nielsen and A. Baun. 2014. Trophic transfer of differently functionalized zinc oxide nanoparticles from crustaceans (*Daphnia magna*) to zebrafish (*Danio rerio*). Aquat. Toxicol. 157: 101–108.

Skjolding, L.M., G. Ašmonaitė, R.I. Jølck, T.L. Andersen, H. Selck, A. Baun et al. 2017. An assessment of the importance of exposure routes to the uptake and internal localisation of fluorescent nanoparticles in zebrafish (*Danio rerio*), using light sheet microscopy. Nanotoxicology. 3: 351–359.

Smith, C.J., B.J. Shaw and R.D. Handy. 2007. Toxicity of single walled carbon nanotubes to rainbow trout (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. Aquat. Toxicol. 82: 94–109.

Song, L., M.G. Vijver, G. de Snoo and W.J.G.M. Peijnenburg. 2015. Assessing toxicity of copper nanoparticles across five cladoceran species. Environ. Toxicol. Chem. 8: 1863–1869.

Sun, L., Y. Li, X. Liu, M. Jin, L. Zhang, Z. Du et al. 2011. Cytotoxicity and mitochondrial damage caused by silica nanoparticles. Toxicol. Vitr. 25: 1619–1629.

Sun, Y., G. Zhang, Z. He, Y. Wang, J. Cui and Y. Li. 2016. Effects of copper oxide nanoparticles on developing zebrafish embryos and larvae. Int. J. Nanomedicine. 11: 905–918.

Tedesco, S., H. Doyle, J. Blasco, G. Redmond and D. Sheehan. 2010. Oxidative stress and toxicity of gold nanoparticles in *Mytilus edulis*. Aquat. Toxicol. 100: 178–186.

Thurn, K.T., E.M.B. Brown, A. Wu, S. Vogt, B. Lai, J. Maser et al. 2007. Nanoparticles for applications in cellular imaging. Nanoscale Res. Lett. 2: 430–441.

Truong, L., S.C. Tilton, T. Zaikova, E. Richman, K.M. Waters, J.E. Hutchison et al. 2013. Surface functionalities of gold nanoparticles impact embryonic gene expression responses. Nanotoxicology. 7: 192–201.

Tsai, Y.-Y., Y.-H. Huang, Y.-L. Chao, K.-Y. Hu, L.-T. Chin, S.-H. Chou et al. 2011. Identification of the nanogold particle-induced endoplasmic reticulum stress by omic techniques and systems biology analysis. ACS Nano. 5: 9354–9369.

Unfried, K., C. Albrecht, L.-O. Klotz, A. Von Mikecz, S. Grether-Beck and R.P.F. Schins. 2007. Cellular responses to nanoparticles: Target structures and mechanism. Nanotoxicology. 1: 52–71.

Valavanidis, A., T. Vlahogianni, M. Dassenakis and M. Scoullos. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64: 178–89.

Van Pomeren, M., N.R. Brun, W.J.G.M. Peijnenburg and M.G. Vijver. 2017. Exploring uptake and biodistrbution of particles in biota. Accepted. Aquat. Toxicol.

Verma, A., O. Uzun, Y. Hu, Y. Hu, H.-S. Han, N. Watson et al. 2008. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. Nat. Mater. 7: 588–595.

Waissi-Leinonen, G.C., E.J. Petersen, K. Pakarinen, J. Akkanen, M.T. Leppänen and J.V.K. Kukkonen. 2012. Toxicity of fullerene (C60) to sediment-dwelling invertebrate *Chironomus riparius* larvae. Environ. Toxicol. Chem. 31: 2108–2116.

Williams, K., J. Milner, M.D. Boudreau, K. Gokulan, C.E. Cerniglia and S. Khare. 2014. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats. Nanotoxicology. 5390: 1–11.

Wu, Y., Q. Zhou, H. Li, W. Liu, T. Wang and G. Jiang. 2010. Effects of silver nanoparticles on the development and histopathology biomarkers of Japanese medaka (*Oryzias latipes*) using the partial-life test. Aquat. Toxicol. 100: 160–167.

Xia, T., M. Kovochich, J. Brant, M. Hotze, J. Sempf, T. Oberley et al. 2006. Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett. 6: 1794–1807.

Xia, T., M. Kovochich, M. Liong, L. Mädler, B. Gilbert, H. Shi et al. 2008. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano. 2: 2121–2134.

Zhang, H., K.E. Burnum, M.L. Luna, B.O. Petritis, J.S. Kim, W.J. Qian et al. 2011. Quantitative proteomics analysis of adsorbed plasma proteins classifies nanoparticles with different surface properties and size. Proteomics. 11: 4569–4577.

Zhang, W., Y. Ji, X. Wu and H. Xu. 2013. Trafficking of gold nanorods in breast cancer cells: Uptake, lysosome maturation and elimination. ACS Appl. Mater. Interfaces. 5: 9856–9865.

Zhao, X., S. Wang, Y. Wu, H. You and L. Lv. 2013. Acute ZnO nanoparticles exposure induces developmental toxicity, oxidative stress and DNA damage in embryo-larval zebrafish. Aquat. Toxicol. 136-137: 49–59.

Zhu, M., G. Nie, H. Meng, T. Xia, A. Nel and Y. Zhao. 2013. Physicochemical properties determine nanomaterial cellular uptake, transport, and fate. Acc. Chem. Res. 46: 622–631.

Zhu, X., J. Wang, X. Zhang, Y. Chang and Y. Chen. 2009. The impact of ZnO nanoparticle aggregates on the embryonic development of zebrafish (*Danio rerio*). Nanotechnology. 20: 195103.