Chapter 8
Potential Ecotoxicological Risk of Nanopharmaceuticals in the Aquatic Environment

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Abstract Nanopharmaceuticals are an emerging innovative domain of research that integrates nanotechnology and biotechnology applications. This technological development will permit producing unique nanopharmaceutical compounds used in the medical field, particularly in drug delivery. This book chapter focuses on organic (polymeric and lipid nanoparticles, dendrimers) and inorganic (magnetic nanoparticles and quantum dots) materials used to produce nanopharmaceuticals with different characteristics such as size, structure, chemical composition, and behavior

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enabling their use in different fields, one of which the drug delivery systems. Within drug delivery systems, special emphasis is given to vesicular (liposomes) and nanoparticulate carriers as they are the most explored at the market level. The biotechnological development, main features, and examples of applications of some types of nanostructures are discussed. Moreover, data available on sources, pathways, and effects of nanopharmaceuticals in the aquatic environment are discussed, with special emphasis on the environmental impact of these nanopharmaceuticals to the aquatic environment. Results indicate that there is no standard protocol for ecotoxicological testing and limited information exists on environmental impact assessment of nanopharmaceuticals. Thus, human and environmental safety guidelines are urgently needed to protect both the human health and the environment.

Keywords Aquatic organisms · Biomarkers · Drug delivery · Ecotoxicity · Environmental risk assessment · Nanopharmaceuticals · Nanotoxicology · Public health

8.1 Introduction

Nanotechnology applications have revolutionized different activities, among them, industry and medicine. Within the medical field, this technology, known as nanomedicine, includes a broad range of nanomaterials that can detect at molecular and cellular level and, at much earlier stages, diseases that affect organs and tissues and help to preserve and restore human health and well-being (Bawarski et al. 2008.). Therefore, nanopharmaceuticals are an emerging domain that integrates nanotechnology, biotechnology, and pharmaceuticals (Jain 2008) and were defined by Rivera Gil et al. (2010) as "pharmaceuticals where nanomaterials play the pivotal therapeutic role or add additional functionality to the previous compound" (Rivera Gil et al. 2010).

Nanomaterials by definition have a size range in the sub-100 nm scale (ISO 2015), different shapes, large surface area, and great reactivity. At this size scale, quantum effects may alter the specific physicochemical properties of the bulk material, which allow them to cross biological barriers and be used in drug delivery, therapy, *in vivo* imaging, *in vitro* diagnostics, biomaterials, active implants, and regenerative medicine (Wagner et al. 2006). Due to this wide range of applications, the size of the individual particles tested for drug delivery and imaging may range from 2 nm to 1000 nm. This led the European Medicines Agency to adopt, in 2010, a broader definition, considering the application of nanomaterials in nanomedicine, in the size range of 1 nm to 1000 nm – even including compounds surpassing these limits – if they are manufactured on purpose of drug delivery (Berkner et al. 2016; Souza and Amaral 2017). A wide range of nanomaterials were created with several therapeutic applications and include particulate and vesicular systems, dendrimers and drug-polymer conjugates, colloidal gold, iron oxide crystals, quantum dots, and

solid nanostructures (fullerenes and carbon nanotubes) (Bawarski et al. 2008). They can be classified in two categories: "hard" and "soft" nanomaterials. "Hard" nanomaterials are formed by ionic or covalent bonds, such as metal and metal oxide nanoparticles, while the "soft" ones are formed via weak interactions, such as liposomes, dendrimers, and micelles (Mahapatra et al. 2013). The main features and examples of application of some types of nanostructures, such as polymeric nanoparticles, magnetic nanoparticles, and liposomes, will be presented in this chapter.

The materials used to produce nanopharmaceuticals have different chemical composition and behavior when present in the aquatic environment. A successful example of a nanopharmaceutical is Abraxane® (Abraxis, Los Angeles, California), an albumin-bound nanoparticle formulation of paclitaxel with application in metastatic breast cancer, which allowed to overcome the limitations associated with drug hydrophobicity while avoiding the need to use toxic organic solvents (Bawarski et al. 2008). Therefore, not only the produced nanopharmaceuticals will be unique, but also their interactions with drug molecules will be distinctive. The impact of their discharge to the aquatic environment will produce interactions with abiotic and biotic components of the aquatic ecosystems that in some cases can be toxic. For example, colloidal gold, iron oxide nanoparticles, and quantum dots generally used in nanomedicine are known to be toxic (Nogueira 2014; Rocha et al. 2015a, 2017; Lefevre et al. 2015; Valdiglesias et al. 2016). Therefore, human and environmental safety guidelines are urgently needed.

Nanopharmaceutical research has focused on drug formulation to improve biodistribution, bioavailability, and pharmacokinetics and on the specific delivery of existing drugs, especially in mammal species (Chen and Guan 2011). After being administered they are excreted from the human body, introduced in hospital waste water sewage or present in industrial effluents, ending up in waste water treatment plants where their elimination is reduced. Clearly, the nanopharmaceutical formulations or their metabolites will be in contact with several other organisms during the elimination process until they reach the aquatic environment. Available data on the behavior and effects of nanoparticles in the aquatic environment, such as aggregation, evidence the need for models that allow predictions, inclusive of their concentrations and potential ecotoxicity. Ecotoxicity effects of nanoparticles in aquatic organisms include oxidative stress, genotoxicity, neurotoxicity, behavior changes and immunotoxicity (Rocha et al. 2017). However, ecotoxicological studies about the behavior, fate, and impact of nanopharmaceuticals in nontarget species remain scarce (Yegin et al. 2017). Given the wide range of applications, nanopharmaceuticals evolved and grew in recent years, but safety issues were not taken into account and possible undesirable effects on humans were not studied properly. In addition, little attention was paid to the potential nefarious effects caused by the starting materials that result in the nanomaterials. As such, the environmental impact assessment of the fabrication process and the problematic effects that may arise from the environmental release of these compounds were also disregarded (Linkov et al. 2008; Berkner et al. 2016). Therefore, the main objective of this book chapter is to highlight the potential effects of nanopharmaceuticals in the aquatic environment as

a result of their applications in drug delivery systems, with special emphasis on liposomes and nanoparticles, as these are the most explored, even at the market level.

8.2 Types and Uses of Nanopharmaceuticals

The pharmaceutical area is, at present, the focus of great innovation. Along with new molecules being discovered and others undergoing chemical modifications to meet specific requirements, several molecules already in clinical practice are being studied in different formulation strategies that include the use of the so-called drug delivery systems (Wu et al. 2017; Tarhini et al. 2017). These systems are gaining increased interest and are developed to fit specific needs that cover a wide range of possibilities. Usually, the delivery systems act as carriers for the molecule of interest and its formulation provides improved stability and protection towards degradation (Zhang et al. 2013; Petros and DeSimone 2010). Additionally, in many cases, the delivery of drugs to a site of interest or the modification of the kinetic profile is envisaged (Almeida and Grenha 2014; Sarwar et al. 2017). Overall, the use of drug delivery systems is also expected to allow the reduction of side effects and potentiate the efficacy of the drug (Zhang et al. 2013; Petros and DeSimone 2010). There are many drug delivery systems, differing in characteristics such as the structure, composition, and size. Nanoscaled carriers are currently gathering much attention, because of several advantages comparing with micron-sized counterparts. These advantages include higher control over drug release (Lopes et al. 2016), increased drug absorption (Csaba et al. 2006), and great ability for surface functionalization (Singh Jr. and Lillard 2009), among others, that make nanocarriers viable therapeutic alternatives.

As of this day, there are several nanopharmaceuticals already approved by the Food and Drug Administration (Weissig et al. 2014) that will be briefly discussed later. Figure 8.1 shows a chronology in which the discovery of different carriers is highlighted, showing some marketed formulations of relevance.

8.2.1 Vesicular Drug Delivery Systems

Vesicular drug delivery systems correspond to liposomes, which were first described by Bangham in 1965 (Bangham et al. 1965) and used in clinic since 1997, when the first products were approved by the Food and Drug Administration (Weissig et al. 2014). Liposomes are vesicles comprised of a lipid bilayer, usually obtained using phospholipids (Lasic 1988). Their amphiphilic structure allows the encapsulation of hydrophilic drugs inside the formed cavity or hydrophobic molecules within the membrane (Gulati et al. 1998). This flexibility is one of the key features of these systems, along with the reported biodegradability and biocompatibility, chemical flexibility, and stability provided to drug molecules, namely, by preventing or

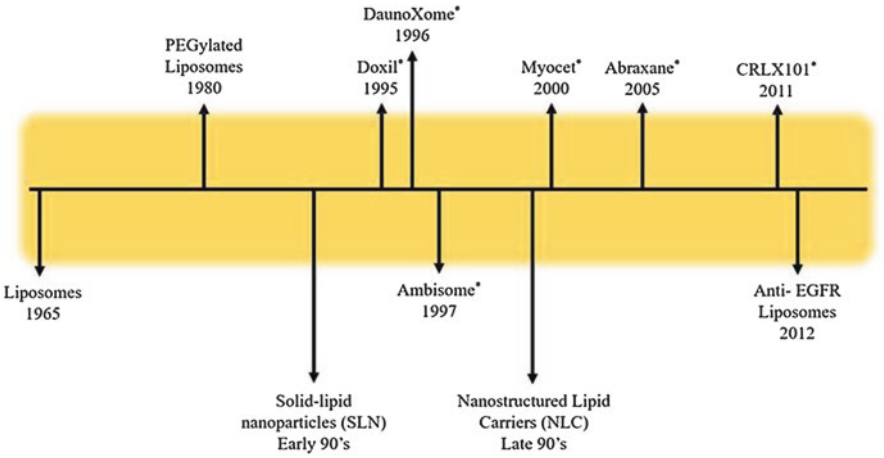

Fig. 8.1 Chronological order of development of several drug nanocarriers and approval of nanopharmaceuticals

delaying their degradation. All these characteristics have fostered their use as drug carriers. Furthermore, liposomes also provide protection to organisms receiving the drugs, as described for amphotericin B. This antifungal drug causes severe toxicity and its liposomal formulation (AmBisome®) soon revealed to be the solution for problems of therapeutic incompliance (Akbarzadeh et al. 2013). Regrettably, liposomes present low solubility and short shelf-life. Furthermore, due to their composition, the possibility of oxidation and hydrolysis of phospholipids needs to be considered, as these compromise liposome usefulness, leading to vesicle disintegration with consequent drug leakage (Akbarzadeh et al. 2013). Fortunately, these problems can be addressed by a process of freeze-drying, which ensures the removal of almost all the water of the formulation, improving liposome's shelf-life and greatly inhibiting oxidation and drug leakage (Miyajima 1997).

Liposomes are mainly composed of phospholipids, being phosphatidylcholine and phosphatidylethanolamine the most commonly used materials for their production (Laouini et al. 2012). Nevertheless, it is very frequent to include other molecules in liposome formulations to confer specific characteristics. Depending on the length and saturation of the lipid chain of the phospholipids, rigid or fluid liposomes may be formed (Akbarzadeh et al. 2013). Cholesterol is included very often, as it makes the liposomal membrane more rigid and less flexible, allowing a better control over the release of the drugs (Tardi et al. 2016). The amount of cholesterol plays a relevant role in this regard (Briuglia et al. 2015). Additionally, it is suggested that cholesterol helps increasing the vesicle's circulation time (Kirby et al. 1980). Polyethylene glycol is also used frequently in liposomal formulations, as it hampers the process of opsonization and, consequently, the detection of the vesicles by the immune system. This delays the elimination of the liposome (Milla et al. 2012; Immordino and Cattel 2006) and, thus, potentiates the drug effect.

Lipid film hydration and the solvent injection methods are two of the most used techniques to produce liposomes. These techniques share two common steps: (1) the dispersion of lipids in organic solvent and (2) the addition of an aqueous solution to form the vesicles (Akbarzadeh et al. 2013; Meure et al. 2008). More recent approaches include the methods of microfluidic channel and of supercritical fluid injection and decompression, but this requires expensive equipment to effectively produce the vesicles (Meure et al. 2008). The lipid film hydration involves solubilization of phospholipids using organic solvents, solvent evaporation to form the lipid film, and subsequent addition of an aqueous solution, either with or without the drug to be encapsulated (Brandelli 2012). Liposomes are formed instantaneously in this case. After their production, further processing is usual to tailor sizes to the desired outcome. A technique of extrusion is frequently applied for this end, as well as sonication (Schroeder et al. 2009). The literature displays a comprehensive review on the methodologies to produce liposomes (Meure et al. 2008) and on techniques to optimize the produced vesicles (Mozafari 2010). In fact, size and zeta potential are two of the most relevant characteristics of nanocarriers. Zeta potential indicates the surface charge of the vesicle and is naturally dependent on its composition. Along with size, it plays an important role on the interaction with involving environment, including epithelial surfaces and proteins in the blood, among others (Manaia et al. 2017).

After production and further refinement, liposomes are classified according to three categories, as shown in Fig. 8.2.

Different types of liposomes can be obtained: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs). Small unilamellar vesicles (SUVs) are formed by a single phospholipid bilayer and can present 100 nm or smaller size; large unilamellar vesicles (LUVs) also have a single bilayer, but range between 200 and 800 nm; finally, multilamellar vesicles (MLVs) are comprised of many concentric bilayers, reaching sizes up to 5000 nm (Torchilin 2008). Fortunately, the myriad of production processes and refinement methods allow the production of vesicles of different sizes and structures that are studied and used depending on the given purpose.

Liposomes are thus efficient drug delivery systems that are strongly used in clinics and are still subject of intense study, as will be addressed in Sect. 8.2.3.

8.2.2 Nanoparticulate Drug Delivery Systems

Nanoparticulate drug delivery systems, along with the vesicular systems revolutionized therapeutics and the field of drug delivery. A great part of research is conducted with several delivery routes being tested, as well as encapsulation strategies that enable and improve the efficiency of certain drugs (Mallipeddi and Rohan 2010; Pachuau 2015).

As for liposomes, size and zeta potential are two of the most relevant characteristics. The International Organization for Standardization (ISO) defines

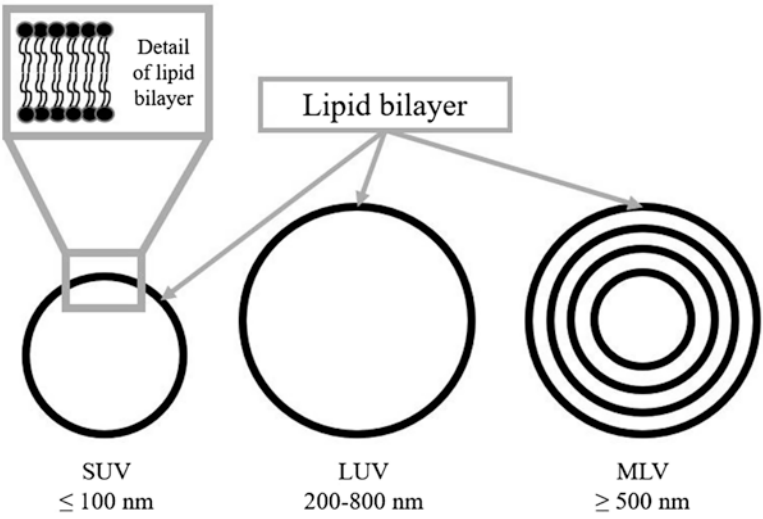

Fig. 8.2 Depiction of the different types of liposomes that can be obtained: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs). Liposomes are vesicular drug delivery systems, mainly composed of phospholipids organized in bilayers. These are represented by the black bold line. Depending on the size, and on the refinement techniques, liposomes can have only one bilayer (the case of SUV and LUV) or more than one concentric bilayer (the case of MLV)

nanoparticles as particles having at least one dimension less than 100 nm (ISO 2015). However, carriers with sizes up to 1000 nm are also considered nanoparticles by most of the scientific community (Wilczewska et al. 2012), including the European Medicines Agency (Berkner et al. 2016). The broader definition is the one considered in this chapter.

Nanoparticulate carriers can have different composition, either organic (polymers or lipids) or inorganic (metals or silica). This chapter will focus on organic nanoparticulate drug delivery systems (polymeric and lipid nanoparticles, and dendrimers), and on magnetic nanoparticles and quantum, which will be detailed below.

Organic Systems

Organic nanoparticulate drug delivery systems enclose two sub-categories: polymeric particles, produced with either synthetic or natural polymers, or lipid particles. These carriers are typically divided in the categories of nanocapsules and nanospheres. Nanocapsules are nano-reservoirs comprised by a shell structure and a core that can be either aqueous or oily and liquid or semi-solid. The core or cavity is the place where the drug of interest is mostly encapsulated/associated. On the other hand, nanospheres are matrix nanoparticles, meaning particles that are solid, having the drug of interest distributed virtually anywhere (Vauthier and Bouchemal 2009).

In the category of polymeric systems, polyesters and, more particularly poly(lactide-co-glycolide) acid (PLGA), are the most used synthetic materials, while polysaccharides and proteins are used as natural ones. PLGA evidences ability to control drug release, along with low toxicity and high biocompatibility, which enable its inclusion in several formulations already in the market for several biomedical applications (Sharma et al. 2016). Chitosan, alginate, and hyaluronic acid are some examples of polysaccharides that are being studied for application in formulations of therapeutic relevance (Huh et al. 2017). Chitosan has been widely studied over the recent decades and is the most explored polysaccharide. This is justified by its unique cationic character, which brings very relevant characteristics such as strong mucoadhesivity. Additionally, it is also reported as biocompatible in many routes of administration and useful in various studies of clinical relevance, as in colon cancer (You et al. 2016), detection of tumors via image diagnosis (Hong et al. 2017), and for the delivery of antibiotics (Madureira et al. 2015), among others. Polymeric nanoparticles have a slight immunomodulatory activity. Thus, when used to carry immunomodulatory vaccines or peptides, they may potentiate the activity of the carried molecules (Amaral et al. 2010; Ribeiro et al. 2015). Despite the exhaustive study of the referred polysaccharides and the demonstration of favorable characteristics such as biocompatibility, low toxicity, and biodegradability (Martínez et al. 2012), there is no nanopharmaceutical formulation approved with these materials. In turn, albumin is one of the most studied proteins as matrix component of drug delivery systems and has one formulation approved (Abraxane®) for the treatment of breast cancer and non-small-cell lung cancer (Bernabeu et al. 2017).

Dendrimers are also an emerging class of polymer-based carriers. These are spherical structures with high surface area derived from the highly branched polymers used in their preparation. Because of this, they present huge internal space to incorporate bioactive molecules (Rimondino et al. 2017). Features such as the spherical shape and symmetrical architecture, coupled with specific physicochemical properties, make these structures fascinating for drug delivery applications. Dendrimers are typically composed of a central component (called core), by several internal cavities created according to the branched polymer used and by a surface that can be formed by different functional groups (Srinageshwar et al. 2017). These groups on the surface promote delivery of dendrimers to different cell types and even simplify their penetration into tissues or cells. Dendrimers are classified according to the number of layers, where each layer is called generation (G): a four-layer dendrimer is called "dendrimer generation 4 (G4)." Most likely because of their small size, usually around 10 nm, and surface charge, dendrimers are able to penetrate diverse tissues of the body (Albertazzi et al. 2010). Upon injection into the carotids of mice, dendrimers with a slight cationic surface were able to cross the blood-brain barrier (Srinageshwar et al. 2017). Dendrimers surface charge strongly correlates with the cell penetration ability, as neutral or negatively charged dendrimers show reduced internalization (Perumal et al. 2008). This attribute can be improved by coupling molecules such as peptides to their surface (Jiang et al. 2016).

Lipid-based nanoparticulate carriers are another category of organic-based systems. Within this category, solid lipid nanoparticles and nanostructured lipid

carriers are those gathering the highest attention. Solid lipid nanoparticles are comprised of a matrix of solid lipids only and were first reported in the early 1990s to avoid some limitations shown by liposomes and polymeric nanoparticles (Nunes et al. 2017). As for the nanostructured lipid carriers, they appeared in the late 1990s as an improvement of solid lipid nanoparticles. A mixture of both solid and liquid lipids is required to prepare nanostructured lipid carriers, which display increased stability provided by the liquid lipids present in the matrix (Li et al. 2017), when compared with solid lipid nanoparticles. The methods of production of solid lipid nanoparticles and nanostructured lipid carriers are described in Lin et al. (2017) and Tamjidi et al. (2013).

Polymeric nanocarriers can be obtained by the polymerization of a monomer or from preformed polymers, the latter being the most used of the approaches. In this context, methodologies involving emulsification such as emulsification/solvent evaporation, emulsification/solvent diffusion, solvent displacement and interfacial deposition, among others, are very frequent and allow the production of both nanocapsules and matrix nanoparticles. Matrix nanoparticles also frequently produced in processes mediated by electrostatic interactions or involving desolvation. The latter consists in adding a desolvating agent, a salt or a non-solvent of the polymer that is miscible with water, to the polymeric solution. Macromolecular aggregation or particle formation is brought about by the partial desolvation of fully solvated polymer molecules (Vila and Lastres 2001). Methods such as polyelectrolyte complexation and ionic gelation are those involving electrostatic interactions, in which nanoparticles form upon interaction between oppositely charged molecules. Several comprehensive reviews exist on the methodologies to produce polymeric nanocarriers, featuring their advantages and disadvantages (Pinto Reis et al. 2006; Vauthier and Bouchemal 2009). To produce solid lipid nanoparticles and nanostructured lipid carriers, the method most commonly applied is of high pressure homogenization. In this method, the solid lipid components are melted and mixed afterwards with the liquid lipids (when applicable) and drugs (if the production of drug-loaded carriers is envisaged). This mixture is then added to a hot aqueous solution containing surfactants, being stirred by a high-shear mixing device, to form an emulsion. Homogenization is repeated until nanodroplets are obtained.

Inorganic Systems

Inorganic systems comprise those nanoparticles that are composed by inorganic materials. Magnetic nanoparticles are one of the most used, with structures with about 7 nm. When close to a magnetic field, they can suffer alterations that influence their behavior (Loebinger et al. 2009; Issa et al. 2013). Metals used to prepare magnetic nanoparticles should be carefully chosen to avoid toxicity. Cobalt, nickel, and neodymium-iron-boron are used. However, they may suffer oxidation during *in vivo* applications (Dias et al. 2011). Iron oxide materials such as maghemite and magnetite are safer and, thus, often used to produce these nanoparticles (Dias et al. 2011). Their interest relies on multifunctional characteristics, such as small size,

supermagnetism, and low toxicity to mammals, easiness of synthesis and functionalization (Wu et al. 2009; Dias et al. 2011). However, from the two materials, maghemite presents iron in the oxidized state, further reducing its toxicity (McBain et al. 2008).

To improve the stability of these structures and to prevent them from clumping, they are functionalized by surface-binding to organic substances, thus forming the magnetic fluids. In this way, the components connected to or incorporated in them will also be influenced by the magnetic field (Shi et al. 2012). Another property of these functional groups is to reduce the toxicity of certain metals and increase their biocompatibility (Lin et al. 2010; Kolhatkar et al. 2013). Gallo et al. (2013) presented an extensive review in which several types of materials used for the coating of magnetic nanoparticles are mentioned, including dextran and bovine serum albumin. According to the nature of the functional groups incorporated in magnetic nanoparticles, they can carry drugs and nucleic acids, as well as substances for contrast in magnetic resonance examinations (Loebinger et al. 2009). They may also be associated with other nanostructures such as liposomes, and, thus, by responding to an external magnetic field, they can be manipulated to be target-directed. Currently, several applications are being focused on these magnetic nanoparticles, but the main applications are in treatment and detection of tumors.

Quantum dots are another group of inorganic systems being strongly explored. They are a class of engineered nanoparticles formed by fluorescent semiconductor nanocrystals with nanometer diameters ranging from 1–10 nm. These nanoparticles are classified in two categories: cadmium-based quantum dots and cadmium-free quantum dots. The quantum dots core can be made of a variety of metal complexes, such as group II–IV series (CdSe, CdTe, CdSeS, ZnS, ZnSe, and PbSe) or group III–V series (InP, InAs, GaAs, and GaN). This core determines their color, while the inorganic shell or ligand(s) can enhance stability, brightness, water solubility, and conjugation capacity (Michalet et al. 2005; Nguyen et al. 2013). The most common quantum dots core used for biological and medical applications are CdSe and CdTe, which can be coated with a shell and additional capping layer or ligands (Michalet et al. 2005; Smith et al. 2008; Rocha et al. 2017).

The quantum dots' shell consists mainly of a second semiconductor material (e.g., ZnS) and protects the core from oxidation and degradation. Surface ligands can be hydrophilic, hydrophobic, or amphiphilic polymers, such as mercaptoacetic acid (MAA), mercaptosuccinic acid (MAS), thioglycolic acid (TGA), dihydrolipoic acid (DHLA), and amphiphyllic polymers like modified polyacrylic acid (PAA). These ligands increase the quantum dots' water solubility and compatibility for applications in biological systems (Maysinger et al. 2007). Furthermore, quantum dots can also be conjugated with biomolecules (e.g., peptides and oligonucleotides), antibodies, and/or drugs for identification and action in specific biological targets (Smith et al. 2008; Rizvi et al. 2010).

Due to their physicochemical properties and biological interactions, quantum dots are applied in many fields. These include electronics (i.e., light-emitting diode (LED), organic light-emitting diode (OLED), photovoltaic, and lasers), solar panels, photo-chemistry (i.e., photoelectrodes), analytical chemistry, pharmacy,

molecular and cell biology (i.e., live cell imaging, co-localization of genes/proteins, multicolor staining, and flow cytometry), and nanomedicine (i.e., molecular profiling of cancer, antimicrobial agents, in vivo tumour imaging, photodynamic therapy, diagnosis, and development of disease- and patient-specific therapies) (Michalet et al. 2005; Deerinck 2008; Rizvi et al. 2010).

Nanoparticulate drug delivery systems comprise a wide topic in the pharmaceutical field. Although their market presence is limited so far, they are considered the future alternative in therapeutics, thus looking for new options on how drugs will be delivered to the human body. The most remarkable achievements will be addressed in the following section.

8.2.3 Nanopharmaceuticals as a Viable Therapy

Many different materials can be used to prepare various carriers that are intended to encapsulate a variety of drug molecules. Vesicular and nanoparticulate drug delivery systems are two of the most studied approaches. As shown in Fig. 8.1, the discovery of liposomes occurred before 1980s. Afterwards, when it was possible to alter the outer membrane of the liposomes by adding specific molecules, the PEGylated liposomes appeared, bringing uncountable advantages regarding drug half-life. From this point on, important liposome-based formulations appeared in the market: Doxil®, DaunoXome®, Ambisome® and Myocet®. Apart from Ambisome®, all the other products encapsulate anticancer drugs, and cancer is, indeed, one of the greatest fields of application of liposomal formulations. Naturally, pharmaceutical formulations comprising advanced technologies result in more expensive products, which find application more easily in diseases permitting higher investment. Ambisome® encapsulates a potent antifungal drug (amphotericin B), enabling the decrease of the strong side effects caused by the administration of the drug per se (Stone et al. 2016). In an interesting approach, thermosensitive liposomes were recently proposed to provide external targeting of drugs to solid tumors, in combination with local hyperthermia or high-intensity focused ultrasounds (Al Sabbagh et al. 2015; Novell et al. 2015).

Another important event in the timeline shown in Fig. 8.1 is the approval of Abraxane® in 2005, the only marketed formulation comprised of nanoparticles. This formulation is composed by albumin conjugated with paclitaxel, currently having an application in metastatic breast cancer and non-small-cell lung cancer. Weissig and Guzman-Villanueva provide a comprehensive review on these products, along with many others that are not subject of this chapter (Weissig et al. 2014).

Nanopharmaceuticals are an alternative approach to conventional therapeutic strategies, also having a role in the field of diagnostics. They can have important roles on smoothing severe adverse effects or on mediating active targeting of the encapsulated molecules to specific cells/tissues. There are several formulations that are currently on clinical trials for a possible approval soon. One example is CRLX101, a cyclodextrin-PEG nanoparticle encapsulating camptothecin, another

anticancer drug. It is an intravenous formulation, and it has finished phase II clinical trials, being proposed for the treatment of rectal, ovarian tubal, and peritoneal cancer (Svenson et al. 2011; Ragelle et al. 2017). Another example included in Fig. 8.1 is the anti-EGFR immune-liposomes, which recently completed phase I clinical trials. This strategy is based on the encapsulation of doxorubicin, to be potentially used in solid tumors (Mamot et al. 2012). Furthermore, several metal-based delivery systems are currently in phase I clinical trials for potential use in radiotherapy and the treatment of prostate cancer by application of a magnetic field (Ragelle et al. 2017). A recent review addresses the market prospects of these nanopharmaceuticals, further referring to many other formulations undergoing clinical trials (Ragelle et al. 2017). Interestingly, it also describes three products currently in phase III: one for hepatocellular carcinoma, another for respiratory syncytial virus infection, and the last one for metastatic breast cancer. The results of these clinical trials will dictate the possibility to obtain marketing authorization. Other strategies, more specific for the cancer therapy area, are described in a novel review by Li et al. (2017), proving that the versatility of this strategy is being taken into consideration for the next generation of therapeutics.

Dendrimers and magnetic nanoparticles are not so advanced in their positioning to market. Nevertheless, there are very interesting applications being reported. Dendrimers formed by polyamidoamine polymer (PAMAM) have a high density of amine groups with empty inner cavities and functional groups that promote high solubility and reactivity (Jiang et al. 2016). Because of this, they are widely used to deliver anticancer drugs, such as doxorubicin. Although toxic, in animal model experiments, doxorubicin was better tolerated by animals when dosed twice the tolerable limit to cause toxicity (Kaminskas et al. 2012). Gene therapy applications have also been reported (Nam et al. 2015; Hemmati et al. 2016). Dendrimers were successfully used to carry siRNA, evidencing ability for incorporation into their internal cavities and to promote cell internalization (Liu and Peng 2016). Highly branched dendrimers comprised of glutamic acid-modified hyperbranched polyamidoamine (HPAMAM) also evidenced efficient gene transfection, with decreased toxicity (Hemmati et al. 2016).

Regarding magnetic nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) have been used in magnetic resonance imaging (Pour and Shaterian 2017; Xiong et al. 2017), benefiting from the attachment of specific receptors to SPIONs surface, which allow greater affinity and precision in detecting tumors. Magnetohyperthermia is also an innovative approach gathering attention, consisting in the generation of heat at different intensities by the application of a magnetic field over magnetic nanoparticles. This technique is applied to destroy tumor cells, which are more sensitive to heat changes (Miranda-Vilela et al. 2014), but it can also be used to promote release of the bioactive compound from magnetic nanoparticles (Tang et al. 2017).

The potential of the systems has been demonstrated in many cases, but regulations have generally tightened up regarding the approval of new drug formulations, with restrictions gaining emphasis for nanopharmaceuticals. For these formulations, it has become even more important to correctly and exhaustively assess the

biological implications of their delivery to and permanence within the human body. In this regard, Kipen and Laskin have stated that *nanotechnology yields nanotoxicology* (Kipen and Lasking 2005). While this is not necessarily true, it reveals the lack of knowledge and information on toxicological effects of engineered nanomedicines. These effects are not restricted to those felt by the organism receiving the formulations but also encompass the effects imposed to the environment. It must be reminded that, after delivery to humans, the formulations or their metabolites end up reaching the environment via debris. The next sections of this chapter address the impact of nanopharmaceuticals on the aquatic environment. In fact, the toxicological effects of engineered nanomedicines are one incredibly important feature in the approval process of a nanopharmaceutical.

8.3 Biotechnology and Production of Nanopharmaceuticals

Nowadays, there are various nanostructured formulations for drug delivery in clinical use. Applications of these nanostructured formulations involve treating various diseases, such as cancer and fungal infections. In Table 8.1 a few examples of drugs at nanoscale currently in clinical use are listed.

The delivery of bioactive molecules, such as drugs, peptides, and nucleic acids, can be performed through different materials, in accordance with their nanoproperties (Amaral and Felipe 2013). When these bioactive molecules are incorporated within nanostructures, they present better stability, improving its therapeutic efficacy (Kaminskas et al. 2012). Drug delivery systems at nanoscale can be prepared by different methods and types of materials, but it is essential to consider the nature of the molecule to be encapsulated and its destination when used for biological purposes.

Table 8.1 Examples of approved clinical nanomedicines and their clinical indications

Medicine	Drug	Composition	Clinical indication	References
Abraxane®	Paclitaxel	Albumin-bound	Breast, lung and pancreatic cancer	Vallo et al. (2017)
Ambisome®	Amphotericin B	Liposome	Fungal and protozoan infections	Stone et al. (2016)
Doxil®	Doxorubicin	Liposome	Ovarian cancer and Kaposi's sarcoma	Kakar et al. (2016)
Epaxal®	Inactivated Hepatitis A virus	Virosome	Hepatitis A infection	Bovier (2008)
Gemzar®	Gemcitabine	Liposome	Several types of solid tumors	Federico et al. (2012)
Inflexal®	Influenza particles	Virosome	Influenza vaccine	Herzog et al. (2009)
Opaxio®	Paclitaxel	Polymer conjugates	Several types of tumors	Galic et al. (2011)

The modern biotechnological techniques to manipulate nucleic acids have allowed the development of drugs with improved pharmacological properties, such as peptides and DNA vaccines (Amaral et al. 2012; Amaral and Felipe 2013). However, because of the physicochemical nature of these molecules, which need to keep their original conformation to preserve the activity, they are easily degraded when in contact with the physiological environment by the action of enzymes. When incorporated into these nanostructures, many drugs may have their concentration increased where the therapeutic activity is needed and thus reducing toxicity (Kaminskas et al. 2012).

In vivo experiments proved to improve peptides and DNA vaccines' bioavailability when incorporated within nanoparticles (Amaral et al. 2010; Ribeiro et al. 2015). Using the murine model of fungal infection *Paracoccidioidomycosis*, it was possible to increase the immunomodulatory activity of a peptide of 10 amino acid residues, called P10, when incorporated into polymeric nanoparticles (Amaral et al. 2010). Similar results were remarked for the same experimental model when a DNA therapeutic vaccine is delivered within polymeric nanoparticles or liposomes (Ribeiro et al. 2015). Both formulations were able to enhance in four times the antifungal activity of the vaccine compared with the DNA vaccine administered in the free form.

8.4 Sources of Nanopharmaceuticals Release into the Aquatic Environment

The sewage effluent is the major source of nanopharmaceuticals in the aquatic environment. Human nanopharmaceuticals are released into the sewage system as a mixture of the unchanged, metabolized or conjugated compounds. The elimination of nanopharmaceuticals by patients occurs via excretory or hepatobiliary system followed by fecal or biliary excretion. In addition, nanopharmaceuticals applied to veterinary medicine are also a potential source of pollution, while the sludge from waste water treatment plants is an additional source of soil and aquatic pollution. The direct or indirect release of nanopharmaceuticals in effluents of wastewater treatment plants from hospital, communities, and industrial facilities will result in the exposure of aquatic organisms to nanopharmaceuticals. Although the concentration of nanopharmaceuticals in the aquatic environment is unknown, the environmental levels of pharmaceuticals is increasing due to an ageing, increase on life expectancy and growing of human population, as well as the increase production and use of new products, indicating that nanopharmaceuticals may follow the same environmental fate of pharmaceutical compounds. Mahapatra et al. (2013) indicated that the release form and environmental fate and exposure of nano-enabled medical products have not been investigated and little or no data exists in the literature, confirming the urgent need to investigate the potential hazards and risks associated to nano-enabled medical products, such as the nanopharmaceuticals.

After its release in the aquatic systems, different processes may influence the environmental behavior and fate of nanopharmaceuticals and their metabolites, as reported for other engineered nanoparticles: physicochemical transformation, aggregation/agglomeration, macromolecular interactions, and biologically mediated reactions (Dwivedi et al. 2015; Rocha et al. 2017). However, these processes have not yet been investigated for freshwater, estuarine, and marine environment.

8.5 Effects of Nanopharmaceuticals in the Aquatic Environment

The potential pathways for ecotoxicological research of nanopharmaceuticals in the aquatic environment are summarized in Fig. 8.3. Nanopharmaceuticals exhibiting novel and multifunctional properties, such as high surface area and saturation solubility, resistant to settling, fast dissolution, and improved adhesion to biological surfaces, may give rise to potentially new ecotoxicological effects and environmental risks.

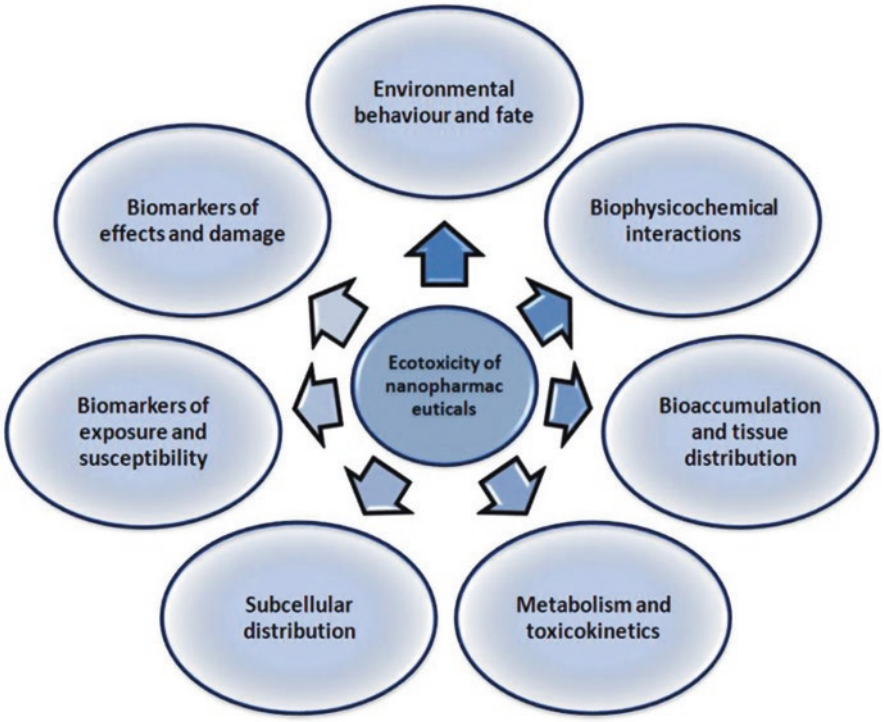

Fig. 8.3 Potential pathways for ecotoxicological research of nanopharmaceuticals

In studies about the mode of action and toxicity of nanopharmaceuticals, a comprehensive knowledge of how these nanomaterials interact with biological systems is fundamental (Fig. 8.3). The interaction between nanomaterials applied to personalized medicine and biological systems is mediated by protein-binding, ligand-mediated interactions and interactions during intracellular processing (Zhang et al. 2012). Furthermore, upon contact with biological fluids (e.g., hemolymph, blood, interstitial fluid, or mucosal secretions), nanomaterials are coated with proteins and/or other molecules, forming the protein corona (bio), which may change their nano-specific properties, such as surface charge (zeta potential) and hydrodynamic diameter. According to Canesi and Corsi (2016) and Canesi et al. (2017), the interaction of nanomaterials with plasma proteins in non-mammal species also induces the formation of the protein corona, changing its uptake and toxicity in target cells. On the other hand, the interaction of nanomaterials with the external environment (i.e., natural organic matter and clays) forms the eco-corona, which changes its environmental behavior and fate in distinct compartments of the ecosystems (aqueous phase, sediments, biota) (Rocha et al. 2015a, 2017; Canesi and Corsi 2016).

The interaction and bioaccumulation of nanomaterials in aquatic organisms are directly related to their mode of action and toxicity (Fig. 8.3). Recently, Yegin et al. (2017) showed that paclitaxel-loaded nanoparticles (84 ± 4 nm; 0.2–16.2 μg ml^{-1}) were adsorbed on cell surfaces of the freshwater algae *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* and decreased the algal growth rate (72 h IC_{50} of 1.6 ± 0.1 μg paclitaxel ml^{-1} for *R. subcapitata* and 120 h IC_{50} of 1.1 ± 0.1 μg paclitaxel ml^{-1} for *C. reinhardtii*), as well as inhibited the photosynthesis efficiency more than molecular (free) paclitaxel after 5 days of exposure. Furthermore, the polymer poly(ethylene oxide)-block-poly(ε-caprolactone) (PEO-*b*-PCL) used in the formation of nanocarriers was nontoxic to both algae species. In this sense, Yegin et al. (2017) indicated that the nanotechnology can increase the ecotoxicity effects of insoluble (lipophilic) drug molecules and that paclitaxel-loaded nanoparticles have algaecide properties. In this sense, the algal community that form the basis of aquatic food web represents an important target for biological interaction and ecotoxicity effects of nanopharmaceuticals. On the other hand, the tissue and subcellular distribution, metabolism and toxicokinetics of nanopharmaceuticals on aquatic organisms deserve further studies.

Fish are considered a suitable model for ecotoxicity assessment of magnetic nanoparticles in the aquatic environment (Table 8.2). The zebrafish *Danio rerio* and *Oryzias latipes* are the main fish species used to assess the environmental impact of magnetic nanoparticles. However, there is limited data for other economically important fish species, such as *Oreochromis niloticus* (Ates et al. 2016) and *Poecilia reticulata* (Qualhato et al. 2017) (Table 8.2).

Li et al. (2009) described oxidative stress and hypoxia in *Oryzias latipes* after exposure to nZVI nanoparticles and nFe-oxide nanoparticles (49 nm; 1–100 mg L^{-1}) for 14 days and revealed that the mortality observed was dependent on the nanoparticle composition [Fe(II) > CMC-nZVI > nFe-oxides]. Recently, Qualhato et al. (2017) showed that ecotoxicity of iron oxide nanoparticles in fish species is exposure time and concentration dependent. In general, data indicate that oxidative stress

Table 8.2 Magnetic NPs effects in fish species

Nanoparticles			Species	Exposure conditions		Accumulation	Cell/ Tissue[b]	Effects[c]	References
Type	Capping layer[a]	Size (nm)		Concentration (mg L^{-1})	Time(h)				
Fe_3O_4	SiO_2 and DTC	100	*Anguilla anguilla*	2.5	72	–	G*	Co-exposure: Hg + NPs; ↓TGSH; ↑GR; GPx↑; ↑GST; ↑LPO. Synergistic response to co-exposure.	Srikanth et al. (2014)
α-Fe_2O_3	–	30	*Danio rerio*	0.1–100	168	–	E	168 h NOEC <50 mg L^{-1}; 168 h LC_{50} = 53.35 mg L^{-1}; ↓embryo-hatching (≥10 mg L^{-1})	Zhu et al. (2012)
Fe_2O_3, Fe_3O_4	–	80–90; 140–160		4, 10	E: 28 days; D: 24 days	x	W	Similar accumulation in both concentrations; elimination (~24 days); uptake by gastrointestinal tract.	Zhang et al. (2015)
γ-Fe_2O_3	DMSA	5.7		4.7–74.4	96	x	Bl, L	↑DNA damage; ↑NAs; low LPO; differential expression gene.	Villacis et al. (2017)
Fe_2O_3	–	100 x 200	*Labeo rohita*	500	25 days	–	G, Bl	↑hemoglobin; ↑red blood cell; ↑hematocrit; ↓cellular volume; ↓white blood cell; ↓Na^+; ↓cl^-; ↓K^+; ↑gill Na+/K + -ATPase	Ribeiro et al. (2015)
α-Fe_2O_3, γ-Fe_2O_3	–	20–90, 40–120	*Oreochromis niloticus*	0.1, 0.5, 1.0	E: 60 days; D: 30 days	x	S, I, K, L, G, Bl, mu	Accumulation (S > I > K > L > G > B > mu); elimination (except in L, S); no changes in haematological parameters and respiratory burst; ↓GLU; ↑GOT; ↑GPT; ↑LDH; ↓LA; ↑MPO.	Ates et al. (2016)

(continued)

Table 8.2 (continued)

Nanoparticles			Species	Exposure conditions		Accumulation	Cell/ Tissue[b]	Effects[c]	References
Type	Capping layer[a]	Size (nm)		Concentration (mg L^{-1})	Time(h)				
nFe	SPA	30	*Oryzias latipes*	0.5–50 μg mL^{-1}	8–14 days	–	E, G, I, L, B	E: ↓SOD, ↑LPO. Adult: ↑histopathological alterations (cell swelling, hyperplasia, granulomas), ↓SOD; no LPO; ↓GSH.	Li et al. (2009)
nZVI; nFe-oxide	CMC	49.3		1–100	12–14 days	x	W, I	Mortality [Fe(II) > CMC-nZVI > nFe-oxides]; ↓dissolved oxygen level (induced hypoxia); ↑ROS; ↑CAT mRNA expression; no histopathological alteration (I)	Chen et al. (2013)
nZVI; nFe_3O_4	CMC	27.1, 30.1		25–200	7 days	x	E	Accumulation (nFe_3O_4 > nZVI); ↑ROS; ↑developmental abnormalities (nZVI > nFe_3O_4); ↓hatchability; ↑SOD; ↓CAT; ↑GR.	Chen et al. (2013)
Fe_2O_3	Citrate	3.97	*Poecilia reticulata*	0.3	3–21 days	–	Bl	↑DNA damage (3–21 days); ↑ NAs (14–21 days). Genotoxicity and mutagenicity: Long-term exposure > acute exposure.	Qualhato et al. (2017)

[a]*CMC* carboxymethyl cellulose, *DTC* dithiocarbamate, *DMSA* meso-2, 3-di-mercaptosuccinic acid, *SPA* sodium polyaspartate
[b]*Bl* blood, *B* brain, *G* gills, *I* intestine, *K* kidney, *L* liver, *M* muscle, *S* spleen, ∗in vitro test
[c]*GLU* glucose, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GST* glutathione sulfotransferase, *LDH* lactate dehydrogenase, *LA* lysozyme activity, *MPO* myeloperoxidase, *NAs* nuclear abnormalities, *TGSH* total glutathione

associated to changes in activities of antioxidant enzymes and oxidative damage (i.e., lipid peroxidation and DNA damage) is one of the main modes of action of toxicity of magnetic nanoparticles in fish species (Table 8.2). *Anguilla anguilla* exposed to SiO_2 and dithiocarbamate (DTC)-coated Fe_3O_4 nanoparticles (100 nm; 2.5 mg L^{-1}; 72 h) showed changes in the activity of glutathione reductase, glutathione peroxidases and lipid peroxidation, as well as a synergistic response after co-exposure to Hg (Srikanth et al. 2014). Genotoxic (DNA damage) and mutagenic effects (nuclear abnormalities) were observed in peripheral erythrocytes of the guppy *Poecilia reticulata* exposed to citrate-coated Fe_2O_3 nanoparticles (3.97 nm; 0.3 mg L^{-1}; 3–21 days) (Qualhato et al. 2017) and in the zebrafish *D. rerio* exposed to meso-2, 3-di-mercaptosuccinic acid (DMSA)-coated γ-Fe_2O_3 nanoparticles (5.7 nm; 4.7–74.4 mg L^{-1}; 96 h) (Villacis et al. 2017), indicating that the comet assay associated to micronucleus test and erythrocyte nuclear abnormalities assessment are a suitable approach to detect the clastogenic and aneugenic effects in fish species after exposure to magnetic nanoparticles (Table 8.2).

Similar to magnetic nanoparticles, the most used fish species in ecotoxicological research to assess the effects of quantum dots is *D. rerio*. The biological effects were assessed in different life stages, such as embryos, adults, and *in vitro* (Rocha et al. 2017). Reactive oxygen species production, lipid peroxidation, and changes in gene expression and in antioxidant enzymes activities in fish species exposed to different types of quantum dots were identified. In addition, mollusc bivalve species, namely *Mytilus galloprovincialis* and *Mytilus edulis*, were indicated as a target group for quantum dots ecotoxicity (Canesi and Corsi 2016; Rocha et al. 2015a, 2017). As filter feeders, the mussels take up quantum dots aggregates/agglomerates from seawater, specially by endocytosis and/or phagocytosis in the digestive system, following tissue distribution and metabolism, wherein the digestive gland is the main organ for storage, metabolism, and elimination of quantum dots (Rocha et al. 2015a, b, 2017). Similar mode of action and toxicity of quantum dots in fish were identified in bivalve species, while the mechanism of genotoxicity for both species remains unknown. In addition, it was demonstrated that hard nanomaterials such as quantum dots and iron oxides nanoparticles are toxic at different trophic levels (Rocha et al. 2015a, 2017; Nogueira 2014; Lefevre et al. 2015; Valdiglesias et al. 2016).

Among the important aspects in NanoEcoSafety, the development of nanomaterial safety standards is a priority. In this context, chitosan (polymer derived from chitin by deacetylation) effectively protected the freshwater crustaceans *Ceriodaphnia cornuta* and *Moina micrura* by enhancing the survival rate and repair of lost parts (Vijayakumar et al. 2016). ZnO nanoparticles (40.9 nm; 160 μg L^{-1}) induce 100 and 76% mortality in *C. cornuta* and *M. micrura* neonates, while the co-exposure to chitosan at 100 μg ml^{-1} significantly reduced the mortality of *C. cornuta* (36%) and *M. micrura* (14%) after 24 h of exposure (Vijayakumar et al. 2016), indicating that chitosan decreases the toxicity of metal-based nanoparticles.

The knowledge of the biological effects of nanopharmaceuticals and of the mode of action in aquatic organisms is limited and no standard protocol for ecotoxicological tests exists. In this sense, bioassays or biomarker assessment should focus on

specific mechanisms of nanopharmaceuticals action on nontarget species. The revised data indicate that there is an urgent need to develop guidelines for ecotoxicological test using aquatic species at different trophic levels, as well as the development of new biomarkers by OMICs technologies (e.g., proteomics, transcriptomics, and metabolomics) to assess the impact of nanopharmaceuticals in aquatic organisms.

8.6 Environmental Risk Assessment of Nanopharmaceuticals

Environmental Risk Assessment (ERA) includes four components: hazard identification, toxicity assessment, exposure assessment, and risk characterization. In the European Union, ERA for medicinal products follows the Guideline of the ERA of Medicinal Products for Human Use (EMA 2011) but is only foreseen for medicinal products within the marketing authorization procedure, not including the conformity assessment for medical devices (EU 2001). The assessment is conducted only taking into account the active ingredient (API) but not excipients. Metabolites and transformation products are covered by the "total residue approach" that assumes the same effects for parent compounds, metabolites, and transformation products (EMA 2011). This still leaves excipients, medical devices, and disposal of the medicinal products out of the current ERA.

ERA consists of a 2-tiered approach. In phase I, the environmental concentration of the API present in the water is measured (MEC) or predicted (PEC). To define PEC it is assumed that APIs are taken up by patients, excreted, and end up in urban sewage, which is then un-treated, partially treated or treated in waste water treatment plants and then introduced in the aquatic environment. For the ERA, it is crucial to determine the amount the patient excretes and in which form, because these nanosized compounds form aggregates/agglomerates in water, particularly in seawater (Berkner et al. 2016). Persistent Bioaccumulation and Toxic (PBT) data must be collected to identify the potential toxicity of these compounds. For this purpose, the octanol/water partitioning coefficient (log K_{OW}) has been used as an indicator of possible toxicity. If log K_{OW} is equal to or above 4.5, within an environmental relevant pH range, information on their fate in aquatic and sediment systems and on bioaccumulation and long-term ecotoxicity are required. Nanopharmaceuticals are formed by a core and a coating. Therefore, information based on partitioning coefficient cannot be used to predict the bioaccumulation potential because it may induce an over estimation (OECD 2014). The assessment of the persistence of the compound can be carried out using the criteria as defined under European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (ECA 2008).

Within ERA, it is assumed that a certain percentage of the population consumes the maximum daily dose of the API. The fraction of inhabitants taking

nanopharmaceutical compounds is either estimated by default (value of 0.01) or based on epidemiological data. The amount of API taken daily is divided by the amount of waste generated (water/inhabitant/day) taking also into account a dilution factor. The obtained PEC is compared with action limit below 10 ng L^{-1}, according to EMA, or to 1 μg L^{-1} according to FDA. If the PEC is below the action limit, there is no risk. However, if PEC is equal or above the established limits, the assessment has to proceed to phase II. Phase II comprises the knowledge of the physicochemical properties of API based on pharmacokinetics (EMA 2011), its fate in the environment, as well as its ecotoxicity in water and sewage sludge. As a result, a non-observed effect concentration (NOEC) is established along with a predicted no-effect concentration (PNEC). To assess if the nanopharmaceutical compound poses a risk to the environment, a risk quotient is calculated based on the ratio between PEC and PNEC. If this ratio is higher than one the compound poses a risk to the aquatic environment and other measures need to be taken to minimize the risk.

Although to date there is no information available on how to conduct an ERA for nanopharmaceuticals, the EMA stated that before marketing a new product, toxicology, and ecotoxicology for a specific nanopharmaceutical need to be assessed (EMA 2006, 2011). For that purpose, the more appropriate methods to assess the fate and toxicology of nanopharmaceuticals need to be established (Berkner et al. 2016). As a prerequisite for a nanopharmaceutical ERA, besides its physicochemical composition, information on size, shape, distribution, morphology, and surface properties (e.g., chemistry, reactivity, surface area) but also aggregation/agglomeration and dissolution behavior need to be taken into account, because normal size ranges are not adapted for nanosized molecules (Gondikas et al. 2012; Tejamaya et al. 2012; Ottofuelling et al. 2011; Misra et al. 2012, Sant'Anna et al. 2013; Rocha et al. 2017). When dissolution of ions occurs, the nano-character of the particles is lost. However, there is a lack of data and some scientific uncertainty (Sant'Anna et al. 2013) regarding all these aspects, namely, what are the particle characteristics that affect toxicity and transport in the different compartments of the environment, their routes of exposure, and the best metric to measure their exposure.

Linkov et al. (2008) proposed that environmental information should be incorporated into engineering nanomaterials and nanomedicine development. In order to avoid the increase on the complexity of the decision, he proposed that this could be achieved combining toxicology, potential health risks, risk assessment modelling, and tools developed in the field of a multi-criteria decision analysis (MCDA). This tool should be used for regulatory decision on nanomaterials and could be used to support the weight-of-evidence approach for evaluating possible health or environmental risks of nanomaterials.

There is a wide variety of nanopharmaceutical compounds, and there is a need to establish guidelines to assess their impact on the marine environment. Therefore, there might be a need to diversify solutions for the correct establishment of an ERA of these compounds.

8.7 Conclusions

There is no doubt that nanostructured systems, more specifically those aimed at health applications for both diagnosis and treatment, represent an important technological advance, bringing many benefits and positive impacts to improve the population quality of life and well-being. However, because these so tiny "entities" are not naturally found in the environment, it is crucial to assess their environmental safety and impact in order to ensure that these nanopharmaceutical compounds do not pose undesirable effects on humans and the environment in the future. For this reason, there is an urgent need to establish appropriate ecotoxicological assays essential for regulatory purposes and environmental and human safety guidelines to protect human health and the environment for the safe use of nanopharmaceutical compounds.

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