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Toxicity of Metal-Organic Framework Nanoparticles: From Essential Analyses to Potential Applications

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In the last two decades, the field of metal-organic frameworks (MOFs) has exploded, and especially MOF nanoparticles are being investigated with increasing interest for various applications, including gas storage and separation, water harvesting, catalysis, energy conversion and storage, sensing, diagnosis, therapy, and theragnostics. To further pave their way into real-world applications, and to push the synthesis of MOF nanoparticles that are 'safe-and-sustainable-by-design', this tutorial review aims to shed light on the importance of a systematic toxicity assessment. After clarifiying and working out the most important terms and aspects from the field of nanotoxicity, the current state-of-the-art of *in vitro* and *in vivo* toxicity studies of MOF nanoparticles is evaluated. Moreover, the key aspects affecting the toxicity of MOF nanoparticles such as their chemical composition, their physico-chemical properties, including their colloidal and chemical stability, are discussed. We highlight the need of more targeted synthesis of MOF nanoparticles that are 'safe-and-sustainable-by-design', and their tailored hazard assessment in the context of their potential applications in order to tap the full potential of this versatile material class in the future.

Key Learning Points

1. General introduction to terminologies and key aspects of the field of nanotoxicity.
2. Evaluation of the state-of-the-art of *in vitro* and *in vivo* metal-organic framework (MOF) nanoparticle toxicity.
3. Identification of the key features affecting the toxicity of MOF nanoparticles.
4. Introduction of the hazard assessment of MOF nanoparticles in the context of their applications.
5. Recommendations of a sustainable workflow for promising MOF nanoparticles from their analysis to potential real-world applications.

Introduction

After the first report of porous crystalline coordination-polymer frameworks, it quickly became apparent that their construction is generalizable owing to the modularity of its constituents: the combination of a metal cluster and an organic linker created a new class of materials called metal-organic frameworks (MOFs, **Figure 1**).[1] This understanding enabled chemists to introduce extensive compositional and structural variations, giving way to a vast structural library of crystalline micro- and meso-porous architectures. The ability to rationally compose extended structures from a library of molecular or extended building blocks introduced a new level of design to solid-state organic and inorganic materials. In addition, different ways of further functionalise MOF materials played a central role to reach their full potential as hybrid organic-inorganic materials.[1] As a result, MOFs represent a highly interesting materials class of porous solids with unprecedented properties that make them promising candidates for numerous potential applications ranging from industrially relevant processes such as gas storage and separation, water harvesting, catalysis, energy conversion and storage, sensing, to biomedicine, e.g. health-care, diagnosis, therapy, theragnostics, among others (**Figure 2**).[1,2] Nevertheless, the design possibilities of MOF materials are far from being limited to the utilisation of different components or the embedding of other functionalities, as the size/shape of the MOF particles themselves can also be used as a tool to achieve new properties, similarly to other materials. Reducing the particle size of a MOF results in a significant increase of their external surface area. In combination with their high internal surface area, this dramatically improves the surface area to volume ratio as well as their chemical reactivity. Therefore, the properties of MOF nanoparticles differ distinctly from those of their bulk analogues with the same chemical composition, which provides access to even further exciting and more specific applications, e.g. highly selective catalysts or sensors, and extremely efficient therapeutics.[3]
However, one material property that is typically strongly affected by downsizing, but often neglected, is the toxicity.

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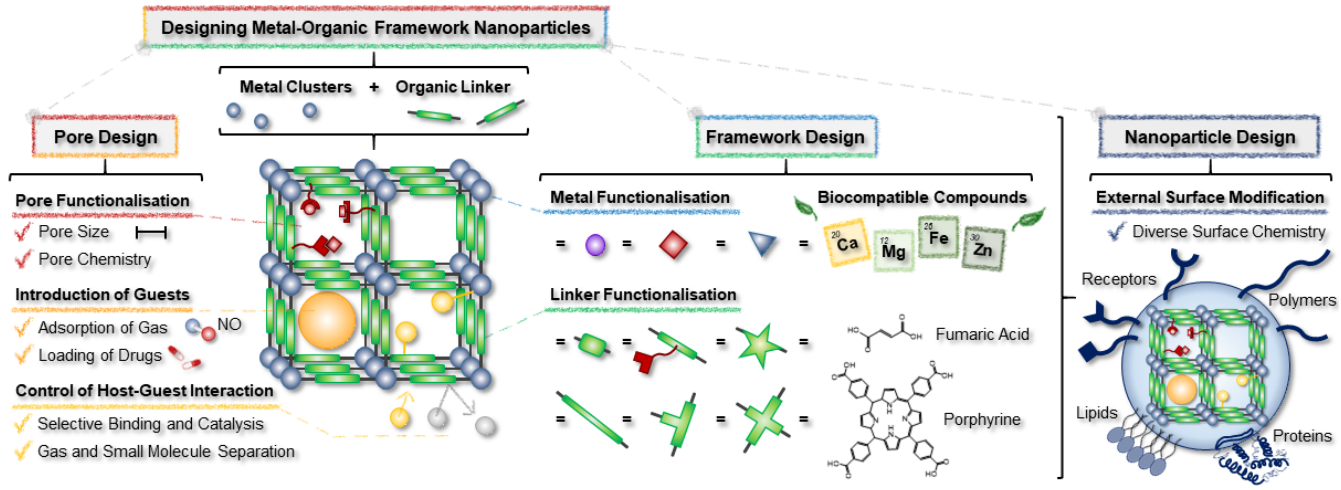

Figure 1: Schematic illustration of the design opportunities in metal-organic framework nanoparticles, i.e. pore design, framework design, and nanoparticle design.

Herein, nanoparticles occupy an exceptional position as they exhibit sizes as small as nano-organisms and viruses, and thus, are able to cross physiological barriers, modifying their biodistribution and elimination, which makes them much more hazardous than their bulk analogues. As a result, scientists tried to address the inevitable – but very important – question of the intrinsic toxicity of a nanoparticle by analysing as many material physico-chemical properties as possible to enable correlation between the key aspects of toxicity and material properties. Various general and specific toxicity-relevant material aspects have been identified, and different *in vitro* toxicity assays as well as *ex vivo* and *in vivo* models have been successfully established. Therefore, this tutorial review aims to clarify the key aspects influencing the toxicity of nanoparticles and to highlight the special role of MOF nanoparticles in this context. A résumé of the state-of-the-art of the evaluation of the toxicity of MOF nanoparticles will be given. Based on this a guideline for future nanosafety evaluations of MOF nanoparticles will be developed and the relevance of MOF nanotoxicity in the context of application will be discussed.

General Introduction to Nanotoxicity

Generally, anything that has the property of being harmful to human beings or animals is considered toxic. Importantly, the resulting toxicity is dose-dependent. Although the knowledge of the toxicity of the isolated organic and inorganic constituents of the MOFs constantly improving, the toxicity of the 'bulk' MOFs remains mostly unknown. Even further, as mentioned above, reducing the particle size from 'bulk' to 'nano' has a huge impact on the properties of a material and on its ability to penetrate physiological barriers. The term 'nanotoxicity' which represents the specific branch of toxicology which addresses the adverse health effects caused by nanoparticles, will be explained in the following.

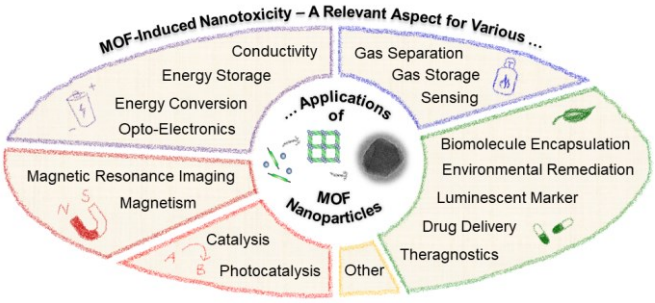

Figure 2: Overview of different applications of MOF nanoparticles for which the aspect of induced nanotoxicity is relevant.

Nanotoxicity specifies the toxic effect of nanoparticles to ecosystems and to living organisms – induced by any route of exposition, such as inhalation, by dermal exposure or by mucosal, oral, intravenous, subcutaneous or intramuscular administration. One must distinguish between acute and chronic toxicity. Acute toxicity is classified as the adverse effect of a substance for a single exposure or multiple exposures within a short period of time (< 24 h; or < 4 h for inhalation exposure), while chronic toxicity occurs only after for a long term exposure (months or years).[4] The assessment of such toxic effects of nanoparticles is mainly performed for two indispensable reasons: *(i)* to evaluate the unintentional hazardous effect of nanoparticles on living organism and ecosystems, which can limit the use of the materials in different industrial applications, e.g. catalysis, separation, sensing, or *(ii)* to determine and verify their intentional utilization as diagnosis and/or therapeutic agent in a new drug. Especially in case of the development of a new drug delivery system (DDS), the toxicity has to be evaluated at different successive stages (**Figure 3**).

In a first step, preclinical studies are carried out with the potential test substance – usually first *in vitro*, i.e. with different cell lines derived from target and off-target tissues, and then *in vivo*, i.e. in living higher organisms. In case of *in vitro* studies, different cell lines are generally incubated for 24 h and 72 h with a range of concentrations of the test substance and subsequently, the inhibition of biological or biochemical functions is evaluated. This allows the assessment of the cell viability in relation to the amount of administered test substance. Herein, the concentration that causes 50% inhibition of cell growth can be expressed by the so-called IC_{50} value, i.e. the half maximal inhibitory concentration. IC_{50} values are given in µM or mg/L. However, it should be noted that the relevance

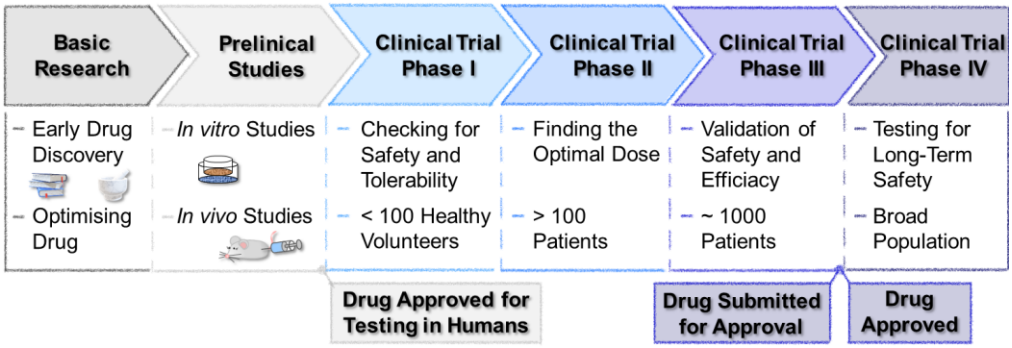

Figure 3: Different phases of the development of novel drugs, as well as their toxicity and efficacy assessment.

of such *in vitro* studies is often questioned, as the administered doses are not readily comparable to real *in vivo* conditions, where the exposure route and the biodistribution of the nanoparticles strongly influences the final concentration in certain tissues. Consequently, for the approval of a new drug the results obtained of *in vitro* studies have to be confirmed by *in vivo* studies. Herein, the so-called LD_{50} value or, more recently to reduce harm to animals, the LD_{10} value is determined, which indicates the lethal dose that kills 50% or 10% of a group of animals. It describes the acute toxicity which arises from a single or short-term exposure, i.e. up to 24 h, of the nanoparticles. For the assessment different doses of the test substance are administered to matched groups of animals, i.e. usually a rodent model (mainly mice and rats, but also rabbits to evaluate the dermal toxicity and eye irritations or pyrogenic effects). To make the respective acute lethal dose of a specific test substance comparable, the LD_{50} or LD_{10} value is always expressed in mg of substance per kg of body weight. However, to cause less pain and suffering to animals, since 2002 most of the LD_{50} or LD_{10} studies were replaced by methods such as the fixed-dose procedure, which yields similar results. When considering a biomedical use, if the first safety preclinical studies on rodents yield promising results, additional safety and efficacy preclinical evaluation is carried out on another animal model, i.e. typically with non-rodents such as dogs or monkeys, suitable for the targeted aim (e.g. therapy, prevention, diagnosis, medical device). If successful, the potential drug would receive the approval for the testing in humans, based on three clinical trial phases, i.e. checking for drug safety, finding the optimal dose and verifying the safety and efficacy, are required (**Figure 3**). And if the drug is approved after this procedure, the monitoring of long-term safety of the drug is continued during the clinical application in the final post-marketing surveillance phase. The journey from the discovery of a drug to its approval is long (several years), labour-consuming and resource intensive. To accelerate this process and to foster the '4Rs'-principles, i.e. replacement, reduction, refinement and rehabilitation, in research in animal experimentation, *in silico* models, i.e. computer based simulations, could become a very useful tool to predict toxicity.[5] Although *in silico* models cannot replace the complexity of an *in vivo* model, they could serve both as a first step in the toxicological evaluation and as a way to understand the main parameters governing the nanoparticles toxicity. Moreover, for more sustainable and efficient research, it is essential that new pharmaceuticals are developed in a targeted manner in order to circumvent biosafety risks *a priori*, for instance by a smart drug design, developing novel strategies for assessment, improving established state-of-the-art solutions – with the overall aim to prevent hazardous effects in later development stages.

To address these issues, the identification of different nanoparticle features or their interplay that could cause the resulting nanotoxicity is absolutely desirable. Various studies with different nanoparticles have elucidated that aspects such as their overall particle size, aggregation state, shape, chemical composition, biostability and their surface chemistry, among others, play an important role (**Figure 5**). Therefore, from a researchers' point of view a full characterisation of these physico-chemical properties of a nanoparticle, which represent the basis for their so-called physical identity, is fundamental for the assessment of the resulting toxicity. By chemical design, the intrinsic nanoparticle properties can be specifically engineered, for instance by systematic surface modifications. However, it is extremely important to note that the intrinsic nanoparticle properties are altered relatively quickly when a nanoparticle is exposed to a biological environment.

Mechanisms of Toxicity

The human body is a highly complex biological system built of trillions of cells, grouped into different tissues, organs and organ systems. The interaction at the different hierarchical levels and the exchange of nutrients, metabolites or information is highly regulated, efficient and specific with the overall aim of keeping homeostasis. Similarly, the field of material toxicity can be sub-classified into toxic effects of a compound on individual cells and sub-cellular compartments as well as superordinate tissue and organ systems. This hierarchical differentiation makes clear, that the mechanisms of toxicity and safety assessments also have to be considered on different levels.

Herein, ADME series, i.e. absorption, distribution, metabolism and excretion, plays an important role in the resulting toxicity of nanoparticles.[6] In consequence of their dimensions and properties, the distribution of colloidal nanoparticles in an organism is different from bulk materials or solutes. This includes the ability to penetrate through organ and tissue barriers, to circulate in the blood stream as well as to being internalized into cells and subcellular compartments. Cellular

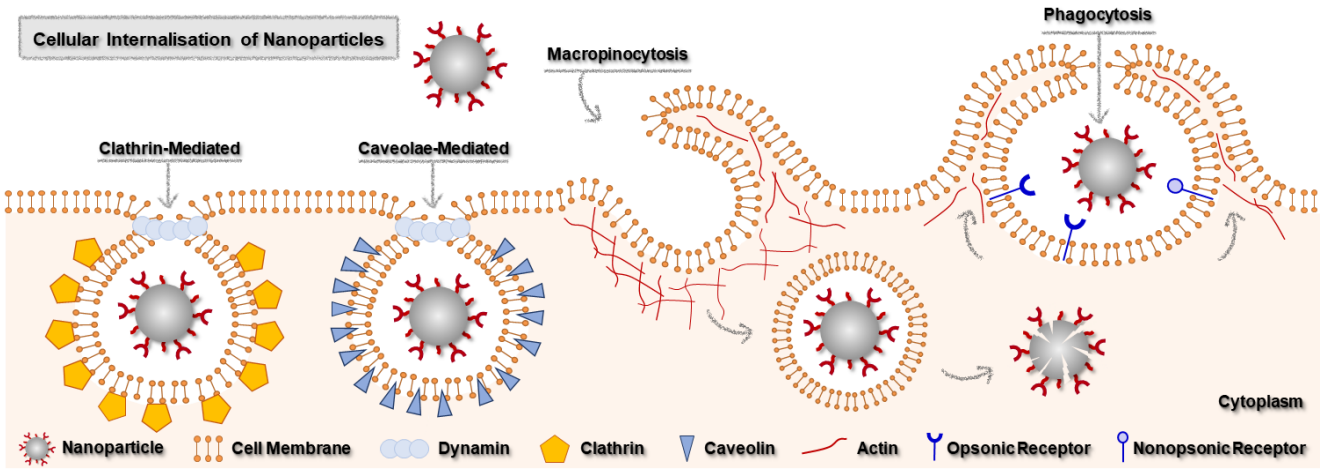

Figure 4: Different cellular internalisations of nanoparticles, i.e. clathrin- or caveolae-mediated, macropinocytosis or phagocytosis.

internalisation of nanoparticles generally occurs via endocytotic uptake mechanisms, such as clathrin- and caveolae-mediated endocytosis, macropinocytosis as well as phagocytosis in macrophages (**Figure 4**).[7] These properties are the basis for the utilization of colloidal nanoparticles as drug carriers, which can change the pharmacokinetics of encapsulated drug molecules and facilitate their transport into areas of the body, which would not be reached otherwise. On the other hand, potential additional biosafety risks are implied, since all reached sites (target and off-target) can be the origin of adverse reactions, eventually with impact on the entire organism. Nanoparticles, which enter the human body, can mediate toxic effects on different hierarchical levels of organisation, for instance via disturbing membrane integrity of cells or organelles, interference with cellular metabolic processes, generation of detrimental reactive molecule species, induction of immune reactions inside or outside cells as well as impairment of tissue and organ function due to nanoparticle deposition.[6,8] Depending on the biodistribution of the nanomaterial, cellular toxicity mechanisms can lead to specific organ toxicities at sites of preferential accumulation.

Synthetic organic components can raise different biosafety issues, due to potential reactivity and interaction with biomolecules, biotransformation into reactive metabolites or phototoxic reactions, depending on the optical properties, the biodistribution and light exposure. Also, excessive doses of metal ions – even those that are essential components of our biological systems – can result in manifold toxicological mechanisms: unbalancing physiological metal homeostasis, interaction with biomolecules (e.g. binding to Lewis bases in peptides and proteins), replacement of physiological metal ions (e.g. co-factors in proteins or calcium in bone mineral) and perturbation of physiological redox processes.

A frequently observed toxicity mechanism induced by nanoparticles containing redox-active metals, is the generation of reactive oxygen (ROS) or nitrogen species (RNS), such as hydroxyl radicals, superoxide anions, hydrogen peroxide or nitric oxide.[9] Although these molecules are also produced and scavenged during metabolism under physiological conditions, a dramatic increase of concentration can unbalance the cellular redox system, mediate oxidative stress and serious damage, if cellular compensation mechanisms are surpassed. Elevated levels of ROS/RNS, which are not scavenged by cellular detoxification mechanisms, can lead to modifications of peptides and proteins, DNA and RNA damage as well as oxidation of unsaturated fatty acids in lipid membranes.[10] Disturbance of membrane integrity by nanoparticles is a general critical mechanism with big impact on cell viability, since the spatial confinement of cells and organelles is essential for keeping homeostasis. Strong lytic effects on cellular plasma membranes leads to direct cell killing, whereas specific damage of the mitochondrial membrane can cause decoupling of the electron transfer chain and release of apoptosis inducing factors, such as pro-apoptotic BCL-2 and cytochrome c.[8] Erythrocytes, leucocytes, and thrombocytes are the first cells exposed to nanoparticles after intravenous administration. The rupture of erythrocytes (hemolysis) can cause anaemia with severe complications and it has been observed that hemolytic activity depends on the size, geometry and surface properties of nanoparticles.[11] On the other hand, non-lytic interactions with blood cells and components may induce pro-coagulant activity with potential risk of thrombosis or embolism.[12] Even if cellular membranes are not destroyed and permeabilised completely, also a selective transduction of impermeable molecules via nanoparticles can lead to an imbalance of chemical gradients between membrane separated milieus and a critical elevation of detrimental factors inside cells. It has been demonstrated that calcium loaded nanoparticles can increase the concentration of the metal species inside cells and lead to calcium-induced apoptosis.[13]

Finally, beside the ability to trigger specific response in individual cells, nanoparticles can modulate the immune system with impact on local tissues and organs, but also the entire organism.[6] Nanomaterials can mediate immunostimulatory effects by interaction with the complement system, by serving as haptens, which bind to endogenous proteins, or by induction of inflammatory responses. Pyrogenicity of nanoparticles, for instance due to contamination with endotoxins, can lead to fever and serious organ damage via immune activation. In contrast, a nanoparticle-mediated cytotoxicity towards immune cells, rather leads to immunosuppression. Although such effects on specific physiological processes can be reason for safety

concern, the knowledge of the involved mechanisms together with tuning of nanoparticle properties also provides opportunities for directed utilization in therapeutic applications, such as by specific induction of programmed cell death in cancer cells or desired immunomodulation in patients. These strategies illustrate the potential to generate designed nanostructures which do not only serve as passive carriers but also as smart nanomaterials with intrinsic functionality based on the targeted selection and combination of suitable building blocks.

Nanoparticle Characterisation – Physical Identity, Synthetic Identity and Biological Identity

In order to discuss and compare the nanotoxicity of different nanoparticles, their full characterisation and the determination of their identity is essential. It turned out that classification of nanoparticles into three different identity types, i.e. physical, synthetic and biological (**Figure 5**), is most useful as these represent the different states of the outermost layers of nanoparticles that are perceived by their environment. Hence, these identities have a strong influence on the biodistribution of nanoparticles and thus, on their overall toxicity.

Physical Identity: As-synthesised nanoparticles that have neither been surface engineered nor exposed to any biological environment have a so-called physical identity. This physical identity includes information about their chemical composition and describes their typical basic functional and intrinsic properties such as their chemical or colloidal stability, reactivity, magnetic behaviour, electrical conductivity, or porosity.

Synthetic Identity: The physical identity of a nanoparticle becomes a so-called synthetic identity when its surface is engineered. In this process, the intrinsic properties of modified nanoparticles are typically preserved, while the surface-dependent properties may differ significantly from those of their non-modified analogues. This is because modified nanoparticles exhibit a different outmost solid-liquid interface between the nanoparticle and its biological environment. As a result, it is possible to favour or inhibit interactive processes, for instance the adsorption of specific biomolecules or tune the biodistribution via certain biological pathways by employing targeted chemical design. In most cases, the surface coatings are designed in such way that the modified nanoparticles show

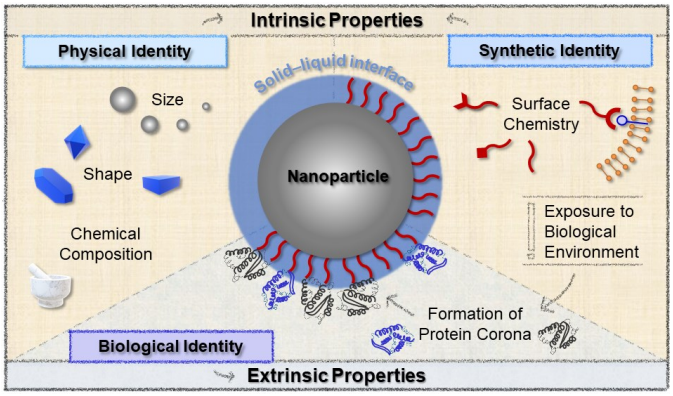

Figure 5: The interplay between the three different nanoparticle identities, *i.e.* physical, synthetic and biologic, and their intrinsic and extrinsic properties.

an improved chemical and colloidal stability and a reduced tendency to form a protein corona. Therefore, the synthetic identity of a nanoparticle is very important for the evaluation of its nanotoxicity.

Biological Identity: The third identity type, the biologic identity, becomes relevant as soon as nanoparticles are exposed to a biological environment. Even though different surface coatings might slow down the adsorption of various biomolecules, e.g. proteins, enzymes, lipids or salts, and thereby, the formation of a protein corona on a nanoparticle, it cannot be inhibited completely. This alters the intrinsic properties of a nanoparticle to the so-called extrinsic properties. Therefore, the biologic identity most likely represents the most important nanoparticle identity in terms of biodistribution across barriers, into target tissues, and cells – but at the same time is also the identity that has been least studied so far. In contrast to the physical and synthetic identity, the biologic identity cannot be analysed with routine physical-chemical characterisation methods but the affinity of any nanoparticle towards the biological entities in a certain biological environment always has to be assessed individually. Moreover, two aspects complicate these analyses: Firstly, the composition of the protein corona and primary influence the resulting nanoparticle kinetics depend strongly on the nanoparticles' portal of entry (administration/exposure route), and secondly, the formation of a protein corona is a dynamic process and thus, the composition of the corona changes over time, and may vary from one individual to another, depending on the physio-pathological situation. This makes it extremely laborious to determine the proteins of the corona and even more complicated to predict the interaction of a nanoparticle with its environment and/or its overall toxicity.

Key Aspects of Nanotoxicity

Different intrinsic material properties, namely the particle size, agglomeration, shape, chemical composition, stability, and surface chemistry, have a major influence on the resulting material toxicity and therefore, their relevance will be briefly presented.

***Particle size*:** Maybe the most important aspect for the toxicity of nanoparticles of any material class and dimensionality (0D, 1D, 2D or 3D) is their particle size. Featuring at least one dimension smaller than 100 nm particles are considered as nanoparticles that potentially cause nanotoxicity. This 'size-induced' nanotoxicity arises from the fact that with sizes as small as nano-organisms, e.g. viruses, nanoparticles could potentially penetrate physiological barriers. By entering the systemic circulation and cells of a human being, they can cause the disruption of physiological functions which in turn can lead to tissue inflammations, altered redox balances, or in the worst case, to cell death. However, a closer look at the critical particle sizes reveals that relatively large (> 200 nm) and very small nanoparticles (< 5-15 nm) tend to be less harmful. This can be attributed to the fact that larger nanoparticles with sizes > 200 nm are typically easily detected by the immune system and removed from the blood circulation by accumulation in the liver and spleen. In case of very small nanoparticles, they can be

excreted through the kidney by renal filtration. Consequently, nanoparticles with sizes from 15-200 nm will last the longest time in the circulatory system which could modify their biodistribution and thus, could generate the greatest harm to the body.

***Agglomeration*:** Following with the particle dimensions, the tendency of nanoparticles to agglomerate in biological fluids is also a highly important physicochemical aspect for the evaluation of their nanotoxicity. The great impact of the agglomeration of nanoparticles on their resulting nanotoxicity can be explained by the direct influence on their overall particle size: agglomerated nanoparticles exhibit a significant increase in particle size and thus, their biodistribution changes and the effective nanotoxicity generally decreases. However, a typical example of severe toxicity associated with the nanoparticle aggregation is the formation of emboli in blood, leading to embolism and even death. On the other hand, when nanoparticles feature a good colloidal stability, i.e. they stay homogeneously dispersed in environmental or biological fluids, their overall particle size is not altered, and neither is their potential nanotoxicity. As a result, one always has to carefully evaluate the colloidal stability of any nanoparticle in various body fluids to gain an impactful insight to their potential nanotoxicity.

***Shape*:** In addition to the size and agglomeration of nanoparticles, also their shape plays an important role for their resulting nanotoxicity. In different studies a nanoparticle's toxic effect and process of endocytosis were systematically evaluated on different inorganic nanoparticles with a special attention paid to their shape, including nanocubes, nanohexagonal prisms, nanospheres, elongated nanospheres, nanodiscs, nanorods, and nanowires, while the other nanoparticle parameters were kept consistent.[14] Even though trends for particular nanoparticles could be observed, a generalization of individual results has not been possible so far. This might be attributed to the fact that the different nanoparticle shapes might affect other nanoparticle features as well as their motion behaviour in biological environments, causing significantly different reactivities, biodistribution and elimination. Therefore, it is highly problematic and usually not reliable to assess the hazardous effect of nanoparticles based on their shape alone, neglecting other nanoparticle features.

***Chemical composition*:** Besides the particle size, its agglomeration behaviour and its shape, of course also the composition of a nanoparticle has a strong influence on its nanotoxicity. In general, nanoparticles are divided into materials based on inorganic and organic building blocks. Inorganic nanoparticles include metals or metal oxides and their toxicity depends on the particular metal, its oxidation state and of course its dose. The high toxicity of metals results, with some exceptions, from their absence of biodegradability, and ability to imitate functions of essential elements in such a way that metabolic processes are severely disturbed. Very often this is the case for heavy metals. The toxicity of organic nanoparticles, i.e. carbon-based materials, is mainly caused by their ability to cross membrane barrier which can induce harmful effects such as inflammation. This behaviour is strongly affected by their structure, size distribution, surface chemistry, and agglomeration state. Actually, the bio interface of both nanoparticle types and the nanoparticles themselves are typically altered relatively quick by their biological environment, i.e. a so-called biotransformation takes place. Thus, no general statements concerning the nanotoxicity of certain material classes can be made, but the nanotoxicity has always to be assessed individually for each nanoparticle formulation.

Surface chemistry: The external surface of a nanoparticle, which represents the area of interaction of a nanoparticle with its environment, is also a very important aspect from the toxicological perspective. Especially in case of nanoparticles, where the decreased particle size causes a significant increase in the outer surface area. An increased surface area leads to a greater proportion of atoms being exposed to the biological environment which typically boosts the chemical reactivity of a nanoparticle. Therefore, nanoparticles immediately adsorb macromolecules (proteins, enzymes, lipids etc.) on their surface and thus, form a so-called 'protein corona'.[15] This can negatively affect biological processes, disturb regulatory mechanisms, and also trigger toxic effects of nanoparticles, for instance by enhancing their cellular uptake or by inducing strong complement activation. In case of nanoparticles with surface charges, the adsorption can be enhanced even further. Positive surface charges are believed to be more hazardous to cells since the negative charge of the cellular lipid bilayers might favour their interaction and uptake.[11] Positively charged nanoparticles, for instance, may trigger hemolysis after intravenous administration.[11] All in all, the boosted chemical reactivity of nanoparticles causes that their original surface chemistry will quickly be altered and thus, the prediction of the corresponding nanotoxicity is very difficult.

State-of-the-Art of the Toxicity Evaluation of MOF Nanoparticles

As the singularity and strength of MOF nanoparticles is derived from their organic-inorganic hybrid feature, one has to elucidate whether this hybrid character of MOFs poses unique challenges when characterising and assessing their toxicity. The opportunity of a targeted design of MOF nanoparticles enables the combination of the best, individual properties, and the compensation of the weaknesses of their different components in one system which makes MOF nanoparticles ideal for special purposes and for various applications – but at the same time, this means that a physicochemical characterisation, including studies with biological fluids, is needed for each MOF nanoparticle formulation as well as its individual building blocks. Similar to the afore mentioned nanoparticles, the different MOF nanoparticle identities have to be considered, when assessing the hazardous potential of MOF nanoparticles. Therefore, their special features will be briefly introduced first and subsequently, the state-of-the-art of the evaluation of the *in vitro* and *in vivo* toxicity of MOF nanoparticles will be given.

MOF Nanoparticle Characterisation – Physical Identity, Synthetic Identity and Biological Identity

Physical Identity: The physical identity of MOF nanoparticles is strongly influenced by the applied MOF synthesis route. Herein, various synthesis parameters can be varied such as the precursors, the solvent, the reaction time, and the temperature. As a result, different as-synthesised MOF nanoparticles can feature the same chemical composition, but exhibit quite different characteristics: *(i)* they can significantly differentiate in their particle size (ranging from nm to µm); *(ii)* the surface chemistry can be influenced by the used media; *(iii)* the integrity of the resulting MOF framework can vary reasonably depending on the employed amount of modulator agents present during the synthesis, which increase the reproducibility and crystallinity of the final product; *(iv)* and the pore content is determined by the used solvent or precursors. Consequently, it is important to emphasize that different MOF synthesis routes can yield MOF nanoparticles with the same chemical composition but with quite different physical identities.

Synthetic Identity: Postsynthetic external surface modifications can be employed to control and manipulate the behaviour of MOF nanoparticles, and subsequently, such modified MOF nanoparticles exhibit synthetic identities. So far, the grafting of surface coatings with polymers such as heparin or polyethylene glycol (PEG) and its modifications with different molecular weights and functional groups or other hydrophilic polymers has shown to successfully minimise the adsorption of proteins, lipids and other biomolecules while not hampering the MOFs' properties.[16,17] Therefore, coatings are often desired when intending medicinal or biological applications of MOF nanoparticles. However, it is not *a priori* predictable, and a careful assessment is required, whether the same surface modification grafted onto different MOF nanoparticles actually results in the same synthetic identity or rather causes different behaviours after exposure to a biological environment, since not only the nature of the coating is important but also the density and the conformation, among others. A special feature of MOF nanoparticle coatings, for example, is that the coating may be located exclusively at the surface of the particle or partly inside the pores.

Biological Identity: Even though surface modifications might minimise or slow down the adsorption of biomolecules, it is still likely to happen to certain extent and then, also functionalized MOF nanoparticles feature biological identities. In general, one can distinguish between the 'hard corona', where molecules with a high affinity are strongly bound to the MOF nanoparticle, and the 'soft corona', where molecules with a low affinity are loosely bound to the surface and can easily be exchanged over time.[18] These surface bound proteins and molecules will have a major impact on the fate of the MOF nanoparticle, and therefore, it is essential to identify those proteins. However, this is relatively complex as the adsorbed biomolecules and proteins are highly dependent on the route of administration of the nanoparticle. For a physico-chemical characterization of the 'protein corona' different analysis techniques can be applied: *(i)* SEM for the particle size distribution and morphology; *(ii)* FT-IR for the qualification and quantification of characteristic bands of the biomolecule; *(iii)* mass spectrometry, gel electrophoresis and immuno-assays for the identification and quantification of adsorbed proteins or biomolecules; *(iv)* TGA for the quantification of the amount and the thermal stability of the adsorbed biomolecules, *(v)* ITC for evaluating the adsorption enthalpy; and *(vi)* fluorescence studies for the conformation of some proteins,[17] the characterisation of the interaction, rearrangement, ground-state complex formation, excited-state reactions, energy transfer and collision in molecular levels. Additionally, cytotoxicity and cell uptake studies can be very insightful for the determination of the influence of the 'protein corona' on the cytotoxic effect and the cell internalisation.

Toxicity Studies of MOF Nanoparticles

To assess the toxicity that is caused by the exposure of MOF nanoparticles on living organisms' different methods have mainly been employed, i.e. *in vitro* and *in vivo* studies. In both cases various parameters, including different concentration ranges and decent exposure times, can be screened, but the two methods enable the assessment of different levels of toxicity: *In vitro* studies facilitate a high-throughput analysis of the cytotoxic effect on different cell types and/or target-organ cells without ethical issues, while *in vivo* studies also elucidate the biodistribution and provide information about the behaviour in the much more complex biological systems of whole organisms. Over the last 15 years, *in vitro* and *in vivo* studies with approximately 50 different MOF nanoparticles have been conducted with various different cell lines and animals – mainly to validate their suitability for medicinal applications. By evaluating the data available in literature, it was possible to present the percentage of the 'top 10 MOF' frameworks which have been analysed in toxicity studies (**Figure 6**) as well as the percentage of different *in vitro* and *in vivo* studies (**Figure 7**). In the following only some highlights of the *in vitro* and *in vivo* studies will be depicted (**Figure 8**). In addition, few *ex vivo* studies have been performed on MOF nanoparticles considering an oral or dermal exposure, evaluating both their physiological barrier bypass, i.e. through the skin and intestine, respectively, and their toxicity over these tissue extracts.[19–21] Finally, although not yet applied to the toxicological evaluation of MOF nanoparticles, *in silico* models could be a very useful tool to predict their toxicity as a function of the multiple and diverse variables of MOFs, e.g. composition, topology, size, and shape.[5]

In vitro Studies: As *in vitro* toxicity studies have shown to be extremely beneficial as they are of low cost, rapid, do not involve animals, and enable high-throughput screenings, different *in vitro* assays, i.e. mainly MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), but also Cell Counting Kit-8 (CCK-8), MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2*H*-tetrazolium), XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide), among other, have been employed to assess the MOF nanoparticles toxicity.

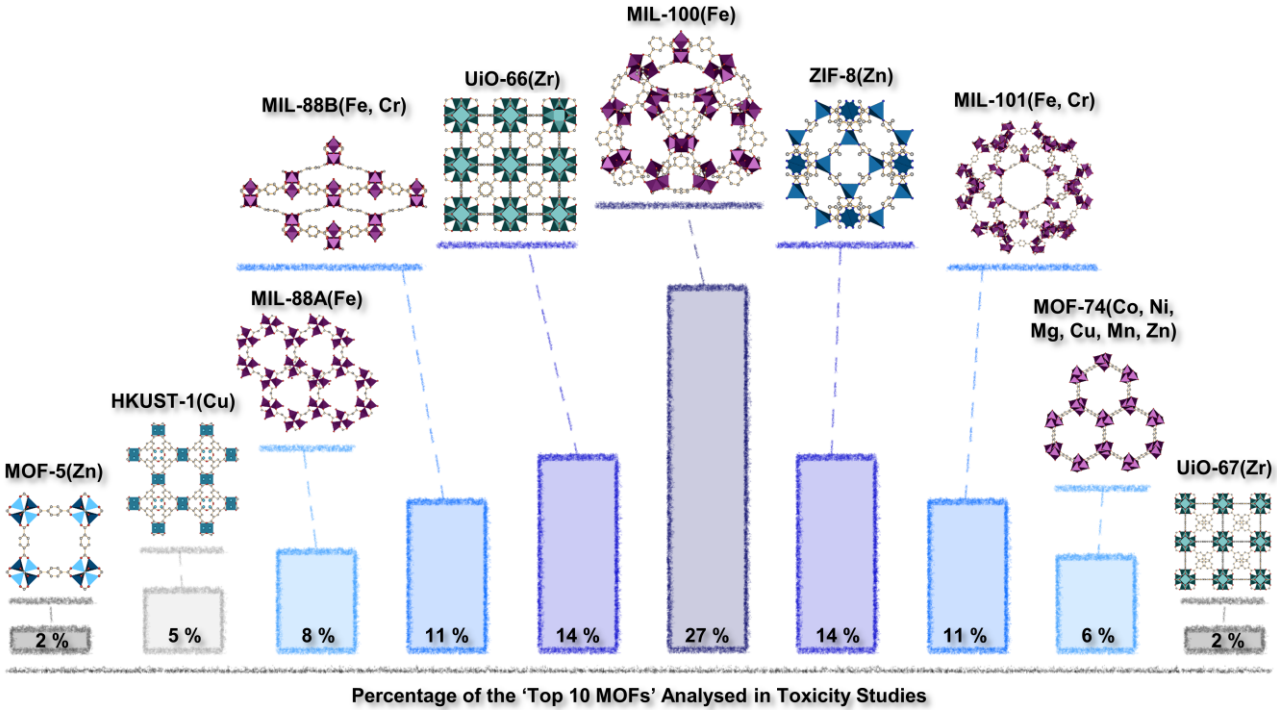

Figure 6: Overview of the percentages of the 'Top 10 MOFs' analysed in toxicity studies, determined by analysing 95 publications.

In general, it is important to be aware of the individual parameters which are determined by the cytotoxicity assays and whether the test samples could interfere with the measurement principles. For instance, MTT, MTS and XTT are based on the reduction of the assay reagents and cases of interference with redox-active test compounds have been reported which generated contradictory results compared to other viability assays.[22]

For all these assays, cell lines are incubated with a range of different MOF nanoparticle concentrations (mg/mL) so that the cytotoxic effect to the different cell lines can be evaluated with respect to the MOF concentration. Usually, after a time period of 24 h, 48 h and 72 h the cell viability is assessed, and typically, the IC_{50} value is determined.

In 2008, Lin *et al.* reported the pioneering work of a nanoscale coordination polymer (NCP) with cytotoxicity towards cancer cells based on a drug containing organic linker. The *in vitro* cytotoxic effect of NCP-1, i.e. a Tb-based MOF with *c,c,t*-(diamminedichlorodisuccinato)Pt(IV) bridging ligands, on the angiogenic human colon carcinoma cell line HT-29 and the human breast carcinoma cell line MCF-7 was analysed.[23]

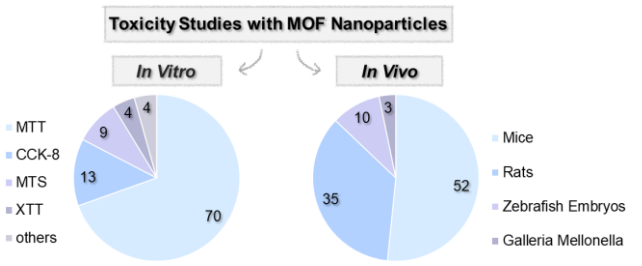

Figure 7: Percentage of different *in vitro* and *in vivo* toxicity studies performed with MOF nanoparticles, determined by analysing 95 publications.

Herein, the authors prepared the Pt-containing MOF nanoparticles, coated them with thin shells of amorphous silica, and subsequently, demonstrated the anticancer efficacies of their Pt-drug containing MOF. The IC_{50} values of 9.7 and 11.9 µM obtained for their two modified NCP-1 samples, were comparable or even slightly lower than the reference of their cisplatin standard with an IC_{50} value of only 13.0 µM. As the cytotoxic effect of the modified NCP-1 nanoparticles on the tumorous tissue was desired, further toxic evaluation with healthy cells was not conducted.

From 2010, Horcajada, Gref, Serre *et al.* systematically evaluated the cytotoxic effects of various Fe-based carboxylate MOFs, i.e. MIL-53 with terephthalic acid, MIL-88A with fumaric acid, MIL-88B-$4CH_3$ with tetramethyl-terephthalic acid, MIL-89 with muconic acid, MIL-100 with trimesic acid, and MIL-101-NH_2 with amino terephthalic acid, MIL-127 with azobenzenetetracarboxylic acid, as well as the Zr-based MOF UiO-66 with terephthalic acid and the Zn-based MOF ZIF-8 with methyl-imidazole. Cytotoxicity of the different MOF species were tested on various cell lines, including human macrophages J774, human leukaemia (CCRF-CEM), human multiple myeloma (RPMI-8226) and human cervical adenocarcinoma (HeLa) cells.[24–27] Due to the great diversity of MOF nanoparticles, the authors succeeded in identifying two main aspects affecting the cytotoxic effect of MOF nanoparticles that can be attributed to the respective MOF components: *(i)* with respect to the nature of the metal within the MOF, the Fe-based MOFs showed less cytotoxicity than the Zr- or Zn-based ones and *(ii)* concerning the organic linker, the hydrophobic–hydrophilic balance turned out to be an important factor. Moreover, cell uptake studies indicated that higher cytotoxic effects are in good agreement with a faster cellular uptake of the respective nanoparticles.[26]

In 2015, Horcajada and Devic *et al.* investigated the toxic effect of Mg-gallate microparticles that is composed solely from well tolerated reagents.[28] Herein, the authors report that Mg-gallate only induces a very low cytotoxicity and that its progressive release of gallic acid in physiological fluids significantly reduced the ROS production, acting thus as a strong antioxidant agent.

In 2015, Fairen-Jimenez *et al.* were the first to compare the influence of the crystallinity of UiO-66(Zr) nanoparticles on their toxic effect to tumorous human HeLa cells.[29] The authors loaded crystalline UiO-66(Zr) with calcein and amorphized the nanoparticles by ball-milling. In doing so, the authors showed that the drug release time can be extremely prolonged by the amorphization, while its cytotoxic effect remained similar as compared to crystalline UiO-66(Zr), ruling out the effect of the crystallinity degree on the MOF cytotoxicity.

In 2017, Wuttke and Meiners *et al.* were the first to consequently follow and monitor the toxic effect of MIL-100(Fe), MIL-101(Cr) and Zr-Fumarate nanoparticles on different cell lines.[30] Herein, the toxic effect on human endothelial and mouse lung cells, human gingiva fibroblasts, human Schwann cells and rat dorsal root ganglion cultures was assessed. All in all, the authors could show that the cytotoxicity strongly varies depending on the effector cell type, and that an evaluation with respect to the particular application of MOF nanoparticles and their interacting with primary cell types always has to be determined carefully.

In 2018, Fairen-Jimenez and Forgan *et al.* proposed the alternative utilization of Zr-Fumarate as DDS instead of UiO-66(Zr). It was shown that dichloroacetate-loaded Zr-Fumarate selectively kill HeLa and MCF-7 cancer cells, while the nanoparticles were well tolerated by HEK293 kidney cells, J774 macrophages, and human peripheral blood lymphocytes.[31] Moreover, the authors reported that Zr-Fumarate induces less ROS than the UiO-66 analogues.

Also in 2018, Ettlinger and Bunzen *et al.* were the first to propose the utilisation of triazolate-based MOF MFU-4l(Zn) nanoparticles as DDS as they feature the ability to directly bind high amounts of highly toxic arsenic trioxide to the MFU-4l framework, and thus, enable a pH-triggered drug release.[32] The corresponding cytotoxicity studies were carried out with tumorous BT12, BT16, CHLA-06, and 311-FHTC cells, in comparison to healthy tissue cells, i.e. fibroblasts and LLC-PK1 cells. Here, it was validated that the arsenic-loaded nanoparticles showed a cytotoxic effect to the tumorous cell lines, while the non-loaded MFU-4l(Zn) nanoparticles as well as its ligand did not cause critical harm for the tested cell cultures.

In 2020, Serre and Pinto *et al.* reported that the tuning of cellular biological function is possible with the carboxylate-based MOF MIP-177(Ti).[33] Herein, the authors designed the MOF in a way that it features a high NO storage capacity and a favourable NO release in biological media. Moreover, the performed *in vitro* studies with HeLa cells revealed that MIP-177(Ti) exhibits an excellent biocompatibility, which makes it a promising candidate for a new NO delivery system for wound healing therapy.

All in all, *in vitro* studies have been established to give a first insight in the potential toxic effects of MOF nanoparticle formulations. It has to be noted that *in vitro* assays have evolved immensely from the initial simple screenings in standard cell lines, such as HeLa and are increasingly tailored towards comprehensive evaluations in more specific models representing diseased and healthy tissues likely to be encountered by the MOF nanoparticles.

In vivo Studies: So far, five different *in vivo* methods were used for the toxicity assessment of MOF nanoparticles on living organisms as well as their biodistribution: mainly mammalian models with mice and rats, but also zebrafish embryos (Dario rerio),[34,35] crustaceans (Gammaropsis atlantica amphipods),[36] and way moths (Galleria Mellonella).[37]

In 2010, Horcajada and Gref *et al.* performed the first pioneering acute and subacute toxicity *in vivo* experiments with different Fe-based carboxylate MOF nanoparticles, i.e. MIL-88A(Fe), MIL-88B-CH_3(Fe), and MIL-100(Fe).[24] These were prepared with an intentional application for drug delivery, theranostic agents, combining their properties and as contrast agents for magnetic resonance imaging (MRI). Very high doses (110-220 mg/kg) were intravenously administered into Wistar rats *via* the jugular vein. In the subsequent three months the animals were monitored with a special attention paid to their body weight, pathologic organ transformations, biochemical parameters (alterations in their cytochrome P-450 activity, ALT, AST transaminases levels, and interkeukine-6 serum concentration, among others) and histopathology. No immune or inflammatory reactions were detected, and the Fe-based carboxylate MOFs can be considered as well tolerated.

In 2014, for the first time an *in vivo* mouse model was investigated with Gd-pDBI (pDBI = 1,4-bis(5carboxy-1H-benzimidazole-2-yl)benzene) by Banerjee *et al*.[38] Herein, concentrations between 0.05-0.15 mg/kg were administrated intravenously, and no significant toxic effects or body weight loss, only minor alterations in the liver associated with the nanoparticle accumulation, were observed.

In 2015, Ruyra *et al.* assessed the toxicity of sixteen different MOFs, i.e. HKUST-1(Cu), MIL-100(Fe), MIL-101(Fe), MOF-5(Zn), MOF-74(Co, Ni, Mg, Cu, Mn, Zn), NOTT-100(Cu), UiO-66(Zr), UiO-66-NH_2(Zr), UiO-67(Zr), ZIF-7(Zn), and ZIF-8(Zn), in an *in vitro - in vivo* correlation approach.[35] Herein, the stability of the MOF nanoparticles in culture medium was analysed, the *in vitro* cytotoxicity of the components and MOF nanoparticles was assessed in 2D cell cultures with human HepG2 and MCF-7 cells, and finally compared to the results obtained from a zebrafish embryo *in vivo* model. The authors found a strong correlation between the *in vitro* and *in vivo* studies for all MOF nanoparticles with the exception of MIL-101(Fe), which exhibited higher toxicity in the *in vivo* model. Moreover, the authors could show that not all MOF nanoparticles that were exposed to biological fluids necessarily degraded by releasing the constitutive metal ions and ligands, but that some MOF nanoparticles became amorphous and underwent structural rearrangements which triggered reactions that modified the inorganic species, which might be responsible for the observed adverse effects.

In 2018, another *in vivo* model, i.e. Galleria Mellonella, was utilised by Tosheva and Wee *et al.* for the evaluation of MOF

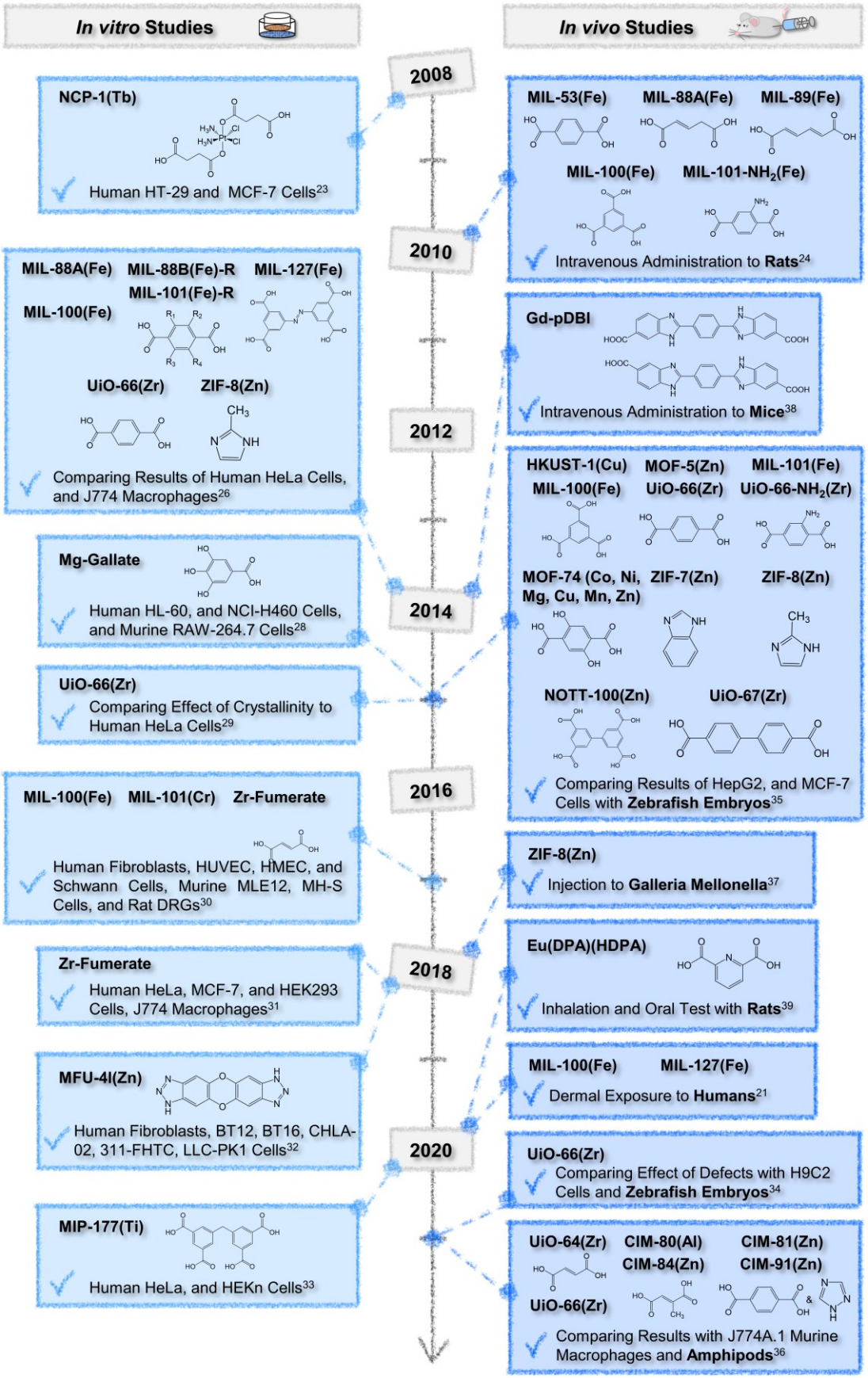

Figure 8: Overview of some *in vitro* and *in vivo* toxicity studies of various different MOF nanoparticles.

nanoparticles.[37] Herein, the authors assessed the toxic effect of ZnO-ZIF-8(Zn) composites on microorganisms, i.e. *K. pneumoniae*, *P. mirabilis*, *E. coli*, and *S. aureus*, as well as by injection into the way moth Galleria Mellonella. Their finding

led to the conclusion that their composite did not show higher toxicity in the *in vivo* model than distilled water.
In 2020, Weber *et al.* conducted acute oral and inhalation tests in rats with the luminescent MOF Eu(DPA)(HDPA), which had previously been found as efficient marker for gunshot residues.[39] The authors could not detect any clinical or pathological signs of toxicity, signs of irritation or inflammation.
Also in 2020, Horcajada *et al.* were the first to investigate the toxic effect caused by the dermal exposure of patches of two different MOFs, namely MIL-100(Fe) and MIL-127(Fe), to human volunteers. The absence of dermal toxicity, either empty or containing a combined antimicrobial therapy (nicotinamide and azelaic acid), proved that a new efficient cutaneous DDS was prepared.[21]
In 2021, also Jodłowski and Boguszewa-Czubara *et al.* revealed a comparison between results for UiO-66(Zr) nanoparticles obtained either from *in vitro* studies with H9C2 cells or from a zebrafish embryo *in vivo* model.[34] The UiO-66(Zr) nanoparticles herein were employed as DDS for chloroquine diphosphate and during the experiments the fertilized zebrafish eggs were monitored with respect to their growth and heartbeat. In comparison to free chloroquine diphosphate, the mortality rate and reduction of heart rate was significantly decreased when chloroquine diphosphate was encapsulated in UiO-66(Zr). indicating that the MOF nanoparticles provide protection and no toxic effect.
Also in 2021, Lorenzo-Morales *et al.* performed a novel *in vitro - in vivo* correlation approach using J774A.1 murine macrophage cell lines for *in vitro* studies, and Gammaropsis atlantica amphipods as an *in vivo* model.[36] The authors compared the results for six MOF microparticles, namely UiO-64(Zr), UiO-66(Zr), CIM-80(Al), CIM-81(Zn), CIM-91(Zn), and CIM-84(Zr), and found strong similarities between both assays. Although this showed great potential of the novel *in vivo* model using amphipods, it is important to note that all MOFs studied were of micrometre size and thus further studies should be conducted with MOF nanoparticles before considering the model for high-throughput toxicity screenings.

In sum, different *in vivo* models have already been established and utilized for the toxicity evaluation of MOF nanoparticles. Beside injections, also oral intake and inhalation, and dermal exposure have been investigated as alternative routes of administration. Such *in vivo* models provide indispensable information on the behaviour of the nanoparticles in the more complex biological systems of whole organisms. In the published examples of *in vitro – in vivo* correlation studies, obtained findings about the toxicity of investigated MOFs were relatively consistent. It is not expected that this is necessarily always the case. Nevertheless, to minimise the ethical issues of animal studies in rodents or other higher organisms, the use of *in vitro* zebrafish cells and embryos, and Gammaropsis atlantica amphipods have been proposed as alternatives to the excessive use of classical *in vivo* tests.[40] But even though a good agreement between *in vitro* and *in vivo* studies has been reported in certain cases,[35,41] there are also examples where the *in vitro* results, e.g. for the linker of MIL-88A(Fe), indicted a high cytotoxicity while the corresponding *in vivo* studies showed no significant harm.[26] In addition, the toxic effects of MOF nanoparticles and their respective components is highly dependent on the cell lines used in the *in vitro* assays. As a result, predicting the toxic effect of the same MOF nanoparticles on different cell lines is extremely complicated and empirical evaluation is indispensable. The same situation is represented by the fact that MOFs are often only investigated in the selected target cells, i.e. cancer cells, as their potential anticancer effect is intended to be proven. However, studies have shown that the results obtained from tumour cells are not directly comparable to healthy cells, as these feature a different cell biology.

Key Factors Affecting the Toxicity of MOF Nanoparticles

Similar to other nanoparticles, also for MOF nanoparticles their dose, physico-chemical properties, biotransformation, and their chemical composition have a huge impact on their resulting toxicity. Therefore, these aspects will be discussed in the following. However, it is important to mention that a direct comparison of all available toxicity data is generally rather complicated or inaccurate because different toxicity assessment set-ups, i.e. different tested concentrations, cell lines or *in vivo* models, have been used. Thus, only main trends can be identified instead of making absolute statements.

Dose Dependency

As Paracelsus stated very well 'the dose makes the poison', i.e. the administered dose of a substance is a fundamental parameter that determines its toxicity. There is no doubt that this statement is also valid for MOF nanoparticles – but it is definitely not sufficient to only rely on the administered dose itself: MOF nanoparticles have the ability to bypass physiological barriers and to accumulate in a specific tissue or organ, which can significantly increase their local concentration, and might subsequently cause severe side-effects. Therefore, toxicity studies are typically performed with higher doses and dose regiments than those used in medical practice.
Furthermore, it is crucial to consider the potential routes of administration of MOF nanoparticles, and to study their ADME series (absorption, distribution, metabolism and excretion) in relevant whole organism *in vivo* models in addition to dose titrations *in vitro*. Additionally, in *ex vivo* models the biodistribution of MOF nanoparticles can be evaluated.

Physico-chemical Properties of MOF Nanoparticles

To achieve a deeper fundamental understanding of a MOF nanoparticle's toxicity, the different physico-chemical properties of MOF nanoparticles that affect their toxicity will be discussed in the following. Special attention will be paid to their size and shape, surface chemistry, colloidal stability, and chemical stability in biological media. It is important to note that

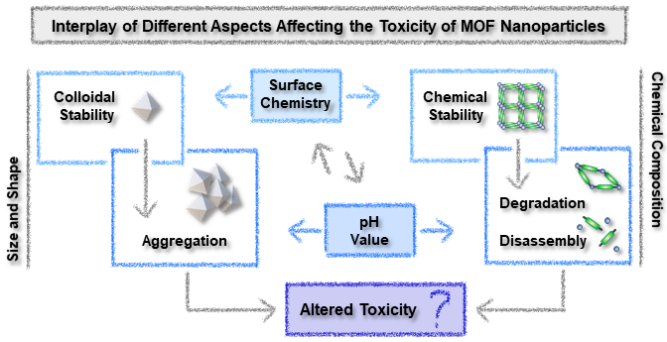

Figure 9: Interplay of the different aspects affecting the toxicity of MOF nanoparticles, i.e. colloidal and chemical stability, aggregation, degradation or disassembly, the influence of the surface chemistry and the external pH value.

they are mutually linked and that they can be strongly influenced under physiological conditions by factors such as varying exterior pH values (**Figure 9**).

Size and shape: In fact, the size of MOF nanoparticles plays a crucial role for their resulting toxicity, since it clearly influences two other physico-chemical properties: first, analogous to other nanoparticles, as particle size decreases, their ability to penetrate physiological barriers, i.e. to enter cells, increases significantly, and thus the likelihood that smaller MOF nanoparticles could cause severe damage is much higher compared to larger analogues. Secondly, a reduction of particle size can also have a negative impact on the stability of MOF nanoparticles in biological media, as smaller particles expose a larger surface area to their microenvironment and diffusion into the nanoparticles is much faster, typically resulting in a faster degradation of smaller particles for a given MOF. However, one must always assess the colloidal stability or agglomeration behaviour of MOF nanoparticles with particle sizes smaller than 200 nm in biological media – and many non-functionalised MOFs tend to agglomerate – before one can make a prediction about a potential toxic effect.[42]

Although one might expect a more or less linear correlation between the decrease in particle size as well as the decrease in stability of MOF nanoparticles, this statement is not generally valid for all MOFs. In fact, stability studies for the ZIF-8(Zn) with 250 nm and 2 µm sized particles in phosphate buffered saline (PBS) media clearly showed the faster decomposition for the smaller ZIF-8(Zn) particles,[43] while similar studies for the Zr-Fumarate MOF with 25 nm and 170 nm sized particles revealed a faster decomposition of the larger particles.[31] Consequently, the stability of MOF nanoparticles is not only affected by their particle size, but one also has to take into account various further parameters and structural features of the respective MOF nanoparticles: the oxidation state of the metal, nature and nuclearity of the metal cluster, the strength of the respective metal to linker coordination bonds the nature and number of the complexing functions, the presence of open metal sites and/or defects, the hydrophilic/hydrophobic balance of the MOF, as well as its pore size(s), among others. and also, the nature of the biological corona which may provide colloidal stability to small nanoparticles.

The influence of the shape or topology of MOF nanoparticles has not yet been investigated in depth. However, the toxicity data available for the MOFs MIL-88(Fe) and MIL-100(Fe)/MIL-101(Fe), which consist of the same constituents but feature a different topology, shows that they cause very similar cytotoxic effects.[26] This suggests that the structure of MOF nanoparticles, unlike other nanoparticles, might only play a minor role for their overall toxicity compared to other aspects, such as their chemical composition. To verify this hypothesis, further studies are definitely needed in the future.

Surface Chemistry: The external surface of MOF nanoparticles is the 'theatre of the interactions' with the living media, and therefore, it has a huge influence on the resulting toxicity. As many researchers have focused on the utilisation of MOF nanoparticles for medical applications, e.g. as DDS, great efforts have been made to study the manipulation of their surface properties.[3] The grafting of polymer coatings has attracted a lot of attention as it offers many advantages: One can fine-tune the colloidal and chemical stability of MOF nanoparticles in diverse biological environments, minimise the adsorption of biomolecules, lipids or proteins, *i.e.* the formation of biological corona, or favour specific physiological pathways. However, it is of the upmost importance to always keep in mind that this synthetic identity of MOF nanoparticles will relatively quickly change to their biological one when exposed to biological media. Consequently, the interactions on the bio-interface will determine the *in vivo* fate of MOF nanoparticles, *i.e.* aggregation, biodistribution or elimination, and thus, their resulting toxicity.[3]

MIL-100(Fe) is the most prominent MOF in toxicity studies as it is one of the most promising MOF for biomedical applications.[3] It is a great example for the effectiveness of surface grafting: non-functionalised MIL-100(Fe) nanoparticles agglomerate after a short period of time under physiological conditions,[44] but when cyclodextrin,[45] heparin,[17] or a crosslinked polyethylene glycols (PEG) have been anchored onto their external surface their colloidal and chemical robustness is significantly enhanced.[46] Besides the improvement of colloidal and chemical stability, surface modifications have also been reported as effective tool for the selective cytotoxicity and targeted delivery of the Zr-based MOF nanoparticles, such as Zr-Fumarate,[31] and UiO-66(Zr).[47] Another interesting aspect of surface functionalisation is their impact on drug release kinetics. Especially for a high efficiency drug delivery in cancer therapy; the coating of different MOF nanoparticles, such as MFU-4l(Zn), UiO-66(Zr) or ZIF-8(Zn) with PEG turned out to be very powerful to gain a better control over drug release kinetics.[32,47,48]

In summary, great success has already been achieved in understanding and controlling the surface chemistry of MOF nanoparticles. Although this knowledge might be helpful to give a first insight to the potential stability of MOF nanoparticles for the different routes of administrations or their most likely biodistribution pathways, it cannot be used to predict the overall toxic effect *a priori*.

Colloidal Stability: As discussed previously, the particle size determines the fate of MOF nanoparticle in the human body, and therefore, their colloidal stability represents an impactful aspect for their resulting toxicity. To analyse the colloidal stability, one has to consider different parameters such as the

pH value as well as the nature and concentration of salts in the test solutions, which might be relevant for physiological conditions.[3]

As mentioned, the colloidal stability of non-functionalised MOF nanoparticles is often poor in biological media, e.g. in studies with simulated physiological fluids MIL-100(Fe) only maintained colloidal stability during the first stages of incubation in representative oral and intravenous media.[44] Depending on the administration route, the required time is usually different, and a manipulation of surface properties is frequently implemented for intended medical applications.[46,49] However, in some cases it was also suggested that a good strategy would be to exploit the poor colloidal stability in biological media as an advantage to favour an intended biodistribution: the agglomeration of MIL-100(Fe) nanoparticles in blood triggers the accumulation in the lung capillaries and thus, a targeted delivery of drugs for pulmonary treatment can be achieved.[42]

To sum up, the extensive assessment of the colloidal stability or agglomeration behaviour of MOF nanoparticles in different biological media is fundamental in assessing their potential toxicity for different routes of exposure.

Chemical Stability in Biological Media: The stability of MOF nanoparticles under biorelevant conditions also strongly affects their resulting toxicity. Since MOFs consist of inorganic and organic building blocks that are connected *via* coordination bonds, it is important to consider the possibility that a fast MOF degradation inside an intracellular compartment and slow diffusion of the degradation species can lead to a significantly higher local concentration of metals or organic linkers, which subsequently might cause toxicity. Notably, the different pH values of the different locations in the human body have a huge impact on the degradation of MOF nanoparticles, i.e. by destabilising the metal to linker bonds. In general, the strength of these coordination bonds is determined by the nature of their respective metals and polydentate organic linkers, and it can be estimated according to the hard and soft acids and bases (HSAB) principle: hard metals, e.g. Ti^{4+}, Fe^{3+}, Cr^{3+}, and Zr^{4+}, form stable bonds with hard organic linkers, e.g. carboxylates, while soft metals, e.g. Zn^{2+}, and Cu^{2+}, form stable bonds with soft organic linkers, e.g. imidazolates, pyrazolates, and triazolates.[50] In the different pH ranges different driving forces will promote the MOF nanoparticle degradation (**Figure 10**): A more acidic environment will foster the competition of metal ions and protons to coordinate with the organic linker, and thus, triggers the decomposition of MOFs based on 'soft' constituents, i.e. ZIF-8(Zn).[51] While a basic environment will lead to a successive replacement of organic linkers by hydroxide ions, and therefore, the degradation of MOFs based on 'hard' constituents is faster, *i.e.* MIL-100(Fe),[44] or UiO-66(Zr).[52]

Another aspect which can enhance the chemical stability of MOF nanoparticles is the nuclearity of their inorganic building unit: ZIF-8(Zn) only features single-metal sites and is only stable at a pH higher 6, while the decomposition of MOF-74(Zn), which forms $Zn_2O_2(CO_2)_2$ 1D-chains, starts at pH values as low as 3.[53] Also the high stability of the Zr-based MOF nanoparticles UiO-66(Zr) and NU-1000(Zr), in the absence of phosphates, can be attributed to the high nuclearity of their Zr_6-metal clusters.[54] The presence of phosphate ions turned out to be a crucial factor for the degradation of MIL-100(Fe): while stable in water, degradation occurred at pH 7.4 when phosphates coordinated to the metal sites, leading to a ligand exchange and its release to the degradation media.[55]

However, the MOF nanoparticle stability can be enhanced by surface modifications, and also, by the incorporation of drugs. MIL-100(Fe) nanoparticles, for instance, could efficiently be stabilised by the loading of topotecan via a 'ship in a bottle' approach, i.e. the drug was allowed to diffuse into the pores of the MOF as individual molecules, and then it formed clusters, which practically filled all the available free volume.[56] This dramatically improved its stability, even in the presence of phosphates.

In conclusion, it is essential to be aware of the chemical stability of MOF nanoparticles, since this will determine at which location of the body and route of administration they may decompose and release their individual components. It is expected that in case of a very unstable MOF nanoparticle degradation is rapid and the biodistribution is mostly be determined by the nature of the leached constituents, whereas a stable MOF nanoparticle is slowly degraded and therefore its biodistribution is mainly influenced by the nature of the MOF nanoparticle (e.g. size, surface chemistry, or route of administration).

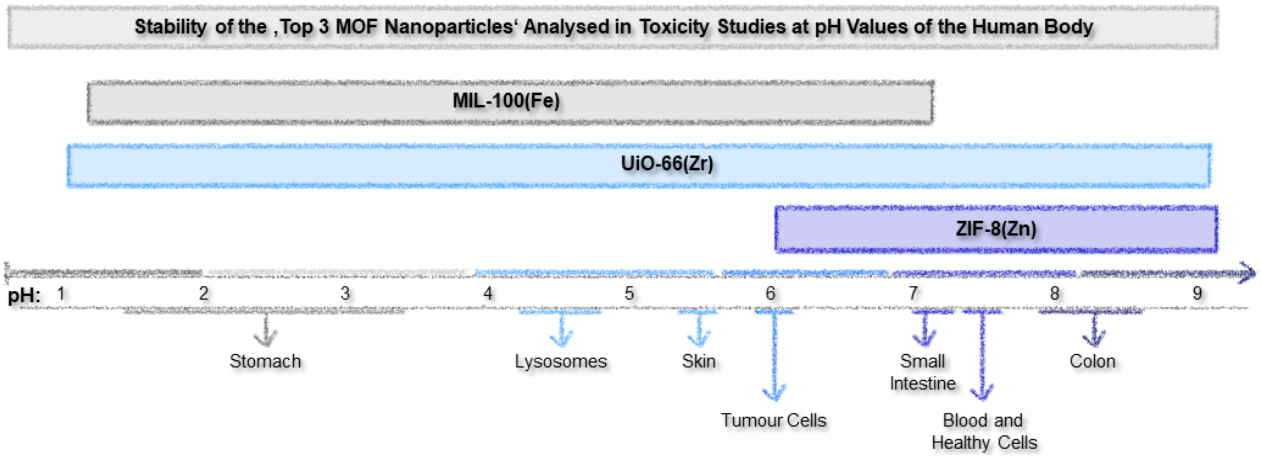

Figure 10: Stability of different MOF nanoparticles with respect to the pH values in the human body.

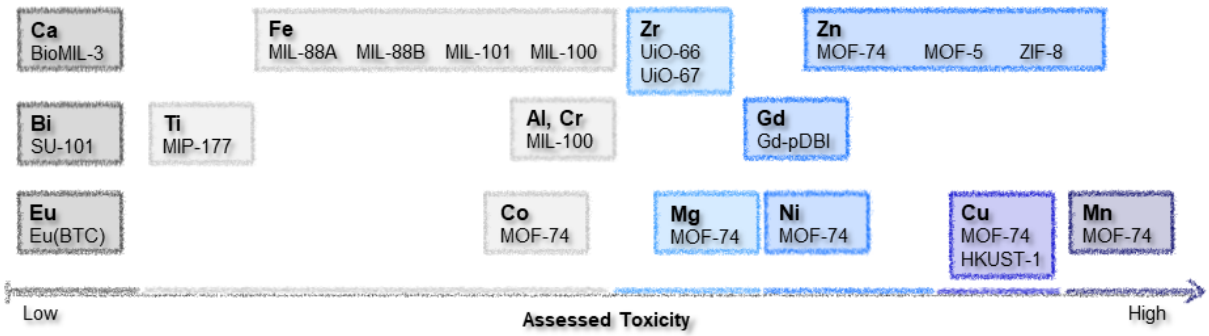

Figure 11: Ranking of MOF nanoparticles with different metals, based on their reported IC_{50} toxicity data.

Chemical Composition of MOF Nanoparticles

As MOF nanoparticles are composed of inorganic and organic building blocks, the toxic effect of both individual constituents is often used to consider the overall toxicity and will therefore be discussed separately.

Effect of the Inorganic Metal Cluster: The 'Top 10 MOFs' analysed in toxicity studies, namely MIL-100, UiO-66, ZIF-8, MIL-88B, MIL-101, MIL-88A, MOF-74, HKUST-1, MOF-5, and UiO-67 (**Figure 6**), contain nine different metals Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Zr, but also various other MOF nanoparticles with metals such as Ca,[57] Ti,[33] Bi,[58] Eu,[39] Gd,[38] and Tb[23] have been studied. Based on their reported toxicity data, these MOFs can roughly be ranked in terms of their cytotoxic effect (**Figure 11**). Herein, the least toxic MOFs contained the metals Ca, Bi, and Eu, followed by only slightly more toxic MOFs with Ti, Fe, Co, Al, and Cr, the moderately toxic ones with Zr, Mg, Gd, Ni, and Zn, while Cu and Mn containing MOFs were the most toxic ones. It is suggested that the toxic effect of the respective MOF nanoparticles is correlated to their degradation in biological media, i.e. the higher the degradation of the MOF, the higher release of its metal which can cause toxicity.[35] Herein, high valence cations have an influence on higher *a priori* stability of the resulting MOFs, but as mentioned before, also the different nature of the complexing groups of the linker impacts the stability. The ranking of Zn-based MOF nanoparticles in **Figure 11** shows that it cannot straightforwardly be assumed that MOF nanoparticles consisting only of metals that are present in significant amounts in the human body anyway, such as Fe and Zn, do not cause any noteworthy harm. In the case of Zn-based MOF nanoparticles, the free Zn^{2+} might compete with Fe^{2+} and Ca^{2+} cations and thereby, cause dysfunctions of transcriptional regulation and synthesis of proteins as well as DNA damage. Consequently, by utilising bio-compatible metals one can only increase the likelihood that the resulting MOF nanoparticles are non-toxic, but it is impossible to predict their actual toxic effect *a priori* solely based on the metal.

Effect of the Organic Linker: To evaluate the effect of the nature of the organic linker (e.g. different complexing groups such as azolates, or carboxylates), it is required to analyse MOF nanoparticles with the same metal that are isoreticular, i.e. that exhibit the same structure type but with ligands of various sizes. A great effort has already been made to compare the Fe-carboxylate MOFs, such as MIL-88, and MIL-100/MIL-101 structures with different functionalisations (**Figure 12**).[26] Herein, endogenous fumaric acid clearly turned out to be the most bio-friendly organic linker, followed by exogenous trimesic acid, amino-terephthalic acid, and terephthalic acid, and the nitro- and tetramethyl-terephthalic acid modifications were the most toxic linkers. Moreover, a more or less linear correlation between the cytotoxicity of the organic linker and the corresponding MOF nanoparticles was observed. Such a 'rule of thumb' can be extremely helpful for the future choice of novel organic linkers for MOF nanoparticles. Furthermore, the importance of the hydrophobic–hydrophilic character of the organic linker was highlighted as this directly influences the excretion rate of the MOF nanoparticles: MOF nanoparticles based on hydrophobic linkers, e.g. tetramethyl terephthalate-based MIL-88B, are only slowly *in vivo* removed likely due to a higher association with lipid droplets in the lysosomes of Kupffer cells in the liver, while MOF nanoparticles that are based on hydrophilic linker, *e.g.* trimesate-based MIL-100, are excreted relatively fast.[59] Thus, already the full characterisation

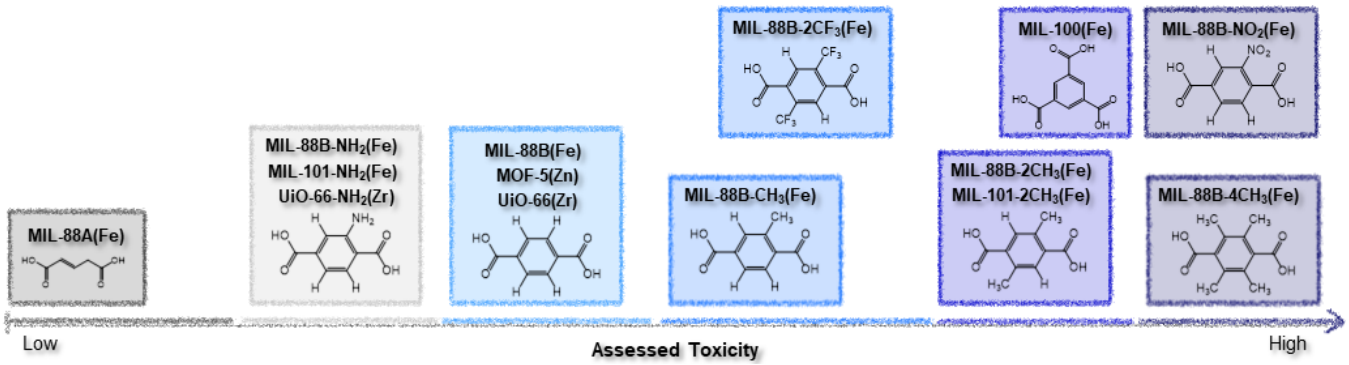

Figure 12: Ranking of different organic linkers, based on their reported IC_{50} toxicity data.

of the organic linker can be really insightful for the subsequent toxicity ranking of MOF nanoparticles.

To sum up, the resulting toxicity of MOF nanoparticles cannot be predicted *a priori* only on the basis of their building blocks and the structure. However, it is recommended to use their reticular design in such way that the final MOF nanoparticles are only composed of non-toxic building blocks as this reduces the overall toxicity. Although the toxicity of the individual components may change after their assembly into MOF nanoparticles, it turned out that it is still very likely that the resulting MOF nanoparticles only causes a low toxicity. In addition, this ensures minimal toxicity after the disassembly of the MOF nanoparticles into its individual inorganic and organic building blocks. However, it is also very important to note that the MOF nanoparticles do not necessarily disassemble into their initial components, but that they form other crystalline or amorphous degradation products or metabolites, e.g. metal oxides, or phosphates. One can only roughly predict such behaviour and must therefore always evaluate it individually for each MOF. Moreover, any solvents or additives from the synthesis that may remain in the structure or porosity of the resulting MOF nanoparticles also have to be considered.

Hazard Assessment of MOF Nanoparticles in the Context of Applications

In evaluating the hazard of MOF nanoparticles in the context of their applications, it is extremely important to be aware of the fact that it is not sufficient to evaluate only the toxicity, but also to assess the actual risk of exposure. The ideal workflow of the hazard assessment is depicted in **Figure 13** that comprises three main parts: (*i*) scientific research, i.e. the identification of the relevant physico-chemical properties and the biokinetics, (*ii*) risk assessment, *i.e.* the assessment of *in vitro* and *in vivo* toxicity as well as exposure assessments, and (*iii*) an appropriate risk management. The exposure to MOF nanoparticles can occur at different stages, i.e. the synthesis, characterisation, packing, transportation, and of course during the application – and certainly the risk can be significantly minimised by employing a safe working environment and good working practices, i.e. working with solutions, nanoparticle masks, and gloves. In the following, some reflections will be given on the hazard assessment in the context of their respective application. From this, an overview will be given for an appropriate, application-dependent MOF nanotoxicity assessment in order to minimise the waste of time, money and resources. Herein, the focus mainly lies on the question: which kind of the toxicity assessment is essential for the intended application of the MOF nanoparticles?

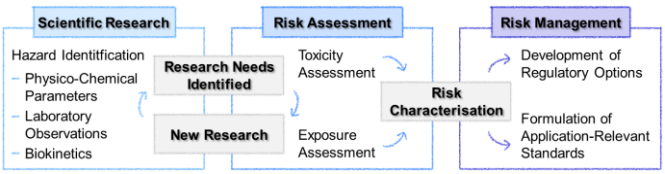

Figure 13: Overview of the hazard assessment process, *i.e.* scientific research, risk assessment and risk management.

As toxicity per se is context-dependent and strongly depends on the route of exposure, i.e. whether the uptake occurs *via* dermal exposure, inhalation, ingestion, or intravenous, in the ideal case one already should have the final application in mind when preparing MOF nanoparticles. Because only in this case it is possible to consider the relevant, potential routes of exposure to these MOF nanoparticles, and thus, to plan an optimal toxicity assessment which ensures that all the cells that will be first encountered are included. After the absorption of MOF nanoparticles, it should be noted that liver, spleen and kidneys should always be included due to metabolism and excretion. **Figure 14** represents an overview of the different routes of exposure, and the corresponding first cell lines or organs which have to be taken into account in case for the *in vitro* and *in vivo* assays, respectively.

In case of ***dermal*** exposure of MOF nanoparticles, i.e. for applications such as in wound healing fabrics[60] or hazard protecting workwear,[61] it is most relevant to first study their toxic effect to cells within the epidermis and dermis in *in vitro* studies, while it is also essential to analyse the MOF nanoparticle permeation using *ex vivo* models. Finally, if particles are able to bypass the skin barrier, the effects on the lymphatic system should be evaluated in *in vivo* models.

The ***inhalation*** of MOF nanoparticles so far plays a major role for applications such as the uptake of luminescent markers for gunshot residues,[39] and drug delivery via pulmonary administration, among others.[62] In case of such applications, local cell lines need to be considered, namely type I and type II alveolar epithelial cells, alveolar macrophages and ciliated epithelia with goblet cell. By uptake via the lungs, MOF nanoparticles can be absorbed and enter the bloodstream in *in vivo* models, so it is important to study the toxic effect on the heart, other secondary organs as well as the lymphatic system.

The ***ingestion*** of MOF nanoparticles is fundamental for applications such as the administration of drugs and detoxifying agents.[20] As the nanoparticles enter the stomach, the cells of the gastric mucosa, i.e. lamina epithelialis mucosea, lamina propria mucosea as well as the tela submucosea, but also intestinal cells are of interest for *in vitro* assays. Additional aspects such as muco-diffusion, -permeation and-adhesion, or the gastro-intestine content (e.g. food, or pancreatin) should also be considered, and could be evaluated using simulated conditions in 2D *in vitro* models. For *in vivo* studies it is necessary to assess the toxic effect to the whole gastro-intestinal system, including esophagus, stomach, duodenum, small intestine, appendix, and colon.

The ***intravenous*** administration of MOF nanoparticles is of special interest for their most prominent applications such as drug delivery and imaging, and represents definitely the by far best studied route of exposure.[24] Circulating in the blood stream, MOF nanoparticles certainly encounter the cells of the blood vessels, white blood cells, red blood cells and platelets first, but *via* the blood stream they can then either enter various organs, including the kidneys, the liver, the spleen and in rare cases also the brain, or accumulate in tumorous tissue. Consequently, an extremely broad spectrum of different cell lines has to be considered.

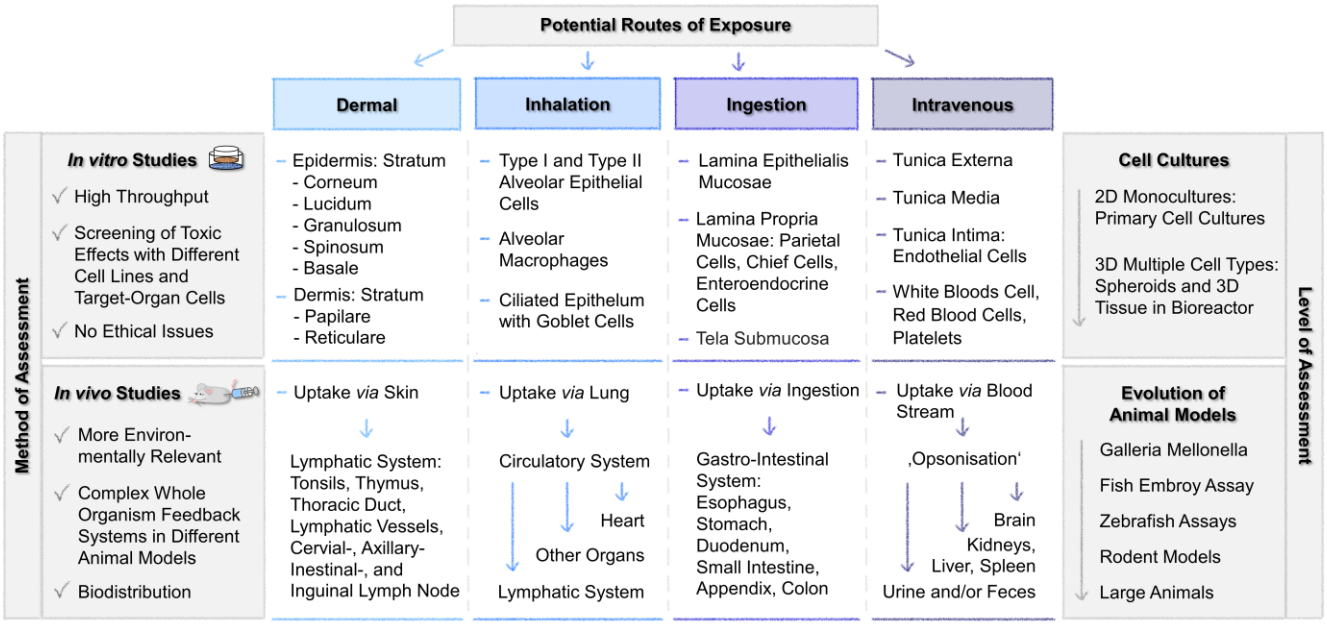

Figure 14: Overview of the different routes of administration and the resulting required levels of toxicity assessment.

The overview in **Figure 14** clearly highlights that for a proper toxicity assessment of MOF nanoparticles in the context of their application a broad range of different cells and different assessment methods has to be investigated. When evaluating the current state of the hazard assessment of MOF nanoparticles it becomes quite evident that it is a relatively young field and still on its way from basic research to real-world application: The different toxicity assessments are already quite well explored, but the corresponding exposure assessments have often been neglected. However, in the last decade already a huge improvement has been made as the utilisation of cell lines in *in vitro* studies has become more and more application-relevant. Analogue to optimised *in vitro* assays, also the *in vivo* models should be further advanced, and one should always strive to make the chosen model as relevant as possible for the realistic hazard assessment of MOF nanoparticles in the context of their application.

To sum up, for the development of a roadmap for the evaluation of the hazard of MOF nanoparticles the following aspect should be pursued in the following order: (*i*) assessing the potential routes of exposure; (*ii*) physico-chemical characterisation, including studies (e.g. chemical and colloidal stability, formation of a biological corona) in contact with simulated biological fluid in each of the routes; (*iii*) *in vitro* cytotoxicity screening of components and assembled MOF nanoparticles; (*iv*) *in vivo* ADME studies; (*v*) *in vivo* toxicity studies evaluating survival, behaviour, animal and organ weight, chemical blood and organ biomarkers, macroscopic aspect of organs and tissues and histopathology, among other relevant parameters.

Towards Safe and Sustainable Design of MOF Nanoparticles

Risk management is the final task of the hazard assessment process (**Figure 13**) and it represents the key that a nanoformulation can be used in society. Herein, regulatory options are being developed to ensure the preparation of safer materials in the future. In particular, the synthesis of materials that are 'safe-by-design' has become highly desirable. As both building blocks of MOFs, i.e. their metal clusters as well as their organic linkers, can be specifically designed, representing one of the most versatile and attractive class of materials to realise this concept. Recently, the European Environment Agency officially decided to extend the 'safe-by-design' approach to 'safe-and-sustainable-by-design' until 2022 to foster the production of materials that are safer for us as well as for the environment. The fundamental chemical strategy herein aims to catalyse the shift towards materials that are inherently safe and sustainable from their production until their end of existence.

This also applies to all the steps involved in the preparation of multifunctional MOF: *(i)* synthesis; *(ii)* drug incorporation; and *(iii)* post-synthetic surface modification. Fortunately, the creation of MOF nanoparticles that are 'safe-and-sustainable-by-design' can also be realised relatively straightforwardly: By only using biocompatible building blocks and reactants, e.g. solvents, acids, bases, mineralizing agents, and modulators, it is very likely that such MOF nanoparticles will also have only low toxicity when disassembled. Reports on the synthesis procedures of MIL-100(Fe) without toxic modulators, or drug loadings and MOF coatings in water without organic solvents show that researchers have already started to use more and more safe and sustainable principles.[45,63,64]

However, as described in the previous chapters, the *a priori* prediction of the toxicity of MOF nanoparticles is not possible. As depicted in **Figure 15** it might rather be described as an iterative approach: MOF nanoparticles are designed, synthesised and characterised including the assessment of their toxicity and based on the result, the material might have to be re-designed and tested again. Subsequent to this

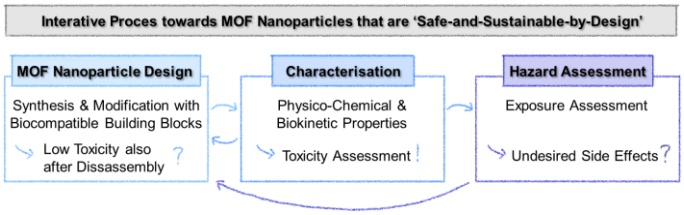

Figure 15: Iterative process towards MOF nanoparticles that are safe-and-sustainable-by-design: design, synthesis, characterisation, and hazard assessment.

characterisation, one has to conduct exposure assessments to evaluate the hazard of the MOF nanoparticles considering important parameters such as administration route and dose.

In order to minimise the steps of this iterative process, it is highly desirable that research is performed in accordance with the so-called MIRIBEL (*M*inimum *I*nformation *R*eporting *I*n *B*io–nano *E*xperimental *L*iterature) guidelines.[65] These strongly encourage provision of at least a minimal set of standardised information with publications of nanoparticles so that transparency and reproducibility is ensured. In addition to regulating the availability of such comprehensive data sets, the European Union became also involved to a safer production and handling of chemicals from another regulatory perspective: The European regulatory framework for chemicals REACH (*R*egistration, *E*valuation and *A*uthorisation of *Ch*emicals) was founded to standardise toxicity testing so that both, workers and consumers, benefit from safer handling with individual chemicals.[66] Moreover, the REACH regulations also clearly emphasise the importance of the gradual substitution of animal testing by alternative models, e.g. *in silico,* or *in vitro*. It would also be desirable to transfer such guidelines to medicinal products, which obey to specific rules as listed by European Medicines Agency and the U.S. Food and Drug Administration.

Consequently, as researchers, we should aim to (*i*) focus on the synthesis of 'safe-and-sustainable' novel MOF nanoparticles, (*ii*) ensure their extensive, standardised synthesis and characterisation, as well as a proper hazard assessment, and (*iii*) guarantee an open access to all the collected data.

Conclusion

Especially over the last years, MOF nanoparticles have become promising candidates for many different relevant socioeconomic applications – and for good reasons: Their reticular design facilitates the synthesis of MOF nanoparticles that are able to address various specific issues, including the preparation of nanoparticles that are 'safe and sustainable-by-design'. Nevertheless, one still has to carefully evaluate the hazard they possess, i.e. their toxicity and the actual risk of potential exposure both to living beings and to the environment.

To date, different *in vitro* and *in vivo* toxicity assessments have successfully been established, and fortunately, they become more and more application-relevant. Based on the toxicity data available for various different MOF nanoparticles, some key aspects affecting their toxicity, e.g. their chemical composition, dose, size, colloidal and chemical stability in biological fluids, could be identified. Evaluation made it obvious that it is not possible to name a single factor which triggers the resulting toxicity but that it is always their interplay: Based on particle size alone, MOF nanoparticles below 200 nm might already be classified as potentially hazardous – however, their resulting toxicity is strongly depended on their administration route as well as their chemical and colloidal stability in the respective biological media, as these will have an impact on their agglomeration and degradation behaviour in the body. Great success has already been achieved in understanding and manipulating the MOF nanoparticle surface, e.g. by grafting polymers, to fine-tune toxicity-relevant properties, such as the colloidal and chemical stability, and to modify their *in vivo* fate, e.g. targeting to the active sites. Nevertheless, one should always be aware of the fact that the synthetic identities of MOF nanoparticles are quickly altered *in vivo*, being strongly dependent on their administration/exposure route. To sum up, the fate of MOF nanoparticles and their biodistribution is not predictable *a priori* and therefore, it is of paramount importance to always consider all aspects relevant to their real-life applications, i.e. their colloidal and chemical stability for different routes of administration as well as the biocompatibility of their individual building blocks. In this sense, the huge versatility of MOF nanoparticles hinders their toxicological evaluation, being limited to few benchmarked MOF nanoparticles. Although complex, *in silico* models, currently used for the toxicological prediction of compounds with different nature, could be a powerful technique in the near future to support a more systematic way to investigate the MOF toxicity, reducing the number of animals and overcoming the large versatility of MOFs.[5]

In addition, reflections on the possible routes of administration should always be made *prior* to extensive toxicity evaluations, as these have a direct influence on the ideal set-up for the required *in vitro* and *in vivo* assessments. Only by incorporating such practices, a meaningful hazard assessment for MOF nanoparticles in the context of their applications can be achieved. To pave the way for MOF nanoparticles towards various applications, one should aim to always address these objectives in ones' workflow: (*i*) Make sure to use bio-compatible building blocks in the first place, as well as other reactants involved in the synthesis; (*ii*) Characterise all physico-chemical properties affecting the MOF nanoparticle toxicity, *i.e.* size and shape, surface chemistry, colloidal and chemical stability; (*iii*) Consider the most likely routes of exposure; (*iv*) Tailor the required toxicity assessment accordingly; and (*v*) Always make all data available with open access. If we as researchers keep these goals in mind, MOF nanoparticles could be an innovative nanomaterial class to tackle many of the upcoming challenges of the 21st century in a way that is safer for us and for the environment.

Author Contributions

We strongly encourage authors to include author contributions and recommend using CRediT for standardised contribution descriptions. Please refer to our general author guidelines for more information about authorship.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 R. Freund, S. Canossa, S. M. Cohen, W. Yan, H. Deng, V. Guillerm, M. Eddaoudi, D. G. Madden, D. Fairen-Jimenez, H. Lyu, L. K. Macreadie, Z. Ji, Y. Zhang, B. Wang, F. Haase, C. Wöll, O. Zaremba, J. Andreo, S. Wuttke and C. S. Diercks, 25 years of Reticular Chemistry, *Angew. Chem. Int. Ed.*, 2021. DOI: 10.1002/anie.202101644.

2 R. Freund, O. Zaremba, G. Arnauts, R. Ameloot, G. Skorupskii, M. Dincă, A. Bavykina, J. Gascon, A. Ejsmont, J. Gościańska, M. Kalmutzki, U. Lächelt, E. Ploetz, C. Diercks and S. Wuttke, The Current Status of MOF and COF Applications, *Angew. Chem. Int. Ed.*, 2021. DOI: 10.1002/anie.202106259.

3 E. Ploetz, H. Engelke, U. Lächelt and S. Wuttke, The Chemistry of Reticular Framework Nanoparticles, *Adv. Funct. Mater.*, 2020, **30**, 1909062.

4 M. Nordberg, J. Duffus and D. M. Templeton, Glossary of terms used in toxicokinetics (IUPAC Recommendations 2003), *Pure Appl. Chem*, 2004, **76**, 1033–1082.

5 A. B. Raies and V. B. Bajic, In silico toxicology: computational methods for the prediction of chemical toxicity, *Wiley Interdiscip. Rev. Comput. Mol. Sci.*, 2016, **6**, 147–172.

6 Y. Zhang, Y. Bai, J. Jia, N. Gao, Y. Li, R. Zhang, G. Jiang and B. Yan, Perturbation of physiological systems by nanoparticles, *Chem. Soc. Rev.*, 2014, **43**, 3762–3809.

7 S. Behzadi, V. Serpooshan, W. Tao, M. A. Hamaly, M. Y. Alkawareek, E. C. Dreaden, D. Brown, A. M. Alkilany, O. C. Farokhzad and M. Mahmoudi, Cellular uptake of nanoparticles: journey inside the cell, *Chem. Soc. Rev.*, 2017, **46**, 4218–4244.

8 S. Hussain, Measurement of Nanoparticle-Induced Mitochondrial Membrane Potential Alterations, *Methods Mol Biol*, 2019, **1894**, 123–131.

9 Z. Yu, Q. Li, J. Wang, Y. Yu, Y. Wang, Q. Zhou and P. Li, Reactive Oxygen Species-Related Nanoparticle Toxicity in the Biomedical Field, *Nanoscale Res. Lett.*, 2020, **15**, 115.

10 Y. Zhang, Cell toxicity mechanism and biomarker, *Clin Trans Med*, 2018, **7**, 34.

11 K. M. de La Harpe, P. P. D. Kondiah, Y. E. Choonara, T. Marimuthu, L. C. Du Toit and V. Pillay, The Hemocompatibility of Nanoparticles: A Review of Cell-Nanoparticle Interactions and Hemostasis, *Cells*, 2019, **8**. DOI: 10.3390/cells8101209.

12 Y. Bian, K. Kim, T. Ngo, I. Kim, O.-N. Bae, K.-M. Lim and J.-H. Chung, Silver nanoparticles promote procoagulant activity of red blood cells: a potential risk of thrombosis in susceptible population, *Part. Fibre Toxicol.*, 2019, **16**, 9.

13 C. von Schirnding, I. Giopanou, A. Hermawan, L. Wehl, G. Ntaliarda, B. Illes, S. Datz, F. Geisslinger, K. Bartel, A.-K. Sommer, M. Lianou, V. Weiß, J. Feckl, A. M. Vollmar, C. Bräuchle, G. T. Stathopoulos, E. Wagner, A. Roidl, T. Bein and H. Engelke, Synergistic Combination of Calcium and Citrate in Mesoporous Nanoparticles Targets Pleural Tumors, *Chem*, 2021, **7**, 480–494.

14 J. T. Buchman, M. J. Gallagher, C.-T. Yang, X. Zhang, M. O. P. Krause, R. Hernandez and G. Orr, Research highlights, *Environ. Sci. Nano*, 2016, **3**, 696–700.

15 M. P. Monopoli, C. Aberg, A. Salvati and K. A. Dawson, Biomolecular coronas provide the biological identity of nanosized materials, *Nat Nanotechnol.*, 2012, **7**, 779–786.

16 A. Zimpel, N. Al Danaf, B. Steinborn, J. Kuhn, M. Höhn, T. Bauer, P. Hirschle, W. Schrimpf, H. Engelke, E. Wagner, M. Barz, D. C. Lamb, U. Lächelt and S. Wuttke, Coordinative Binding of Polymers to Metal-Organic Framework Nanoparticles for Control of Interactions at the Biointerface, *ACS nano*, 2019, **13**, 3884–3895.

17 E. Bellido, T. Hidalgo, M. V. Lozano, M. Guillevic, R. Simón-Vázquez, M. J. Santander-Ortega, Á. González-Fernández, C. Serre, M. J. Alonso and P. Horcajada, Heparin-engineered mesoporous iron metal-organic framework nanoparticles: toward stealth drug nanocarriers, *Adv. Healthc. Mater.*, 2015, **4**, 1246–1257.

18 N. Gan, Q. Sun, L. Zhao, P. Tang, Z. Suo, S. Zhang, Y. Zhang, M. Zhang, W. Wang and H. Li, Protein corona of metal-organic framework nanoparticals, *International journal of biological macromolecules*, 2019, **140**, 709–718.

19 A. García-Márquez, T. Hidalgo, H. Lana, D. Cunha, M. J. Blanco-Prieto, C. Álvarez-Lorenzo, C. Boissière, C. Sánchez, C. Serre and P. Horcajada, Biocompatible polymer-metal-organic framework composite patches for cutaneous administration of cosmetic molecules, *J. Mater. Chem. B*, 2016, **4**, 7031–7040.

20 S. Rojas, T. Baati, L. Njim, L. Manchego, F. Neffati, N. Abdeljelil, S. Saguem, C. Serre, M. F. Najjar, A. Zakhama and P. Horcajada, Metal-Organic Frameworks as Efficient Oral Detoxifying Agents, *J. Am. Chem. Soc.*, 2018, **140**, 9581–9586.

21 S. D. Taherzade, S. Rojas, J. Soleimannejad and P. Horcajada, Combined Cutaneous Therapy Using Biocompatible Metal-Organic Frameworks, *Nanomaterials*, 2020, **10**. DOI: 10.3390/nano10122296.

22 P. Wang, S. M. Henning and D. Heber, Limitations of MTT and MTS-based assays for measurement of antiproliferative activity of green tea polyphenols, *PloS one*, 2010, **5**, e10202.

23 W. J. Rieter, K. M. Pott, K. M. L. Taylor and W. Lin, Nanoscale coordination polymers for platinum-based anticancer drug delivery, *J. Am. Chem. Soc.*, 2008, **130**, 11584–11585.

24 P. Horcajada, T. Chalati, C. Serre, B. Gillet, C. Sebrie, T. Baati, J. F. Eubank, D. Heurtaux, P. Clayette, C. Kreuz, J.-S. Chang, Y. K. Hwang, V. Marsaud, P.-N. Bories, L. Cynober, S. Gil, G. Férey, P. Couvreur and R. Gref, Porous metal-organic-framework nanoscale carriers as a potential platform for drug delivery and imaging, *Nat. Mater.*, 2010, **9**, 172–178.

25 P. Horcajada, R. Gref, T. Baati, P. K. Allan, G. Maurin, P. Couvreur, G. Férey, R. E. Morris and C. Serre, Metal-organic frameworks in biomedicine, *Chem. Rev.*, 2012, **112**, 1232–1268.

26 C. Tamames-Tabar, D. Cunha, E. Imbuluzqueta, F. Ragon, C. Serre, M. J. Blanco-Prieto and P. Horcajada, Cytotoxicity of nanoscaled metal-organic frameworks, *J. Mater. Chem. B*, 2014, **2**, 262–271.

27 R. Grall, T. Hidalgo, J. Delic, A. Garcia-Marquez, S. Chevillard and P. Horcajada, In vitro biocompatibility of mesoporous metal (III; Fe, Al, Cr) trimesate MOF nanocarriers, *J. Mater. Chem. B*, 2015, **3**, 8279–8292.

28 L. Cooper, T. Hidalgo, M. Gorman, T. Lozano-Fernández, R. Simón-Vázquez, C. Olivier, N. Guillou, C. Serre, C. Martineau, F. Taulelle, D. Damasceno-Borges, G. Maurin, Á. González-Fernández, P. Horcajada and T. Devic, A biocompatible porous Mg-gallate metal-organic framework as an antioxidant carrier, *Chemical communications (Cambridge, England)*, 2015, **51**, 5848–5851.

29 C. Orellana-Tavra, E. F. Baxter, T. Tian, T. D. Bennett, N. K. H. Slater, A. K. Cheetham and D. Fairen-Jimenez, Amorphous metal-organic frameworks for drug delivery, *Chem. Comm.*, 2015, **51**, 13878–13881.

30 S. Wuttke, A. Zimpel, T. Bein, S. Braig, K. Stoiber, A. Vollmar, D. Müller, K. Haastert-Talini, J. Schaeske, M. Stiesch, G. Zahn, A. Mohmeyer, P. Behrens, O. Eickelberg, D. A. Bölükbas and S. Meiners, Validating Metal-Organic Framework Nanoparticles for Their Nanosafety in Diverse Biomedical Applications, *Advanced healthcare materials*, 2017, **6**. DOI: 10.1002/adhm.201600818.

31 I. Abánades Lázaro, S. Haddad, J. M. Rodrigo-Muñoz, R. J. Marshall, B. Sastre, V. Del Pozo, D. Fairen-Jimenez and R. S. Forgan, Surface-Functionalization of Zr-Fumarate MOF for Selective Cytotoxicity and Immune System Compatibility in Nanoscale Drug Delivery, *ACS Appl. Mater. Interfaces*, 2018, **10**, 31146–31157.

32 R. Ettlinger, M. Sönksen, M. Graf, N. Moreno, D. Denysenko, D. Volkmer, K. Kerl and H. Bunzen, Metal-organic framework nanoparticles for arsenic trioxide drug delivery, *J. Mater. Chem. B*, 2018, **6**, 6481–6489.

33 R. V. Pinto, S. Wang, S. R. Tavares, J. Pires, F. Antunes, A. Vimont, G. Clet, M. Daturi, G. Maurin, C. Serre and M. L. Pinto, Tuning Cellular Biological Functions Through the Controlled Release of NO from a Porous Ti-MOF, *Angew. Chem.*, 2020, **132**, 5173–5181.

34 P. J. Jodłowski, G. Kurowski, Ł. Kuterasiński, M. Sitarz, P. Jeleń, J. Jaśkowska, A. Kołodziej, A. Pajdak, Z. Majka and A. Boguszewska-Czubara, Cracking the Chloroquine Conundrum: The Application of Defective UiO-66 Metal-Organic Framework Materials to Prevent the Onset of Heart Defects-In Vivo and In Vitro, *ACS Appl. Mater. Interfaces*, 2021, **13**, 312–323.

35 À. Ruyra, A. Yazdi, J. Espín, A. Carné-Sánchez, N. Roher, J. Lorenzo, I. Imaz and D. Maspoch, Synthesis, culture medium stability, and in vitro and in vivo zebrafish embryo toxicity of metal-organic framework nanoparticles, *Chem. Eur. J.*, 2015, **21**, 2508–2518.

36 I. Sifaoui, I. Pacheco-Fernández, J. E. Piñero, V. Pino and J. Lorenzo-Morales, A Simple in vivo Assay Using Amphipods for the Evaluation of Potential Biocompatible Metal-Organic Frameworks, *Front. Bioeng. Biotechnol.*, 2021, **9**, 584115.

37 J. Redfern, L. Geerts, J. W. Seo, J. Verran, L. Tosheva and L. H. Wee, Toxicity and Antimicrobial Properties of ZnO@ZIF-8 Embedded Silicone against Planktonic and Biofilm Catheter-Associated Pathogens, *ACS Appl. Nano Mater.*, 2018, **1**, 1657–1665.

38 T. Kundu, S. Mitra, P. Patra, A. Goswami, D. Díaz Díaz and R. Banerjee, Mechanical downsizing of a gadolinium(III)-based metal-organic framework for anticancer drug delivery, *Chem. Eur. J.*, 2014, **20**, 10514–10518.

39 A. L. R. Talhari, M. A. M. Lucena, F. G. M. Mauricio, M. F. L. Oliveira, F. H. Veiga-Souza, S. A. Junior and I. T. Weber, Luminescent Marker for GSR, *ACS Appl. Bio Mater.*, 2020, **3**, 3049–3056.

40 E. Lammer, G. J. Carr, K. Wendler, J. M. Rawlings, S. E. Belanger and T. Braunbeck, Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test?, *Comp. Biochem. Physiol. C*, 2009, **149**, 196–209.

41 G. Tan, Y. Zhong, L. Yang, Y. Jiang, J. Liu and F. Ren, A multifunctional MOF-based nanohybrid as injectable implant platform for drug synergistic oral cancer therapy, *Chem. Eng. J.*, 2020, **390**, 124446.

42 T. Simon-Yarza, M. Giménez-Marqués, R. Mrimi, A. Mielcarek, R. Gref, P. Horcajada, C. Serre and P. Couvreur, A Smart Metal-Organic Framework Nanomaterial for Lung Targeting, *Angew. Chem. Int. Ed.*, 2017, **56**, 15565–15569.

43 M. d. J. Velásquez-Hernández, R. Ricco, F. Carraro, F. T. Limpoco, M. Linares-Moreau, E. Leitner, H. Wiltsche, J. Rattenberger, H. Schröttner, P. Frühwirt, E. M. Stadler, G. Gescheidt, H. Amenitsch, C. J. Doonan and P. Falcaro, Degradation of ZIF-8 in phosphate buffered saline media, *CrystEngComm*, 2019, **21**, 4538–4544.

44 E. Bellido, M. Guillevic, T. Hidalgo, M. J. Santander-Ortega, C. Serre and P. Horcajada, Understanding the colloidal stability of the mesoporous MIL-100(Fe) nanoparticles in physiological media, *Langmuir*, 2014, **30**, 5911–5920.

45 V. Agostoni, P. Horcajada, M. Noiray, M. Malanga, A. Aykaç, L. Jicsinszky, A. Vargas-Berenguel, N. Semiramoth, S. Daoud-Mahammed, V. Nicolas, C. Martineau, F. Taulelle, J. Vigneron, A. Etcheberry, C. Serre and R. Gref, A "green" strategy to construct non-covalent, stable and bioactive coatings on porous MOF nanoparticles, *Sci. Rep.*, 2015, **5**, 7925.

46 M. Giménez-Marqués, E. Bellido, T. Berthelot, T. Simón-Yarza, T. Hidalgo, R. Simón-Vázquez, Á. González-Fernández, J. Avila, M. C. Asensio, R. Gref, P. Couvreur, C. Serre and P. Horcajada, GraftFast Surface Engineering to Improve MOF Nanoparticles Furtiveness, *Small*, 2018, **14**, e1801900.

47 I. Abánades Lázaro, S. Haddad, S. Sacca, C. Orellana-Tavra, D. Fairen-Jimenez and R. S. Forgan, Selective Surface PEGylation of UiO-66 Nanoparticles for Enhanced Stability, Cell Uptake, and pH-Responsive Drug Delivery, *Chem*, 2017, **2**, 561–578.

48 R. Ettlinger, N. Moreno, D. Volkmer, K. Kerl and H. Bunzen, Zeolitic Imidazolate Framework-8 as pH-Sensitive Nanocarrier for "Arsenic Trioxide" Drug Delivery, *Chem. Eng. J.*, 2019, **25**, 13189–13196.

49 A. Zimpel, T. Preiß, R. Röder, H. Engelke, M. Ingrisch, M. Peller, J. O. Rädler, E. Wagner, T. Bein, U. Lächelt and S. Wuttke, Imparting Functionality to MOF Nanoparticles by External Surface Selective Covalent Attachment of Polymers, *Chem. Mater.*, 2016, **28**, 3318–3326.

50 R. G. Pearson, <bold>Hard and Soft Acids and Bases</bold>, *J. Am. Chem. Soc.*, 1963, **85**, 3533–3539.

51 C.-Y. Sun, C. Qin, X.-L. Wang, G.-S. Yang, K.-Z. Shao, Y.-Q. Lan, Z.-M. Su, P. Huang, C.-G. Wang and E.-B. Wang, Zeolitic Imidazolate framework-8 as efficient pH-sensitive drug delivery vehicle, *Dalton Trans.*, 2012, **41**, 6906–6909.

52 C. G. Piscopo, A. Polyzoidis, M. Schwarzer and S. Loebbecke, Stability of UiO-66 under acidic treatment: Opportunities and limitations for post-synthetic modifications, *Microporous Mesoporous Mater.*, 2015, **208**, 30–35.

53 J. Schnabel, R. Ettlinger and H. Bunzen, Zn-MOF-74 as pH-Responsive Drug-Delivery System of Arsenic Trioxide, *ChemNanoMat*, 2020, **6**, 1229–1236.

54 Y. Chen, P. Li, J. A. Modica, R. J. Drout and O. K. Farha, Acid-Resistant Mesoporous Metal-Organic Framework toward Oral Insulin Delivery: Protein Encapsulation, Protection, and Release, *J. Am. Chem. Soc.*, 2018, **140**, 5678–5681.

55 I. Christodoulou, T. Bourguignon, X. Li, G. Patriarche, C. Serre, C. Marlière and R. Gref, Degradation Mechanism of Porous Metal-Organic Frameworks by In Situ Atomic Force Microscopy, *Nanomaterials*, 2021, **11**. DOI: 10.3390/nano11030722.

56 M. R. Di Nunzio, V. Agostoni, B. Cohen, R. Gref and A. Douhal, A "ship in a bottle" strategy to load a hydrophilic anticancer drug in porous metal organic framework nanoparticles: efficient encapsulation, matrix stabilization, and photodelivery, *J. Med. Chem.*, 2014, **57**, 411–420.

57 S. R. Miller, E. Alvarez, L. Fradcourt, T. Devic, S. Wuttke, P. S. Wheatley, N. Steunou, C. Bonhomme, C. Gervais, D. Laurencin, R. E. Morris, A. Vimont, M. Daturi, P. Horcajada and C. Serre, A rare example of a porous Ca-MOF for the controlled release of biologically active NO, *Chem. Comm.*, 2013, **49**, 7773–7775.

58 E. S. Grape, J. G. Flores, T. Hidalgo, E. Martínez-Ahumada, A. Gutiérrez-Alejandre, A. Hautier, D. R. Williams, M. O'Keeffe, L. Öhrström, T. Willhammar, P. Horcajada, I. A. Ibarra and A. K. Inge, A Robust and Biocompatible Bismuth Ellagate MOF Synthesized Under Green Ambient Conditions, *J. Am. Chem. Soc.*, 2020, **142**, 16795–16804.

59 T. Baati, L. Njim, F. Neffati, A. Kerkeni, M. Bouttemi, R. Gref, M. F. Najjar, A. Zakhama, P. Couvreur, C. Serre and P. Horcajada, In depth analysis of the in vivo toxicity of nanoparticles of porous iron(iii) metal–organic frameworks, *Chem. Sci.*, 2013, **4**, 1597.

60 L.-Q. Fu, X.-Y. Chen, M.-H. Cai, X.-H. Tao, Y.-B. Fan and X.-Z. Mou, Surface Engineered Metal-Organic Frameworks (MOFs) Based Novel Hybrid Systems for Effective Wound Healing: A Review of Recent Developments, *Front. Bioeng. Biotechnol.*, 2020, **8**, 576348.

61 H. S. Jhinjer, A. Singh, S. Bhattacharya, M. Jassal and A. K. Agrawal, Metal-organic frameworks functionalized smart textiles for adsorptive removal of hazardous aromatic pollutants from ambient air, *J. Hazard. Mater.*, 2021, **411**, 125056.

62 C. Fernández-Paz, S. Rojas, P. Salcedo-Abraira, T. Simón-Yarza, C. Remuñán-López and P. Horcajada, Metal-Organic Framework Microsphere Formulation for Pulmonary Administration, *ACS Appl. Mater. Interfaces*, 2020, **12**, 25676–25682.

63 V. Agostoni, P. Horcajada, V. Rodriguez-Ruiz, H. Willaime, P. Couvreur, C. Serre and R. Gref, 'Green' fluorine-free mesoporous iron(III) trimesate nanoparticles for drug delivery, *Green Mater.*, 2013, **1**, 209–217.

64 V. Agostoni, T. Chalati, P. Horcajada, H. Willaime, R. Anand, N. Semiramoth, T. Baati, S. Hall, G. Maurin, H. Chacun, K. Bouchemal, C. Martineau, F. Taulelle, P. Couvreur, C. Rogez-Kreuz, P. Clayette, S. Monti, C. Serre and R. Gref, Towards an improved anti-HIV activity of NRTI via metal-organic frameworks nanoparticles, *Adv. Healthc. Mater.*, 2013, **2**, 1630–1637.

65 M. Faria, M. Björnmalm, K. J. Thurecht, S. J. Kent, R. G. Parton, M. Kavallaris, A. P. R. Johnston, J. J. Gooding, S. R. Corrie, B. J. Boyd, P. Thordarson, A. K. Whittaker, M. M. Stevens, C. A. Prestidge, C. J. H. Porter, W. J. Parak, T. P. Davis, E. J. Crampin and F. Caruso, Minimum information reporting in bio-nano experimental literature, *Nat Nanotechnol.*, 2018, **13**, 777–785.

66 *Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency*, 2006.