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Teaser The review examines the stratified regulatory framework concerning the use of nanomaterials in healthcare products intended to be marketed in the European Economic Area, and highlights the current criticisms associated with the framework.

Is the European regulatory framework sufficient to assure the safety of citizens using health products containing nanomaterials?

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The growing application of nanomaterials in healthcare products (i.e., cosmetics, medical devices, and medicinal products) has encouraged the upgrade of the regulatory framework within the European Community to better control their use and manage the risk of negative effects on human health and environment. Unfortunately, despite the efforts of European Authorities, the current legislation is still stratified and several criticisms remain because of the lack of well-established scientific knowledge on nanomaterials. Although the regulatory framework for cosmetic products is almost complete, the efficacy and/or safety assessment of nanomaterials in medicinal products and medical devices is still based on case-by-case evaluation because of the complexity of such systems.

Introduction

Over the past few decades, the rapid diffusion onto the market of healthcare products containing nanomaterials, namely cosmetics, medical devices, and medicinal products [1] has raised concerns about their possible impact on human health and the environment [2,3]. The nanoscaling process modifies the physicochemical properties of bulk materials, conferring new magnetic, optical, mechanical, and biological properties in addition to a high surface:volume ratio. As a consequence, nanomaterials are widely used for several healthcare applications. For example, colloidal silver has been used in the treatment of wound antisepsis since 1891 [4] or, more recently, drug-loaded liposomes and superparamagnetic nanoparticles (NPs) were developed as cancer therapeutics [5] and as diagnostic tools [6], respectively. Starting from the first empirical evidence for the clinical use of nanomaterials (e.g., colloidal silver), scientific progress in biology, material science, and engineering has increased the interest of the wider scientific and industrial community in the use of such materials to fulfill currently unmet clinical and technological needs. However, the same physicochemical properties responsible for the technological success of nanomaterials can be hazardous, with potential toxic effects on humans and the environment [7–9]. Concerns about 'nanotoxicity' first arose in initial reports showing that the toxicological profile of particles with dimensions of less than a micron, such as carbon nanotubes, asbestos fibers [10], and ultrafine particles in air pollution, differs from that of the corresponding materials

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with macro- or microscale dimensions. Indeed, nanoscale particles can cause diseases in humans because of the disruption of the physiological functionality of tissues and induction of inflammation processes, as a result of the ability of such particles to penetrate biological and cellular structures and to react with intra- and extracellular targets, causing changes in metabolic pathways and altering the redox balance [11,12].

Thus, there is a need for national and international regulatory agencies to regulate the use of nanotechnology applications in market-available products and, consequently, to not only encourage sustainable technological progress, but also to guarantee the safety of workers, consumers, patients, and the environment. Unfortunately, because of the lack of a unique definition of nanomaterial in Europe, several versions are available in the European legislation according to the nanomaterial type, application, or industrial sector. Such a terminological kaleidoscope generates confusion in both operators and consumers because the same material can be considered nanostructured or not on the basis of the legal references connected to its proposed application.

In this review, we discuss the current European regulatory framework on nanomaterials as ingredients of products used by humans for healthcare purposes (i.e., cosmetics, medical devices, and medicinal products).

European legislative framework

The widespread interest of the industrial sector in nanotechnologies encouraged the European Commission (EC) to stimulate research in nanoscience and nanotechnology to maintain the technological competitiveness of researchers operating in the European Economic Area (EEA) against their American and Japanese competitors. In a specific Communication issued in 2004, the EC underlined the need to encourage the cooperation and technological transfer between European academic and industrial sectors for developing commercially available products and industrial processes based on nanomaterials [13]. In 2005, the Communication was followed by an action plan aimed to implement studies of nanoscience and to investigate its impact on human health and environment, with the aim to harmonize knowledge about the effective hazard of nanomaterials [14]. Such a plan included a series of interconnected actions to be carried out from 2005 to 2009. Both these acts were evaluated by the European Parliament (EP), which issued a specific Resolution on nanoscience and nanotechnology in 2006 [15]. Embracing the EC interventions, the EP expressed concerns about the technological and health risks for consumers, workers, and the environment throughout the life cycle of nanotechnology products. Moreover, for the first time, the EP recommended that manufactured nanomaterials should be clearly identifiable by the consumer in the product ingredients list. In 2008, the EC responded to the EP Resolution with another Communication [16], in which it stated the regulatory aspects applicable to all nanomaterials used in the EEA and confirmed the effectiveness of the established law to cover potential health, safety, and environmental risks of nanomaterials; it also launched a review and implementation of the normative framework.

An upgrade of the European legislation was driven in 2009 by the EP Resolution on regulatory aspects of nanomaterials [17]. Given both the advantages of the use of nanomaterials and their potential nanotoxicity, the EP was mainly concerned with the absence of a harmonized definition of the term 'nanomaterial' in the EEA and the lack of clear information about the presence of nanomaterials in the labeling of a product. Such criticisms promoted the diffusion of miscellaneous interpretations about the nanomaterial classification and permitted companies to use the claim related to 'nanoscale' only for marketing purposes, without informing consumers clearly about the real presence of nanomaterials in their products. Following the EP Resolution, the European legislative framework was implemented. The Regulation (EC) No 1223/2009 introduced specific requirements for the use of nanomaterials in cosmetic products [18] and the European Medicines Agency (EMA) issued several guidelines for supporting the development of medicinal products containing nanomaterials, which has been in place since 2009. Nevertheless, only in 2011 a harmonized definition of nanomaterials valid for all goods marketed in the EEA was released by the EC. Furthermore, in 2012, the EC published a proposal for a Regulation on medical devices [19], which was reviewed in June 2016 [20] and which introduced requirements for the use of nanomaterials.

Nanomaterial definition

The current legal reference in the European Union (EU) about nanomaterials was established in 2011, when the EC published Recommendation 2011/696/EU [21]. Although the Recommendation is not legally binding and is not compulsory enforced in all EU countries, it introduces the first definition of 'nanomaterial' that should be used as reference in EU for legislative and policy purposes. Based on standards established by the International Organization of Standardization (ISO) [22], the definition was implemented by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) [23] and by the European Commission Joint Research Centre (JRC) [24] for adapting the ISO standard to the European regulatory framework.

In particular, a nanomaterial is defined as 'a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%'. As a consequence, the size, distribution, and physical state of material particles are indicated by the EC as univocal parameters with which to identify a nanomaterial. In addition, a material can also be considered 'nano' if its specific surface area by volume is greater than 60 m^2/cm^3 [21].

The defined size range aimed to facilitate the easy and uniform identification of nanomaterials. As evidenced in a JRC Reference Report [24], the lower limit is helpful to distinguish nanomaterials from atoms or molecules. By contrast, the upper limit of 100 nm was established by general consensus, even though there is no scientific evidence to support such threshold on the basis of changes in the physicochemical properties of a material [22]. Moreover, to avoid misleading interpretations, the EC specified that more than 50% of the particles of a nanomaterial must fall within the proposed size range. However, the Recommendation

also stated that fullerenes, graphene flakes, and single-wall carbon nanotubes must be considered nanomaterials because of concerns about their effects on the environment, health, and safety, even if their size is <1 nm.

Finally, the physical state of the particles (i.e., aggregates, agglomerates, and unbound particles) was included in the criteria for defining a nanomaterial, because: (i) nanomaterials tend to aggregate or agglomerate because of the attractive forces, producing structures >100 nm; and (ii) materials with a particle size >100 nm can, during their life cycle, release particles that have a diameter within the range of the definition.

Besides defining criteria, another critical issue is represented by the development of harmonized measurement methods that should ensure the correct application of the aforementioned definition. Indeed, published studies have demonstrated that measurement methods used for determining NPs size do not provide comparable results because of differences in recording and elaborating analytical data [25–27]. Therefore, in July 2012, the JRC published a reference report describing the requirements for particle size measurements of nanomaterials, underlining the existing issues and trying to suggest the most appropriate measurement methods for a specific nanomaterial type among those currently available [27]. Nevertheless, in 2014, the JRC confirmed the existence of pending relevant gaps among the available validated methods; these cannot be resolved via legislative interventions without establishing stronger scientific and technical cooperation among the European scientific community [28].

Nanomaterials in cosmetics

Cosmetic products can be directly applied to the external parts of the body (e.g., epidermis, hairs, nails, teeth, or buccal mucosa) for maintaining them in good condition, or protecting or cleaning them [29]. As a consequence, the safety assessment of their components is mandatory. Nanomaterials can be widely used in the cosmetic field [30,31] for improving the properties of the formulation (e.g., organoleptic or texture), its stability [32,33], and performance, such as enhancing substance accumulation in the upper layer of skin [34,35] or improving the protection from ultraviolet (UV) radiation [36]. Examples of nanomaterials incorporated in cosmetic products include silver NPs as antibacterial and/or antifungal agents, titanium and zinc oxide NPs as sunscreen agents, liposomes and solid lipid NPs as skin enhancers, and calcium salt NPs containing hydroxyapatite for dental care products [30,37,38]. However, such applications of nanomaterials require an in-depth re-evaluation of the risk assessment procedures related to their use in cosmetic products. For example, the reduction of the dimensions of a material to the nanoscale can improve its skin permeability profile, increasing its potential systemic exposure with respect to the corresponding bulk material.

The increased application of nanomaterials in the cosmetic field and the increasing concern about consumer safety [39] encouraged European Authorities to regulate their production and use. Given that a harmonized definition of nanomaterials was not available in 2009, the Regulation (CE) No 1223/2009 included the following definition of a nanomaterial: 'an insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm' (Article 2, Subsection 1, letter k) [18]. Although it is under revision, this definition is valid for cosmetic purposes.

The cosmetic regulation uses the same size range of Recommendation 2011/696/EC; however, it considers as nanomaterials only those that are intentionally made, excluding all the nanoscale materials that are accidentally produced or can be found in nature. Moreover, only materials that are insoluble or biopersistant (e.g., metals, metal oxides, carbon materials, etc.) are considered nanomaterials, whereas all those that are soluble, degradable or nonpersistent in a biological system (e.g., liposomes, solid lipid NPs, and structured lipid carriers) are not included in this definition. Such differences with respect to the harmonized definition proposed by the EC are justified by the fact that cosmetic regulation has been specifically created for guaranteeing product safety; by contrast, the EC definition tried to be universally applicable to all products marketed in the EEA, regardless of the industrial field of interest. Insoluble or biopersistant nanomaterials have a higher risk of toxicity because of acute and chronic exposure compared with those that are degradable or can be quickly eliminated by the organism [40]. Indeed, the exposure to insoluble or biopersistant nanomaterials might lead to harmful effects both at the local and/or systemic levels because of unwanted skin permeation and biodistribution. Moreover, the determination of nanomaterial biopersistence is particularly challenging: the current *in vitro* testing methods used in the risk assessment process of conventional chemicals are ideally applicable to nanomaterials, but they are not validated or appropriate for determining the dose–response profile of nanomaterials with respect to their particular properties [41].

Regulation (CE) No 1223/2009 introduced specific requirements for marketing cosmetic products containing nanomaterials. In particular, manufacturers have to preventively notify their intention to use nanoscale ingredients to the EC, transmitting product-related information to Cosmetic Products Notification Portal (CPNP) 6 months before placing the product on the market [Article 16, Regulation (CE) No 1223/2009]. The notification should include information regarding the identification of the nanomaterial, its specifications (e.g., particle size, and physical and chemical properties), an estimation of the amount of nanomaterial in the cosmetic product that is intended to be marketed per year, a toxicological profile, and, according to the category of the cosmetic product and its exposure conditions, safety data of the nanomaterial as used in such a product. In addition, the Scientific Committee on Consumer Safety (SCCS) was asked to enlarge the list of information needed to be notified and to intervene whenever the EC has concern regarding the safety of a specific nanomaterial. In 2012, the SCCS released guidance specifying the criteria to conduct the physicochemical characterization and to determine the toxicological profile and the reasonably foreseeable exposure conditions of a nanomaterial (Table 1) [42]. Furthermore, for guaranteeing the safer use of cosmetic products containing nanomaterials by consumers, the Regulation states that producers have to clearly identify nanomaterials in the label, adding the claim 'nano' after the INCI name of the ingredient [Article 19, Regulation (CE) No 1223/2009]. However, the effectiveness of such a legislative requirement remains controversial. The indication of nanomaterial presence in a cosmetic product does not provide to consumers any information about its safety

TABLE 1

Notification requirements for cosmetic products containing nanomaterials according to Regulation (EC) No 1223/2009 and to the SCCS

Notification Requirement according to Regulation (EC) No 1223/2009 [18]	Clarifications of Notification Requirement by SCCS/1484/12 [42]	
Identification of the nanomaterial and its specification	Physicochemical characterization (of the NPs as produced, as added to the cosmetic product, and as present during exposure for toxicological assessment)	Chemical identity (structural formula/molecular structure, CAS number)
		Chemical composition (including impurities, coatings, or surface moieties, encapsulating materials)
		Size (primary and secondary particle size, number size distribution, particle mass size distribution)
		Morphology (physical form and crystalline phase/shape)
		Surface characteristics (zeta potential)
		Solubility and dissolution rate in water
		BET surface area and volume-specific surface area
		Catalytic activity
		Concentration in terms of particle mass and particle number per volume
		Dustiness
		Density and pour density
		Redox potential (for inorganic nanomaterials)
		pH for aqueous suspension
		Viscosity
		Stability
		Other relevant information (e.g., UV absorption)
Toxicological profile of the nanomaterial	Toxicological end-points assessed for safety evaluation	Dermal/percutaneous absorption
		Toxicokinetics, determining ADME parameters to understand the fate and behavior of the nanomaterial in the body
		Acute toxicity
		Irritation and corrosivity
		Skin sensitization
		Mutagenicity/genotoxicity
		Repeated dose toxicity over prolonged periods, followed up by histopathological investigations
		Carcinogenicity
		Reproductive toxicity
		Photo-induced toxicity if expected or intended for use in sunlight-exposed skin
		Human data (extremely useful, but ethical concern because of the lack of information about the frequency and severity of adverse effects)
Reasonably foreseeable exposure conditions	Factors to assess reasonable foreseeable exposure	Class of cosmetic products in which the nanomaterial might be used
		Method of application (e.g., rubbed on, sprayed, applied and washed off)
		Concentration of nanomaterial in the finished cosmetic product
		Quantity of the product used at each application
		Frequency of use
		Total area of skin in contact
		Duration of exposure
		Foreseeable misuse, which could increase exposure
		Consumer target groups where specifically required
		Fraction absorbed by the body
		Application on skin areas exposed to sunlight
		Use area (indoors/outdoors) and ventilation
		All routes of exposure (dermal, oral, and inhalation exposure) should be considered in view of the intended use of the product

TABLE 1 (*Continued*)

Notification Requirement according to Regulation (EC) No 1223/2009 [18]	Clarifications of Notification Requirement by SCCS/1484/12 [42]	
	Exposure assessment (strictly depending on the reasonably foreseeable exposure conditions)	Determination of Systemic Exposure Dosage (SED) and Margin of Safety (MoS), according to the exposure route of administration (e.g., dermal, oral, or inhalation exposure)
		Qualitative determination of local effects (e.g., on skin after dermal application, on respiratory tract after spray application)

profile, even if detailed investigations are available for the notification process [43]. In other words, the current regulation transfers to the consumer the responsibility of the risk related to product use, without providing any data to support them in a conscious choice, and promoting uncertainties about the means beyond the 'nano' claim [44].

Nanomaterials in medical devices

As stated by Directive 2007/47/EC [45], a medical device is any instrument, apparatus, appliance, software, material, or other article intended to be used on humans for the diagnosis, prevention, monitoring, treatment, or alleviation of a disease or handicap, replacement or modification of the body anatomy or a physiological process, or control of conception by a mechanical or physical mechanism. Therefore, except for the mechanism of action, medical devices are similar to medicinal products in terms of claims and criticisms with respect to human health and safety. In addition, medical devices can also be designed to deliver medicinal products (e.g., syringe) or to incorporate (e.g., coronary stent) an active substance or a nanomaterial. When an active substance is incorporated in the medical device, its function must be ancillary, otherwise the product has to follow the more stringent regulation of medicinal products. Examples of medical devices containing nanomaterials reported in the SCENIHR guideline in 2015 [46] and available on the market are: antibacterial nanosilver incorporated in wound dressings (e.g., Acticoat®) and catheters as antibacterial agents (e.g., SilvaGard™), silicon dioxide NPs in composites for dental restoration, hydroxyapatite NPs contained in injectable bone-filling products (e.g., Nanogel®), catheters containing carbon nanotubes to strengthen the wall structure, nanostructured ceramic for stent coating (e.g., Debiostent™), and superparamagnetic NPs for cancer treatments (e.g., NanoXray, NanoTherm™) [47–49].

When the therapeutic activity is due to the nanomaterial itself, the classification can be difficult. For example, polymer-based dental filling composites [48] or iron oxide NPs administered to the patient for hyperthermia therapy against cancer [50] can be classified as medical devices because of the predominance of a mechanico-physical action. In particular, superparamagnetic iron oxide NPs have been studied for *in situ* treatments of solid tumors, taking advantage of their ability to vibrate in the presence of a magnetic field, producing heat (e.g., NanoTherm™, NCT02033447). When superparamagnetic NPs reach the tumor mass, the localized increase in temperature because of their vibration induces the consequent death of surrounding cells, damaging the inner structures of the cancer. However, in addition to such a physical mechanism of action, literature evidence suggests that the therapeutic and toxicological profiles of such nanosystems are mediated by other biological effects on cancer cells [51]. In addition, the functionalization of the particle surface with chemotherapy agents has been studied for improving their anticancer efficacy [52].

According to the legislation, medical devices are subdivided into classes (class I, IIA, IIB, and III) based on how the product use can be considered critical for human health and safety [53]. To be marketed in the EEA, a medical device must have the CE mark, which can be obtained after a certification assessment by the manufacturer or by a Certification Authority, called the 'Notified Body'. The requirements for obtaining the CE mark vary in terms of the function of the medical device class: from a simple self-certification by the manufacturer regarding the quality control of the device manufacturing process for class I, to a complete risk assessment of the product and manufacturing process made by the Notified Body for class III. To guarantee the harmonization of assessment procedures within the EEA, the EC also provides specific guidelines (i.e., MEDDEVs) to assist certification authorities and manufacturers in implementing directives related to medical devices [54].

The need for a revision of the Regulation on medical devices arose in 2011, when concerns about the control of the production and classification of medical devices were raised after a scandal in the use of industrial silicone instead of medical-grade silicone for the manufacture of breast implants [55,56]. In 2012, the EC published a *Proposal for a Regulation of the European Parliament and of the Council on Medical Devices* [19], which overhauled the regulatory framework of medical devices by enhancing the assessment procedures for obtaining the CE mark and by creating a centralized Medical Device Coordination Group (MDCG) to improve collaboration among European Notified Bodies. The Proposal of the EC also aimed to review the regulatory framework regarding the use of nanomaterials as, or in, medical devices. In June 2016, the General Secretariat of the Council of the EU released the last correction of the Regulation Proposal [20], which included further regulatory innovations on nanomaterials in medical devices. Unlike cosmetics, the definition of a nanomaterial for medical devices is that reported in European Recommendation 2011/696/EU [21]. Moreover, the revision process of the initial EC proposal yielded a more stringent classification for nanomaterial-containing devices on the basis of the risk of internal exposure to nanomaterials: devices with a high or medium potential risk should be included in Class III, with a low risk in Class IIB, and if the risk is negligible, in Class IIA. However, the Proposal does not indicate any methodological approach for performing the risk assessment to evaluate internal exposure, referring to the opinion

of unspecified 'relevant' scientific committee. Therefore, when the regulation needs to be enforced, manufacturers should test hypothetical hazards for the internal exposure of nanomaterials contained in the medical devices before defining the class to which the device belongs, aside from all the further studies that would be required by the Notified Body according to the peculiarities of the particular device and nanomaterial used. Thus, the SCENIHR underlined the need for manufacturers to study the potential toxicity pattern of nanomaterials according to the absorption route (e.g., ingestion, inhalation, parenteral, or dermal) and the exposure time both for devices containing nanomaterials and those that can release them following device wear-and-tear, even if the device does not nominally contain any NPs [57]. However, given that the release and toxicity are influenced by both the nanomaterial state (e.g., free, fixed on surfaces, or embedded in a matrix) and the methodological procedure used in the assessment, European Authorities underlined the need to identify sensitive analytical procedures for providing the biological safety evaluation. At the moment, no harmonized guidelines are available for supporting Notified Bodies in the evaluation of the documentation produced by manufacturers of a medical device containing nanomaterials. Waiting for the ISO guideline on 'Biological evaluation of medical devices—Part 22: Guidance on nanomaterials (ISO/TR 10993-22)', which is currently in preparation, the SCENIHR [46] proposed an interesting approach based on four evaluation phases for driving the safety assessment of invasive nanomaterial-containing devices. In Phase 1, the producer is asked to evaluate the potential risk connected to the release of nanomaterial for a device, including the possibility that a nanomaterial-free device can release them in in-use conditions. In this case, the producer should perform physicochemical tests to establish the nature of the released particles and should estimate the potential exposure of the human body to such nanomaterials, including risks of local effects occurring in the contact area with the device. Phase 2 aims to determine the biodistribution of the released nanomaterials and their persistence in the body. In the case of non-invasive devices, the determination of nanomaterial concentration in systemic circulation and in tissues is recommended. By contrast, more detailed toxicokinetic studies for establishing the ability of the nanomaterial to reach, and be retained in, specific tissues should be performed for invasive devices. Based on the obtained results, Phase 3 studies should focus on determining the hazard profile of nanomaterials based on specific toxicity tests, which are selected according to the observed exposure and potential organ persistence. The information gathered will be included in the final risk characterization (Phase 4). For determining the risk–benefit balance of a nanomaterial-containing device, the risk profile estimated by Phase 4 should be compared with those of nanomaterial-free devices designed for comparable applications. Such a comparison should include also the potential benefit to the patient.

If a nanomaterial-containing medical device is classified as class III or class IIB on the basis of Phase 4 results, the manufacturer has to performed further clinical evaluations to assess the clinical performance and safety of the medical device [20]. The clinical documentation produced is then examined by the Notified Body in collaboration with a relevant expert panel, which is provided by the EC in consultation with the MDCG (Annex VIII of Regulation Proposal). In particular, the expert panel has to provide a scientific opinion on the clinical documentation based on the following criteria: (i) novelty of the device or the related clinical procedure involved, with a possible major clinical or health impact; (ii) significant adverse change in the risk–benefit profile of the device category or group because of scientifically valid health concerns in terms of the components, source materials, or the impact on health in the case of failure; and (iii) significantly increased rate of serious incidents reported by the postmarketing vigilance system in terms of the device category or group. Even though the opinion of the expert panel is not mandatory for the Notified Body, it should be taken in consideration during the certification procedure, otherwise the Notified Body should provide a full justification.

Nanomaterials and medicinal products

The interest in the application of nanomaterials to medicine has increased so much that the term 'nanomedicine' was coined to identify a new interdisciplinary field of research, which involves physics, chemistry, engineering, biology, and medical sciences [58]. Different nanotechnology-based products, including nanocrystals, nanoemulsions, liposomes, protein NPs, polymer–drug conjugates, polymeric micelles, and inorganic NPs, have been authorized and marketed in Europe and the USA for nanomedicine applications.

Most nanotechnology-based products currently available on the European market were authorized by centralized or mutual recognition procedures [59]. Thus, the EMA issued a reflection paper on nanotechnology-based medicinal products for human use in 2006 [60], which also included an official definition of nanomedicine. As stated, nanomedicine is defined as 'the application of nanotechnology in view of making a medical diagnosis or treating or preventing diseases. It exploits the improved and often novel physical, chemical and biological properties of materials at nanometre scale', namely from the atomic level at around 0.2 nm (2 Å) up to around 100 nm.

However, nanotechnology-based products investigated for pharmaceutical applications turned out to be more broad than the proposed definition, inducing the EMA to also include all the 'structures' with size less than 1000 nm that are designed to have specific properties [61]. Indeed, most of the investigational and approved nanomedicine products contain nanocomponents with a mean size of 0–300 nm and nanocrystal dispersions that resulted in sizes up to 2000 nm [62]. The nanosize of the material is only one of the critical attributes that influences its capacity to interact at the molecular level with structures in the human body, overcoming the most common criticisms of drug biopharmaceutics (e.g., drug solubility, administration, distribution, metabolism, and elimination). For example, the possibility for nanomaterial to be functionalized with different targeting moieties (e.g., small molecules, antibodies, proteins, or aptamers) can improve its effectiveness in delivering drugs to the therapeutic site and can alter significantly its toxicological profile [63,64]. Therefore, Regulatory Agencies required manufacturers to perform accurate preauthorization studies for assessing the quality, safety, and efficacy profile of a new drug product. Given that nanomedicine products can be innovative, the typology and entity of such studies can be evaluated on a case-by-case basis

and the EMA suggests that companies seek product-specific scientific advice regarding questions related to the necessary studies.

Despite the huge effort of the scientific community and private pharmaceutical companies in developing new nanoscale drug products, the success rate of approving novel nanomedicine products has not exceeded 10%, mainly because of failures in terms of safety and efficacy profiles during nonclinical and clinical studies [65].

Moreover, the increase in nanomedicine products and the recent expiration of patents have also pushed regulatory agencies to release specific guidelines and reflection papers for supporting manufacturers in developing nanomedicine products with specific properties (e.g., surface coating) or generic products of authorized nanoformulations (e.g., liposomal systems, polymeric micelles, and coated iron NPs). For nanomedicine products intended to be commercialized in the EEA as generic products, the EMA applies a similar approach to that with biotechnological products [66]. In addition to a detailed characterization of drug product properties and identification of the critical attributes of products and the manufacturing process, the EMA requires applicants to conduct in-depth studies of how such quality aspects influence the safety and efficacy profiles of their product. The authorization of 'generic' nanomedicine products should be supported by appropriate comparability studies, because the therapeutic equivalence versus an originator nanomedicine product cannot be assessed by a bioequivalence study alone [59]. Given that the nanoworld is variegated, the EMA released different guidelines for helping manufactures to develop specific classes of nanomedicine product (Table 2).

Coated nanomedicine products

A 'coated nanomedicine product' is a nanomedicine product with modified surface properties that alter its interactions with the biological environment after administration. The surface of many approved nanosystems was engineered to minimize aggregation (e.g., coated iron NPs for treating anemia) and interactions with circulating proteins to reduce the reticuloendothelial system (RES) and macrophage clearance and to prolong the plasma circulation time. Furthermore, surface modifications can impact significantly the biodistribution of nanosystems and their interactions with cellular targets, which are therapeutically and toxicologically relevant. A nanosystem can be modified by attaching a 'simple' molecule, such as drug or poly-(ethylene glycol) chains (PEG), or more complex ligands such as proteins or antibodies, to its surface via covalent or noncovalent bonds.

The position of the EMA on the requirements for coated nanomedicine products was summarized in a reflection paper issued by the Committee for Medicinal Products for Human Use (CHMP) in 2013 [67]. As stated, the impact of coating on the product stability and the pharmacokinetic profile should be thoroughly studied during the pharmaceutical development, including any potential new interaction (i.e., specific or non-specific) with biomolecules and cells, triggered by any ligand conjugated on the nanosystem surface. In addition, the reflection paper indicated several critical points that should be considered for assessing the quality, safety, and efficacy of the final drug products (Table 2). In particular, the coating process and the starting materials should be fully characterized and validated. The influence of the coverage heterogeneity of the coating surface should be investigated in terms of pharmacokinetics profiles with respect to the proposed use. Indeed, it has been demonstrated that the density of PEG chains on polymeric NP surfaces critically affects the control of the nanosystem interactions with the complement cascade [68]. Finally, the physicochemical stability of coating, its premature detachment, or any degradation pattern should be exhaustively studied for predicting the *in vivo* fate of all formulation components. If coating degrades or detaches from the nanosystem, appropriate *in vitro* and *in vivo* studies should be performed to determine the presence of new functional groups on the nanosystem surface and the biodistribution and metabolism of all the released components.

Micellar systems

The use of micellar systems has been widely applied for improving the biopharmaceutical performances of low-soluble drugs in several administration routes (e.g., oral or intravenous) [69]. In general, such medicinal products are lyophilized powders or concentrated suspensions that should be diluted before administration. However, their development and production show some similarities to other nanomedicine products, given that micellar systems are nanoscale products in many cases.

The quality attributes of micellar systems required by the EMA are those that guarantee the high in-use stability of the dosage form and the rapid release of the drug after intravenous administration, which results from the rapid loss of micelle integrity in physiological fluids (Table 2). Given that researchers mainly aim to avoid drug precipitation in reconstructed vehicles before infusion, complete physicochemical characterization of the drug and its excipients has to be performed during pharmaceutical development to identify all the critical quality parameters affecting the performances of the drug products. In addition, the nonclinical and clinical part of the authorization dossier should include suitable studies for rationalizing the time and condition of infusion to guarantee the breakdown of micelles after administration and to determine the risk of adverse effects (e.g., hemolysis). Finally, the reflection paper reports specific requirements for conducting clinical studies required for generic products. Given that the EMA considers micellar systems as 'complex' parenteral products, the bioequivalence between the generic and the originator product should be demonstrated. The type of studies required by the EMA varies according to the similarity in formulation composition between the test and originator product. Indeed, if their compositions are identical or if they are similar (i.e., they have the same surfactant), a biowaiver is accepted for assessing the equivalence of the drug products. By contrast, when formulations differ in surfactant type, a bioequivalence study should be carried out, at least to exclude whether the behavior of micellar systems during the early stages of a slow infusion can alter the drug distribution significantly.

Block copolymer micellar systems

Block copolymer micellar systems (bPMS) are nanomedicine products comprising amphiphilic self-assembling polymers [70]. Unlike other micellar systems, bPMS dissociate slowly *in vivo* and can

TABLE 2

Additional quality, nonclinical, and clinical studies required for Common Technical Document (CTD) of nanomedicine products according to reflection papers of the EMA.

Quality studies	Non-clinical studies	Clinical studies
Coated nanomedicine products (EMA/325027/2013) [67]		
- Complete characterization of coating materials; - Definition of physicochemical nature of surface to which the coating adheres; - Complete validation of coating steps, including detailed analyses of the chemistry beyond; - Additional information (e.g., conformational state, protein consistency) are required for complex ligands (e.g., protein or antibody) intended to active targeting; - Coating stability during storage and in use; - Premature detachment and release of coated ligands and/or their degradation.	- Impact of surface coverage heterogeneity and coating physicochemical stability on safety and efficacy of drug product; - In vivo impact of different coating materials/surface coverage on pharmacokinetics and biodistribution of drug product; - Biodistribution and metabolism of coating ligands.	
Micellar systems (EMA/CHMP/QWP/799402/2011) [69]		
- Physicochemical characterization of active substance (e.g., lipophilicity, pH-solubility, pH-stability, LogP, and LogD); - Physicochemical characterization of excipients (e.g., surfactant polydispersity, purity); - Impact of pH and ionic strength on micelle properties; - Critical micelle concentration (CMC) in model of reconstitution vehicles; - Carrier solubility capacity; - Physical stability in diluted infusion solutions at different temperature; - Compatibility with the common injection and infusion devices; - Mean size and distribution of dispersed micelles; - Estimation of micelle concentration; - Determination of entrapped/free drug factions.	- In vitro studies for investigating the influence of infusion on the breakdown of micelles in plasma-based models; - In vivo studies for determining the risk of persistence of micelles in animal models.	- Determination of time and condition of infusion, for excluding risk of adverse effects (e.g., haemolysis); - Tracking of micelles in plasma to investigate their loss of integrity and their distribution; - Biowaiver or bioequivalence studies (only for generic products).
Block co-polymer micellar systems (bPMS) (EMA/CHMP/13099/2013) [70]		
- Complete characterization of all bPMS components (e.g., active substance, block copolymer, stabilizing agent); - Content of block copolymer and active substances in final drug product; - Determination of unloaded/loaded drug in bPMS; - Complete characterization of polymers used in the synthesis of block copolymer; - Impact of copolymer modification of morphological properties of bPMS - Impact of micellar systems (e.g., osmolality, drug fraction adsorbed on the micellar surface) on the drug release; - Validation of manufacturing process and identification of the key steps, including block copolymer synthesis; - Accurate control of raw materials and intermediates; - Physical stability of bPMS; - Chemical stability of drug and block copolymer; - Degradation profile of bPMS in physiological fluids.	- Pharmacological profiles of placebo bPMS ; - Influence of bPMS on the drug pharmacokinetics (e.g., rate of clearance, distribution, interaction with plasma proteins); - Pharmacodynamics studies for investigating the impact of chemical composition and physicochemical properties of bPMS on its mechanism of action using in vitro and in vivo models; - Full toxicity characterization of bPMS, including toxicokinetic investigation in circulating fluids, target tissues and toxic relevant organs.	

TABLE 2 (*Continued*)

Quality studies	Non-clinical studies	Clinical studies
Liposomal systems (EMA/CHMP/806058/2009/rev.02) [74]		
- Complete characterization of all liposome components, including the quality and the purity of lipids; - Morphological properties of liposomal systems; - Drug fraction encapsulated and its distribution in liposome; - Determination of lipid bilayer phase transition behaviour; - pH of internal compartments (only for pH-gradient loaded liposome) - Stability in physiological fluids (e.g., plasma); - In vitro drug release from the liposome in physiologically/clinically relevant media; - Full characterization of ligand should be carried out according to guideline on coated nanomedicine products, if liposome is functionalized; - Stress tests for determining physical and chemical degradation profiles (comparative studies for generic products) - Identification of the key steps of manufacturing process; - Stability of drug, lipids, and other critical excipients in the finished product; - Stability of liposomal systems during storage and in-use conditions; - Robustness of reconstitution process.	- Comparative studies of pharmacokinetics, toxicology and pharmacodynamics (only for generic products); - Interaction between liposome and cellular lines that are pharmacologically and toxicologically relevant; - In vitro or in vivo immune reactogenicity assays; - CARPA tests; - Organ function tests.	- Comparative pharmacokinetics studies for assessing the equivalence of generic and originator products (e.g., systemic exposure of total, unencapsulated and encapsulated drug, similar distribution and elimination profiles).
Iron-core nanoparticles (EMA/CHMP/SWP/100094/2011; EMA/CHMP/SWP/620008/2012) [84,85]		
- Complete characterization of physicochemical properties of raw materials (e.g., carbohydrate characterization, polymorphism of iron core); - Morphological properties of iron core and iron-carbohydrate complexes; - Ratio between bound carbohydrate to iron; - Impact of physicochemical properties of carbohydrate matrix on the nanoparticle stability during storage; - Impact of physicochemical properties of carbohydrate matrix in vivo pharmacokinetics and toxicokinetics; - Amount of labile iron released from the product when administered - Impurities (e.g., ratio of iron-(II) and iron(III); - Degradation profiles; - Measurement of amount of iron-(III) released by the systems; - Stress tests for determining physical and chemical degradation profiles (comparative studies for generic products)	- Biodistribution studies on compartments involved in pharmacological action (e.g., plasma, RES, spleen) and in therapeutic (e.g., bone marrow) and toxic target tissues (e.g., kidney, liver, lungs, heart).	- Bioequivalence studies (only for generic products).

be used for controlling the drug biodistribution in specific compartments, such as solid tumors.

In collaboration with the Japanese Ministry of Health, Labor, and Welfare (MHLW), the EMA issued in 2014 a specific reflection paper on bPMS, which included all the quality, nonclinical, and clinical requirements for marketing authorization (Table 2). In particular, the critical quality parameters, which are able to influence strongly the pharmacokinetic and pharmacodynamic properties of the bPMS, have to be provided to assess its efficacy and safety profiles. The EMA requires a complete characterization of the formulation and its components (e.g., active substance, block copolymer, and stabilizing agent) to predict better the biopharmaceutical performances of drug-loaded bPMS and its toxicological profile. However, given the low regulatory experience of such nanomedicine products and the absence of validated analytical methods in the *European Pharmacopoeia*, the guideline emphasizes the necessity of developing biorelevant methods for studying *in vitro* bPMS performances, such as how the drug can be released by the nanosystem. Furthermore, given that the bPMS itself can be biologically active, the EMA requires accurate studies to determine the pharmacological and toxicological profiles of the bPMS and how its physicochemical properties can impact on those. Finally,

the reflection paper contains specific indications to help applicants to design first-in-human clinical studies.

Liposomal systems

Liposomes are lipid-based nanosystems comprising one or more phospholipid bilayers enclosing aqueous compartments. Given the analogies with biological membranes, lipid vehicles were first used as simple models for studying lipid bilayers [71] and, second, as drug-delivery systems [72]. Indeed, several liposomal-medicinal products have been investigated and authorized over the past decades [73]. Among those, Doxil®, a doxorubicin-loaded PEGylated liposome approved in 1995, is perhaps the most famous liposomal medicinal product and one of the most-characterized nanosystems *in vitro* and *in vivo* [5]. Encouraged by the expiration of the Doxil® patent in 2010, regulatory agencies started to work toward identifying general indications to help pharmaceutical companies in the development of generic products. In 2012, the EMA released the latest revision of the reflection paper to support the marketing authorization of intravenous liposomal systems that are generics of products already authorized in the EEA [74]. The requirements for such systems in terms of quality, nonclinical, and clinical data are specified in Table 2.

The quality and purity profiles of lipids and other critical excipients should be extensively characterized during their pharmaceutical development because their features impact strongly the liposomal functionality. Moreover, if the liposomal system was functionalized with specific ligands, additional studies are requested in accordance with the specific reflection paper on coated nanosystems [67]. For generic products, the tested product should demonstrate that its composition is qualitatively and quantitatively comparable to that of its originator under both normal and stressful conditions. Furthermore, the test formulation should also be comparable with that of the originator in terms of the physical and chemical degradation profiles after proper comparative stress tests. Nonclinical and clinical requirements include extensive comparative *in vitro* and *in vivo* studies for demonstrating superimposable biopharmaceutics and toxicological profiles between generic and the originator liposomal product. Given that acute hypersensitivity infusion reactions are common for such nanomedicine systems [75], appropriate toxicological studies, such as *in vitro* or *in vivo* immune reactogenicity assays and complement activation-related pseudoallergy (CARPA) tests, are mandatory to exclude potential risks of adverse effects. If the toxicological studies highlight an increased risk of the tested product compared with the originator, the pharmaceutical development should be completely re-evaluated.

Iron-core nanoparticles

Iron-based nanosystems have been widely studied for various applications and several nanomedicine products are available in the EEA for clinical use [76]. On the one side, iron-oxide NPs (IONs) have been studied for diagnostic purposes as imaging agents, taking advantage of their superparamagnetic properties [77]. According to the literature and clinical evidence, IONs appear to be more versatile than other conventional agents and to improve the image accuracy under some physiological and pathological conditions [78]. As a consequence, several products have been authorized in the EEA, inducing the EMA to upgrade the guidelines regarding the clinical evaluation of diagnostic agents to include superparamagnetic NPs [79,80]. However, only a few nanomedicine products are currently available in Europe. Most of the medicinal products previously authorized were withdrawn from the market by the holders of the marketing authorization. Moreover, other ION-based products (e.g., Sinerem®) were withdrawn during the authorization process after CHMP expressed concerns about their effectiveness [81].

On the other side, IONs also found applications in the treatment of anemia and severe iron deficiency in adults with chronic kidney disease. In particular, the nanomedicine formulation (e.g., ferumoxytol) was able to improve the iron pharmacokinetics and to reduce the adverse effects compared with traditional iron treatments [77]. As a consequence, several nanomedicine products are authorized in the EEA and, despite some withdrawals (e.g., Rienso®) [81], most remain available [59,73]. Comprising an iron core stabilized by a carbohydrate complex (e.g., dextran, gluconate, or sucrose), such IONs were designed to be administered intravenously and be internalized by the RES, which degrades the IONs and releases free iron into the blood stream. However, their clinical use has received some criticisms. First, although most of the iron in the human body circulates in association with proteins, such systems can induce an increase in labile iron [iron-(III)] concentration that can trigger toxicity effects, such as oxidative cellular damage [82]. Second, serious adverse effects (e.g., hypersensitivity reactions) were reported by pharmacovigilance systems in various European countries, inducing a re-evaluation of the benefit–risk assessment. In 2013, CHMP concluded that the 'benefits of these medicines are greater than their risks' and introduced proper recommendations to manage the risk of allergic reactions [83].

In this context, the patent expiration of IONs originator products induced the EMA to issue specific reflection papers for helping applicants to develop generic products [84,85]. In particular, the EMA requires extensive characterization of the physicochemical properties of the carbohydrate matrix, the iron core, and the final formulation, including the identification of all the critical quality aspects that influence the safety and efficacy profiles. A 'quality-in-process' approach is suggested for the manufacturing process of such nanosystems, including a detailed validation of the manufacturing process and the key step controls.

Nonclinical comparative studies should be focused on the determination of the biodistribution of iron-core nanoparticles in model animals. Particular emphasis was given in the EMA reflection paper to the accumulation and retention in the compartment involved in the pharmacological action of IONs, such as plasma, RES (especially spleen), and therapeutic (e.g., bone marrow) and toxic target tissues (e.g., kidney, liver, lungs, and heart). Toxicity studies were not required because the current animal models were not sensitive enough to accurately assess differences in the safety profiles.

Finally, clinical studies should focus primarily on the comparison of the pharmacokinetics of the tested and originator products. The therapeutic equivalence of products should be demonstrated by comparing efficacy and safety profiles, but only if comparative studies have highlighted significant differences.

Concluding remarks

The current European regulatory framework supervising the presence of nanomaterials in healthcare products is stratified and far from being harmonized. Despite the efforts of various European Authorities, several criticisms inevitably remain because of the lack of well-established scientific knowledge of nanomaterial properties and their characterization. First, the definition of nanomaterials currently enforced in the EEA is mainly based on particle size, without considering that some materials can exhibit physicochemical properties close to accurately defined nanomaterials even if their particle size is bigger than the proposed size range (i.e., 1–100 nm). The EC has approached the nanomaterial issue in a comprehensive manner to assure the safety of consumers, workers, and the environment, resulting in regulatory initiatives that aim to be applicable to all industrial sectors. As a consequence, healthcare, chemical, and electronic products are under the same regulatory umbrella. However, the specific features of healthcare products, such as the higher risk for consumer safety because of direct internal or external exposure to nanomaterials during the final product use, demonstrate the need for new regulatory initiatives. Guidelines and requirements more focused on characterizing the most critical physicochemical, technological, and biological properties of nanomaterials as a function of their performances in final commercialized healthcare products are desirable. The regulatory framework should also include harmonizing factors to avoid the contrasting interpretations of nanomaterial classification, led by the differences in current regulatory frameworks for healthcare products. A liposome (particle size >100 nm) is not considered a nanomaterial on the basis of the current cosmetic regulation, but it does if it is authorized as a medicinal product. By contrast, a drug-loaded nanomedicine product (particle size >100 nm) might not be classified as a nanomaterial if it is included in an invasive medical device for absolving an ancillary function. Furthermore, the distinction between a medical device and a medicinal product is difficult to draw in some cases, affecting the risk assessment of the nanomaterial-containing product. For example, IONs can be classified as medical devices if they are designed as hyperthermia treatments for tumors because of their physical mechanism of action (e.g., heat produced by the vibration induced by a magnetic field; NCT02033447). However, they might also fall within the definition of medicinal product, given that they can have a direct biological effect on cancer cells [51] or they can be functionalized with chemotherapy agents for improving the anticancer efficacy [52].

The current regulations of medical devices and medicinal products are not as thorough as those for cosmetic products, although such products have proven to be even more critical in terms of their possible toxicity to humans. In any case, unlike cosmetic products, the quality of a medical device or a medicinal product and any relevant risk to the safety of users need to be evaluated in-depth before the product is launched in the EEA.

Unlike European authorities, American authorities released a specific guidance for healthcare products in 2014, taking advantage of the presence of a unique regulatory agency (i.e., the US Food and Drug Administration; FDA) for food, cosmetic, medicinal products, and medical devices [86]. Although similar to the European approach in many aspects, the initiative of the FDA is based more on the properties of the nanomaterial. The nanomaterial definition proposed by the FDA states that the size range used to define a nanomaterial (i.e., 1–100 nm) can be enlarged up to 1000 nm if the material exhibits specific properties or phenomena that are attributable to its small dimensions.

Although the current European regulatory framework on nanomaterials use in healthcare products has several strengths, its upgrade is desirable, a need that is even more urgent for medical devices. Even though the latest revision of the regulatory framework includes attempts to improve the cooperation among European Certification Authorities (e.g., the creation of MDCG), harmonized guidelines to support Certification Authorities and expert panels in the evaluation procedures for CE marking are still not available. In addition, on the one hand, the creation of a unique European Regulatory Authority might be desirable to centralize the evaluation of the most critical medical devices, overcoming the criticisms related to the current Notified Body-based system. On the other hand, the improvement in international cooperation between regulatory experts is desirable to implement the existing ICH and ISO guidelines or to create specific internationally harmonized guidelines for helping manufacturers during product development and characterization and for limiting differences in regulatory requirements among regulatory authorities. In this context, it is worth noting the collaboration between the EMA and MHLW that resulted in the release of the reflection paper on bPMS [70] and the creation of specific nanomedicine working groups by international networks of regulatory experts, such as the International Pharmaceutical Regulatory Forum (IPRF) [87].

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