A state of play study of the market for so called "next generation" nanomaterials

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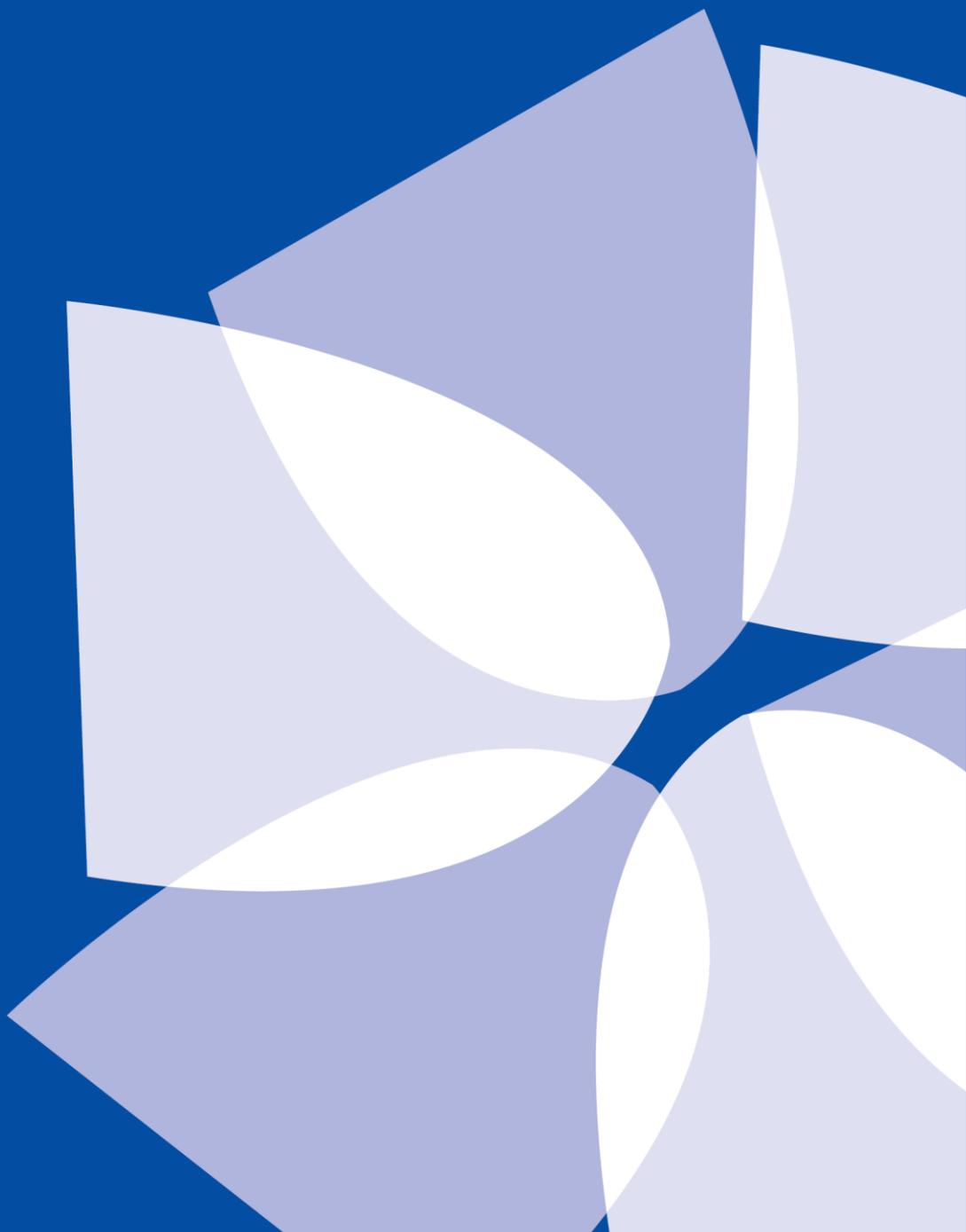
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This study was commissioned by the European Union Observatory for Nanomaterials (EUON) and was carried out by Risk & Policy Analysts Ltd and RPA Europe Srl.

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Abbreviations

CLP	Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures
DNA	Deoxyribo-Nucleic Acid
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EUON	European Union Observatory for Nanomaterials
ISO	International Organisation for Standardisation
KET	Key Enabling Technology
MRL	Market Readiness Level
NBIC	Nano-Bio-Info-Cognitive
NEMS	Nano-Electro-Mechanical Systems
nm	Nanometre
PEG	(Poly)Ethylene Glycol
RDG	Arginylglycylaspartic acid
REACH	Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
RES	Reticulo-Endothelial System
ROS	Reactive Oxygen Species
S&T	Science & Technology
SPIO	SuperParamagnetic Iron Oxide
SPR	Surface Plasmon Resonance
TNF	Tumour Necrosis Factor
TRL	Technology Readiness Level
UVCB	Unknown or Variable composition, Complex reaction products or of Biological materials
WP	Work Package

Abstract

This study presents the analysis of the definitions of next generations of nanomaterials proposed in the literature and suggests some refinements to allow for the creation of an inventory of second and higher generation nanomaterials on the market or expected on the market in the next five years. It also details the assessment of the suitability of the REACH and CLP terms "substance", "mixture" and "article" and of the characterisation and identification parameters included in the amended REACH Annexes with regard to next generation nanomaterials.

The review of the relevant literature has been complemented with an experts' consultation and with data scraping from nanotechnology inventories and companies' websites.

Rather than being used for regulatory purposes, the concept of different "generations" of nanomaterials was introduced to highlight the possibilities of nanotechnology and attract private and public investments. In this context, the word "nanomaterials" referred to nanotechnology products, including objects, such as nanostructures and nanostructured materials, that go beyond the European Commission's recommended definition of nanomaterials. Some refinements to the definitions proposed in the literature have been suggested referring to the concepts of energy, work and thermodynamic equilibrium. Only few examples of second generation "active" nanomaterials have been found as already on the market, mainly in medicines and electronics applications. Given the increasing complexity and integration between organic and inorganic components, further guidance would benefit prospective REACH registrants.

Executive Summary

Several publications have discussed the problem of governance for "next generation", "second generation" or "future" nanomaterials. However, these different generations are not well defined or are not commonly agreed terms and therefore the precise identification of regulatory challenges that may be associated with such materials is not possible.

The objectives of the study were:

- To collect existing definitions of generations of nanomaterials;
- To refine these definitions in order to allow the sorting of different nanomaterials into different generations unambiguously;
- The establishment of an inventory of second or higher generation nanomaterials on the market or close to the market;
- The assessment of the suitability of the terms "substance", "mixture" and "article" and of the identification and characterisation parameters included in the revised Annex VI of the REACH Regulation to describe the next generation nanomaterials found.

In order to fulfil these objectives, the project team carried out a literature review complemented with a targeted consultation with experts from industry, academia and regulatory authorities.

The concept of subsequent generations of nanotechnology applications was introduced by Mihail Roco in 2004 and further refined in subsequent publications. Roco envisaged six overlapping generations from an engineering perspective, based on an increasing level of sophistication and complexity of systems at the nanoscale and of a convergence of different disciplines of science and engineering. These generations are:

- Passive nanostructures: the nanostructures have stable behaviour during their use;
- Active nanostructures: the nanostructures change their composition and/or behaviour during their use;
- Systems of nanosystems: three-dimensional nanosystems frequently incorporated into other systems and using various syntheses and assembling techniques such as bio-assembling, robotics with emerging behaviour, and evolutionary approaches;
- Heterogeneous molecular nanosystems, where each molecule in the nanosystem has a specific structure and plays a different role. Molecules will be used as devices and from their engineered structures and architectures will emerge fundamentally new functions;
- Nano Bio Info Cogno (NBIC) integrated technology platforms;
- Nanosystem convergence networks.

An alternative attempt to outline the ongoing nanotechnology research at the time was done by James Tour in 2007. Tour describes:

- Passive nanotechnologies: The nano part does nothing particularly elaborate. Its presence alone adds a significant increase to the performance of the system;
- Active nanotechnologies: In this case, the nano entity does something elaborate such as absorbing a photon and releasing an electron, thereby driving a device, or moving in a specific and definable fashion across a surface. An example could be a "nanocar", a nano-engineered molecule that can be used to move atoms from one place to another;
- Hybrid nanotechnologies: The complementing of a known platform through the attachment of a nano-sized entity but where the platform carries the bulk of the burden. An example would be using a silicon platform to carry out electronics, but making the silicon work with higher performance through the attachment of a surface layer of organic molecules that donate or accept charge.

No other alternative definitions have been found.

The project team has identified some issues around the Roco's and Tour's definitions. These are:

- The definitions were proposed to highlight the possibilities of nanotechnology and to attract public and private investments and, as such, were never meant to be used in a regulatory context. Roco himself clarified that the defined generations are overlapping. In turn, Tour's definitions, although simpler, use too vague terms (i.e. to do something elaborate) to distinguish between the first and second generation. They are therefore difficult to use for regulatory purposes or for sorting different nanotechnology applications into different generations unambiguously;

- The definitions are not used by researchers, who rather refer to other categories, such as the composition of the nanomaterial or areas of technological application;

- The recommended definition of nanomaterial of the European Commission is more specific and quantitative than most other (often non-regulatory) definitions. It is explicitly limited to particulate matter and its provisions are designed and tailor-made to specifically address this type of material, based on human and environmental exposure considerations. Roco refers to different generations of nanotechnology products or nanotechnology applications and uses terms such as nanostructures (passive and active), nanosystems (system of and molecular), NBIC integrated technology platforms and nanosystem convergence networks. Tour refers to different generations of nanotechnologies. There is no specific definition recommended by the European Commission for nanostructured materials, but the International Organisation for Standardisation (ISO) defines these as material[s] having internal or surface structure in the nanoscale. Whether nanostructured materials and nanostructures are nanomaterials according to the EC recommended definition depends on whether they are materials consisting of particles and whether 50% or more of these particles are in the size range from 1 nm to 100 nm (particle number size distribution)[1];

- The definitions suggested by Roco and Tour do not allow an unambiguous distinction between passive and active nanostructures.

In light of these issues, the project team suggested to refer to generations of nanomaterials, nanostructures and nanostructured materials and to refine the definitions using the concepts of energy, work and thermodynamic equilibrium. Energy is the capacity for doing work, where work is the quantity of energy transferred from one system to another. Thermodynamic equilibrium is the condition or state of a thermodynamic system, the properties of which do not change with time and that can be changed to another condition only at the expense of effects on other systems. On this basis, the proposed refined definitions are:

- First generation of nanotechnology applications – Passive nanomaterials, nanostructures and nanostructured materials: These are materials and structures, with constituent parts in the nanoscale, which are in thermodynamic metastable equilibrium with the surrounding system. No intentional changes of state are occurring at a measurable rate during their use, beyond agglomeration and aggregation;

- Second generation of nanotechnology applications – (Re)active nanomaterials, nanostructures and nanostructured materials: These are stimuli-responsive materials and structures, with constituent parts in the nanoscale, which during their use absorb, receive or harvest energy from their surroundings and transduce it to engage in a

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[1] See https://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm#12

variety of non-equilibrium activities, usually, but not solely, connected to motility, growth or replication. These non-equilibrium activities usually imply a change in the energy level of the system and in the conformation / molecular structure. They qualify as (re)active nanodevices, meaning that they are artificial constructs designed to perform a predefined function in an environment by exploiting externally-supplied energy, in the form of a stimuli, and which have an overall size or components in the nanoscale. The stimuli-responsiveness can originate from the organic ligands on the surface or from their inorganic cores. Stimuli can be chemical (e.g. solvents, acid/base signals, metal ions, gases, bio-macromolecules and redox signals) as well as physical (e.g. temperature, magnetic fields and light);

- Third generation of nanotechnology applications - Multifunctional nanosystems: Stimuli-responsive nanoparticles and nanostructures are the building blocks for constructing complex chemical reaction networks and synthetic life-like systems and materials. They are characterised by an increased integration between organic and inorganic components. In multifunctional nanosystems, multi-functional nanodevices are integrated and can function as living species with capabilities of responding, sensing, controlling, communicating and actuating.

As expected and suggested by Roco, each generation is characterised by an increased integration between organic and inorganic components and by the convergence between different technological fields: active nanostructures are often functionalised with proteins or enzymes or based on micelles or liposomes; multifunctional nanosystems aim to mimic living cells and may use some of their components, such as proteins and nucleic acids. At this level, nanotechnology overlaps with synthetic biology.

According to two papers published in 2010 and 2016 and to the bibliometric analysis carried out by the project team, there has been some increase in recent years towards research on active (second generation) nanostructures, but the absolute share in all nanotechnology research is still modest. The search for second and third generation nanotechnology applications for the establishment of an inventory has yielded 48 examples of (re)active (second generation) nanomaterials, nanostructures and nanostructured materials found on the market or expected on the market in the next 5-10 years. These are mostly medical and nanoelectronics applications. Although not fully comprehensive, they represent the types of second-generation nanotechnology applications currently on the market. The inventory also lists eight examples of third generation nanotechnology applications. Research on multifunctional systems (3rd generation) is still at the conceptual phase.

The identification and characterisation parameters required by the revised Annex VI of the REACH Regulation allow to unambiguously identify and characterise different nanoforms of a substance. Further guidance is required (and currently being developed) on whether coatings and functionalisation with organic and inorganic compounds are to be considered for determining whether the particle size regulatory threshold included in the EC recommended definition is exceeded. Some criteria for distinguishing between solid and fluid materials would add further clarity. Moreover, given the increasing integration between organic and inorganic components and the functionalisation with proteins or enzymes, some additional suggestions on the information to be provided on the surface treatment would facilitate registrants while ensuring information coherence across different registration dossiers. The current use descriptors included in the use description system, and in particular the technical functions, do not adequately represent the uses and functions of nanomaterials on the market or which could be placed on the market in the near future. An ad-hoc list of use descriptors for nanomaterials would facilitate the provision of relevant information. Finally, the guidelines on how to determine whether an object is an article according to the REACH Regulation could be complemented with specific examples on different nanomaterials, from simple nanoparticles to more complex assembly structures.

The Annex VI identification and characterisation parameters are designed to characterise the

nanomaterial as manufactured, and do not capture the dynamic dimension of the second and third generation of nanotechnology applications, including of second and third generation nanomaterials. The parameters were thought to identify and characterise nanoforms of substances rather than more complex technologies. To capture such dynamics, information would be needed on:

- The type of energy input/external stimulus (e.g. light, pH, temperature, magnetic field);
- The intended function/work to be carried out (e.g. remotely activated drug release, thermal ablation, fluorescence imaging);
- The changes occurred to the nanomaterial, nanostructure or nanostructured material following the external stimulus (e.g. change in composition, shape, surface area).

The identification and characterisation parameters apply to nanomaterials, i.e. solid particulate matter consisting of particles with the majority of its particle size number distribution below the 100 nm threshold. The generations of nanotechnology applications, as conceived by Roco and refined in this report, include nanostructures and nanostructured materials which may not be covered by the EC recommended definition of nanomaterials. Increasingly complex and functional particulate materials are currently used in the medical sector only, and as such may be out of the scope of the REACH Regulation. As noted by some authors, innovation depends on the understanding of nanomaterial interactions with the surrounding material systems. Nano-enabled materials entering the market are currently enabled by employing internal nanostructures designed for, synthesised in and integrated with material systems, rather than by embedding in the product matrix particulate nanomaterials previously synthesised and functionalised.

New metrological methods are necessary for the characterisation of nanostructured materials. These need to go beyond particle size and shape and look into 3D-resolved composition analysis of inorganic and organic structures, interface/surface chemistry, surface reactivity, and transformational/reactive processes. Work is currently ongoing on the development of OECD test guidelines addressing some of these areas.

1. Introduction

1.1 Study objectives

Nanotechnology has been identified by the European Commission as a key enabling technology (KET) and it is widely predicted that it will play a crucial role in future economic growth in the EU (TNO, 2014). Although nanomaterials offer significant technical and commercial opportunities, the rapid increase in the use of nanomaterials combined with their specific properties raises questions about their potential effects on health and the environment.   As such, there is a need to adequately assess and manage any potential risks that these new materials may have (SCENIHR, 2006).

In April 2018 the European Union's Member States representatives on the REACH Committee gave a positive opinion on the revised REACH Annexes, which have been adopted by the European Commission on December 2018 and which will apply from 1 January 2020[2]. The revised REACH Annexes clarify what information companies placing on the market substances in nanoform need to provide in their registration dossiers.

Nevertheless, some authors have argued that the adaptation of the information requirements and the specification of the test methods in REACH, in light of the experience accumulated so far, will take at least 10 years and therefore *a more future proof approach for securing the safety of new nanomaterials must be explored* (Van Teunenbroek, Baker, & Dijkzeul, 2017). Oomen et al. (2018) note that the current risk assessment framework for nanomaterials is based on the knowledge gained from the "first generation" of nanomaterials, *which comprise relatively simple inorganic and carbon-based nanomaterials*. They recommend monitoring the ongoing research and upcoming innovations in nanotechnology to determine the suitability of the regulatory risk assessment framework in identifying and dealing with the potential impacts on the human health and the environment.

This study focuses on so-called "next generation" nanomaterials, and as such it can be considered as a horizon scanning exercise to allow for anticipation of future challenges. The study has been divided into five work packages, namely:

- WP1: Collection and refinement of commonly agreed definitions of different generations of nanomaterials (Section 2);

- WP2: Creation of an inventory of existing 2nd or higher generation nanomaterials currently on the market or that may be expected on the market in a time span of five years into the future (Section 3);

- WP3: Assessment of the suitability of the key terms "substance", "mixture", and "article" against the results of WPs 1 and 2 (Section 4);

- WP4: Assessment of the suitability of characterisation or identification parameters against the results of WP1, 2 and 3 (Section 4); and

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[2] Commission Regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances.

- WP5: Market assessment (Section 3).

2. Definition of Generations of Nanomaterials

2.1 Introduction

The specific objectives of the first work package were:

- The collection of definitions of different generations of nanomaterials proposed in the scientific literature; and
- The refinement of the existing definitions or the proposal of new definitions to ensure that these are sufficiently precise to enable sorting different nanomaterials into different generations.

2.2 The concept of "generations of nanomaterials"

In discussing the suitability of current risk assessment approaches on more "advanced nanotechnologies", some authors (e.g. Van Teunenbroek, Baker, & Dijkzeul, 2017; Oomen, et al., 2018) have referred to the concept of "first generation" versus "next generation" or "future generation" of nanomaterials. However, a definition of what are the next generation nanomaterials is not provided in those papers.

The concept of generation is routinely applied to describe different stages of technological development (e.g. five generations of computers, five generations of wireless telephone technologies). The passage from one generation to another is characterised by a breakthrough which fundamentally changes the way a technology operates. The concept of subsequent generations of nanotechnology applications was introduced by Mihail Roco in 2004 and further refined in subsequent publications (Roco, 2004; Renn & Roco, 2006; Roco, 2011; Roco, 2018).

In 1997-1999, the US Interagency Working Group on Nanoscience, Engineering and Technology chaired by Roco set a long-term plan for its research and development (laid out in Roco, Williams & Alivisatos, 1999), which led to the creation of the National Nanotechnology Initiative, the United States federal government program for the research and development of nanotechnology. The Working Group adopted the following definition of nanotechnology:

> *Nanotechnology is (1) the creation of useful materials, devices, and systems through the control of matter on the nanometer-length scale, and (2) the exploitation of novel properties and phenomena developed at that scale.*

According to Roco (2018), nanotechnology is a foundational general-purpose technology for all sectors of the economy, which along with information technology, biotechnology, cognitive sciences and artificial intelligence, is the most dynamic knowledge field at the starting of the 21st century. These five foundational Science & Technology (S&T) platforms generate spin-offs and recombine in inter-platforms which create topical S&T platforms (e.g. photonics, semiconductors, genomics, biomedicine). Each topical platform has different application domains which, in turn, provides different services and products. Roco sees the development of nanotechnology as a convergence-divergence process that can be separated into three stages, characterised by six overlapping generations of nanoproducts (two generations per stage). Each stage is defined by:

- *Type of investigative methods and synthesis/assembling techniques;*
- *Level of nanoscale integration and complexity of their respective products;*
- *Typical application areas;*

- *Education needs; and*
- *Risk governance.*

In the first stage (roughly the decade 2000-2010), the focus was on the investigation of the phenomena at the nanometre scale and on the semi-empirical synthesis of nanoscale components. In the second stage (decade 2010-2020), *the focus is on integration at the nanoscale and science-based creation of devices and systems for fundamentally new products*. In the third stage (decade 2020-2030), the focus will be on the integration of nanoscience and nanotechnology with the other foundational knowledge fields, technology domains and applications. The convergence of nanotechnology with information technology, biotechnology, cognitive science and artificial intelligence will lead to Nano-Bio-Info-Cognitive (NBIC) integrated platforms. The convergence-divergence process leads to the establishment of new fields, such as synthetic biology, and the creation of new products. Examples are nanoelectromechanical systems (NEMS), which integrate nanoelectronics components such as nanotransistors with mechanical nanoactuators to form physical, biological and chemical sensors. Furthermore, to overcome the limitations of carbon-based materials, which exhibit large variance in their electronic properties when exposed to oxygen, new bio-hybrid systems are being investigated, raising from the convergence of nanotechnology with biotechnology and synthetic biology. This convergence leads to the combination of biological elements, such as DNA and proteins, with nanostructured electromechanical elements. Another example is the creation of "genetic hard drives", which apply DNA synthesis and sequencing to encode and store digital information in DNA nanochips. DNA nanochips are, at the moment, the storage medium with the highest known information density (Church, Gao, & Kosuri, 2012). Roco et al. (2013) presents an in-depth discussion on convergence platforms and their implications.

The main characteristics and some examples of nanotechnology products and productive processes within the first two generations, along with broad descriptions of the following four generations, are provided in Roco (2018) and reproduced in Table 2-1.

Table 2-1: Generations of nanotechnology products and productive processes, and the corresponding interval for beginning commercial prototypes

Stage	Generation	Main characteristics
Nano 1 Component basics	G1: Passive nanostructures (~2000-2005)	The nanostructures have stable behaviour during their use. They typically are used to tailor macroscale properties and functions. a) Dispersed nanostructures, such as aerosols, colloids, and quantum dots on surfaces. b) Contact nanostructures, such as in nanocomposites, metals, polymers, ceramics, and coatings.
	G2: Active nanostructures (~2005-2010)	The nanostructures change their composition and/or behaviour during their use. They typically are integrated into microscale devices and systems and used for their biological, mechanical, electronic, magnetic, photonic, and other effects. a) Bioactive with health effects, such as targeted drugs, biodevices, and artificial muscles b) Physico-chemical active, such as amplifiers, actuators, adaptive structures, and 3-D transistors.

Stage	Generation	Main characteristics
Nano 2 System integration	G3: System of nanosystems (~2010-2015)	Three-dimensional nanosystems frequently incorporated into other systems and using various syntheses and assembling techniques such as bio-assembling, robotics with emerging behaviour, and evolutionary approaches. A key challenge is networking at the nanoscale and hierarchical architectures. Research focus will shift toward heterogeneous nanostructures and supramolecular system engineering. This includes directed multiscale self-assembling, artificial tissues and sensorial systems, quantum interactions within nanoscale systems, processing of information using photons or electron spin, and assemblies of nanoscale electromechanical systems (NEMS).
	G4: Molecular nanosystems (~2015-2020)	Heterogeneous molecular nanosystems, where each molecule in the nanosystem has a specific structure and plays a different role. Molecules will be used as devices and from their engineered structures and architectures will emerge fundamentally new functions. Designing new atomic and molecular assemblies is expected to increase in importance, including macromolecules "by design", nanoscale machines, and directed and multiscale self-assembling, exploiting quantum control, nanosystem biology for healthcare, and human–machine interface at the tissue and nervous system level. Research will include topics such as atomic manipulation for design of molecules and supramolecular systems, controlled interaction between light and matter with relevance to energy conversion among others, exploiting quantum control mechanical–chemical molecular processes, nanosystem biology for healthcare and agricultural systems, and human–machine interface at the tissue and nervous system level.
Nano 3 Technology divergence	G5: NBIC integrated technology platforms (~2020–2025)	Converging technology platforms from the nanoscale based on new nanosystem architectures at confluence with other foundational emerging technologies. This includes converging foundational technologies (nano-bio-info-cogno) platforms integrated from the nanoscale.
	G6: Nanosystem convergence networks (~2025–2030)	Distributed and interconnected nanosystem networks, across domains and interacting at various levels (foundational, topical, application, products/service), for health, production, infrastructure, and services. This includes networks of foundational technologies (nano-bio-info-cogno) platforms and their spin-offs including for emerging nano-biosystems.

Source: reproduced from Roco (2018)

Roco defines the six **overlapping** generations of nanotechnology applications from an engineering perspective, based on an increasing level of sophistication and complexity of systems at the nanoscale and of a convergence of different disciplines of science and engineering. He places these generations in a timeline according to the expected prototyping and commercialisation date. Importantly, the six generations are part of the vision designed by Roco and colleagues to attract public and private investments on nanotechnology.

An alternative attempt to outline the future of nanotechnology by categorising the ongoing

research and the types of applications using the concept of different generations of technology has been done by James Tour. Tour (2007) describes:

- Passive nanotechnologies: *The nano part does nothing particularly elaborate. Its presence alone adds a significant increase to the performance of the system*;
- Active nanotechnologies: In this case, *the nano entity does something elaborate such as absorbing a photon and releasing an electron, thereby driving a device, or moving in a specific and definable fashion across a surface*. An example could be a "nanocar", a nano-engineered molecule that can be used to move atoms from one place to another;
- Hybrid nanotechnologies: *The complementing of a known platform through the attachment of a nano-sized entity but where the platform carries the bulk of the burden. An example would be using a silicon platform to carry out electronics, but making the silicon work with higher performance through the attachment of a surface layer of organic molecules that donate or accept charge*.

The definition of passive and active nanotechnologies proposed by Tour broadly coincides with Roco's first and second generations. Importantly, Tour's generations have also been defined to support the evaluation of the technology time horizon for investment purposes.

2.3 Use of the concept of generation of nanotechnology applications in the literature: a bibliometric analysis

Subramanian et al. (2010) and Suominen et al. (2016) conducted bibliographic analyses of the research literature referring to the generations defined by Roco. Subramanian et al. (2010) concluded that, in 2009, the following categories of active nanostructures were emerging:

- *Remote actuated active nanostructure: nanotechnology whose active principle is remotely activated or sensed.* Examples include *magnetic, electrical, light and wireless tagged nanotechnologies,* such as sensors with tin oxide nanoparticles integrated with a patch antenna for the detection of ethylene gas emitted by ripening climacteric fruits (Agarwal et al., 2012);

- *Environmentally responsive active nanostructure: nanotechnology that is sensitive to stimuli such as pH, temperature, light, oxidation-reduction, certain chemicals, etc. Examples of environmentally responsive active nanostructures include sensors, light-driven molecular motors, responsive drug delivery, and environmentally responsive actuators*;

- *Miniaturised active nanostructure: nanotechnology which is a conceptual scaling down of larger devices and technologies. Examples include synthetic molecular motors, molecular machines, and molecular electronics structures*;

- *Hybrid active nanostructure: nanotechnology that involves uncommon combinations (biotic- abiotic, organic-inorganic) of materials. Subramanian et al. (2010) discuss two classes: biotic–abiotic hybrid and silicon–organic hybrid nanostructures.* Examples of biotic–abiotic hybrid nanostructures include *(a) an enzyme responsive hydrogel which comprises of an enzyme immobilized in a three-dimensional polymer network which shrinks on enzyme catalysis, and (b) motor proteins or whole organisms containing functional motor proteins* [which] *can be tethered to surfaces to produce linear and rotary motions (that they produce in living systems) in hybrid devices.* Examples of silicon–organic hybrid nanostructures are electro-optic nanomodulators (Wolf et al., 2018) and silicon nanocrystal-organic light-emitting devices for infrared electroluminiscence (Cheng et al., 2010);

- *Transforming active nanostructure: nanotechnology that changes irreversibly during*

some stage of its use or life. Examples include self-healing materials such as metal and plastic coatings which on specific triggers, repair damage caused by corrosion and mechanical damage.

Subramanian et al. (2010) noted that active nanostructures, as defined by Roco and Tour, do not necessarily fall into one of the proposed categories only. Nanostructures belonging to more categories suggest more complexity and dynamic behaviour. It should be noted that the definitions proposed by Tour and Subramanian for hybrid nanotechnologies are substantially different and other scientists may use the qualifying term "hybrid" with different purposes.

Suominen et al. (2016) built on the results of Subramanian et al. (2010). The objective was the development of a bibliometric definition able to distinguish active next-generation nanotechnologies, to capture nanotechnology concepts that respond to the environment as well as system concepts combining dynamic non-uniform devices and structures. They found that *transistor*, *biosensing/ biosensor* and *motor* were the terms which yield the highest number of active nanotechnology publications. When comparing number of publications with number of patents, the ratio was 6:1, i.e. six active nanotechnology papers for every one patent record. Over 40% of the patent records related to *transistors*, 20% to *biosensors*, 15% to *motors* and the rest to terms such as *valve*, *actuator* and *rotor*. They concluded that, although there was a higher growth in the number of publications on active nanotechnologies (the average annual growth rate in the period 1991-2010 for active nanotechnology was 130% compared to 55% for all nanotechnology publications), the absolute share in all nanotechnology research was still modest (from 3% in 1991 to 8% of all nanotechnology publications in 2010).

For the purpose of collecting alternative definitions of generations of nanomaterials or nanotechnologies and for verifying the uptake of the definitions proposed by Roco and Tour, bibliometric searches were run through different peer-reviewed literature databases. The findings of a preliminary bibliometric search performed through Google Scholar® for the categories proposed by Roco, Tour and Subramanian (Table 2-2) suggest that some of these definitions have been more widely adopted than others. "Active" and "hybrid" are qualifying terms increasingly used in association with "nano-". "Miniaturised" and "environmentally responsive" also rank high in the findings, although the terms may not necessarily refer to a device miniaturised to the nanoscale or to an environmentally responsive nanoobject. "Nano-bio-info-cogno" cumulates a considerable number of results, but these may refer in large part to articles discussing the four reigns of technologies rather than NBIC integrated platforms described by Roco. The third, fourth and sixth generation of nanomaterials as defined by Roco do not have many references in the literature. This may be for different reasons:

- The definitions are not widely shared in the scientific community;
- The qualifying terms are not necessarily used by scientists in publications to describe their studies; and/or
- These generations are still speculative and research on these nanotechnology products is still very limited.

Table 2-2: Generations of nanomaterials: number of articles and patents in 2004, 2011, 2018 and in total (Google Scholar)

	2004	2011	2018	Total
"Passive nano"	5	18	23	277
"Active nano"	95	208	383	3,800
"3-D nanosystems"	1	3	4	30
"Systems of nanosystems"	2	21	4	12

	2004	2011	2018	Total
"Heterogenous molecular nanosystems"	2	6	1	93
"Hybrid nano"	105	458	1,450	9,560
"nano-bio-info-cogno"	28	98	77	1,560
"Nanosystem convergence network"	0	0	1	2
"remote actuated" AND "nano"	1	2	13	119
"Environmentally responsive" AND "nano"	90	292	481	5,030
"miniaturised" OR "miniaturised" AND "nano"	496	1,110	1,100	7,900
"Transforming nano"	2	1	17	75

Further searches have been run on Scopus®, focusing on titles, abstracts and keywords of (review) papers published after 2011, in three broad subject areas:

- References which cite the key papers by Roco, Tour and Subramanian;
- General forwarding looking nano* papers with some relevance to definitions/categories; and
- Specific terms relating to future nano* generations.

A first search was run to collect the number of citations of the following papers:

- Roco, M. C. (2004). Nanoscale Science and Engineering: Unifying and Transforming Tools. AIChE Journal. Vol. 50, No. 5, 890-897.
- Roco, M. C. (2011). The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. *Journal of Nanoparticle Research, 13*(2), 427-445.
- Tour (2007): Nanotechnology: The Passive, Active and Hybrid Sides—Gauging the Investment Landscape from the Technology Perspective. Nanotechnology Law and Business, Fall: 361-373.
- Subramanian et al (2010): Is there a shift to "active nanostructures?", J Nanopart Res (2010) 12:1–10.

This search gave 125 hits for Roco, M. C. (2004): 45 articles, 4 books, 36 book chapters, 18 conference papers, 2 editorials, 1 note, 19 reviews; 198 hits for Roco, M. C. (2011): 125 articles, 24 books/book chapters, 9 conference papers, 4 notes/editorials and 36 reviews; 3 hits for Tour (post 2007): 1 article, 1 conference paper and 1 review; 37 hits for Subramanian (post 2010): 17 articles, 5 books/book chapters, 2 conference papers, 1 notes/editorials and 12 reviews. With regard to forwarding looking nano* papers, the search terms used were:

future of nano* OR emerging nano* OR nano* AND generation OR next generation nano* OR smart nano* OR	AND	definition OR *disciplinary OR categor*

nano* AND state		

This search returned 77 hits (post 2011) including 16 articles, 20 books/book chapters, 7 conference papers and 33 reviews. The search on specific terms relating to future nano* generations was carried out using the following terms:

selfassembl* OR active OR hybrid OR NBIC OR nano-bio-info-cogno	AND	nano*
nano*bot		

This search returned 118,061 hits (post 2011) including: 96,945 articles, 2,392 books/book chapters, 10,374 conference papers and 5,682 reviews (Figure 2-1). The number of publications found by searching for the qualifying terms also used by Subramanian et al. (2010) keeps growing every year, but the quadratic curves seen for data between 1995 to 2008 (Figure 2-2 overleaf) are not being continued – the growth is now linear.

All the searches generate a downloadable excel file which allows to explore the results by the following fields: authors, author(s) ID, Title, Year, Source title, Volume, Issue, Art. No., Page start, Page end, Page count, Cited by, DOI, Link, Abstract, Author Keywords, Index Keywords, Publisher, Document Type, Publication Stage, Access Type, Source, EID.

These further searches confirmed that the concept of generations of nanomaterials is not widely used in the literature, but it is rather confined to the field of regulatory science and the discussion on the future of nanotechnology and the appropriateness of the current risk assessment frameworks. Roco's categories seem the most widely cited when compared to the categories proposed by Tour. Nevertheless, as already noted, the definition of passive and active nanotechnologies proposed by Tour broadly coincides with Roco's first and second generations. The articles by Subramanian et al. (2010) and Suominen et al. (2016) conclude that there is an increasing number of publications on active nanotechnologies.

Figure 2-1: Number of hits per year for "active" and "hybrid" nano* 2012-2018
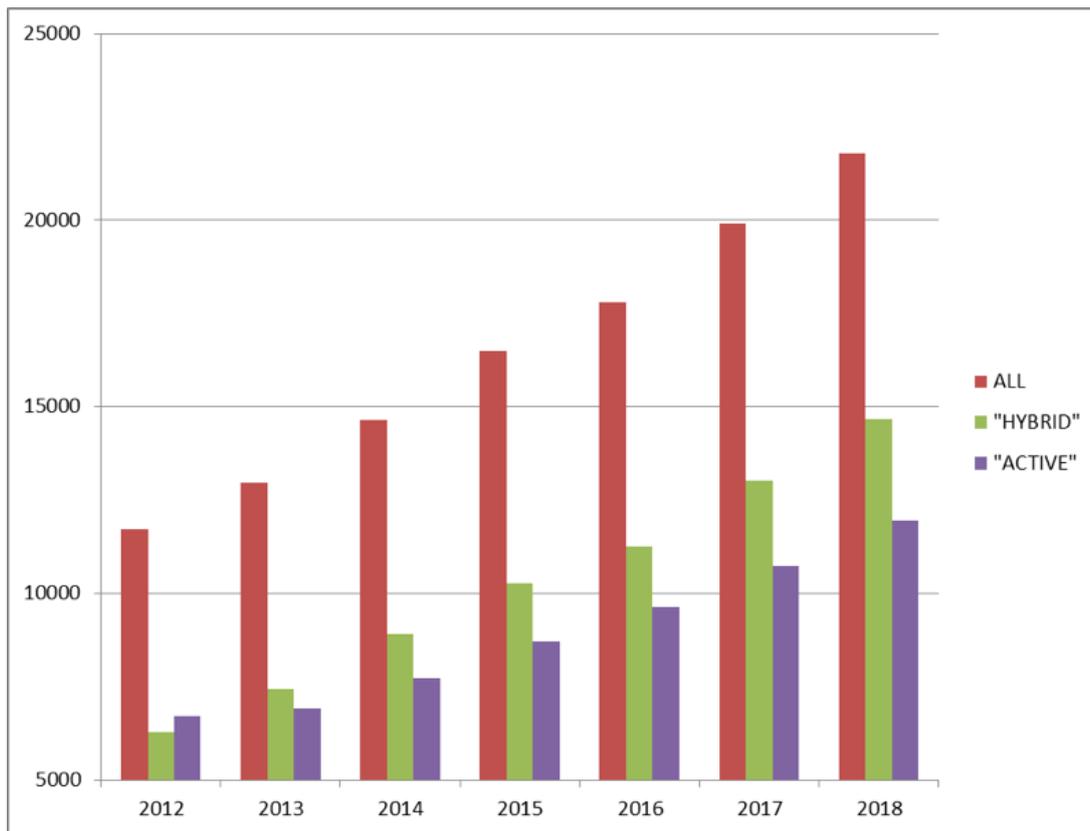
Figure 2-2: Number of hits for "active nanostructures" 1995 – 2008 - *Source: Subramanian et al. (2010)*
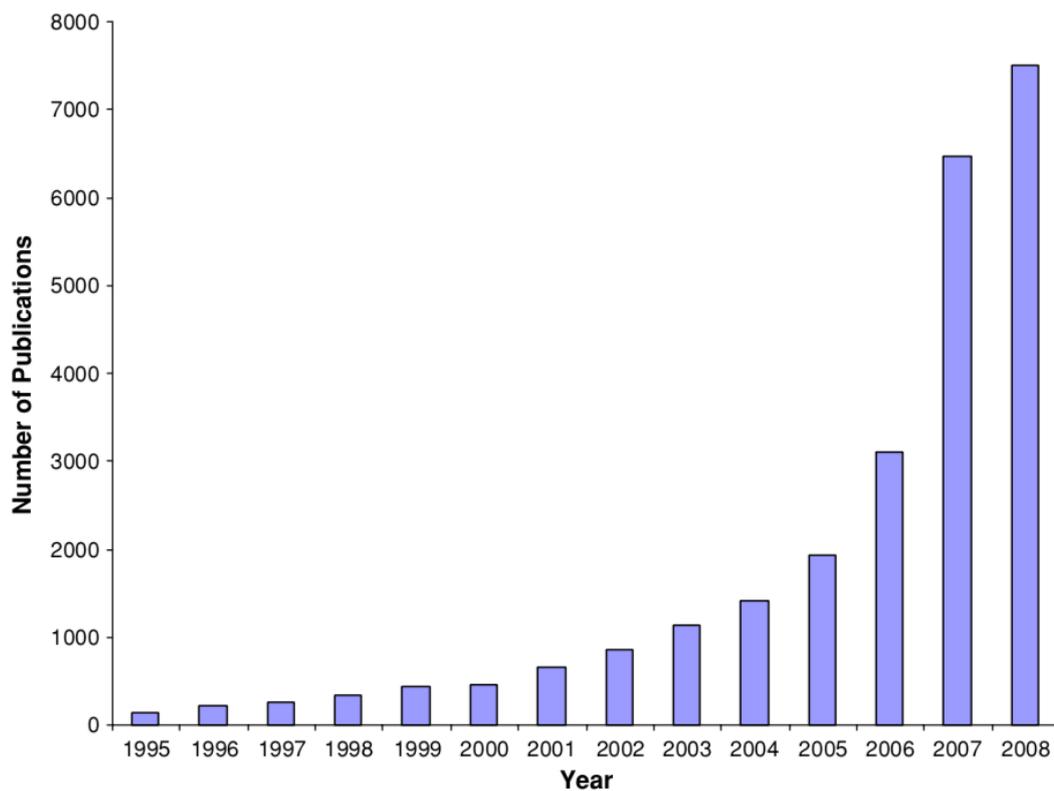
2.4 Expert consultation

To complement the analysis of the literature, we carried out semi-structured interviews[3] with eight experts from academia, research institutes, public authorities and industry stakeholders:

- Dr. Simina Dreve, Technical and Regulatory Affairs Manager, European Association of Chemical Distributors (FECC);
- Dr. Scott Brown, Principal Investigator at The Chemours Company;
- Dr. Hans-Jürgen Klockner, Head of Section Science and Research, Department Science, Technical and Environmental Affairs, German Chemical Industry Association (VCI);
- Dr. Maria Antonietta Loi, Professor of Photophysics and Optoelectronics at the Faculty of Science and Engineering of the University of Groningen;
- Dr. Alexandra Latnikova, Research Division Synthesis and Polymer Technology, Fraunhofer Institute for Applied Polymer Research (IAP);
- Dr. Marie Zimmer, Head of Product Stewardship, France Chimie;
- Dr. Kathrin Schwirn, German Federal Environment Agency (Umweltbundesamt – UBA); and
- Dr. Gert Roebben, Policy Officer, European Commission Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs.

The experts were provided with the indicative list of questions prior to the interviews. They were presented with the definitions and examples provided by Roco and Tour and enquired about their acquaintance with the definitions, whether they ever used or referred to the concept of next generation of nanomaterials and their knowledge of any other categorisation that would allow monitoring nanotechnology research. They were then asked about the usefulness of the definitions from a risk management and market assessment perspectives. Finally, they were enquired about next generation nanomaterials that may be already on the market and whether these would qualify as substances, mixtures or articles according to the REACH Regulation and whether they would be properly characterised according to the revised Annex VI of the REACH Regulation.

All experts were acquainted with the concept of "next generation" of nanomaterials and with the definitions proposed by Roco and, to a lesser extent, Tour. They however confirmed that the suggested definitions are not used by researchers, which rather refer to other categories (e.g. carbon-based nanomaterials, inorganic-based nanomaterials, organic-based nanomaterials, nanocomposites). One expert noted that both Roco and Tour refer to next generations of nanostructures and referring to nanomaterials is therefore problematic, in particular if referring to the European Commission's Recommendation on the definition of nanomaterial. Another noted that third and fourth generations of nanotechnology applications are likely to be over the threshold limit of 100 nm. Another suggestion when referring to next generations of nanotechnologies / nanotechnology applications was to categorise these by their fields of application (e.g. optics, optoelectronics, quantum electronics). An alternative suggestion was to define next generations of nanotechnologies in terms of the number of components and/or functions and whether these have unique and novel properties.

All experts agreed that the definitions are not unambiguous, and it was noted that Roco himself refers to overlapping generations of nanotechnology products. While the distinction between passive and active nanostructures intuitively makes sense and helps in imaging new applications, its use for categorising different nanotechnologies is difficult: the experts named conductors, sensors and catalysts as examples of nanostructures for which it is not entirely clear whether they should be classified as passive (the nanostructures have stable behaviour during their use) or active (the nanostructures change their composition and/or behaviour during their use). The examples provided by Roco do not allow such unambiguous

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[3] The indicative list of questions is provided in Annex I.

categorisation. One expert pointed to the fact that all nanomaterials/nanostructures are subject to degradation (e.g. photochemical transformations, oxidation and reduction, dissolution, precipitation, adsorption and desorption, combustion, abrasion and biotransformation) during their use and therefore defining the first generation of passive nanostructures in these terms is not possible. Another noted that passive nanostructures could be components of active nanostructures or nanosystems. The subsequent generations of nanotechnology applications suggested by Roco are rather speculative and while they are useful to imagine the possible future of nanotechnology research, they do not allow the categorisation of different applications. There was consensus on the fact that while the defined generations may be useful for the assessment of the market situation for different nanotechnologies, they are not fit for the purpose of regulation.

Beyond the ambiguity on the distinction between passive and active nanostructures, the experts noted that second generation nanostructures are on the market as well as Roco's third and fourth generations, although mainly in medical applications and in very small quantities.

The experts that were more familiar with the EU legislation were enquired on the suitability of the REACH terminology and the characterisation parameters of the revised Annex VI in dealing with active nanostructures. The REACH Regulation defines:

- substance as *a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition*;

- mixture *as a mixture or solution composed of two or more substances*; and

- article as *an object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition*.

The revised Annex VI requires manufacturers and importers of nanoforms of a substance to provide, in particular, *2.4.1. Names or other identifiers of the nanoforms or sets of similar nanoforms of the substance; 2.4.2. Particle number size distribution with indication of the number fraction of constituent particles in the size range 1 nm – 100 nm; 2.4.3. Description of surface functionalization or treatment and identification of each agent including IUPAC name and CAS or EC number; 2.4.4. Shape, aspect ratio and other morphological characterisation; information on assembly structure including e.g. shell like structures or hollow structures, if appropriate; 2.4.5. Surface area (specific surface area by volume, specific surface area by mass or both).*[4]

The opinion was that the revised Annex VI ensures the proper characterisation of nanomaterials, but whether active nanostructures can be considered nanomaterials or indeed substances, mixtures or articles would have to be considered on a case by case basis. One expert observed that if, within the definition of article, the term "production" includes synthesis too, then many nanostructures, either passive or active, may be considered articles.

In addition, the initial findings of the literature review and of the expert consultation were discussed during a one-day meeting with experts from the Dutch National Institute for Public Health and the Environment (RIVM):

- Eric A.J. Bleeker, risk evaluator and ecotoxicologist at the Centre for Safety of Substances and Products;

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[4] More details are provided in Section 4.

- Agnes Oomen, senior researcher on pharmacokinetics and risks of nanomaterials; and
- Adrienne Sips, research co-ordinator for risks of nanotechnology.

The discussion revolved around the concept of generation of nanotechnologies and on the definitions proposed by Roco and Tour. It was concluded that, while these cannot be used to set a risk management framework, they are nevertheless useful to understand what new technologies may be on the horizon and where the risks could be.

Significant time was dedicated to how to distinguish between passive and active nanostructures and how to refine the proposed definitions. Such distinction however is not trivial, in particular if to provide water-tight definitions for regulatory purposes. It was concluded that since the concept of next generations of nanotechnologies is not fit for regulatory purposes, such definitions are not needed, and the definitions proposed by Roco and Tour work fine for the purpose of horizon scanning. During the discussions, it was considered that the examples of next generations of nanotechnologies provided (such as hybrid active nanostructures which involve uncommon combinations of biotic- abiotic, organic-inorganic materials) do highlight the fact that the current EC recommended definition of nanomaterial and the REACH and CLP terminology are not all-encompassing and may not be future-proof: the REACH Regulation was conceived to deal with chemical substances and the legislator, at that time, did not consider the problems posed by their nanoforms. Nanomaterials are as much about physics as they are about chemistry: their novel properties are the result of their size, larger surface areas and underlying energy fluctuations at the nanoscale. While the characterisation parameters required by the revised Annex VI address the characterisation of nanomaterials falling within the EC definition, the problem in interpretation of whether certain nanomaterials or nanotechnologies should be considered substances, mixtures or articles is a separate challenge. The EC recommended definition of nanomaterial focuses on solid particles having one or more external dimension in the size range 1 nm to 100 nm, but the next generations refer to structures, devices and systems which may well be beyond the threshold of 100 nm. The International Organisation for Standardisation (ISO) provides some definitions which may be used in this context (e.g. for "nanostructure" and "nanostructured material"), but the REACH Regulation does not include this terminology and only refers to "nanomaterials" and "nanoforms" in the Annexes.

In addition, the convergence between nanotechnology and other fields of research such as biotechnology and synthetic biology is creating hybrid structures and systems made of solid and soft matter (bio), the latter not covered by the EC definition or the characterisation parameters of the revised Annex VI. Moreover, active nanostructures and nanosystems add a dynamic dimension that is not currently captured by the risk regulatory framework: the characterisation parameters of the revised Annex VI describe a nanoform of a substance as manufactured or imported on the EU market but they do not describe the intended changes which environmental responsive and transformative nanostructures undergo during their use nor do they capture the complexity and dynamism of devices and systems such as synthetic molecular motors or functional motor proteins, i.e. *molecules that can undergo changes in shape in response to external stimuli and thereby perform mechanical work* (Davis, 1999)[5].

However, it should be noted that the results of this project found only a few examples of next generation nanomaterials on the market, mainly nanomedicines. These are outside the scope of REACH, and in the remit of the European Medicines Agency (EMA). EMA has a working definition of nanomedicines, defining them as *purposely designed systems for clinical applications, with at least one component at the nanoscale, resulting in reproducible properties and characteristics, related to the specific nanotechnology application and characteristics for the intended use (route of administration, dose), associated with the expected clinical*

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[5] See the pioneering work of Kelly, De Silva, & Silva (1999) and Koumura, Zijlstra, van Delden, Harada, & Feringa (1999).

advantages of nano-engineering (e.g. preferential organ / tissue distribution). These systems need to meet the definition as medicinal product according to the European legislation (Hernán Pérez de la Ossa, 4 April 2014)*.* The relevant European Agencies (in particular ECHA, EMA and EFSA[6]) recognise the scientific and regulatory challenges posed by nanotechnology and have set ad-hoc expert groups with the objective of keeping up-to-date with the progress in the field. In addition, they organise international conferences and workshops on the topic, maintain public information portals and carry out horizon-scanning exercises, which this study is part of. Finally, they offer technical support to companies on the regulatory framework and develop specific guidance on certain aspects once sufficiently focused and identified sub-technologies have emerged and sufficient scientific experience is established.

2.5 Issues around the existing definitions

This section discusses the different issues around the definitions of generations of nanomaterials or nanotechnology applications.

2.5.1 Purpose of the definitions

The first issue is the purpose of the use of the concept of different generations of products in nanotechnology. Roco's and Tour's definitions were proposed to highlight the possibilities of nanotechnology and to attract public and private investments. They were never meant to be used in a regulatory context. Roco himself clarified that the defined generations are overlapping. In turn, Tour's definitions, although simpler, use vague terms (i.e. to do something elaborate) to distinguish between first and second generation and are therefore difficult to use for regulatory purposes or for sorting different nanotechnology applications into different generations unambiguously. Tour's third generation used the qualifying term "hybrid", which seems to refer to the presence and use of nano-sized technologies together with micro-sized platforms or technologies. However, the term "hybrid" is used in the literature by different authors for different purposes, mainly to qualify nanotechnologies composed of different materials.

According to Davies (2009), the definitions provided by Roco for the second, third and fourth generations (the last two are more recent) have generated perplexities among some nanotechnology experts, who could not understand some of the examples provided by Roco. The expert consultation has confirmed these perplexities.

In nanotechnology, regulatory definitions have focused on the component or dimensional size, overlooking functionality; *where regulatory definitions exist, they operate at the level of the nanoparticle* (Suominen et al., 2016)*.* Some regulatory and advisory definitions do refer to novel or unique properties (i.e. those by: the Australian Government Department of Health and Ageing; Health Canada; the United States Food and Drug Administration; the United States Environment Protection Agency; the European Parliament and the Council of the European Union on the Provision of Food Information to Consumers; the Taiwan Council of Labour Affairs), however, they *provide little or no guidance as to which properties should be considered novel and unique for hazard assessment purposes and fail to adequately consider the difficulties entailed in accurately measuring these properties* (Boverhof, et al., 2015).

Ricardo Energy & Environment (2016) has gone as far as stating that, based on the review of the scientific and non-scientific literature as well as the stakeholder consultations performed, discussing generations of nanotechnology may no longer be as meaningful as it once was, as research is not so much focused on novel nanomaterials but rather on integrated systems and solutions. According to Wohlleben et al. (2016), this means that rather than adding novel

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[6] European Food Safety Authority.

nanomaterials to enhance the mechanical, optical and electrical properties of the products, nanomaterials and their characteristics have to be designed to interact with the surrounding matrix into an integrated material system in order to be functional and achieve the desired properties. Switching the focus on developing nanostructures (such as nanopores and surfaces with nano-thickness patterns) to achieve superior material properties allows one to move away from nanoparticles while enhancing safety and reducing regulatory uncertainty. Suominen et al. (2016) arrive to a similar conclusion, observing that the appearance of active nanotechnologies marks a significant shift from an emphasis on individual engineered nanomaterials toward conceptualisations of more complex nanotechnology devices and structures, dynamism in use, and the interlinking of non-uniform components.

2.5.2 Nanomaterials, nanostructures and nanostructured materials

A second issue regards the existence of different definitions of what is a nanomaterial, with the EC recommended definition being *more specific and quantitative than most other (often non-regulatory) definitions* (Rauscher et al., 2019). In addition, Roco refers to different generations of nanotechnology products or nanotechnology applications and uses terms such as nanostructures (passive and active), nanosystems (system of and molecular), NBIC integrated technology platforms and nanosystem convergence networks. Tour refers to different generations of nanotechnologies.

In this context, it may not be appropriate to refer to different generations of nanomaterials. In the European Union, 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 nm - 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 %.*
>
> *By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.[7]*

The definition is currently under review, but *the Commission is not considering any major alterations or changes in scope but rather minor clarifications in the text and ways to facilitate its implementation*.[8]

According to JRC (2015), the EC definition *is explicitly limited to particulate matter and its provisions are designed and tailor-made to specifically address this type of material*, based on human and environmental exposure considerations. SCENIHR (2006) stated that human and environmental exposure to particulate materials with a nano-specific character is more likely than exposure to materials with 'embedded' nanostructural features, or particles embedded in a solid matrix. The definition keeps the focus on those materials that are relevant in a regulatory context, but it leaves out certain nanostructured materials (not all) which are instead covered by other definitions, such as the ISO definition[9]. JRC (2014) discusses

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[7] Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32011H0696
[8] http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm
[9] https://www.iso.org/obp/ui

nanostructured materials and clarifies that *there is no specific definition recommended by the EC for this term*. ISO/TS 80004-1:2010 defines nanostructured material as a

> *material having internal or surface structure in the* **nanoscale**

ISO/TS 80004-4:2001 notes that

> *materials with a grain size distribution having a significant fraction of grains in the nanoscale (nanocrystalline), voids and pores in the nanoscale, or precipitations in the nanoscale (i.e. nano-objects in a solid matrix) are sufficient features for materials to be classified as "nanostructured". Similarly, almost all materials always have surfaces with morphological and chemical heterogeneities in the nanoscale. Only surfaces that have been intentionally modified or textured to have morphological or chemical heterogeneities in the nanoscale identify materials as "nanostructured".*

The ISO Technical Specification distinguishes between five categories of nanostructured materials:

- Nanostructured powders;
- Nanocomposites;
- Solid nanofoam;
- Nanoporous material;
- Fluid nanodispersion.

Nanostructure is instead defined as a

> *Composition of inter-related constituent parts in which one or more of those parts is a nanoscale region* [where] *a region is defined by a boundary representing a discontinuity in properties.*

Whether nanostructured materials and nanostructures are nanomaterials according to the EC recommended definition depends on whether they are materials containing (or consisting of) particles and whether 50% or more of these particles are in the size range from 1 nm to 100 nm (particle number-based particle size distribution). JRC (2014) specifies that *aggregates and agglomerates of constituent particles that fall under the EC definition are nanostructured materials according to ISO terminology. Therefore, the EC definition already covers certain types of nanostructured materials, including several types of nanostructured powders which consist of nanostructured agglomerates and nanostructured aggregates in the ISO sense*. In addition, *if a nanostructured powder consists of particles which have internal structures at the nanoscale but external dimensions larger than 100 nm it is not covered by the EC definition*.

ISO defines nanocomposite as a *solid comprising a mixture of two or more phase-separated materials, one or more being nanophase*. Composite materials are a combination of a filler material, either particles or fibres, and a matrix material, either a polymer, a ceramic or a metal, that surrounds the filler. Ultrahigh performance concrete is an example of nanocomposite, where the concrete matrix contains silica nanoparticles and other fibres which improve strength and durability.[10] JRC (2014) clarifies that nanocomposite materials *are not covered by the EC definition unless they consist of particles with external dimensions in the nanoscale* and adds that these materials *are usually regulated according to the purposes they are used for (construction, aviation) and under the General Product Safety Directive in case of consumer products*. During normal wear, nanocomposites may release particles in the nanosize, but these are covered by the EC definition. Layered materials with layer thickness in the nanoscale are also nanocomposites. These include core-shell particles which may have

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[10] https://www.uhpcsolutions.com/blog/what-is-ultra-high-performing-concrete

external dimensions above the nanoscale (and therefore not covered by the EC recommended definition) but a core or a shell with a diameter or thickness at the nanoscale (JRC, 2014).

Also not covered by the EC recommended definition are nanoporous materials (e.g. zeolites) and solid nanofoams, unless the materials consist of particles with external dimensions at the nanoscale (e.g. silica gels).

Finally, fluid nanodispersions are defined by ISO as materials *in which nano-objects or a nanophase are dispersed in a continuous fluid phase of a different composition*. Examples of fluid nanodispersions are nanosuspensions and nanoemulsions. According to JRC (2014), nanosuspensions are covered by the EC definition (in the sense that the dispersed phase is a nanomaterial). Nanoemulsions contain at least one liquid nanophase, which may consist of droplets, micelles, liposomes or natural vesicles.

JRC (2015), in debating whether to extend the definition to non-particulate matter and include nanostructured materials refers to the concept of next generation nanomaterials, recognising that the EC definition does not include them. No definition or reference to a definition is provided, but only one example: *hybrid polymeric / multifunctional molecular systems purposely designed for medical applications*.

Examples of objects which are not covered by the EC recommended definition of nanomaterial are micelles and liposomes, which may have external dimensions above the nanoscale, but shells' thickness at the nanoscale and can transport and release substances. A concrete example of active nanostructures based on liposomes is ThermoDox®, which is currently undergoing clinical trials in the US for the treatment of breast and primary liver cancer. The technology is based on thermosensitive liposomes which selectively release a chemotherapeutic agent, doxorubicin, when triggered by temperature.[11] Another example is biopolymer-based supramolecular micelles with diameter of 25 ± 5 nm, which are investigated for their drug release behaviour, with a time-span of 700 hours (Du et al., 2012). These materials are of regulatory relevance as they are being used in applications for cosmetics, food or for drug delivery.

Even some first generation of nanostructures proposed by Roco (*dispersed nanostructures, such as aerosols, colloids, and quantum dots on surfaces, and contact nanostructures, such as in nanocomposites, metals, polymers, ceramics, and coatings*) may not be covered by the EC definition. Therefore, it seems more appropriate to refer to generations of nanotechnology applications rather than generations of nanomaterials, where the former include nanomaterials, nanostructures, nanodevices and nanosystems. This has implications on the assessment of the suitability of the key terms "substance", "mixture" and "article" (work package 3) and on the assessment of the suitability of the characterisation and identification parameters (work package 4).

2.5.3 Passive and active

The third issue is how to distinguish between what is "passive" and what is "active". Suominen et al. (2016) acknowledge that there is inevitably a certain degree of subjectivity in determining what should be categorised as active nanotechnology and the challenge is to distinguish active nanotechnologies from those which may be characterised as passive nanotechnologies. For the first generation, Renn & Roco (2006) refer to "steady or quasi-steady structures and functions" and Roco (2018) refers to "stable behaviour during use". For the second generation, Renn & Roco (2006) refer to changes in "state in time during its operation" and Roco (2018) refers additionally to composition: "nanostructures change their composition and/or behaviour during their use". Furthermore, he adds: "they typically are

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[11] http://celsion.com/thermodox/

integrated into microscale devices and systems" [which would make them hybrid and therefore third generation according to Tour's definition] "and used for their biological, mechanical, electronic, magnetic, photonic, and other effects". But passive nanostructures are used, at least, for their capacity to enhance the mechanical properties of materials. To substantiate further, Roco adds *a) Bioactive with health effects, such as targeted drugs, biodevices, and artificial muscles; b) Physico-chemically active, such as amplifiers, actuators, adaptive structures, and 3-D transistors*.

Defining biological activity, however, is not a trivial matter. Jackson et al. (2007) point to the fact that there is a dichotomy between the perspectives of chemists and biologists. While *a biologist is mainly concerned about what the entity does*, (…) *a chemist is mainly interested in what the entity is and how much of it is present*. This has resulted in confusion on how to measure biological entities and on how to improve *comparability, traceability and equivalence of the results of many biological assays*. To ensure comparability of results over space and time and therefore comply with metrological principles (fitness for purpose, validation, uncertainty and traceability), they proposed to define biological activity as *the ability of any entity to effect a change in a biological process.* More specifically, biological activity can be considered as the product of concentration and a parameter called inherent activity, which is the slope of the linear dose-response curve in a diagram with "concentration" as horizontal axis and "activity" as vertical axis (Jackson et al., 2007). Molecular entities with biological activity are therefore defined by what they are, what they do and how much of them is present.

Regarding physico-chemical activity, no definition is available and it is not clear what are the properties common to the examples provided (amplifiers, actuators, adaptive structures and 3-D transistors).

The Appendix for nanomaterials to the guidance on information requirements and chemical safety assessment prepared by ECHA[12] provides the list of key physicochemical parameters (Figure 2-3 overleaf) that are possibly relevant for grouping and read-across of nanoforms and which may provide some hints on how to distinguish between passive and active nanostructures. It should be noted that this is not the intention and function of the guidance, though these parameters may nevertheless be useful for this purpose.

A first chemical parameter which is used by Roco in the definition of second-generation nanostructures is composition, which is defined in the ECHA guidance for identification and naming of substances under REACH and CLP[13] as the combination of the main constituents, additives and impurities. A change in the composition results either from the change of one or more of the nanostructure's constituents, additives or impurities or from the change in their percentages. In terms of behaviour, the other term used by Roco to distinguish between passive and active nanostructures, ECHA (2017a) lists five key parameters for determining where nanoforms go:

- Solubility;
- Hydrophobicity;
- Zeta potential;
- Dispersibility; and
- Dustiness.

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[12] ECHA (2017a): Guidance on information requirements and chemical safety assessment, Appendix R.6-1 for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals, European Chemicals Agency.
[13] ECHA (2017b): Guidance for identification and naming of substances under REACH and CLP, European Chemicals Agency.

Importantly, ECHA (2017a) identifies two key parameters determining the (re)activity of nanoforms (what they do):

- Biological (re)activity; and
- Photoreactivity.

With regard to biological (re)activity (or surface reactivity), Appendix 1 to ECHA (2017a) states:

- The biological (re)activity or surface reactivity of a nanoform of a substance appears to generate reactive oxygen species (ROS) which induce inflammation, and thus may elicit cellular toxicity.

Regarding photoreactivity:

- Photoreactivity refers to activity that enables substances to participate in or to initiate a reaction due to light. "Photo" indicates the energy source causing the activity (light). If the molecule itself becomes a radical it may easily react and be transformed. If oxygen radicals are induced (i.e. reactive oxygen species or ROS), they may easily react with other molecules, which in some cases may lead to severe effects (e.g. reaction with DNA leads to genotoxicity).

Figure 2-3: Key physicochemical parameters for grouping and read-across of nanoforms. *Source: ECHA (2017a)*
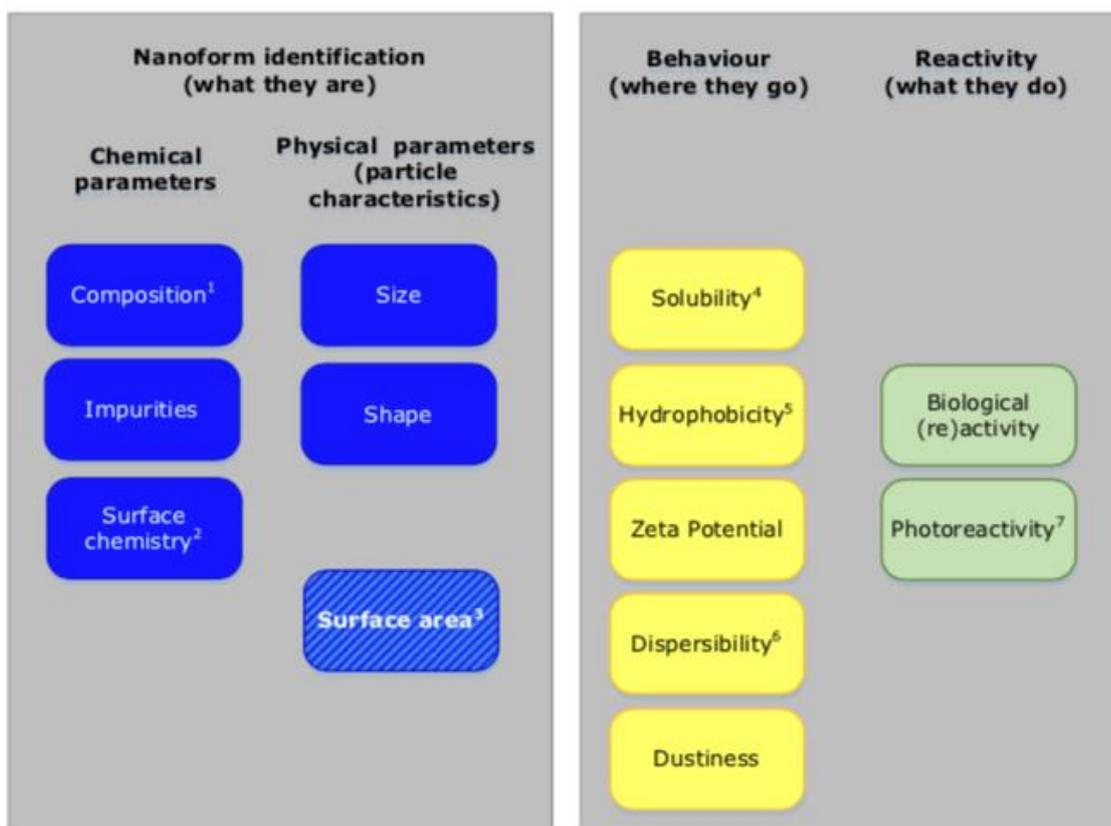
It is important to stress again that the parameters related to the behaviour and reactivity of the nanoforms suggested in ECHA (2017a) are the parameters used to determine physicochemical similarity and to substantiate whether nanoforms can be grouped for hazard assessment purposes and are not used to determine the passivity/activity of nanostructures as defined by Roco. Changes in these parameters, which may be determined by the surrounding

environment (e.g. temperature, pH), affect the toxicokinetics of the nanomaterials (i.e. the dissolution rate in biological media, surface reactivity and dispersibility). They could also be used to distinguish between passive and active nanostructures, although Roco does not indicate any particular parameter or rate of change that would determine to which generation the nanostructures belong.

In summary, Roco's definitions were conceived to sketch the possible future of R&D and commercialisation of nanotechnology products and to describe the application of nanotechnology to other science and technology domains and its ultimate convergence with them. They do not refer to nanomaterials only, encompassing nanostructures, nanostructured materials and the interactions between these elements, and characterising each generation on the basis of the level of control researchers can exert over these systems. In defining the first and second generations, Roco refers to changes in composition and behaviour but does not provide any more specific parameter, or the rate of change, that could be used to differentiate between passive and active nanostructures. This makes the use of the concept of a first generation of passive nanotechnology products and a second generation of active nanotechnology products challenging from a regulatory perspective, as it leaves a certain degree of subjectivity in the categorisation.

Nevertheless, Roco's generations, in foreseeing the convergence of nanotechnology with other science and technology domains and the increasing integration between organic and inorganic components in nanotechnology products, are useful for the assessment of the suitability of the terminology used in the REACH and CLP Regulations and of the characterisation and identification parameters included in the revised Annex VI of REACH. In order to perform such assessment, and to forecast the emergence of such generations of nanotechnology products on the EU market (the objectives of this study), some refinements to the definitions are proposed in the following subsection.

2.6 Proposed refinements

As discussed in the previous sections, Roco's generations are not regulatory concepts, lacking well-defined and measurable qualifying characteristics which would be necessary for an unambiguous implementation in a legislative framework. They also refer to nano-objects, nano-systems and concepts from other science and technology domains that go beyond the EC recommended definition of nanomaterial and the chemical legislative framework of which the REACH and the CLP Regulations are the cornerstones.

Nonetheless, Roco's generations can be used by regulators to frame the research and development of the products of nanotechnology and to monitor and forecast their emergence on the market, allowing for sufficient lead time to design or amend regulatory measures, if necessary. For the threefold objective of this study (the suitability assessment of the REACH and CLP terminology, the suitability assessment of the characterisation and identification parameters and the market assessment), some more clarity is necessary, in particular on how to better distinguish first from second generation nanotechnology products and therefore between what is "passive" and what is "active".

The project team proposes to use the physics notions of energy, work and thermodynamic equilibrium. **Error! Reference source not found.** provides the definitions of the concepts introduced and used for the refinements of the definitions of generations of nanotechnology products.

   Table 2-3: Definitions of the concepts used for the refinement of the definitions of

generation of nanotechnology products

Definitions
Energy is the capacity for doing work.
Work is the quantity of energy transferred from one system to another.
Thermodynamic equilibrium is the condition or state of a thermodynamic system, the properties of which do not change with time and that can be changed to another condition only at the expense of effects on other systems.
Active matter are natural or artificial systems that are out of thermodynamic equilibrium because of energy input to, or, by individual particles [and] active matter extracts energy from its surroundings at the single particle level and transforms it into mechanical work.[*]
A **nanodevice** can be defined as an artificial construct with an overall nanoscale size, designed to perform a predefined function in an environment (…) Nanodevices require at least a power supply, in order to differentiate them from completely passive constructs like nanoparticles. In this context, "energy supply" is more accurate than "power supply", where power is defined as work per unit time. Nanodevices consist of one mandatory components (the energy supply) and a set of optional components.[**]
A **nanomachine** is a nanodevice with two mandatory components (actuator and energy supply) and a set of optional components.[**]
A **nanosensor** is a nanodevice with two mandatory components (energy supply and sensor) and a set of optional components. [**]
A **nanorobot** is a nanodevice that is reprogrammable, has a degree of autonomy and operates in an environment. It consists of five mandatory components (actuator, information processing, memory, energy supply and sensor) and a set of optional components. [**]
A **nanonetwork** is formed when nanodevices collaborate to achieve a given task by communicating to each other.[**]
Nanonodes are nanodevices with two mandatory components (component for communication and energy supply).[**]
Nanosystems are the evolution of the nanonetworks, where multi-functional nanodevices are integrated and can function as living species with capabilities of sensing, controlling communicating and actuating/responding. [**]
Notes and sources: [*] Doostmohammadi et al. (2018)   [**] Formal definition of nanodevice, nanomachine and nanorobot adapted by Büther et al. (2017) from the definitions of machines and robots at the macroscale given by Xie (2003) and Moon & Lee (2012). They identify two sets of relevant components, which correspond to the parts the respective device is made of. The first set of components deals with interaction, namely sensors, actuators, component for locomotion and a component for communication with other devices. The second set of components facilitates complex behaviour and includes components for information processing, memory and measurement of time. Finally, the device will need an energy supply for its operation.

On this basis, the project team proposes the following refinements to the definitions of generations of nanotechnology applications:

- **First generation of nanotechnology applications – Passive nanomaterials, nanostructures and nanostructured materials**: These are materials and structures,

with constituent parts in the nanoscale, which are in metastable thermodynamic equilibrium with the surrounding system. No intentional changes of state are occurring at a measurable rate during their use, beyond agglomeration and aggregation. Examples are: polymer composites with carbon nanotubes or other nanofibers that provide increased strength and light weight to the materials; automobile tires containing carbon black or silica for improved mechanical and thermal properties; cement containing calcium silicate hydrate platelets which favour rapid crystallisation and improve strength; "first generation" of passive nanovectors, such as liposomes, which serve as containers of active principle and localise in the tumour thanks to enhanced permeability and retention (Riehemann et al., 2009);

- **Second generation of nanotechnology applications – (Re)active nanomaterials, nanostructures and nanostructured materials**: These are stimuli-responsive materials and structures, with constituent parts in the nanoscale, which during their use absorb, receive or harvest energy from their surroundings and transduce it to engage in a variety of non-equilibrium activities, usually, but not solely, connected to motility, growth or replication[14]. These non-equilibrium activities usually imply a change in the energy level of the system and in the conformation / molecular structure. They qualify as (re)active nanodevices, meaning that they are artificial constructs designed to perform a predefined function in an environment by exploiting externally-supplied energy, in the form of a stimuli, and which have an overall size or components in the nanoscale. The stimuli-responsiveness can originate from the organic ligands on the surface or from their inorganic cores. Stimuli can be chemical (e.g. solvents, acid/base signals, metal ions, gases, bio-macromolecules and redox signals) as well as physical (e.g. temperature, magnetic fields and light). Examples are: gold-silica nanoshells for cancer treatment; metal–polymer hybrid nanosensors for plasmonic ultrafast hydrogen detection; quantum dots in electronics; graphene plasmons for gas identification in a wide range of applications, such as high-quality chip fabrication in semiconductor technology, detection of explosives and medical diagnostics;

- **Third generation of nanotechnology applications – Multifunctional nanosystems**: Stimuli-responsive nanoparticles and nanostructures are the building blocks for constructing complex chemical reaction networks and synthetic life-like systems and materials. They are characterised by an increased integration between organic and inorganic components (Grzelczak, Liz-Marzán, & Klajn, 2019). In multifunctional nanosystems, multi-functional nanodevices are integrated and can function as living species with capabilities of responding, sensing, controlling, communicating and actuating[15]. Examples are: triboelectric nanogenerators, which are able to convert mechanical movement (such as body motion, muscle stretching and blood pressure), vibrations (such as acoustic and ultrasonic waves) and hydraulic movement (such as flow of body fluid and blood or the contraction of blood vessels) into electrical energy to power specific functions (Wang, 2011); molecular, self-assembled and hybrid inorganic nanomachines for medical applications, scavenging environmental toxins, molecular computation and beyond (Ellis, Moorthy, Chio, & Lee, 2018); artificial photosynthetic cell producing energy for protein synthesis (Berhanu, Ueda, & Kuruma, 2019).

With regard to the definition of the first and second generations of nanotechnology products, it is important to note that any given collection of particles in a solution tends towards a macro-state that minimises the free energy of the whole system. Ostwald ripening is the phenomenon in which smaller particles dissolve and deposit on larger particles in order to reach a more thermodynamically stable state wherein the surface area to volume ratio is minimised. Any

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[14] Adapted from De Magistris & Marenduzzo (2015)
[15] Adapted from Wang (2011).

system with a positive surface tension or surface energy aggregates, grows or adheres other materials to it. The dynamic physicochemical interactions, kinetics and thermodynamic exchanges between nanomaterial surfaces and the surfaces of biological components (such as proteins, membranes, phospholipids, endocytic vesicles, organelles, DNA and biological fluids) constitute the so-called "nano-bio" interface (Nel, et al., 2009), which can influence the toxicokinetics of the nanomaterials (ECHA, 2017a). Nevertheless, a metastable thermodynamic equilibrium is reached when the pro-forma surface tension is zero, i.e. changing the surface area does not come with a gain or loss of energy or matter. In condensed matter physics, the underlying microscopic processes are active even with no external perturbation. This means that after the relaxation time, i.e. the time required by a perturbed system to return in equilibrium, the system should remain in the corresponding macroscopic state.

Moreover, it should be noted that the reactive properties of nanomaterials and nanostructures can be used during the production of first generation nano-enabled products to obtain, for example, a better dispersion of nanoparticles in a matrix, for the creation of self-assembled monolayers or to achieve the required characteristics as in, for example, cement containing calcium silicate hydrate platelets (Delhorme et al., 2016).

Each generation is characterised by increased integration between organic and inorganic components and, as expected and suggested by Roco, by the convergence between different technological fields: active nanostructures are often functionalised with proteins or enzymes or based on micelles or liposomes; multifunctional nanosystems aim to mimic living cells and may use some of their components, such as proteins and nucleic acids. At this level, nanotechnology overlaps with synthetic biology (see for example: Jungmann, Renner, & Simmel, 2008).

The proposed refinements aim to support a more rigorous categorisation of nanotechnology applications without revolutionising the definitions suggested by Roco. The proposed three generations allow to present the ongoing research, to survey the market nanotechnology products and to explore any issues with the REACH and CLP terminology and with the characterisation and identification parameters.

The search for second or higher generation nanotechnology applications for the establishment of an inventory (Work Package 2 in Section 3) has yielded 48 examples of active (second generation) nanomaterials, nanostructures and nanostructured materials found on the market or expected on the market in the next 5-10 years. These are mostly medical and nanoelectronics applications. The inventory presents also 8 examples of multifunctional nanosystems (3rd generation). These aim to replicate the same functions and behaviours of natural systems, such as self-healing, signal amplification, homeostasis and camouflage (Grzelczak, Liz-Marzán, & Klajn, 2019) through the integration of building blocks, such as (re)active nanoparticles, into complex chemical reaction networks. Research on this nanotechnology applications is still at the conceptual phase, although prototypes of nanomachines and synthetic living cells have been produced in labs.

3. Establishment of an Inventory of Second and Higher Generation Nanomaterials and Market Assessment

3.1 Introduction

The objective of the second work package was the establishment of an inventory of second and higher generation nanomaterials currently on the market or that may be expected on the market in a timespan of five years into the future. As discussed in Section 2, Roco defined six subsequent and overlapping generations in reference to nanotechnology applications/products rather than nanomaterials. Roco's definitions are not unambiguous and imply a certain degree of subjectivity in determining to which generation a nanotechnology product should belong. In particular, distinguishing between the first passive generation of nanotechnologies and the second active generation of nanotechnologies poses a challenge, given the lose non-regulatory definitions and the few examples provided. The definitions of three generations of nanotechnology applications proposed by the project team aim to support a more rigorous categorisation, while maintaining the logic and framework envisaged by Roco.

As observed by Suominen et al. (2016), there has been an increase in publications on the reactive properties of nanomaterials and nanostructures during their use. However, the bulk of research is still on the first generation of nanotechnology applications, with the investigation on the properties of nanoparticles of different chemical substances with different shapes and functionalisations, on their integration and interaction with matrix materials and on their application in a virtually endless list of sectors, far to be exhausted. Indeed, most of the innovative nano-enabled products recently brought to market still belong to the first generation (Wohllenben & et al., 2016). The fourth, fifth and sixth generations, as envisaged by Roco, are still a long way to come, if the research will actually ever go in that direction.

This section also presents the results of work package 5, the specific objectives of which were:

- The forecast or the expected timeframe for the emergence of the different generations of nanotechnology applications on the market, focusing specifically on the EU market. The aim is to allow authorities sufficient lead time in order to design/amend regulatory measures if necessary;
- The estimate of the number of such materials belonging to each generation currently on the market and, where possible, the estimate of the number of such materials that are expected to emerge in the coming five years;
- The proposal of methods/tools for the purpose of tracking the emergence of such materials on the EU market.

3.2 Methodology

As noted by Subramanian et al. (2010) and Suominen et al. (2016), the complexity of the concept "active nanostructures" means that a simple bibliometric search strategy is not fit for purpose, but it needs to be complemented with the review of the most relevant titles and abstracts. By inspection, searches of the abstracts of review papers for the term "potential applications" may provide a short cut to identifying those areas where products may come onto the EU market. This strategy allowed the team to screen the number of review papers found by the search described in Section 2.3 from 5,682 down to 285, allowing inspection of

the abstracts.[16]

The abstracts of review papers have been scanned to check whether the full paper was likely to inform:

- The commentary on the classification of future generations of nanotechnology applications; and/or

- The inventory of those new nanotechnology applications coming onto the EU market in the next five years.

If this preliminary screening was positive, (indicated as yes (y) or possibly (p) in the spreadsheet), the full paper has been downloaded and reviewed. The results of this investigation strategy indicate that most papers relate to developments within the medical field (particularly related to oncology). In parallel to the literature search and review, the databases listed in Table 3-1 have been screened, starting with the Nanotechnology in City Environments (NICE) maintained by the Center for Nanotechnology in Society of the Arizona State University, which is already organised according to the generations proposed by Roco. As noted by Wohlleben et al. (2017), available databases *all share a strong focus on particle-based systems, and predict rapidly increasing numbers of consumer products that are being brought into the market.*

Table 3-1: List of nanotechnology databases and inventories

Nanotechnology databases and inventories
*Nanomaterial Biological Interactions Knowledgebase - The knowledgebase serves as a repository for annotated data on nanomaterial characterization, synthesis methods, and nanomaterial-biological interactions. http://nbi.oregonstate.edu/
*InterNano - InterNano brings together resources related to advances in applications, devices, metrology, and materials to facilitate the commercial development of nanotechnology. http://www.internano.org/
*Nano-EHS Database Analysis Tool - This web tool provides a quick and thorough synopsis of the Environment, Health and Safety Database. http://icon.rice.edu/report.cfm
*Nanoparticle Information Library - The goal of the NIL is to help occupational health professionals, industrial users, worker groups, and researchers organize and share information on nanomaterials, including their health and safety-associated properties. http://nanoparticlelibrary.net/
*Nanomaterials Registry - The Registry was established to provide researchers with a convenient data management and sharing plan. https://www.nanomaterialregistry.org
*National Toxicology Program Database - The goal of this database is to develop and apply modern toxicology and molecular biology tools to identify substances in the environment that may affect human health. http://tools.niehs.nih.gov/ntp_tox/
*Nano-HUB database - Nano-HUB offers a searchable online database of nanoBIO tools. http://nanohub.org/resources/database
*National Center for Biomedical Ontology Bioportal - It uses biomedical ontologies to aid in the management and analysis of data derived from complex experiments. http://www.bioontology.org/

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[16] The results of the search strategy have been provided in the spreadsheet named "Reviews_abstracts reviewed".

Nanotechnology databases and inventories
*ISA-TAB-Nano - It specifies the format for representing and sharing information about nanomaterials, small molecules, and biological specimens along with their assay characterization data using spreadsheet or TAB-delimited files. https://wiki.nci.nih.gov/display/ICR/ISA-TAB-Nano
*caNanoLab - A data-sharing portal designed for the biomedical nanotechnology research community to expedite and validate the use of nanotechnology in biomedicine. https://cananolab.nci.nih.gov/caNanoLab/
*Toxicology Data Network - Includes databases on toxicology, hazardous chemicals, environmental health, and toxic releases.   http://toxnet.nlm.nih.gov/
*Nanotechnology Characterization Laboratory - It performs and standardizes the preclinical characterization of nanomaterials intended for cancer therapeutics and diagnostics developed by researchers. http://ncl.cancer.gov/objectives_ncl_obj5.asp
*Collaboratory for Structural Nanobiology - A nanoinformatics service dedicated to the collection, curation, and correlation of structural, physicochemical, and biological and biomedical data. http://csn.ncifcrf.gov/Advanced_Structure_Analysis/HOME.html
EUON NanoData database – maintained by the European Chemicals Agency
Nanotechnology in City Environments (NICE) – maintained by the Center for Nanotechnology in Society, Arizona State University https://nice.asu.edu/article/nano-mechanism
StatNano - https://statnano.com/aboutus
Nano.Nature https://nano.nature.com
Nanowerk https://nanowerk.com/nanocatalog/
the Nanodatabase http://nanodb.dk
the Project on Emerging Nanotechnologies http://www.nanotechproject.org/inventories/
DaNa https://www.nanopartikel.info/en/
StatNano https://statnano.com/nanomaterials
Catalogue of nanomaterials on the French market https://www.r-nano.fr
Espacenet European Patent Office database https://www.epo.org/searching-for-patents/technical/espacenet.html#tab-1
*Source: Panneerselvam & Choi (2014)

The Espacenet Patent search tool of the European Patent Office has been consulted to identify nanotechnologies that are either close to market or on the market. A number of searches were performed, using the qualifying terms of the technologies described in the NICE database and the terms used by Subramanian et al. (2010) and Suominen et al. (2016) in their bibliometric searches.   The websites of the companies with relevant patents were then searched to establish whether the patented nanotechnology application is already commercialised or near commercialisation and could therefore be added to the inventory. **Error! Reference source not found.** presents the search results.

Table 3-2: Search terms used for the Espacenet Patent search tool of the European Patent Office

Search term	Number of patents
Smart AND nano in the title or abstract	182
hybrid nanostructure in the title or abstract	114
nanocarrier in title	76
active nanomaterials in the title or abstract	67
molecular switch nano in the title or abstract	34
hybrid nanotechnology in the title or abstract	16
biosensing and biosensor nano in the title or abstract	11
environmentally responsive nano in the title or abstract	7
remote activated nano in title or abstract	6
molecular nanosystems in the title or abstract	3
miniaturised nano in the title or abstract	2
systems of nanosystems in the title or abstract	2
molecular tweezer AND nano in the title or abstract	2
nanoelectromechnical system in the title or abstract	1
nano-bio-info-cogno in the title or abstract	1
nanobot in the title or abstract	1
remote activated nanostructure in title or abstract	0
miniaturised nanotechnology in the title or abstract	0
selfassembly AND nano in the title or abstract	0

Scientific articles from the bibliometric analysis (Section 2.3) that were identified as relevant (through the screening of titles and abstracts) were also subject to full text screening to identify entries for the inventory.

To complement the searches through nanomaterials and nanoproducts databases, the project team consulted the Nanowerk nanotechnology company database[17]. The ideal methodology entails the use of application programming interfaces (APIs) provided by the specific websites, however not all have this option. In order to digest the huge amount of data available on the internet in the absence of APIs, an alternative approach has been used to structure the data and facilitate its further use. With the help of a Python script, and a library (Beautiful Soup) that makes it easy to parse information from web pages, it is possible to analyse HTML code from various web domains and systematically access their complete structure to investigate

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[17] https://www.nanowerk.com/nanotechnology/nanomaterial/commercial_all.php

their product portfolio. The idea behind this methodology is to transform a convoluted HTML document into a complex tree of Python objects, such as tags, strings, or references. This allows the team to access the information, but in a scalable way, that hence facilitates to collect, organise, and analyse it in subsequent steps as pictured in **Error! Reference source not found.**. In a nutshell, the project team identified various databases that might contain relevant information (step 1). After extracting all the web addresses of a database (step 2), the project team created a pipeline of data (step 3) that then received requests from the scrape engine (step 4) which checked if the terms used by Subramanian and Suominen (see used list below) were present in the companies' websites, creating a structured data (step 5) with possible candidates. This was again processed (step 6) to assure coherence (step 7). If the result was positive for any of the terms, a team member (step 8) opened the link and reviewed the website content to verify (step 9) if the companies are offering next generation nanotechnologies as well as the level of development of those.

Figure 3-1: Methodology followed for the screening of nanotechnology company databases
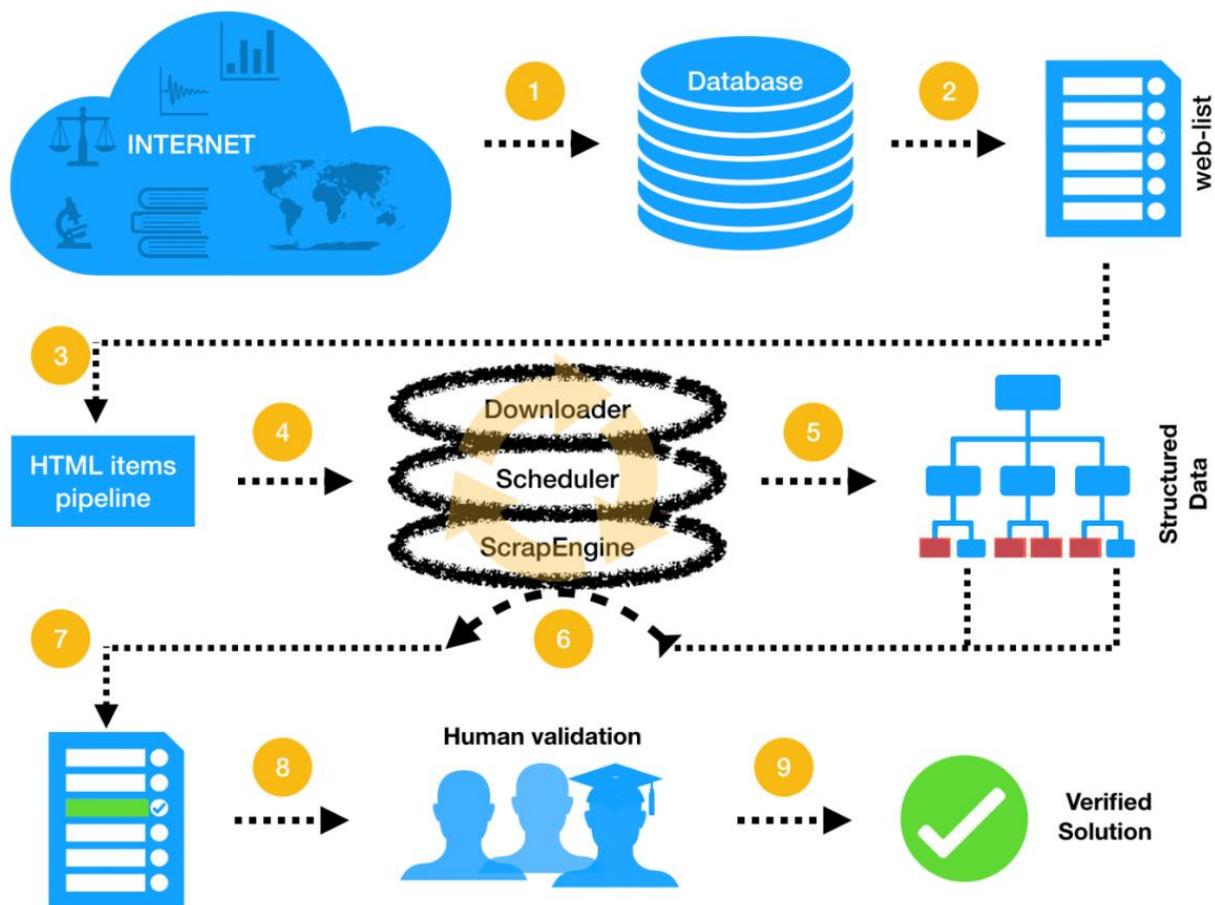
Table 3-3: Search terms used for the Nanowerk company database and linked websites

Search terms		
active system	sensing	rotaxenes
nano system*	nanoelectromechanical	Smart devices
molecular machine	adaptive	catenanes
self-replicat	self-healing	kinesin
hybrid system	biosensor	dynein
amplifiers	molecular switch	dendri*
actuators	tweezer	self-assembl*
adaptive structure	nanovalve	motor
emerging nanotechno*	system	smart
transistor	NEMS	antenna
nanodevice	Nanorobot*	plasmon
nano device	responsive	logic gate
molecular assembly	nanocar*	self-healing
active nanostructure	molecular switch	Intelligent
nanosystem*	logic gate	Active nano

For the second or higher generation nano-enabled products that have been found through the search strategy described above, technology readiness levels (TRL) and the manufacturing readiness level (MRL) have been estimated. The TRL scale is:

- TRL 1 - Basic principles observed: Research begins to be translated into applied research and development. Scientific papers start to be published on the technology's basic properties;
- TRL 2 - Technology concept formulated: Practical inventions start to be invented. Applications are speculative and there may be no proof or detailed analysis to support the assumptions. The first analytical studies are published;
- TRL 3 - Experimental proof of concept: Active R&D is initiated. This includes analytical studies and laboratory studies to physically validate analytical predictions of separate elements of the technology. Studies on components that are not yet integrated or representative are published;
- TRL 4 - Technology validated in lab: Basic technological components are integrated to establish if they work together;
- TRL 5 - Technology validated in relevant environment: Components are integrated with supporting elements to test the technology in a simulated environment;
- TRL 6 - Technology demonstrated in relevant environment: Prototypes start to be tested in a high-fidelity laboratory environment or in simulated operational environment;
- TRL 7 System prototype demonstration in operational environment: Prototypes reach planned operational functionalities and stability;
- TRL 8 - System complete and qualified: The technology is proven to work in its final form and under expected conditions;
- TRL 9 - Actual system proven in operational environment: the technology, in its final form, is used in "real-life" conditions;[18]

or

- TRL 1 – 4 Technology assessment and proving
- TRL 5 – 6 Pre-production

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[18] TRLs' descriptions adapted from: Nolte, W. L., Kennedy, B. C., Dziegiel, R. J. J. (undated): Technology Readiness Calculator.

- TRL 7 – 9 Production implementation.

The MRL scale is:

- MRL 1 Basic manufacturing implications identified: At this stage, the focus is to address manufacturing shortfalls. Basic research begins in the form of studies.
- MRL 2 Manufacturing concepts identified: At this level, an understanding of manufacturing feasibility and risk is emerging.
- MRL 3 Manufacturing proof of concept developed: Analytical or laboratory experiments validate the manufacturing concepts. Materials and processes are characterised for manufacturability and availability.
- MRL 4 Capability to produce the technology in a laboratory environment: Quality processes are in place and are sufficient to produce prototypes in laboratory. This allows to identify possible manufacturing risks, special tooling, facilities, material handling, skills required and associated costs.
- MRL 5 Capability to produce prototype components in a production relevant environment: Technologies should have matured to at least TRL 5. Manufacturing processes and procedures have been demonstrated but are still in development.
- MRL 6 Capability to produce a prototype system or subsystem in a production relevant environment: Technologies should have matured to at least TRL 6. Preliminary design has been completed and producibility assessments and trade studies of key technologies and components are complete.
- MRL 7 Capability to produce systems, subsystems, or components in a production representative environment: Technologies should be on a path to achieve TRL 7. The supply chain and supplier quality assurance have been assessed and procurement plans are in place. Manufacturing plans and quality targets have been developed. Production tooling and test equipment design and development have been initiated.
- MRL 8 Pilot line capability demonstrated; Ready to begin Low Rate Initial Production: The manufacturing system design is complete and sufficiently stable to enter low rate production.
- MRL 9 Low rate production demonstrated; Capability in place to begin Full Rate Production: Technologies should have matured to TRL 9. This level of readiness is normally associated with readiness for entry into Full Rate Production (FRP).
- MRL 10 Full Rate Production demonstrated and lean production practices in place: This is the highest level of production readiness.

Nano-enabled products that have been identified as being commercially available from manufacturer websites/scientific literature are at TRL 9 and MRL 10 stage. They have been proven in an operational environment and production is in place. For nano-enabled products which are not commercially available, the TRL and MRL status have been identified from recent articles on the technology in the scientific literature. For those products that are discussed in the literature as having significant challenges to commercialisation (i.e. technical barriers with regards to performance), these products are likely to take a number of years to come onto the market (TRL 1-4, MRL 1-4). Nano-enabled products, where barriers have been identified from the literature, but can be overcome and potentially be on the market in the coming years (i.e. scaling up of production needs to be solved) are TRL 5-6 as the technology has been validated and between MRL 5-7 depending on the technology. A number of nano-enabled products have been identified as being in the process of commercialisation (MRL 9) from manufacturer websites.

For nano-enabled products in medical applications, the TRL and MRL can be derived on the basis of whether the product is on the market, in clinical trials or in the research and development phase. Those nano-enabled products that are on the market have had the system proven in an operational environment (TRL 9) and full rate production has been demonstrated (MRL 10). For those products in clinical trials, these are likely to be on the market in the near future dependent on the outcome of these trials (TRL 7-9, MRL 7-9).

Generally, patents provide limited TRL and MRL information. In many cases, patents indicate the technology is at the TRL 4 and MRL 3-4 stage of development. It must be noted that holding a patent does not necessarily mean that the intellectual property will be incorporated into a specific product or that the product is close or will reach the market. Nano-enabled products described in the scientific literature are, in the majority of cases, in the early stages of development (TRL 1-4).

3.3 Results

The inventory is presented in **Error! Reference source not found.**. References for the entries are provided in Annex II. As concluded by similar exercises (e.g. Wohlleben et al., 2017), the project team expects the inventory not to be fully comprehensive, partly due to the fact that nano-enabled functionality in products is often not broadly advertised for reasons including marketing considerations. In addition, the publicly available databases are not particularly informative with regards to the mechanism of action of the nanotechnologies presented, and thus allocation to the second or third generations has been possible only for those examples for which the project team was able to access other sources on the same technologies and cross-link the information.

There are several companies specialised in the commercialisation of nanoparticles and nanostructures to be utilised in Life Sciences. For example:

- Micromod Partikeltechnologies GmbH, which offer more than 1,000 different particles types (magnetic iron oxide particles, silica / polystyrene / poly(lactic) acid / albumin particles with fluorescent dyes for optical visualisation, magnetic iron oxide particles with fluorescent dyes, white particles such as silica or albumin particles, coloured particles with blue, red or black dyes) with particle size ranging from 10 nm to 100 μm and different functionalisations;[19]

- Avanti Polar Lipids, Inc., which specialises in the commercialisation of different lipids, liposomes, micelles and other (nano)particles;[20]

- Sigma-Aldrich® has an extensive catalogue of active nanoparticles and nanostructures which can be used in electronics, photodynamic therapy, therapeutic agent delivery, sensors, probes, diagnostics and catalysis (e.g. gold nanoparticles, nanorods and nanourchins, with different functionalisations and particle size ranging from 2 nm to 400 nm; fluorescent alloyed, core-type or core shell type quantum dots; iron oxide nanoparticles).[21]

These are nanomaterials and nanostructures to be used in labs or for the manufacturing of other products. Moreover, no mechanism of action is specified. For the inventory, only final nano-enabled products were considered.

The project team found 48 examples of active nanotechnology applications (2nd generation) on the market or close to the market. The inventory also lists 8 examples of multifunctional nanosystems (3rd generation of nanotechnology applications). For some entries, the project team was able to find size and shape of the nanoparticles or nanostructures. For the latter, in some cases the particle size of the components has been provided. There is a paucity of detailed parameters for already commercialised technologies from companies' websites. For nanotechnology applications still under research the specification of such parameters may not

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[19] https://www.micromod.de/en/product-overview-10.html
[20] https://avantilipids.com/products
[21] https://www.sigmaaldrich.com/technical-service-home/product-catalog.html

be possible, as the researchers may still be investigating the behaviours and performances by varying substances and relevant parameters, such as surface functionalisation or shape.

Since 2000, when the US National Nanotechnology Initiative was founded, nanotechnology has been cherished as revolutionary technology with Roco anticipating six generations of nanotechnology products. Market estimates predicted around one trillion US$ revenue for 2010. No matter how one defines the market of nano-enabled products, these expectations were clearly not met. The majority of nanomaterials currently commercialised is not truly novel.

According to Wohlleben et al. (2017), the hype generated and exploited by industry and academia at the beginning of the research on nanotechnology has now ceased. Investigation efforts have moved from the sophistication of the structures of nano-objects to the analysis and understanding of nano-objects' interactions with the surrounding systems. Novel nanomaterials, such as quantum dots or carbon nanotubes, compete with alternative and more established technologies. The additional costs for the manufacturing of sophisticated nanomaterials are justified for very specialised market niches only. Indeed, among the (re)active nanotechnology applications (2nd generation) on the market or close to the market, there are nanomedical applications (drug delivery systems, imaging, theragnostic), nanosensors, nanoelectronics components (e.g. nanotransistors) and optoelectronics (e.g. quantum dots).

In particular, inorganic nanoparticles are used and investigated for the treatment, diagnosis and detection of many diseases (Anselmo & Mitragori, 2015). Inorganic compounds have been used in therapeutic applications for a long time (for example, lithium carbonate for the treatment of bipolar disorders, platinum in cancer treatment applications and silver ions as antibacterial agents), while research on their applications at the nanosized is relatively more recent. Research on inorganic nanoparticles focuses on improving targeting of damaged tissues, organs or cells, drug loading and immune system evasion, with most of the ongoing investigation on gold, iron oxide and silica nanoparticles. By leveraging the use of certain characteristics, inorganic nanoparticles can perform or deliver additional functions, such as thermal heating through surface plasmon resonance (gold nanoparticles), enhanced magnetic imaging or on-demand drug-release by the response to magnetic fields or near-infrared light (iron oxides nanoparticles). In addition, inorganic nanoparticles can incorporate ligands and polymers to enhance their biological function. These nanoparticles can therefore be considered as belonging to the second generation of nanotechnology applications (active nanomaterials, nanostructures and nanostructured materials). They are (re)active because they are responsive to external stimuli and use these to carry out a specific function (do work).

Research on the third generation of nanotechnology applications is still ongoing, with researchers demonstrating in labs the potential for engineering and medical applications (e.g. biological computers, synthetic DNA for data storage, synthetic enzymes). It is likely that the first multifunctional nanosystems will be applied in the medical sector and will be ready to undergo clinical trials in the next 5 to 10 years. For these technologies, market forecast is beyond the scope of this study.

3.4 Recommendations for monitoring the emergence on the market of second or higher generations nanomaterials

Given the relatively small number of second/third generation materials on or approaching the market, it is suggested that the simplest approach would probably be to undertake an update of the inventory every two years using the range of methods outlined in this Section, with a clear focus on the most recent developments. To summarise, the methods followed are:

- A bibliometric search of the scientific literature, using Boolean operators with some broad key qualifying terms such as "nano" AND "active" AND/OR "stimuli responsive"

AND/OR "environment responsive" AND/OR "remote actuated" AND/OR "biotic-abiotic" AND/OR "organic-inorganic", etc.

- A screening of the abstracts to identify the most frequent qualifying terms for the technologies described (e.g. "actuators", "molecular motors", etc.);
- An analysis of nanotechnology products inventories and of nanotechnology patents;
- An automated data scraping, to import information from companies' websites, to identify those companies which may be already placing on the market some of these technologies.

The combination of these methods could be supplemented with a survey of nanotechnology researchers, enquiring about nanotechnologies characterisation, possible and investigated applications, TRLs and possible risks.

Clearly, the inventory could be adapted/revised to reflect any future changes to the legal requirements for nanomaterials and associated definitions.

Table 3-4: Inventory of next generation nanotechnology applications

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
Second generation of nanotechnology applications – (Re)active nanomaterials, nanostructures and nanostructured materials			
2	Sebacia Microparticles for the Treatment of Mild to Moderate Inflammatory Acne	Substances: Gold and Silica Surface functionalisation: (poly)ethylene glycol (PEG) (PEGylation) Particle size: Gold shell ~150 nm Shape: Spheroidal-like particles, nanoshells Mechanism: The particles are applied topically pores and glands. A laser is then applied to the skin, causing the gold particles to heat up. This in turn, results in less acne lesions. Not a nanomaterial according to the EC definition (>100nm)	On the market in the US TRL9, MRL10
3	Mono-layered capped gold nanoparticle chemiresistors for testing lung cancer	Substances: Gold, silica, conducting polymer Particle size: Gold nanoparticles 5nm Shape: Spheroidal-like particles on silica chips (or wafers) Mechanism: Surfaces of cancerous tissues emit volatile organic compounds that can be detected by gold nanoparticles through the electron density change in the particle surfaces, which causes a maximum shift in the plasmon absorption. The gold nanoparticles, which are nanomaterials according to the EC definition, are synthetized in a two-phase system to form monolayers. Mono-layered capped gold nanoparticle films are then deposited on micro-sized thermal oxide silicon wafers by using an electron beam evaporator.	TRL1-4, MRL4
4	Aurora®-DSG Nanoparticles used as contrast agents in imaging applications	Substances: Gold - Au_{55} (fifty-five gold atom nanoparticles) Particle size: 1.39 nm (core) Shape: Spheroidal-like particles Surface functionalisation: capped with triphenylphosphine ligands and diglyceride ligand Mechanism: Gold nanoparticles in the visible and near-infrared region have strong absorption and they also scatter light when exposed to electromagnetic radiation. Gold nanoparticles are also good contrast agents as they possess both a large scattering cross-section and a tuneable optical resonance wavelength. Nanomaterial according to the EC definition	On the US market

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
5	Aurora®-PLC nanoparticles for the development of novel molecular delivery strategies or intracellular biosensor applications	Substances: Gold, Dodecane-1-thiol (stabiliser) Particle size: 3-5 nm Shape: Spheroidal-like particles Surface functionalisation: 1-Myristoyl-sn-glycero-3-phosphocholine Nanomaterial according to the EC definition Mechanism: On-demand release of anti-cancer agents by using the surface plasmon resonance triggered by light	On the US market
6	Aurimune®	Substances: Gold Surface functionalisation: PEG thiols loaded with tumour necrosis factor (TNF) proteins Particle size: 27 nm Shape: Spheroidal-like particles Nanomaterial according to the EC definition Mechanism: on-demand release of anti-cancer agents by using the surface plasmon resonance triggered by light	Ongoing clinical trials
7	Feraheme® / Rienso® (Ferumoxytol injections) - Superparamagnetic iron oxide nanoparticles coated with PSC for the treatment of iron deficiency anaemia in adult chronic kidney disease patients and as contrast agents in Magnetic Resonance Imaging (MRI) for evaluating hepatic lesions	Substance: Iron oxide (Fe_2O_3) Shell: Carbohydrate shell of polyglucose sorbitol carboxymethylether (PSC) Particle size: mean diameter 3.25 nm Shape: Cubic maghemites Mechanism: Iron oxide nanoparticles create microscopic field gradients in a strong microscopic field. Superparamagnetism is a form of magnetism which appears in ferromagnetic or ferromagnetic particles which involves an external magnetic field being used to magnetise the particles Nanomaterial according to the EC definition	Approved on the EU market in 2012 but withdrawn from the market in 2015. On the US market.
8	Ferumoxtran-10 (Combidex®/Sinarem®) - Superparamagnetic iron oxide nanoparticles used as contract agents in Magnetic Resonance Imaging (MRI) for evaluating hepatic lesions	Substance: Iron oxide (Fe_2O_3) Particle size: 4-6 nm and hydrodynamic diameter of 20-40 nm Surface functionalisation: coated with dextran or other polysaccharide Mechanism: Superparamagnetic iron oxide (SPIO) nanoparticles coated with carboxydextran are accumulated by phagocytosis in cells of the reticuloendothelial system (RES). Most malignant liver tumours do not contain RES cells and therefore do not uptake the iron particles. The resulting imaging effect is an improved contrast between the tumour (bright) and the surrounding tissue (dark). Nanomaterial according to the EC definition	Withdrawn from the market in 2007. Now undergoing clinical trials in the EU TRL7-9, MRL7

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
9	Endorem® (Feridex®) - Iron oxide nanoparticles coated with dextran for MRI applications	Substance: Iron oxide Surface functionalisation or treatment: dextran Particle size: 6-9 nm Mechanism: Magnetic nanoparticles can be used as contrast probes and used in magnetic resonance imaging. They induce hyper-intensities or hypo-intensities on MRI areas and highlight areas that are concentrated with the particles. Magnetic NPs (iron oxide NPs) create microscopic field gradients in a strong microscopic field. Nanomaterial according to the EC definition	On the market
10	Resovist®- Superparamagnetic iron oxide nanoparticles for liver contrast	Substance: Iron oxide Surface functionalisation: dextran Particle size: hydrodynamic diameter of 45-60 nm Mechanism: As per entry 8 Nanomaterial according to the EC definition	Approved on the EU market in 2001, discontinued in 2009.
11	VSOP C184 by Ferropharm - Superparamagnetic iron oxide nanoparticles coated with citrate for MRI contrast agents	Substance: Iron oxide Surface functionalisation: Citrate (coating) Particle size: core diameter 4 nm – total diameter: 7 nm +/- 0.15 nm Mechanism: As per entry 8 Nanomaterial according to the EC definition	Stopped from further development.
12	GastroMARK® - Iron oxide nanoparticles as contrast agent for bowel and abdominal MRI	Substance: Iron oxide Surface functionalisation: poly [N-(2-aminoethyl)-3-aminopropyl] siloxane Particle size: 400 nm Other: in suspension Mechanism: As per entry 8 Not a nanomaterial according to the EC definition (>100nm)	On the US market
13	NanoTherm®- Superparamagnetic iron oxide nanoparticles for the treatment of brain tumour (glioblastoma)	Substance: Iron oxide Surface functionalisation or treatment: aminoslaine (coating) Particle size: ~12nm diameter Mechanism: Stimulated by external alternating magnetic field generate heat Nanomaterial according to the EC definition	On the EU market (approved in 2011)

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
14	Sienna+®- Superparamagnetic iron oxide nanoparticles as a tracer for medical imaging	Substance: Iron oxide Surface functionalisation or treatment: organic substances (coating) Other: Suspension Particle size: Size distribution ~60 nm Mechanism: Superparamagnetic iron oxide particles which respond to a magnetic field Nanomaterial according to the EC definition	On the market TRL9, MRL10
15	CELLSEARCH - Iron oxide nanoparticles for detecting circulating tumour cells (CTCs)	Substance: Fe_3O_4 Particle size: clusters of 120-200 nm Surface functionalisation: coated with antibodies (anti-EpCAM) Mechanism: based on specific molecular recognition, magnetic separation and immunofluorescence. The CellSearch system isolates CTCs upon binding to the beads in blood specimens by applying an external magnetic field Nanomaterial according to the EC definition	On the market TRL9, MRL10
16	Nanosensors for electronic nose environmental applications (gas detection)	Substances under investigation: metal oxides, carbon, silicon oxide Particle size: Shape: nanotubes, nanobelts, nanowires Mechanism: The conductivity of the surface changes when a gas is absorbed/desorbed Some maybe nanomaterials according to the EC definition	TRL5-6, MRL7 The manufacturing requires expensive equipment so that economic requirements still limit commercial development
17	Nanoscale Zero Valent Iron (nZVI) Particles in Contaminated Groundwater Treatment (e.g. Nanofer 25, Nanofer 25S and Nanofer Star®)	Substance: Iron Particle size: <80-200 nm Surface functionalisation or treatment: iron oxides/hydroxides Shape: core-shell structure Mechanism: Zero Valent Iron is oxidised to its +2 and +3 state and during this process reduces organic and inorganic impurities Nanomaterial according to the EC definition	On the market TRL9, MRL10

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
18	Nanoenergetic materials for use in explosives and propellants	Substances: Nanothermite systems composed of hydroxide-aluminium and various metal hydroxides such as bismuth, copper, nickel, cerium hydroxides Particle size examples: 40-80 nm (copper oxide) 150-200 nm (final product) Shape: aluminium nanoparticles, copper oxide nanowires Mechanism: Material stores energy which is released when initiated by an external source (mechanical, thermal or shock wave) Some may be nanomaterials according to the EC definition	On the market TRL9, MRL10
19	Quantum Dot nanoparticles in LCD displays for electronics	Substance: Varies (e.g. cadmium, perovskite quantum dots and lead sulfide) Particle size: 2-10 nm Mechanism: Absorb short wavelengths of light and emit a narrow spectrum of light at a slightly longer wavelength Nanomaterial according to the EC definition	On the market TRL9, MRL10
20	Complementary Metal-Oxide Semiconductor (CMOS) for integrated circuits	Substances: Carbon, Gallium, Iron and Silicon Size: Currently around 12/14/16 nm chips. 5 nm chips expected in 2020. Mechanism: Basis of integrated circuits and work by varying the resistance of current flow within a circuit. The width is controlled by voltage. Not a nanomaterial according to the EC definition	On the market TRL9, MRL10
21	Silicon nanophotonics in optoelectronic devices	Substances: silicon Shape: Spheroidal-like particles, semiconductor core Mechanism: optical (create a dielectric waveguide for light) Not a nanomaterial according to the EC definition	On the market TRL9, MRL10
22	Silver nanoparticles / carbon nanotubes infused ink for radiofrequency identification tags that could be easily printed on paper, plastic, textile, glass and metallic surfaces and in flexible and wearable technologies.	Substances: Carbon, Various metals (e.g. silver) and polymers Surface functionalisation or treatment: polymer sheath (coating) Shape: single walled carbon nanotubes, metallic nanoparticles Mechanism: Information from radiofrequency identification is captured by a reader through radio waves	On the market TRL9, MRL10

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
23	SageGlass® - Electrochromic nano-film smart coatings for reducing thermal losses and glare in glass	Substances: Indium tin oxide and others Shape: nanometre film foils Mechanism: When low voltage is applied to the electrodes, a charge is created between the layers which changes the optical properties of the glass	On the market TRL9, MRL 10
24	Power Chips - Power generation	Substance: Carbon, silicon oxide and titanium oxide CMOS Size: 65 nm Mechanism: Heat is applied to create energetic electrons to generate power	TRL5-6, MRL9
25	Double walled silicon nanotubes for lithium ion battery anodes	Substance: Silicon Surface functionalisation or treatment: coated with silicon oxide Size: 30 nm (wall thickness) Shape: nanotubes Mechanism: oxidation reaction at the anode; electrochemical process	TRL5-6, MRL5
26	EAI SuperGel - Hydrogel for the surface removal of radioactive contamination	Substances: Various polymers Shapes: nanoparticles and gel Mechanism: absorbs radioactive material. The polymer material when exposed to a wetting agent form a structural scaffold that allows absorption	On the market TRL9, MRL10
27	ThermoDox® - Smart nano drug delivery system for the treatment of breast and liver cancer	Liposomes Mechanism: responds to a temperature trigger to release the drug (exo-trigger)	TRL7-9 MRL7 Currently in phase III clinical trials
28	Opaxio® - Smart nano drug delivery system for cancer treatment	Substance: Polymer based Shape: nanoparticles Mechanism: responds to tumour enzyme (endo-trigger) to release the drug	Approval withdrawn in 2009
29	Carbon materials for flexible and wearable sensors (e.g. SimplECG™ - Cloth-based nanosensor technology for cardiac monitoring)	Substance: Carbon Shape: fibres, films and monoliths Mechanism: convert physical or environmental stimuli into detectable signals. SimplECG collects continuous multi-channel Electrocardiography (ECG), heart rate and respiratory rate data from the garment and transfers it to a web-based portal	On the market

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
30	ClearOhm® - nanowire transparent conductive film for displays (electrical devices with transparent conductors)	Substance: Silver Shape: nanowires	On the market TRL9, MRL10
31	Grat-FET™ - Graphene Field-Effect Transistor	Substances: Graphene and Silver oxide Size: 300 nm (Back-gate oxide thickness) 40-50 nm (contact metal thickness) Mechanism: uses a nearby electrical field and the associated voltage to modulate the flow.	On the market TRL9, MRL10
32	CFQD® - Cadmium Free Quantum Dots for displays, lightings and biomedical applications	Substances: Various (core can include: indium, magnesium and phosphorus ions; shell can include zinc, sulfur, selenium, iron and/or oxygen) Particle size: ~10-100 nm Shape: nanoparticles with a core and shell Mechanism: an external light source excites the particle and it absorbs this energy and re-emits the light in a different colour	On the market TRL9, MRL10
33	Nanoweb® - transparent conductive film for use on the surfaces of plastic and glass	Substance: Various including aluminium, copper, nickel, platinum and silver Shape: nanowires Mechanism: conductive and transparent for display applications (transparent conductor)	On the market TRL9, MRL10
34	Bikanta® - fluorescent nanodiamonds for biomedical imaging probes	Substance: diamond Particle size: <~100 nm Mechanism: Fluorescence properties from negatively charged nitrogen-vacancy centres. By applying laser, radio frequency radiation and a zero magnetic field to the nitrogen-vacancy centre results in optical excitation of electrons. The electrons decay to ground state by emitting a photon. Nanomaterial according to the EC definition	On the market TRL9, MRL10
35	QDot® nanocrystals - for medical imaging and optical imaging	Substances: Cadmium mixed with selenium or tellurium Surface functionalisation or treatment: zinc sulfide (shell) Shape: nanocrystals consisting of nanometer-scale atom clusters Size: 10-20 nm Mechanism: absorb photons of light and re-emit the photons at different wavelengths Nanomaterial according to the EC definition	On the market TRL9, MRL10

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
36	Visudyne® - treatment of age-related macular degeneration (AMD) and choroidal neovascularisation	Substance: Verteporfin Confirmed at the nanoscale, no further information is available Mechanism: Light activated drug (photosensitiser)	On the market TRL9, MRL10
37	Targeted core-shell silica nanoparticles (Cornell or C dots) for medical imaging of nodal metastases	Substance: Silica Shape: Spheroidal Size: ~6-7 nm diameter Surface functionalisation or treatment: polyethylene glycol (PEG) with peptide ligands, cyclic arginine-glycine-aspartic acid-tyrosine Mechanism: Imaging agent by fluorescence Nanomaterial according to the EC definition	TRL7-9, MRL7 Currently undergoing clinical trials
38	Hensify® - NBTXR3 (Nano X-ray) nanoparticles - for cancer treatment	Substance: Hafnium oxide Shape: nanoparticles Other: aqueous suspension Mechanism: The nanoparticles are exposed to ionising radiation with the hafnium oxide generating a large number of electrons. This amplifies the dose of energy inside the tumour which increases the efficacy of radiotherapy.	On the EU market. Received authorisation in April 2019.
39	Vitoss® - Bone graft substitute	Substance: Calcium phosphate Particle size: ~100 nm Shape: nanoparticles arranged in a 3D scaffold Mechanism: resembles human cancellous bone. Calcium phosphate resorbs during the natural remodelling of bone and 3D bone regeneration is facilitated	On the market
40	nanOss®- advanced bone graft substitute	Substance: Hydroxyapatite Mechanism: provides a bone void filler that is resorbed and replaced by bone during healing	TRL7-9, MRL9 Approved for use in the EU
41	MaioRegen® - nanostructured implant that can be used for orthopaedics	Substances: Collagen and Magnesium hydroxyapatite Shape: Multi-layered matrix Fibres (collagen) Mechanism: based on the nucleation of hydroxyapatite nanocrystals onto self-assembled collagen fibre	On the market TRL7-9, MRL9

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
42	Biodegradable Nanopolymer Bone and Tissue Scaffolds	Substances: Various Shape: nanoporous materials with nanoparticles integrated in the scaffolds Mechanism: allow tissue cell attachments to a biodegradable matrix which then acts as an intermediate extracellular matrix	On the market TRL9, MRL10
43	Nanolog® Audio molecular junction device for amplification in guitars	Substance: Carbon Size: 4 nm, 6 nm and 8 nm Mechanism: Quantum mechanical tunnelling	TRL9, MRL10 On the market
44	Florescent silica nanobeads for imaging and sensing	Substance: Silica Particle size: 25-150 nm Mechanism: Emit light in the blue, cyan, and green light spectrum	TRL9, MRL10 On the market
45	CANdot® fluorescent quantum dots for solar cells, LEDs and for fluorescent labels in biomedical research.	Substances: Sodium yttrium fluoride: Ytterbium; Thulium-doped Particle size: 13nm Mechanism: Absorb short wavelengths of light and emit a narrow spectrum of light at a slightly longer wavelength Nanomaterial according to the EC definition	On the market TRL9, MRL10
46	Carbon-Based quantum dots for imaging	Substance: Carbon and Graphene Particle size: 1-4 nm (graphene) Mechanism: fluorescence Nanomaterial according to the EC definition	On the market TRL9, MRL10
47	Trilite™ Fluorescent Nanocrystals for nanoelectronics and opto-electronics	Substances: Cadmium sulfide selenium/zinc sulfide Surface functionalisation or treatment: oleic acid, carboxylic or amine Particle size: 6.5 nm diameter Mechanism: Absorb short wavelengths of light and emit a narrow spectrum of light at a slightly longer wavelength. The emission wavelength is tuneable. Nanomaterial according to the EC definition	On the market TRL9, MRL10
48	Alloyed Quantum Dots for various applications including display, lighting and biomedical applications	Substances: Cadmium Sulfide/Zinc Sulfide Shape: nanocrystals Particle size: 6 nm diameter Mechanism: Absorb short wavelengths of light and emit a narrow spectrum of light at a slightly longer wavelength Nanomaterial according to the EC definition	On the market TRL9, MRL10

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
Third generation of nanotechnology applications - Multifunctional nanosystems			
49	Fully-Implantable Cochlear Device Enabled by Piezoelectric Sensor for medical applications	Substance(s): Lead Zirconate Titanate Mechanism: The piezoelectric sensor is located at the side of the chip that is positioned at the middle ear; this receives acoustic stimuli (sound waves). The piezo ceramic material bends in response to the pressure variations creates a change in voltage and convert sound waves to electric signals. The transducer is made of a film with multiple nanolayers.	TRL1-4, MRL4
50	Cyberplasm for targeting diseases and advanced prosthetics in healthcare	Substance(s): Silicon and Carbon Mechanism: Environmental stimulus captured by the neurons are processed by an electronic nervous system and converted into electrical signals. The electrical signals produced by the nervous system generate movements	TRL1-4, MRL2-3
51	Magnetosprillum Magneticum bacteria in data storage for the production of micro hard disks	Substance(s): Magnetite Magnetite crystals form on the sections of a surface covered by a protein	TRL1-4, MRL3-4
52	DNA for storing digital information	Substance(s): Synthetic DNA Mechanism: The system integrates fluidics, electronics and infrastructure to create a full automated DNA storage device	TRL5-6, MRL4
53	Use of graphene dots in quantum computing	Substance(s): Graphene Mechanism: The graphene quantum dots, for 98% of the time do not have a spin. This decreases the tendency to interact with the spin of neighbouring atom's nuclei which dismantles their undefined superposition state.	TRL5-6, MRL6-7
54	Biomimetic enzyme nanocomplexes for treating alcohol induced liver damage	Substance(s): Acrylamide as monomer, bis-methylacrylamide as the crosslinker and ammonium persulphate /tetramethylethylenediamine as the initiator), protein, DNA, nanocapsules (glucose oxidase, alcohol oxidase) Size: Average diameter of 30 nm ± 7 nm Mechanism: Two or more enzymes with complementary functions are encapsulated within a thin polymer shell to form enzyme nanocomplexes, which exhibit improved catalytic efficiency and can be used as antidote in cases of alcohol intoxication.	TRL5-6, MRL6-7

No.	Description and use application	Identification and characterisation parameters / mechanism of action / EC definition	Market forecast
55	Molecular, self-assembled and hybrid inorganic nanomachines for medical applications, scavenging environmental toxins, molecular computation and beyond	Substance(s): Gold, silica, DNA and synthetic biological material Size: ~1-100 nm Mechanism: Supramolecular interactions within multicomponent molecular structures can be used to perform non-trivial mechanical movement and put into widespread use in a variety of nanoscale molecular devices.	TRL4, MRL4
56	Artificial photosynthetic cell producing energy for protein synthesis	Substance(s): purified ATP synthase and bacteriorhodopsin Mechanism: The photo-synthesized ATP is consumed as a substrate for transcription and as an energy for translation, eventually driving the synthesis of bacteriorhodopsin or constituent proteins of ATP synthase.	TRL4, MRL4

4. Assessment of the Suitability of the Key Terms "Substance", "Mixture" and "Article" and of the Identification and Characterisation Parameters

4.1 Introduction

The REACH Regulation (Regulation (EC) No 1907/2006) and the CLP Regulation (Regulation (EC) No 1272/2008) are the cornerstones of the European chemical legislative framework. The prerequisite of a functioning regulatory framework is that the objects regulated can be clearly defined. To ensure that the regulatory processes work properly, correct and unambiguous terminology and substance identification is essential. The REACH and CLP Regulations provide the legal definitions of substance, mixture and article and Annex VI of REACH provides a list of parameters for defining substance identity.

Substances within the scope of REACH and CLP are typically the result of chemical reactions as part of the manufacture of the substance and may contain multiple distinct constituents. Substances, as defined in REACH and CLP, also include substances chemically derived or isolated from naturally occurring materials, which may comprise a single element or molecule (e.g. pure metals or certain minerals) or several constituents (e.g. essential oils, metal mattes that are formed when sulphide metal ores are smelted). However, substances which are regulated by other Community legislation are in a number of cases exempted from registration under REACH (see Article 2 of REACH). In addition, substances listed in Annex IV of REACH and substances fulfilling certain criteria which are specified in Annex V of REACH are exempted from registration.

The table below provides some important definitions to be considered.

Table 4-1: Definitions

Term	Definition
Additive	A substance that has been intentionally added to stabilise the substance (stabiliser).
Article	An object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition.
Component	Substance intentionally added to form a mixture.
Constituent	Any single species present in a substance that can be characterised by its unique chemical identity.
Impurity	An unintended constituent present in a substance as manufactured. It may originate from the starting materials or be the result of secondary or incomplete reactions during the manufacture process. While it is present in the final substance it was not intentionally added.
Main constituent	A constituent, not being an additive or impurity, in a substance that makes a significant part of that substance and is therefore used in substance naming and detailed substance identification.
Mixture	Mixture or solution composed of two or more substances.
Monomer	A substance which is capable of forming covalent bonds with a sequence of additional like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process.

Term	Definition
Mono-constituent substance	As a general rule, a substance, defined by its composition, in which one main constituent is present to at least 80% (w/w).
Multi-constituent substance	As a general rule, a substance, defined by its composition, in which more than one main constituent is present in a concentration ≥ 10% (w/w) and < 80% (w/w).
Polymer	A substance consisting of molecules characterised by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. A polymer comprises the following: 1. (a) a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant; 2. (b) less than a simple weight majority of molecules of the same molecular weight. In the context of this definition a 'monomer unit' means the reacted form of a monomer substance in a polymer.
Substance	A chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.
Source: ECHA (2017b)	

It is important to note that *the difference between mixture and multi-constituent substance is that a mixture is obtained by blending of two or more substances without chemical reaction. A multi-constituent substance is the result of a chemical reaction* (OECD, 2018).

Under the REACH Regulation, when a registration is required it shall include information on the identification of the substance as specified in Section 2 of Annex VI. The table below provides the substance identification parameters listed in REACH Annex VI section 2.

Table 4-2: Substance identification parameters in REACH Annex VI section 2

No.	Paragraph
2.	IDENTIFICATION OF THE SUBSTANCE. For each substance the information given shall be sufficient to enable each substance to be identified. If it is not technically possible or if it does not appear scientifically necessary to give information on one or more items below, the reason shall be clearly stated.
2.1	Name or other identifier of each substance - Name(s) in the IUPAC nomenclature or other international chemical name(s); Other names (usual name, trade name, abbreviation); EINECS or ELINCS number (if available and appropriate); CAS name and CAS number (if available); Other identity code (if available)
2.2	Information related to molecular and structural formula of each substance - Molecular and structural formula (including SMILES notation, if available); Information on optical activity and typical ratio of (stereo) isomer (if applicable and appropriate); Molecular weight or molecular weight range

No.	Paragraph
2.3	Composition of each substance - Degree of purity (%); Nature of impurities, including isomers and by-products; Percentage of (significant) main impurities; Nature and order of magnitude (......ppm, ...%) of any additives (e.g. stabilising agents or inhibitors); Spectral data (ultra-violet, infra-red, nuclear magnetic resonance or mass spectrum); High performance liquid chromatogram, gas chromatogram; Description of the analytical methods or the appropriate bibliographical references for the identification of the substance and, where appropriate, for the identification of impurities and additives. This information shall be sufficient to allow the methods to be reproduced.
Source: ECHA (2017b)	

ECHA (2017b) notes that *a substance is completely identified by its chemical composition i.e. the chemical identity and the content of each constituent in the substance. Although such straightforward identification may be possible for most substances, for certain substances it is not feasible or not adequate within the scope of REACH and CLP. In those cases, other or additional substance identification information is required.*

Thus, substances can be divided into two main groups:

- "Well defined substances": Substances with a defined qualitative and quantitative composition that can be sufficiently identified based on the identification parameters of REACH Annex VI section 2.
- "UVCB substances": Substances of Unknown or Variable composition, Complex reaction products or Biological materials. These substances cannot be sufficiently identified by the above parameters.

The guidance however recognises that the identification of substances in the nanoform may require additional information.

Commission Regulation of 3 December 2018 amending REACH has made explicit that nanoforms of substances need to be covered by the registration dossiers through the provision of specific information. The revised Annex VI provides the definition of nanoform, which mirrors the Commission Recommendation of 18 October 2011 on the definition of nanomaterial. Nanoforms must be characterised as provided by section 2.4 (**Error! Reference source not found.**). The same tonnage trigger requirements apply. For registrants of a substance in nanoforms and non-nanoforms, the total volume determines the need for registration and the information to be provided.

Table 4-3: Nanoforms characterisation parameters

No.	Paragraph
2.4	Characterisation of nanoforms of a substance: For any of the characteristics, the information provided may be applicable to individual nanoforms or sets of similar nanoforms provided that the boundaries of the sets are clearly specified. A justification shall be provided to demonstrate why the sets are appropriate for the hazard assessment, exposure assessment and risk assessment of the individual nanoforms that are manufactured and placed on the market. The information in points 2.4.2 – 2.4.5 shall be clearly assigned to the different nanoforms or sets of similar nanoforms identified in point 2.4.1.
2.4.1	Names or other identifiers of the nanoforms or sets of similar nanoforms of the substance
2.4.2	Particle number size distribution with indication of the number fraction of constituent particles in the size range 1 nm – 100 nm.

No.	Paragraph
2.4.3	Description of surface functionalization or treatment and identification of each agent including IUPAC name and CAS or EC number.
2.4.4	Shape, aspect ratio and other morphological characterisation; information on assembly structure including e.g. shell-like structures or hollow structures, if appropriate
2.4.5	Surface area (specific surface area by volume, specific surface area by mass or both)
2.4.6	Description of the analytical methods or the appropriate bibliographical references for the identification of the information elements in this sub-section. This information shall be sufficient to allow the methods to be reproduced.
Source: ECHA (2017b)	

In February 2019, ECHA published a draft public version of the appendix for nanoforms applicable to the Guidance on Registration and substance identification.[22] In the following subsections, some case studies are presented in order to discuss the suitability of the terminology and of the characterisation parameters of the revised REACH Annex VI.

4.2 The generations of nanotechnology products, the key terms "substance", "mixture" and "article" and the identification and characterisation parameters

As discussed in Section 2, Roco's generations refer to nanotechnology products rather than nanomaterials as defined by the European Commission. The recommended definition focuses on solid particulate matter within the 1 to 100 nm size. The proposed refined definitions of nanotechnology generations aim to support a more rigorous categorisation of nanotechnology applications without revolutionising Roco's logic and framework. In surveying the market for second and third generation nanotechnologies, the project team identified some active second-generation nanomaterials, mainly gold-silica nanoparticles and superparamagnetic iron oxide nanoparticles in medical applications and quantum dots in electronics. Most can be identified as nanoforms of substances. However, for assembly structures like core-shell particles, there may be some room for interpretation on whether these nanostructures are substances or articles. Core-shell particles are a specific form of nanocomposites, which may have external dimensions above the nanoscale but a core or a shell with a diameter or thickness, respectively, at the nanoscale. JRC (2014) notes that *there is a discussion among regulators and scientists whether such materials, i.e., particles with external dimensions larger than the nanoscale but with deliberately engineered surface structures in the nanoscale, should be considered as nanomaterials. On the one hand, this would include a group of materials considered as true products of nanotechnology, but on the other hand it would widen the scope of the definition. If such materials were to be included in the definition it would also be necessary to define a specific limit (larger than 100 nm) for their external dimensions, up to which they would be covered by the definition of nanomaterial. Such materials could for example be called "nanostructured particulate materials".* This is further discussed in the case study on gold-silica nanoshells.

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[22] ECHA (2019). Available at:
https://echa.europa.eu/documents/10162/23047722/appendix_nanoforms_draft_to_peg_en.pdf/bb84f0ca-7688-5293-604e-fb43982c7afd

With regard to the third generation of nanotechnology products, multifunctional nanosystems, researchers are investigating and developing molecular motors with sizes below 100 nm (Ellis, Moorthy, Chio, & Lee, 2018) and synthetic living cells (for example, see: Berhanu, Ueda, & Kuruma, 2019). The determination as articles or complex articles for molecular motors seems straightforward. This is further discussed in the specific case study. Synthetic living cells instead may go beyond the discussed categories, as they do not qualify as objects but living things.

In determining whether the examples of second or third generation of nanotechnology products included in the inventory are articles as defined by the REACH Regulation, the project team assessed the technologies' functions, their shapes, surfaces and designs, following the guidelines provided in the guidance on requirements for substances in articles by the European Chemicals Agency (ECHA, 2017c). According to the guidelines, the function(s) of an article should be interpreted as the intended purpose(s) for which the object is to be used. Shape, surface and design represent the physical form of the object, with the shape being its three-dimensional form, the surface being the outermost layer and the design being the arrangement or combination of elements to best accomplish a particular purpose of the object. Importantly, the *shape, surface or design must be deliberately determined and given during a production step*. The guidance provides a workflow to decide on whether an object is an article or not. This is reproduced in **Error! Reference source not found.**. The assessment has been possible only for a limited number of the inventory entries, as the necessary information is not always available.

Figure 4-1: Decision-making workflow on whether an object is an article or not –

Source: ECHA (2017c)
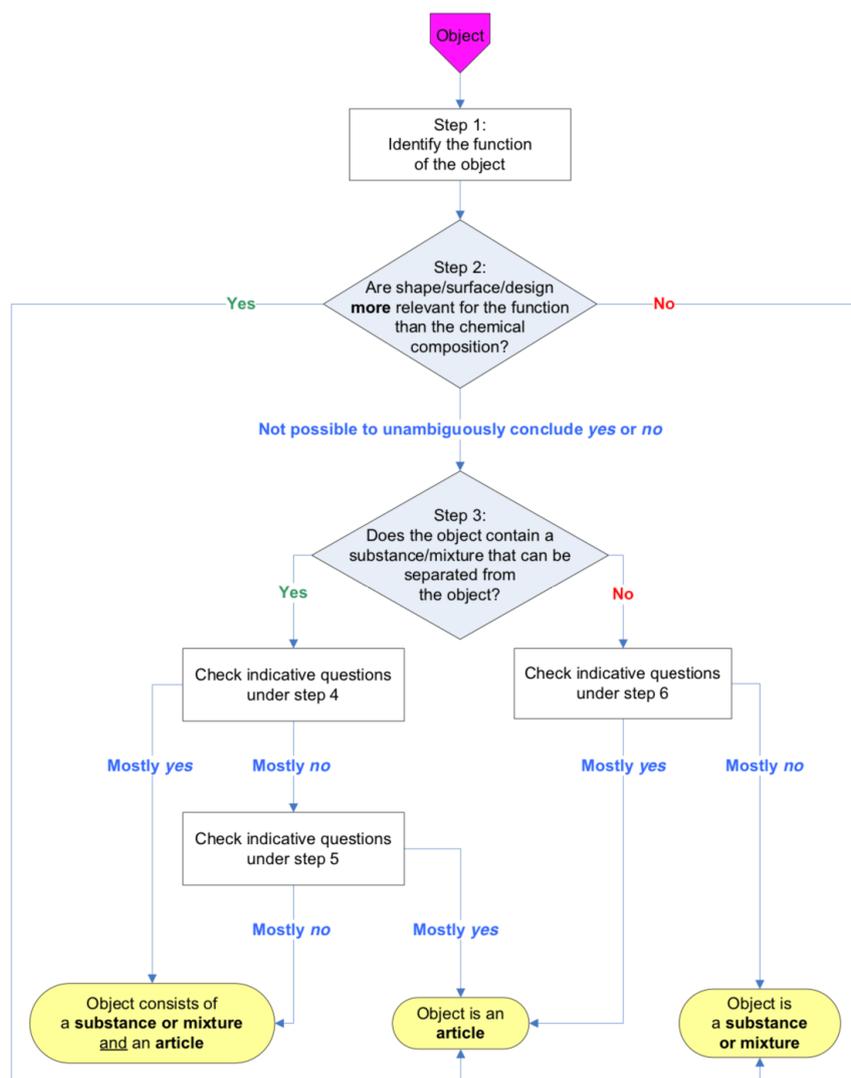
There are six steps:

- Step 1: Define the function of the object;
- Step 2: Compare the importance of the physical form and chemical characteristics for achieving the function of the object and determine what is more relevant. If it cannot be concluded unambiguously, proceed with step 3;
- Step 3: Determine if the object contains a substance or mixture that can be physically separated from the object. The substance or mixture can be enclosed or carried on the surface of the object. If a substance or mixture can be separated from the object, proceed with Step 4, otherwise proceed with step 6;
- Step 4: Answer to three questions for determining whether the chemical content of the object is an integral part thereof or if it is a substance or mixture for which the rest of the object functions as a container or carrier. If the answers are predominantly affirmative (i.e. 2 or 3 out of 3), then the object should be regarded as a combination of an article and a substance/mixture. If the answers are predominantly negative, proceed with step 5.

  - *Question 4a: If the substance/mixture were to be removed or separated from the object and used independently from it, would the substance/mixture still be capable*

> *in principle (though perhaps without convenience or sophistication) of carrying out the function defined under step 1?*
> – *Question 4b: Does the object act mainly (i.e. according to the function defined under step 1) as a container or carrier for release or controlled delivery of the substance/mixture or its reaction products?*
> – *Question 4c: Is the substance/mixture consumed (i.e. used up e.g. due to a chemical or physical modification) or eliminated (i.e. released from the object) during the use phase of the object, thereby rendering the object useless and leading to the end of its service life?*

- Step 5: Answer to further three questions for clarifying whether the object as a whole should be considered as an article. If the answers are affirmative, the object should be regarded as an article.

  – *Question 5a: If the substance/mixture were to be removed or separated from the object, would the object be unable to fulfil its intended purpose?*
  – *Question 5b: Is the main purpose of the object other than to deliver the substance/mixture or its reaction products?*
  – *Question 5c: Is the object normally discarded with the substance/mixture at the end of its service life, i.e. at disposal?*

- Step 6: Answer to four questions (not all questions may apply to all objects) for supporting the evaluation of the importance of the chemical composition versus the physical form of the object. If the answers are predominantly affirmative, the object is an article.

  – *Question 6a: Does the object have a function other than being further processed?*
  – *If the object predominantly has other functions (i.e. end-use functions), then this may be an indication that it is an article according to the definition of REACH.*
  – *Question 6b: Does the seller place the object on the market and/or is the customer mainly interested in acquiring it because of its shape/surface/design (and less because of its chemical composition)?*
  – *If the object is mainly put on the market or acquired because of its shape/surface/design, this is an indication that the object is an article.*
  – *Question 6c: When further processed, does the object undergo only "light processing", i.e. no gross changes in shape?*
  – *"Light processing", such as drilling, surface grinding or coating, may improve or modify an object's shape, surface or design for carrying out a function and is thus frequently applied to objects which are already articles. Thus, if only "light processing" is applied, this is an indication that the object is an article.*
  – *Processes leading to gross changes in shape, meaning changes of depth, width and height of an object, are not regarded as "light processing". These can for example be primary shaping processes (such as casting or sintering) or forming processes (such as extrusion, forging or rolling). If the object preserves at least one of its characteristic dimensions (depth, width and/or height) when further processed, the process can be regarded as "light processing".*
  – *Question 6d: When further processed, does the chemical composition of the object remain the same?*
  – *A change of the chemical composition in the next processing steps may indicate the object being a mixture. However, some treatments of an object which is an article may result in a change in its overall chemical composition, but not in the status of the object being an article. Examples are printing onto the surface, painting, applying coatings, dyeing etc.*

With regard to the identification and characterisation parameters, the amendments of the REACH annexes are designed to apply to nanomaterials as defined by the EC recommendation.

Most of the entries in the inventory are nanostructures and nanostructured materials not covered by the EC recommended definition and for which different characterisation parameters should be used. This is further discussed in Section 4.3.

4.2.1 Gold nanoparticles and gold-silica nanoshells

The first case study regards gold nanoparticles and gold-silica nanoshells. Their complex structure, the reaction triggered by external stimuli, the use of multiple substances/components and different polymeric and organic surface coatings allow the discussion of all the issues relevant for the assessment of the suitability of the terminology and of the characterisation parameters.

Gold nanoparticles are widely investigated due to their relatively simple preparation and functionalisation, low toxicity and unique photothermal and optical properties (including a unique surface plasmon resonance (SPR))[23] which can be tuned and controlled precisely by changing parameters such as size, shape, structure and composition (Anselmo & Mitragori, 2015). Synthesis techniques (e.g. chemical reduction of gold salts and seed-mediated growth) (Kodiha et al., 2015) are available to size the particles precisely. For example, particle size ranging between 15 and 50 nm are perfect for immunotherapeutic site (Surendran et al, 2018). Gold nanoparticles are investigated in a wide range of shapes, including spheres, shells, rods, diamonds, flowers and stars, and their surface can be modified through covalent and non-covalent interactions. Gold nanoparticles have been functionalised with proteins (such as the Epidermal Growth Factor), peptides (e.g.: the tripeptide Arg-Gly-Asp (RDG) consisting of arginylglycylaspartic acid, which is often used for its cell-adhesive activity; octa-arginine, which penetrates the cell and inhibit proteasome activities), other substances and polymers (CTAB, which is a surfactant used in the nanoparticles synthesis to control their surface energy; polyvinylpyrrolidone, a homopolymer which works as a partial inhibitor and has a disaggregation effect on protein amyloids, which are responsible of several degenerative diseases).

The first AuNPs-based products undergoing clinical trials are drug delivery systems, diagnostic and therapeutic technologies. Gold nanoparticles can be loaded with a variety of drugs through surface modification and can be used for on-demand release by external stimuli (e.g. light). Table 4-4 lists the examples of active nanotechnology applications based on gold nanoparticles and gold-silica nanoshells.

Table 4-4: Examples of gold nanoparticles from the inventory

No.	Description and use application	Identification and characterisation parameters	Market forecast
1	Aurolase® - Silica-gold nanoshells (Auroshells®) coated with (poly)ethylene glycol (PEG) for the thermal ablation of cancer cells	Substances: Gold and Silica Surface functionalisation: (poly)ethylene glycol (PEG) (PEGylation) Particle size: - Silica core of ~120 nm - Gold shell ~15 nm Shape: Spheroidal-like particles, nanoshells Mechanism: Nanoshells convert light into heat and thermally destroy cancer cells Not a nanomaterial according to the EC definition (>100nm)	TRL7-9, MRL7 Ongoing clinical trials in the US

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[23] Surface plasmon resonance (SPR) is an optical technique that measures changes in index of refraction caused by changes in mass near specific metallic surfaces, and changes in the bulk refractive index of the fluid above the surface. – Source: (LaFleur & Yager, 2013)

No.	Description and use application	Identification and characterisation parameters	Market forecast
2	Sebacia Microparticles for the Treatment of Mild to Moderate Inflammatory Acne	Substances: Gold and Silica Surface functionalisation: (poly)ethylene glycol (PEG) (PEGylation) Particle size: Gold shell ~150 nm Shape: Spheroidal-like particles, nanoshells Mechanism: Nanoshells absorb Near Infra-Red (NIR) light and laser irradiation Not a nanomaterial according to the EC definition (>100nm)	On the market in the US TRL9, MRL10
3	Mono-layered capped gold nanoparticle chemiresistors for testing for lung cancer	Substances: Gold, silica, conducting polymer Particle size: Gold nanoparticles 5nm Shape: Spheroidal-like particles on silica chips (or wafers) Mechanism: Surfaces of cancerous tissues emit volatile organic compounds that can be detected by gold nanoparticles through the electron density change in the particle surfaces, which causes a maximum shift in the plasmon absorption. The gold nanoparticles, which are nanomaterials according to the EC definition, are synthetized in a two-phase system to form monolayers. Mono-layered capped gold nanoparticle films are then deposited on micro-sized thermal oxide silicon wafers by using an electron beam evaporator.	TRL1-4, MRL4
4	Aurora®-DSG Nanoparticles used as contrast agents in imaging applications	Substances: Gold - Au_{55} (fifty-five gold atom nanoparticles) Particle size: 1.39 nm (core) Surface functionalisation: capped with triphenylphosphine ligands and diglyceride ligand Nanomaterial according to the EC definition	On the US market
5	Aurora®-PLC nanoparticles for the development of novel molecular delivery strategies or intracellular biosensor applications	Substances: Gold, Dodecane-1-thiol (stabiliser) Particle size: 3-5 nm Surface functionalisation: 1-Myristoyl-sn-glycero-3-phosphocholine Nanomaterial according to the EC definition	On the US market
6	Aurimune®	Substances: Gold Surface functionalisation: PEG thiols loaded with tumour necrosis factor (TNF) proteins Particle size: 27 nm Shape: Spheroidal-like particles Mechanism: on-demand release of anti-cancer agents (TNF) by using the surface plasmon resonance triggered by light Nanomaterial according to the EC definition	Ongoing clinical trials

An example of AuNPs-based product which is on the EU and US markets is the Sebacia Acne Treatment. This is a formulation of gold particles with silica core and particle size around 150 nanometres coated with (poly)ethylene glycol (PEG) for increased stability and tuned in order to absorb near infra-red light and laser irradiation used for the treatment of moderate to moderately severe inflammatory acne vulgaris. Other two examples of gold-silica nanoshells

coated with PEG are Aurolase®[24], used for cancer ablation, and gold-silica nanoshells being developed by the Wiesner Group[25] for imaging (of melanoma and malignant brain tumours through intraoperative sentinel lymph node mapping) through the use of AuNPs' light-scattering properties. AuNPs have strong absorption in the visible and near-infrared region and scatter light when exposed to electromagnetic radiation and they can therefore be used for cancer detection. If exposed to a light source at a specific wavelength, their conduction band electrons become excited and begin to oscillate (surface plasmon resonance) and convert laser radiation to thermal energy, killing cancer cells in the proximity (cancer ablation). Both products are undergoing clinical trials in the US.

Gold nanoparticles can also be used as therapeutics delivery systems, where the therapeutics (e.g. proteins, peptides and other active substances) can be incorporated through surface modification. The SPR can then be used to facilitate the on-demand release of the therapeutics via externally triggering stimuli (e.g. light). An example is Aurimune®, which is made by binding a cancer-killing protein called tumour necrosis factor (TNF) to the surface of gold nanoparticles with particle size of 27 nm. The size is optimised to leverage the inherent leakiness of tumour blood vessels. The objective is to kill cancer cells without harming healthy tissue. The particles are coated with PEG to prevent the removal by the reticuloendothelial system (RES). In addition, the particle surfaces have been functionalized with linkers or ligands to attach anti-cancer agents and to improve docking at the tumour cell membrane, including Epidermal Growth Factor (a protein made up of 50 or more amino acids) and peptides (RDG, made up of 2-50 amino acids). These linkers are unique and can be adjusted to work with a variety of agents targeting multiple tumour types.

Most of these technologies are composed of two substances: gold and silica. They are used because of their simple preparation, photothermal and optical properties and low toxicity (inert materials which is the opposite of bioactive materials). Nevertheless, they can be easily functionalised with polymeric and organic materials to carry out certain functions. They are (re)active nanostructures, because they (re)act to external stimuli to do work.

Some of these nanotechnology applications can be considered nanomaterials, because their particle size is in the range 1-100 nm. This is true for entries no. 4 to 6 in Table 4-4. In these cases, the identification and characterisation parameters included in the revised Annex VI of REACH allow the identification and characterisation of the nanomaterial. Indeed, ECHA (2019) specifies that, for assembly structures like nano-onions spherical nanoparticles with concentric multiple shell structure, information on the number of multiple shells need to be provided.

However, once coated and functionalised, the particle size of these nanotechnology applications can exceed the 100 nm threshold. In these cases, it is not completely clear whether the coating or functionalisation should be considered to determine whether the 100 nm threshold is exceeded or not and therefore whether the technologies can be considered nanomaterials according to the EC recommended definition. An interpretation could be that if the coating or functionalisation is solid at normal temperature and pressure (NTP), i.e. 298.15 K and 101,325 Pa, then it should be considered for determining the compliance with the size threshold. If the coating is instead 'fluid', able to deform or flow under an applied shear stress, it should not be considered.

Further guidance is required on whether coatings and functionalisation are to be considered for determining whether the particle size regulatory threshold is exceeded and, if the 'solid' vs 'fluid' suggestion is uptaken, some criteria for distinguishing between solid and fluid materials (as, for example, those suggested by Rauscher et al., 2019). Moreover, some additional

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[24] https://nanospectra.com
[25] https://wiesner.mse.cornell.edu/res_nanoparticles.htm

suggestions could be given on the information to be provided on the surface treatment.[26]

With regard to entry no. 3, the 5nm-sized gold particles are further synthesised in films and deposited on silicon wafers. These are nanostructured materials which are not covered by the EC recommended definition.

But are gold-silica nanoshells substances, mixtures or articles? Are gold, silica and the other substances used to functionalise the particles to be considered different substances, different constituents of a multi-constituent substance, different components of a mixture or different materials used in an article?

ECHA (2017b) and OECD (2018) clarifies that the difference between a mixture and a multi-constituent substance is that a mixture is obtained by blending of two or more substances without chemical reaction. Nanoshells, which have a dielectric core (silica) and a thin noble metal shell (gold), are obtained through synthesis[27], which is a chemical reaction. Thus, gold-silica nanoshells are not mixtures. But are they multi-constituent substances or articles?

According to ECHA (2017c), the first step is to determine the function of the object. Auroshells® and Sebacia microparticles are both gold-silica nanoshells used for, respectively, the thermal ablation of tumour tissues and the thermal treatment of acne. The second step is to determine whether the shape/surface/design is more relevant for the function than the chemical composition. In this case, it is not possible to conclude unambiguously whether the physical form is more relevant than the chemical composition for the thermal treatment of acne or the thermal ablation of tumorous cells. Gold-silica nanoshells have special surfaces and designs which determine their function to a great degree. At the same time, their chemical composition, gold, silica and the other substances used for their functionalisation (PEG, proteins, etc.) play a big role too. What plays a bigger role and determines the function to a greater degree is open to interpretation. Step 3 requires determining whether the object contains a substance or mixture that can be physically separated from the object. Neither Auroshells® nor Sebacia microparticles contain substances or mixtures that could be easily separated; therefore, the assessment should proceed with step 6. However, the questions listed at step 6 to support the evaluation are not relevant for these types of objects.

The steps to be followed for determining whether an object is an article according to the REACH Regulation presented in ECHA (2017c) could be complemented with specific examples on different nanomaterials, from simple nanoparticles to more complex assembly structures.

It should be noted that the revised Annex VI, at point 2.4.4. states that *information on assembly structure including e.g. shell like structures or hollow structures* should be provided if appropriate. It seems therefore that the legislator has taken the practical approach to require this information for all those materials which, given their characteristics (solid particulate matter with particle size below 100 nm and particle size number distribution above 50%), are covered by the EC recommended definition of nanomaterial, regardless any ambiguity in the determination of whether a nanomaterial could be considered an article according to the definition provided at Article 3(3) of the REACH Regulation.

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[26] ECHA (2017b), in discussing UVCBs, suggests some identifiers for the complex biological macromolecules, such as enzymes and proteins, that can be used for the functionalisation of the nanoparticles: standard enzyme index, genetic code, stereo configuration, physical properties, function/activity, structure, amino acid sequence. In addition, it is suggested to identify enzymes according to the international system for enzyme nomenclature, IUBMB (International Union of Biochemistry and Molecular Biology).

[27] For example, see Pham et al. (2002)

4.2.2 Iron oxide nanoparticles

Iron oxide nanoparticles exhibit unique electrical, optical and magnetic properties and find applications in inorganic pigments, drug delivery systems, magnetic data storage, biosensing, imaging (Ali et al., 2016), catalytic reactions for the removal of heavy metals in wastewater (Cheng et al., 2012) and for improved thermal combustion in ballistic additives in propellant formulations (Fujimura & Miyake, 2010). Iron oxide nanoparticles currently in use consist of maghemite (γ-Fe2O3), magnetite (Fe3O4) and hematite (α-Fe2O3). **Error! Reference source not found.** lists the iron oxide nanoparticles applications classifiable as second-generation nanotechnology applications which have been included in the inventory.

Table 4-5: Examples of iron oxide nanoparticles from the inventory

No.	Description and use application	Identification and characterisation parameters	Market forecast
7	Feraheme® / Rienso® (Ferumoxytol injections) - Superparamagnetic iron oxide nanoparticles coated with PSC for the treatment of iron deficiency anaemia in adult chronic kidney disease patients	Substance: Iron oxide (Fe_2O_3) Shell: Carbohydrate shell of polyglucose sorbitol carboxymethylether (PSC) Particle size: mean diameter 3.25 nm Shape: Irregular cubic maghemites Mechanism: Iron oxide nanoparticles create microscopic field gradients in a strong microscopic field. Superparamagnetism is a form of magnetism which appears in ferromagnetic or ferromagnetic particles which involves an external magnetic field being used to magnetise the particles. Nanomaterial according to the EC definition	Approved on the EU market in 2012 but withdrawn from the market in 2015. On the US market.
8	Ferumoxtran-10 (Combidex®/Sinarem®) - Superparamagnetic iron oxide nanoparticles used as contract agents in Magnetic Resonance Imaging (MRI) for evaluating hepatic lesions	Substance: Iron oxide (Fe_2O_3) Particle size: 4-6 nm and hydrodynamic diameter of 20-40 nm Surface functionalisation: coated with dextran or other polysaccharide Mechanism: Superparamagnetic iron oxide (SPIO) nanoparticles coated with carboxydextran are accumulated by phagocytosis in cells of the reticuloendothelial system (RES). Most malignant liver tumours do not contain RES cells and therefore do not uptake the iron particles. The resulting imaging effect is an improved contrast between the tumour (bright) and the surrounding tissue (dark). Nanomaterial according to the EC definition	Withdrawn from the market in 2007. Now undergoing clinical trials in the EU TRL7-9, MRL7
9	Endorem® (Feridex®) - Iron oxide nanoparticles coated with dextran for MRI applications	Substance: Iron oxide Surface functionalisation or treatment: dextran Particle size: 6-9 nm Mechanism: As per entry 10 Nanomaterial according to the EC definition	On the market
10	Resovist®- Superparamagnetic iron oxide nanoparticles for liver contrast	Substance: Iron oxide Surface functionalisation: dextran Particle size: hydrodynamic diameter of 45-60 nm Mechanism: As per entry 10 Nanomaterial according to the EC definition	Approved on the EU market in 2001, discontinued in 2009.

No.	Description and use application	Identification and characterisation parameters	Market forecast
11	VSOP C184 by Ferropharm - Superparamagnetic iron oxide nanoparticles coated with citrate for MRI contrast agents	Substance: Iron oxide Surface functionalisation: Citrate (coating) Particle size: core diameter 4 nm – total diameter: 7 nm +/- 0.15 nm Mechanism: As per entry 10 Nanomaterial according to the EC definition	Stopped from further development.
12	GastroMARK® - Iron oxide nanoparticles as contrast agent for bowel and abdominal MRI	Substance: Iron oxide Surface functionalisation: poly [N-(2-aminoethyl)-3-aminopropyl] siloxane Particle size: 400 nm Other: in suspension Mechanism: As per entry 12 Not a nanomaterial according to the EC definition (>100nm)	On the US market

Most of the products listed are superparamagnetic iron oxide (known as "SPIO") nanoparticles designed and tested as magnetic resonance contrast agents in imaging of tissue lesions. They have different functionalisations and particle sizes and shapes to obtain different pharmacokinetics profiles and target different types of tissues. According to Wang Y.-X. J. (2015), SPIO were developed for liver imaging when computed tomography (CT) scan and multiple-slice magnetic resonance imaging (MRI) were slow. Due to progress in these technologies, different types of contrast agents are now leading for the diagnosis and staging of hepatocellular carcinoma. As a result, many of SPIO-based products have been withdrawn from the market.

Another application of iron oxide nanoparticles is for the reduction or oxidation of contaminants in soil and groundwater. The functionalisation of the particles has allowed to reduce the rate of aggregation and control the release rate of Fe^{2+}, leading to the development of stable commercial products. In particular, the aggregation rate is higher for uncoated nZVI (Nanofer 25®) when compared to polymer coated nZVI (Nanofer 25S®) and nZVI capped with a 2nm shell of FeO (Nanofer STAR®) (Keller, Garner, Miller, & Lenihan, 2012).

The identification and characterisation parameters required by the REACH Annex VI allow the unequivocal identification and characterisation of different iron oxide nanoparticles, but not of the technology (i.e. how the superparamagnetism of the particles is used to achieve their function and what changes this provokes on the nanoparticles). As for gold-silica nanoshells, additional guidance on the information to be provided for the description of the surface functionalisation (2.4.3 of Annex VI) would ensure the notification of consistent information across different nanoforms. The revised Annex VI requires to provide separate information on the manufacture and use(s) of the nanoforms of the substances registered. In particular, point 3.5. requires the provision of a brief description of the identified use(s). Use description is the basis for exposure assessments, for the communication on safe use down the supply chain, for the authorities' decision making and for the dissemination on information to the general public. The current use descriptors included in the use description system (ECHA, 2015), in particular the technical functions, do not adequately represent the uses and functions of nanomaterials on the market or which could be placed on the market in the near future. An ad-hoc list of use descriptors for nanomaterials could fill in this information gap.

4.2.3 Molecular motors

The development and characterisation of structures able to perform controlled functions at the nanoscale is one of the frontiers of nanotechnology research. The 2016 Nobel prize in

Chemistry has been awarded to Jean-Pierre Sauvage, Fraser Stoddart and Ben Feringa for their work on molecular machines. These can function as rotors, shuttles and pumps and, recently, DNA nanorobots have been designed and prototyped which are able to serve as switches, rotors, joints and logic gated carriers. Molecular machines can be categorised according to *(1) the types of motion they perform, including rotors, switches, propellers, shuttles, walkers, ratchets and tweezers and (2) the types of energy input used to operate the machine, including chemical, light and electrochemical inputs* (Ellis, Moorthy, Chio, & Lee, 2018). Molecular nanosystems can perform useful work in solution, on interfaces and in the solid state. An additional level of complexity is presented by self-assembled nanomachines, i.e. molecular machinery which rely on the autonomous organisation of an ensemble of molecules. Important well-established capabilities of self-assembled nanomachines are cargo delivery and stimuli-responsiveness, but research is ongoing on self-propelling nanomotors and DNA nanomachines able to perform molecular computation and nanoscale walkers.

While research is predominantly on organic nanomachines, nanomachines based mostly on inorganic materials have been reported (Ellis, Moorthy, Chio, & Lee, 2018). Examples are Janus nanomotors made of platinum and gold and chiral nanomotors, such as ferromagnetic nanohelices, made of gold, silica, cobalt and nickel. Inorganic nanomachines are stronger catalysts and therefore can more easily harvest chemical energy from the surrounding environment. Moreover, they have stronger interactions with external stimuli and can therefore be controlled precisely and powered remotely. However, when compared to organic nanomachines, they are less biocompatible. Numerous examples of hybrid organic-inorganic nanomachines have also been reported in the literature.

The paper by Ellis, Moorthy, Chio, & Lee (2018) focuses on molecular machines and systems with feature size between 1 and 100 nm. As clarified by Rauscher et al. (2019), *the EC NM definition limits the scope of the term nanomaterial to materials containing (or consisting of) particles, i.e. particulate materials.* Molecular machines consist of particles, i.e. *minute piece[s] of matter with defined physical boundaries*.[28] Rauscher et al. (2019) further clarify that *the EC NM definition is (…) restricted to solid particles*. Inorganic and hybrid molecular machines with external dimensions within the 1 – 100 nm range are therefore nanomaterials as defined by the European Commission.

The revised Annex VI identification and characterisation parameters for nanoforms of registered substances and, in particular, *2.4.4. Shape, aspect ratio and other morphological characterisation; information on assembly structure including e.g. shell-like structures or hollow structures, if appropriate*, would ensure the provision of information on the chemical composition and the physical form of molecular machines. No information, however, is required on, for example, the type of motion performed or the type of energy harvested.

As specified by Ellis, Moorthy, Chio, & Lee (2018), *when designing and constructing artificial nanomachines, one typical approach is to specify the function of each individual component and then build precisely what has been designed*. In most cases, it can be concluded unambiguously that the shapes, surfaces or designs of the components of the molecular machines are more relevant for the function than their chemical composition. In these cases, it would seem uncontroversial to regard such molecular machines as complex objects, i.e. objects made up of more than one article. However, in Janus nanomotors, which are nanoparticles with two chemically distinct faces, the motility is fuelled by the catalytic decomposition of hydrogen peroxide by the platinum-gold nanoparticles. In this case, an unambiguous conclusion over whether the physical form or the chemical composition is more relevant for the function is not possible.

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[28] ISO 26824:2013, Particle characterisation of particulate systems – Vocabulary, international Organisation for Standardisation, 2013.

Although prototypes of molecular motors have been developed, the commercialisation of this type of nanotechnology application is still far in time.

4.3 Conclusions on the identification and characterisation of passive vs active nanomaterials, nanostructures and nanostructured materials and on the suitability of the REACH and CLP terminology

The identification and characterisation parameters required by the revised Annex VI of the REACH Regulation allow to unambiguously identify and characterise the different nanoforms of substances identified during the study. Further guidance is required on whether coatings and functionalisation are to be considered for determining if the particle size regulatory threshold included in the EC recommended definition is exceeded. Some criteria for distinguishing between solid and fluid materials (as, for example, those suggested by Rauscher et al., 2019) would add further clarity. Moreover, given the increasing integration between organic and inorganic components and the functionalisation with proteins or enzymes, some additional suggestions on the information to be provided on the surface treatment would benefit registrants.[29] Since the current use descriptors included in the use description system (ECHA, 2015), and in particular the technical functions, do not adequately represent the uses and functions of nanomaterials on the market or which could be placed on the market in the near future, an ad-hoc list of use descriptors for nanomaterials would facilitate the provision of relevant information. Finally, the guidelines on how to determine whether an object is an article according to the REACH Regulation presented in ECHA (2017c) could be complemented with specific examples on different nanomaterials, from simple nanoparticles to more complex assembly structures.

The Annex VI identification and characterisation parameters do not capture the dynamic dimension of the second and third generation of nanotechnology applications, including of second and third generation nanomaterials. The parameters were thought to identify and characterise nanoforms of substances rather than more complex technologies. For these, additional identification and characterisation parameters should require information on:

- The type of energy input/external stimulus (e.g. light, pH, temperature, magnetic field);
- The intended function/work to be carried out (e.g. remotely activated drug release, thermal ablation, fluorescence imaging);
- The changes occurred to the nanomaterial, nanostructure or nanostructured material following the external stimulus (e.g. change in composition, shape, surface area).

The identification and characterisation parameters apply to nanomaterials, i.e. solid particulate matter consisting of particles with the majority of its particle size number distribution below the 100 nm threshold. The generations of nanotechnology applications, as conceived by Roco and refined in this report, include nanostructures and nanostructured materials which may not be covered by the EC recommended definition of nanomaterials. Wohlleben et al. (2017) noted that *advanced generations of nanotechnology were imagined to consist of increasingly complex, increasingly functional particulate materials*. So far however, this is the case only for

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[29] ECHA (2017b), in discussing UVCBs, suggests some identifiers for the complex biological macromolecules, such as enzymes and proteins, that can be used for the functionalisation of the nanoparticles: standard enzyme index, genetic code, stereo configuration, physical properties, function/activity, structure, amino acid sequence. In addition, it is suggested to identify enzymes according to the international system for enzyme nomenclature, IUBMB (International Union of Biochemistry and Molecular Biology).

nanotechnologies being developed and used in the medical sector (e.g. Aurora®-DSG Nanoparticles). Since 2000, nano-enabled materials entering the market have been enabled by employing internal nanostructures synthesised in the material rather than by embedding in the product matrix nanoparticles or nanofibers previously synthesised and functionalised (*original conception of a "nano value chain"*).

More specifically, Wohlleben et al. (2017) observed three main trends of industrial innovation:

- The first is the shifting from novel nanomaterials (such as quantum dots and graphene) to integrated material systems and solutions. The trend encompasses both chemistry in general and nanotechnology more specifically. During the first decade of nanotechnology research, it has been learned that nanoparticles (nanomaterials as defined by the EC) are rarely a drop-in additive that enhance the properties of materials and products. Instead, the shape, size and interaction between the nanomaterials and the surrounding matrix need to be designed together into an integrated material system;

- The second trend sees products manufactured by a reactive pathway that induces or consumes nanostructures, abandoning the intermediate step of particulate nanomaterials;

- The third trend is *the notion that nano-enabled products should be designed from a perspective of the entire material system that balances performance, cost, safety, and sustainability*. Wohlleben et al. (2017) validate these arguments referring to the commercialisation of carbon nanotubes. These are novel nanomaterials which enable a wide range of applications. However, it has been observed that those start-up companies with higher number of applications are more likely to have significantly lower revenue than those companies focusing on fewer applications. This is because of two reasons: *specific particulate nanomaterial rarely performs in a variety of nano-enabled products* and clients *balance performance, cost, safety, and sustainability*. In addition, Suominen et al. (2012) observes that *treating engineered nanomaterials as separate elements can lead to a misunderstanding of how they will be used in integrated devices and systems*.

Due to the tonnage threshold of one tonne per manufacturer/importer per year and to the above trends, it seems unlikely that the European Chemicals Agency will receive specific information for second- or third-generation nanomaterials. In particular, the third trend described by Wohlleben et al. (2017) highlights that companies manufacturing/importing second or third generation nanomaterials are likely to be specialised in few applications and therefore are unlikely to be manufacturing/importing significant quantities of nanomaterials or the bulk form of the substances used.

Finally, Wohlleben et al. (2017) notes that innovation depends on the understanding of nanomaterial interactions with the surrounding material systems. The characterisation of nanostructured materials therefore requires metrological methods which go beyond size and shape of particles and look into *3D-resolved composition analysis (also for organic structures), interface/surface chemistry, surface reactivity, and transformational/reactive processes. However, most existing methods for the characterization of such properties do not properly reflect the heterogeneity within the system, in particular not at the nanoscale. Further, it is fair to state that no in-line metrology exists for such properties.*

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Appendix 1. Questions for the Expert Consultation

1) Are you familiar with the nanomaterials' categorisation into six subsequent generations proposed by Roco?

2) Have you ever referred to this categorisation?

3) Do you find the definitions unambiguous and specific? Could you provide examples of nanomaterials for each generation? Could you provide examples of nanomaterials that fall outside these categories?

4) Roco defined these six overlapping generations of nanomaterials from an engineering perspective, on the basis of an increasing level of sophistication and complexity of systems at the nanoscale and of a convergence of different disciplines of science and engineering. Do you find this categorisation useful, for example, from a risk management perspective? In your opinion, to different generations correspond different potential risks?

5) Do you find this categorisation useful from a market assessment perspective? In your opinion, nanomaterials belonging to different generations are to be expected on the market at different times?

6) An alternative categorisation, based on the type of research ongoing in 2007 and on the type of applications of the technology, has been put forward by Tour:

   – *Passive nanotechnologies: The nano part does nothing particularly elaborate. Its presence alone adds a significant increase to the performance of the system;*
   – *Active nanotechnologies: In this case, the nano entity does something elaborate such as absorbing a photon and releasing an electron, thereby driving a device, or moving in a specific and definable fashion across a surface. An example could be a "nanocar", a nano-engineered molecule that can be used to move atoms from one place to another;*
   – *Hybrid nanotechnologies: The complementing of a known platform through the attachment of a nano-sized entity but where the platform carries the bulk of the burden. An example would be using a silicon platform to carry out electronics, but making the silicon work with higher performance through the attachment of a surface layer of organic molecules that donate or accept charge.*

7) Are you familiar with this alternative categorisation and did you use or refer to any of these definitions? Do you find them unambiguous and specific? Could you provide examples of nanomaterials for each generation? Could you provide examples of nanomaterials that fall outside these categories?

8) Are you aware of any alternative categorisation that would allow monitoring the ongoing research on nanotechnology?

9) How could the definitions of Roco's generations be improved so to allow sorting nanomaterials by generation? Are the first two generations (passive and active nanomaterials) sufficient or, based on some of the ongoing research, there is the need to define additional generations?

10) Are you aware of any next (second and beyond) generation nanomaterials already on the market? Are you aware of any next (second and beyond) generation nanomaterials close to be commercialised? Would you be able to estimate the Technology Readiness Level?

11) Article 3 of the REACH Regulation and Article 2 of the CLP Regulation define

   – *a substance*

   means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.

   – *A preparation or mixture*

   means a mixture or solution composed of two or more substances

   – *An article*

   means an object which during production is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition

   In your opinion, could 2nd or higher generation nanomaterials have their functions determined by special shapes, surfaces or designs to a greater degree than their chemical compositions, therefore falling within the definition of articles?

12) In your opinion, does the revised Annex VI of the REACH Regulation ensure the proper characterisation of 2nd or higher generation nanomaterials?

13) Are you aware of any source of information that would help us in compiling an inventory of second or higher generation nanomaterials?

Table A2-1: Inventory of next generation nanotechnology applications - References

No.	References
Second generation of nanotechnology applications – (Re)active nanomaterials, nanostructures and nanostructured materials	
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