The social context of nanotechnology and regulating its uncertainty: A nanotechnologist approach

Vincent Jamier¹, Ignasi Gispert², Victor Puntes³

¹ Centre for NanoBioSafety and Sustainability (CNBSS), Campus UAB, 08193 Bellaterra (Barcelona), Spain
² Institute of Law and Technology-UAB, Campus UAB, 08193 Bellaterra (Barcelona), Spain
³ Catalan Institute of Nanotechnology (ICN), Campus UAB, 08193 Bellaterra (Barcelona), Spain. Institut Català de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Vincent.jamier@cnbss.eu

Abstract. With an increasing number of consumer’s products containing nanomaterials (NMs), Nanotechnology is already a reality on the market despite it may only be the emerging part of an iceberg. The potential exponential increase of nanotechnology uses is starting to alert major public bodies due to the uncertainty inherent of every emerging technology. According to the number of publications on safety/toxicity of NMs, major concern seems to be focused on NMs that may be released as free nano-objects. From a nanotechnologist point of view, major identified aspects on inorganic nano-objects are: i) Corrosion of metallic nano-objects, ii) Aggregation to sizes that trigger immune exacerbation, iii) Presence of toxic bystanders, iv) Biological membrane/barrier perturbation, v) Protein association & protein alteration. While the description of phenomena occurring at the nano-scale has not been classified yet, added to the contradictory studies on “unknown” samples, regulatory agencies have no clear idea on what should be considered “nano” or which NMs might pose a threat for health, safety or the environment. In addition to provide full and accessible information on new nano-product, it would seem advisable to plan research and innovation in a responsible and precautionary manner in order to be pro-active towards (responsive) NMs regulation.

1. Introduction

With 1317 consumers’ products already registered on the database of the Project on Emerging Nanotechnology (Figure 1), and little less on European Consumers’ Organisation (Figure 2), the use of Nanotechnology is a reality on the market despite that number may represent only the emerging part of an iceberg. As every emerging technology where the balance between risks and benefits is not clearly identified, the exponential increase of nanotechnology entering the consumer market is starting to alert major Non-Governmental Organisation (NGO’s), other organization and regulatory agencies due to the uncertainty inherent to novelty.
By definition, Nanotechnology is a technology that permits the manipulation of matter on an atomic and molecular scale, generally with materials with at least one dimension sized from 1 to 100 nanometres. At contrary of other emerging technologies, the nanotechnology cannot be easily defined as an independent technology, it is a field that cover most of the technology already existing (i.e. Physics, Chemistry, Biology, Electronic, etc.). With a significant number of publication covering the safety/toxicity of NMs, it seems that a majority of the health concerns related to nanotechnology are focused on nano-objects, which may enter the environment via the air or water and be inhaled or ingested. Probably for this reason, there is much less concerns in the development of nano-electronics, probably because these electronic components are less likely to be in direct contact with the consumer. Similarly as many toxic components of electronic apparatus, the isolation of the toxic product from consumer’s exposure allow its safe use (or at least, risk is limited to the production -worker's H&S and final disposal).

2. Identification of major concerns
Exposition to such nano-objects has made of importance the nanotoxicology field. It is known that, at contrary of their analogue “bulk” or “molecular” material, the toxicology of nano-objects could not only be established through the evaluation of mass composition doses (i.e. the same mass of nano-object will not have the same toxicity depending on particle size, shape,\(^1\) surface state, stabilization process, etc.). Here, the major question is why a nanoparticle (NP) of gold may be different from another NP of gold. Now, it is becoming more and more evident that the precise determination of physico-chemical properties of the investigated nano-object is primordial to be further correlated with others\(^2\) and to understand what make a nano-object less toxic than another one. Here, we can cite some parameters of importance: mean size, shape (if controlled), size range, surface state, surrounding...
coating (naked, electrostatic or steric), concentration (in solution), crystallinity. Moreover, the instability and short-lived evolution of NPs is also another aspect to be considered as it is common to any of the inorganic NPs, which may often be considered as evolving objects. This makes their toxicity evaluation more complex to understand. This behaviour can be principally attributed to their reduced size, where the surface energy is of great importance. Thus, in their search to minimize this energy, they have the tendency to interact with their environment and/or themselves by aggregation or by dissolution toward more stable phases, and engender the consequent associated concerns.

2.1. Corrosion of metallic nano-objects
Following a simple physical process describes by the Gibbs-Thompson effect and the Noyes-Whitney equation, NPs will dissolve as their surface energy increases (and therefore their size decreases and/or shape evolve toward spherical forms). Due to their reduced size, high surface-to-mass ratio, high radii of curvature, and the corresponding low coordinated atoms at the surface, dissolution is enhanced by chemical reactions with its surrounding or simply themself if it is thermodynamically or kinetically favourable. The last is often the case happening while stocking colloidal solutions for a certain amount of time, morphological modifications are occurring through the physical Ostwald ripening phenomenon. As the concentration of ions increases moderately after the purification and, unless free atoms are removed from the equilibrium, the following consequences should be devised: i) ion concentration remains constant while the size distribution of NPs broadens, ii) NPs shape is modified most probably via selective etching, iii) the number of NPs decreases as the smaller ones are completely dissolved.

2.2. Aggregation to sizes that trigger immune exacerbation
Competitively, NPs have also the tendency to aggregate following the Derjaguin and Lanadau, Verwey and Overbeek (DLVO) rules. It is known that the aggregability of NP suspensions depends on parameters such as the surface charge or coating and on the medium in which they are dispersed (i.e. highly conductive cell culture medium, physiological buffers, serum and plasma). For example, it has been reported that dramatic change in the state of aggregation, dispersability and charge (through zeta-potential measures) of NPs is occurring while transferring from a buffered aqueous solution to commonly used cell culture medium. This is even more evident while transferring nanopowder (with “naked” surface coating) to aqueous solutions. Those cases enhance the production of agglomerates that precipitate/deposit by weight accordingly to the Stokes Law. As NPs are destabilized in biological media, their special physicochemical properties are progressively/partially lost and may not be more consider as “nano” (neither properties nor the dynamics are any longer the same). Of course, NPs aggregation is a time-dependent process which may takes from a couple of second to months, and this has to be taken in consideration when performing biological tests, in particular in toxicological study attempting to investigate a particular type of NPs, ending up evaluating its micrometric aggregates (some time a source of confusion).

2.3. Presence of toxic bystanders
Under realistic conditions, NPs will frequently come in contact with cells as a component of a complex mixture. While most bystander substances will not affect the interaction between NPs and cells, negative synergistic mechanisms may also occur, including weakening of barrier cell layers, changes in membrane permeability, and changes of the cell activation status by bacterial compounds. Those bystander substances often originate from NP formulations or from ubiquitous bacterial fragments that will directly stimulate immune receptors. It is therefore of interest to understand to what extent the presence of contaminants affects the formation of a bio-corona, and how transcription of key genes is affected by bystander substances. In another hand, sterilisation of NPs may not sufficient to eliminate the bioactive contamination where bacterial fragments by triggering the defence reaction by binding to pattern recognition receptors (i.e. Toll-like receptors, Nucleotide Oligomerization Domain receptors, etc.) and enhancing immune reactions. Controlling and
understanding such contamination is of key importance, as it has been shown that contamination with lipopolysaccharide can be fully responsible for the "inflammatory" effect of some NPs, leading to false positive results.[5] In some cases, the association of bacterial fragments bound to NPs may also change the effects of each one in unexpected synergetic ways. Therefore, two key questions should be addressed: i) which assays are suitable to rule out contamination with bacterial substances? ii) to what extent can NPs exacerbate the level of "danger" of bacterial compounds? While bacteria are recognized by the human innate immune system through the repetitive patterns of their moieties (i.e. flagellin, CpG DNA), such patterns may be disrupted by NPs (altering the capacity of bacteria to induce usual inflammatory reaction). Emerging from such patterns, one may ask how NPs can modify the current immune-stimulating effects of bacterial fragments (e.g. antigen organization influences B cell tolerance).[6]

2.4. Biological membrane/barrier perturbation
NPs may cross barriers like the cell membrane, skin and mucosal barriers in order to fully make their biological impact. Biological membranes have a major significance in mechanisms of toxicity both because of the function as a cell barrier (plasma membrane) and because they are involved in many important physiological mechanisms. Any disruption of the membrane's structure by NP binding will lead to a modification of its barrier properties and physiological processes associated with them. It is important to tackle the present lack of understanding on the interaction of NP with bio-membranes and its mechanistic significance in NPs' bioactivity and toxicity. This interaction is fundamental to assess cell toxicity and translocation (transcytosis) potential.

2.5. Protein association & protein alteration
For many of the previous physicochemical reactivity reasons, the surface of NPs readily interacts with other particles, and in particular with biological molecules, such as proteins. Proteins are the predominant way for communicating to the exterior (i.e. living entities or environment) of the living cell. While hydrophobicity and surface charge have been the factors taken in account to explain protein adsorption to functionalized inorganic surfaces,[7] the interaction with NPs surface is greater as protein and NPs are in the same size range and therefore similarly distributed in solution. Those old concepts have been more recently grouped under the well-known name of Protein Corona formation (that may evolve also with time)[8]. Major interests have been focused on this formation because the proteins forming the corona remain associated with nanoparticles in in vitro and in vivo exposure, thereby conferring their biological identity to the NP-Protein Corona complex and determining the interactions between NPs and the host. Despite this association with proteins may indeed biocompatibilize NPs (i.e. albuminization of potentially toxic drugs, such as Abraxan©), there is the potential of creating a “Trojan horse” effect, enhancing the accumulation or vectorization of the toxic components of NPs that will slowly release their toxic cargo. Additionally, it has been postulated that the creation of this protein layer may trigger the depletion of specific protein; however, the amounts of NPs or the NP selectivity for a determined property should be much larger than the normal used NP concentrations and observed NP specificity.

Concluding, we do know that the NPs interact with biological systems and molecules in a non-very specific manner and that the existing protective barriers in the body designed to avoid invasion are normally effective preventing NP uptake and distribution. However, if these barrier are crossed (as by intravenous injection), then the NPs have the ability of both, activate the immune system, leading to NP phagocytosis and/or inflammation, or to interfere in the biomolecular machinery what can have long term undesired effects or negative effects in the case of disease. The subtle but prolonged effects that NPs may produce after repeated and chronic exposure are still a subject of research. This knowledge already allow us to initially classify NPs and NP features regarding they hazard, as, for instance, the use of NPs that may aggregate into macroparticles, specially anisotropic, that results in chronic inflammation are subject of concern, as much as metallic semiconductor NPs that leaches
toxic ions as CdSe NPs, or NP degradation (corrosion) effects that results in the induction of generation of reactive oxygen species (free radicals) that have been correlated with the REDOX potential of the material in the NP. This knowledge, combined with strategies for reducing exposure, by embedding and employing the NPs embedded in solid or liquid matrices, allows for the first time the safety by design approach, and the most likely, materials will be classified in families depending of their size and morphological ranges and other properties as valence state, electronegativity and surface state. It is therefore expected that in the future the toxicity of a NP will be easily predicted by the knowledge of which NP features result in an increased toxicity at the nanoscale. For advanced and more complex materials, protocols to assess their potential toxicity and strategies to decrease the exposure and dispersability of those NPs will be determined.

3. Regulating uncertainty: a nanotechnologist approach

Taking in consideration those scientific concerns, it is understandable that uncertainty dominates the field of nanotoxicology (aren’t we aiming to investigate the unknown?). By waiting a scientific clear answer to generate a general regulatory approach, it is evident that we may now be facing a “paralysis by analysis” situation. The description of phenomena occurring at the nanoscale has not been classified yet, thus while there is contradictory studies on “unknown” samples regulators agencies have no idea where “nano” starts or even if it exists, and have no precise strategy to address these issues yet (except a case by case approach). To answer social concerns the development of Nanotechnology need to provide full and accessible information on new product, establish the safety at the first stage of nanomaterial design, attempt to restrict the risk at the production site (where safety monitoring is easily controllable) and control their dispersions in the environment.

3.1. General regulation framework

There are no specific regulations for nanotechnologies or NMs at EU level. Instead, the manufacture, use and disposal of NMs are covered, at least in principle, by a complex set of existing regulatory regimes: i) at a “vertical” level with regulation on Chemical Products (under REACH), pharmaceutical products, pesticide and biocide; Medicines and medical devices, cosmetic, novel foods, food additive, food contact materials and etc. ii) at a “horizontal” level with regulation related to Health and safety of workers and also environmental legislation (like air quality, water, waste, soil protection, IPPC, Seveso II) as well as with environmental liability, product liability or general product safety.

3.1.1. Effectiveness and Regulatory gaps. Regulatory regimes are designed (almost by definition) to handle regulatory concerns existing at the time of promulgation and, as a consequence, emerging technologies often exacerbate or create regulatory gaps. In other words, regulatory systems are badly equipped to respond to NMs. In fact, it could be seen as logical to expect that the emergence of nanotechnology creates regulatory gaps. For instance, the Monash Centre for Regulatory Studies has reported six main types of regulatory gaps for the families of NMs and applications available or likely to become available in Australia before 2017:

- the issue of whether changing the particle size of a substance creates a "new" substance;
- regulatory triggers based on mass or volume;
- regulatory triggers that assume knowledge of risk;
- regulatory triggers reliant on risk assessment protocols;
- regulations that contains exceptions for R&D;
- regulatory triggers reliant on international or external documents that do not themselves address the risk of NMs.

The appraisal as to how important those gaps are is contested: while the Commission considers that "current legislation covers in principle the potential health, safety and environmental risks in relation to NMs", others, like Carl Schlyter (the responsible for the European Parliament resolution on regulatory aspects of NMs) clearly highlighted the limitation of such system when stating that
“(Current) rules are about as effective in addressing nanotechnology as trying to catch plankton with cod fishing net”.[13]

3.1.2. Regulation explicitly “Nano”. Regardless of the assessment that the Commission or other institutions, organizations and scientific literature have done on the degree of adequacy of the legal framework, the fact is that since 2008 we have seen an increasing regulatory activity related with NMs: basically, nano specific Recommendations (horizontal measures) and Sectorial Regulations (within the above explained framework of non-specific nano regulation). The nano specific Recommendations are the Code of Conduct for responsible research in Nanotechnology and Nanoscience,[14] and more recently, the Recommendation on the definition of nanomaterial.[15] On the Sectorial Regulation regulatory processes has been developed in different areas such as REACH (excluding carbon and graphite from the Annex IV),[16] food, food packaging and food contact materials,[17-19] cosmetic,[20] hazardous substances in electrical and electronic equipment (postponing possible nano regulation at Recital 16),[21] and biocides.[22] Generally speaking, sectorial regulation identifies the need to differentiate nanomaterial from “bulk” material, the need to identify them (labelling) and the need for a case by case approach. It is therefore not surprising that the recent second appraisal on NMs regulation from the Commission endorses the case by case approach.[23, 24] What remains an open question is whether the case-by-case approach is efficient and can be handled with the exponential increase of NMs (or discovery of already existing NMs) in the marketplace.

3.2. Beyond actual framework
From a legal standpoint, the lack of scientific certitude on the possible effects of NM over health and safety must be seen as a trigger for the implementation of preventive measures, economically reasonable, to avoid, or as a default mitigate, the potential risks associated with its use. Moreover, the existence of regulatory gaps and the existence of open legal concepts that imposes general legal obligations imply that companies no longer satisfy their obligations by complying with (non-existent nano-) standards, but by outlining a proactive behaviour in the context of responsive regulation.[25] The regulators’ interest in opening up the regulatory process to all stakeholders and the need for a regulatory framework that is flexible (adaptation to uncertainty and new scientific data) is shaping a new regulatory landscape for NMs where the hard law mechanisms (traditional command-and-control measures) will be increasingly complemented with the so called soft law mechanisms (those non legally binding but that might have certain indirect legal effect).[26] Among those soft law mechanisms for NMs we can mention: mandatory/voluntary reporting schemes, Codes of Conduct, Risk Management Systems or Schemes and Guidelines and Standards (such as ISO, etc.). Overall, it can be said that voluntary measures share similar principles and actions (due respect for precaution; priority on safety; raise/consider stakeholder awareness; inclusive approach) and goals (build trust and confidence in the technology; promoting health and environmental safety; gathering information).[27]

3.3. Principles of precaution
A precautionary approach is not only the cornerstone of responsive regulation, but also the basic strategy toward the use of NMs in industry, as shown in Figure 3.

---

[1] Following Senden, soft law mechanisms can be defined as rules of conduct that are laid down in instruments which have not been attributed legally binding force as such, but nevertheless may have certain (indirect) legal effects, and that are aimed at and may produce practical effects.
3.3.1. Safety-by-design. The integration of Safety within the design of NMs should not be limited to the investigation of the possible advert effect of used NMs but should be considered in the first step of the material design. It means to evaluate from the actual data available which nanomaterial is less likely to possess toxic properties with the desired final properties. In this search, it is necessary to cover the wide spectrum of available scientific investigation (however the polemic subject and judgement of the publication quality) and reduce by all mean the exposure to consumers as well as establishing a road guideline of the full lifecycle of the NM from cradle to grave assessment. To permit a such safety-by-design there is a need to stopping the “seek and try” evaluation techniques and start the effective and potential “bottom up design” where the properties (and principally safety features) could be controlled from the design of the material directly from the nano-scale as well as help to calibrate/understand the risk and benefit of advanced NMs. A strong inventory database of properties/parameters may therefore grow supported by high throughput toxicity screening of engineered NMs.\textsuperscript{2}\footnote{With integration of the regulatory pyramid from Mantovanni, E., Porcari, A. “Hard e Soft Regulation per le Nanotecnologie” 4a Conferenza Nazionale del Programma NIC. Milano 2 Decembre 2012.}

3.3.2. “ALARA (As Low As Reasonably Achievable)” Principle and H&S. Adoption of preventive and protective measures similar to those used to control exposure to hazardous (principally aerosols one) is necessary to protect workers manipulating NMs. The main measures can be regrouped in three area: i) the technical measures such as substitution of substances, processes, equipment, isolation or enclosure of the process, local exhaust; ii) the organisational measures such as cleanliness of the work area by using wet sweeping systems (and vacuum systems equipped with high efficiency filters), reduction of exposure time and limit the number of workers exposed; iii) the personal protective equipment such as the use of clothing chemical protective, gloves, glasses with side protection.
4. Conclusion
Similarly as the two approaches on nano-object production, the bottom-up and top-down approach, regulation of nanotechnology needs similar processes, like we have seen in the "regulatory pyramid" of Figure 3. The presence and use of naturally transformed, accidental and engineered nano-objects (such as processed proteins, ultrafine particles and powders grinded to the nano-scale) is not new. Such "low tech" nanotechnology has been surrounding us or used since centuries for example pigmentation in cosmetics (since the pharaohs time) and in ceramics processes as well as their possible adverse effects is well estimated allowing us to use a body of knowledge that while it has not been collected with a nanosafety perspective, it can be extremely helpful to frame the risks and limits of exposure to NPs. This is linking knowledge from microparticles toxicity (sarcoidosis, asbestosis, etc.) and metal compounds toxicity.

It is important that regulatory bodies are able to cover the actual use of NPs that was unnoticed, as in food additives, pigments, catalysis and consumer products as talc or polymeric materials and also the use of next and advanced generation of new NPs which will be each time more artificial than already existing as natural, unintended and unnoticed ones. For that it may be needed to establish complex matrices of calibrated properties (mean size with low size range, shape, surface state, stabilization process, interaction behaviour) covering the toxicology including majors concerns (corrosion of metallic nano-objects, aggregation to sizes that trigger immune exacerbation, presence of toxic bystanders, biological membrane/barrier perturbation, protein association & protein alteration) of existing and foreseen upcoming NPs. Nanoinformatics, to deal with the existing and proposed plethora of useful NPs and their evolution in different matrices of different complexity will surely be a determining tool for NP risk assessment.

For being able to do so the regulatory system must be focused on risk (avoiding regulation based on hazard) and the burden of proof must be shifted from public administration to NMs producers (in line with a responsive regulation and following the REACH model). From the research and innovation stage to marketplace new strategies needs to be implemented, based on selecting those low hazard NMs and designing NP and NPs that minimize exposure during the whole life cycle (so responsible research and innovation strategies). The regulatory system, accordingly, should promote that strategy, but, for doing so, regulation cannot rely solely on command-and-control measures. To complement them, soft law mechanisms are playing and increasing important role in nanotechnology regulation. All stakeholders might need to analyse how they are integrating soft law mechanisms into their organizations and how they are shifting towards pro-active behaviour for legal compliance.

References
[1] L.C. Stoehr, E. Gonzalez, A. Stampfl, E. Casals, A. Duschl, V. Puntes, G.J. Oostingh, Shape matters: effects of silver nanospheres and wires on human alveolar epithelial cells, Part Fibre Toxicol, 8 (2011).
[2] P. Rivera Gil, G. Oberdorster, A. Elder, V. Puntes, W.J. Parak, Correlating Physico-Chemical with Toxicological Properties of Nanoparticles: The Present and the Future, Acs Nano, 4 (2010) 5527-5531.
[3] E. Casals, T. Pfaller, A. Duschl, G.J. Oostingh, V.F. Puntes, Hardening of the nanoparticle-protein corona in metal (Au, Ag) and oxide (Fe3O4, CoO, and CeO2) nanoparticles, Small, 7 (2011) 3479-3486.
[4] E. Casals, E. Gonzalez, V.F. Puntes, Reactivity of inorganic nanoparticles in biological environments: insights into nanotoxicology mechanisms, J. Phys. D: Appl. Phys., 45 (2012) 443001.
[5] G.J. Oostingh, E. Casals, P. Italiani, R. Colognato, R. Stritzinger, J. Ponti, T. Pfaller, Y. Kohl, D. Ooms, F. Favilli, H. Leppens, D. Lucchesi, F. Rossi, I. Nelissen, H. Thielecke, V.F. Puntes, A. Duschl, D. Boraschi, Problems and challenges in the development and validation of human cell-based assays to determine nanoparticle-induced immunomodulatory effects, Part Fibre Toxicol, 8 (2011) 8.
[6] M.F. Bachmann, U.H. Rohrer, T.M. Kundig, K. Burki, H. Hengartner, R.M. Zinkernagel, The influence of antigen organization on B cell responsiveness, Science, 262 (1993) 1448-1451.

[7] S. Goy-Lopez, J. Juarez, M. Alatorre-Meda, E. Casals, V.F. Puntes, P. Taboada, V. Mosquera, Physicochemical characteristics of protein-NP bioconjugates: the role of particle curvature and solution conditions on human serum albumin conformation and fibrillogensis inhibition, Langmuir : the ACS journal of surfaces and colloids, 28 (2012) 9113-9126.

[8] E. Casals, T. Pfaller, A. Duschl, G.J. Oostingh, V. Puntes, Time Evolution of the Nanoparticle Protein Corona, Acns Nano, 4 (2010) 3623-3632.

[9] S.F. Hansen, A. Baun, When enough is enough, Nat Nanotechnol, 7 (2012) 409-411.

[10] G.N. Mandel, Regulating Emerging Technologies, Law, Innovation & Technology, 1 (2009) 75-87.

[11] D. Ludlow, D. Bowman, G. Hodge, A Review of Possible Impacts of Nanotechnology on Australia's Regulatory Framework, in. 2007.

[12] Communication of the EC of 17/06/08 on Regulatory Aspects of Nanomaterials (COM(2008)366).

[13] On the explanatory statement from the Resolution. Available at http://www.europarl.europa.eu/oeil/FindByProcnum.do?lang=en&procnum=INI/2008/2208. (Accessed February 2011). in.

[14] COMMISSION RECOMMENDATION of 07/02/2008 on a code of conduct for responsible nanosciences and nanotechnologies research, in.

[15] COMMISSION RECOMMENDATION of 18.10.2011 on the definition of nanomaterial (2011/696/EU) OJ L275/38 of 20.11.2011, in.

[16] COMMISSION REGULATION 987/2008 of 08.10.2008. Carbon and graphite are excluded from REACH Annex IV. OJ L268/14, in.

[17] REGULATION (EC) 1333/2008 of 16.12.2008 on food additives. Article 12 OJ L345/16, in.

[18] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (OJ L12, 15.1.2011, page 1), in.

[19] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/31/EC, Directive 1999/10/EC and of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2005/74/EC and Commission Regulation (EC) No 608/2004 (OJ L304, 22.11.2011, page 18), in.

[20] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Text with EEA relevance). in.

[21] DIRECTIVE 2011/65/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment, in.

[22] Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products, in.

[23] EUROPEAN COMMISSION - PRESS RELEASE of the 03/10/12 - Nanomaterials: Case by case safety approach for breakthrough technology, in.

[24] Communication of the EC of 03/10/12 on the Second Regulatory Review on Nanomaterials (COM(2012) 572 final), in.

[25] M. Fuhr, K. Bizer, REACH as a paradigm shift in chemical policy - responsive regulation and behavioural models, Journal of Cleaner Production, 15 (2007) 327-334.

[26] L. Senden, Soft Law in European Community Law, Hart Publishing, 2004.

[27] E. Mantovani, The role of voluntary measures in the governance of nanotechnologies: the case of the European Code of Conduct, in: AIRI/Nanotec IT. Euronanoforum 2011, Budapest, 2011.
[28] S. George, T.A. Xia, R. Rallo, Y. Zhao, Z.X. Ji, S.J. Lin, X. Wang, H.Y. Zhang, B. France, D. Schoenfeld, R. Damoiseaux, R. Liu, S. Lin, K.A. Bradley, Y. Cohen, A.E. Nal, Use of a High-Throughput Screening Approach Coupled with In Vivo Zebrafish Embryo Screening To Develop Hazard Ranking for Engineered Nanomaterials, Acs Nano, 5 (2011) 1805-1817.