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Towards Nanotechnology Regulation – Publish the Unpublishable

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***Graphical Abstract**

In an ideal world, regulation should seek to facilitate and harmonize the identification, characterization and control of all hazards, exposures and risks associated with substances and products, to protect human health and the environment, while at the same time enhancing industrial competitiveness and innovation. To date, this is the center of current global debates on nanotechnology and its products. A challenging situation now occurs. Nanotoxicology data are required, many studies are ongoing and yet the questions remain open as to whether the obtained data are appropriate and what existing data can be used here. A critical aspect, perhaps even the most critical aspect of nanotoxicology, is the availabilty of no-effect data. We propose here a framework that includes all scientific data on nanomaterials which will contribute to the development of harmonized guidelines for nanomaterial safety studies.

Research Highlights

Nanotechnology > novel regulations > nanotoxicology > crucial no effect data > framework to publish the unpublishable

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Nanomaterials have the potential to be active and dangerous for human health and the environment, depending upon their concentration, species, use and exposure. Regulation seeks to facilitate and harmonize the identification, characterization and control of hazards, exposures and risks associated with substances and products, to protect human health and the environment, while at the same time enhancing industrial competitiveness and innovation. We argue that the evidence-base of peer-reviewed toxicological literature, upon which regulators and those charged with protecting human and environmental health draw opinion and conclusions, is biased towards data reporting positive (adverse) results, and that all toxicological data should be published.

To address the bias and limitations of the evidence base, we suggest that the scientific principle of **positive and negative results carrying equal importance** be adopted and promulgated by two of the key 'gatekeepers' of research, i.e. the funding bodies and the publishers. They reach the wider community of stakeholders that utilizes scientific data, and thus have a crucial role in upholding the value and quality standards of research to provide as robust and meaningful data as possible. The obvious caveat is that these data are obtained within a robust scientific framework, including the use of adequate positive and negative controls.

Maintaining a meaningful regulatory framework for nanomaterials requires a variety of data, from basic material/product properties to biological effect information. The current Information Requirements for substances within the scope of the European Union's

REACH regulation are provided in Annexes VII to X of the legal text with further description in the accompanying guidance documents [1]. In nanotoxicology, a challenging situation now occurs. Whilst it is accepted that data is desperately required, and many initiatives such as EU Framework Programme projects (e.g., ENNSATOX, InLiveTox and ENPRA) are aimed at streamlining experiments and providing wider access to data (e.g., through NapiraHub), questions remain open as to whether these data are appropriate, whether new data are needed specifically for regulatory purposes, and what data already published in the peer-reviewed literature can be used in this context. This latter point is a critical issue that has far-reaching consequences. To aid regulators in setting appropriate guidelines, and industry to develop safe nanotechnological innovations, we propose a nanosafety data repository, preferably integrated in the current initiatives, that includes significant biological effects and no-effect data.

The need for complete data reporting

A problem encountered by most researchers is that important details are not described in the majority of the scientific literature. Nanomaterials are by no means "ready to use" in biological testing [2], thus they must be accurately characterized and tested under the conditions they are used in biological assays. As an example, data are often incomplete regarding the properties of nanomaterials upon contact with biological systems [3-5]. It is now evident that nanomaterials undergo modifications directly dependent on the biological molecules encountered in the body fluids or at the body surfaces [6-9]. These interactions may also lead to various forms of modification of the nanomaterial physical status, including degradation/dissolution and aggregation. Therefore, it is important to

characterize the particles in the situation they are applied in the biological tests, in contrast to their status "as received". In the absence of such information, most of the data, either positive or negative, may be misunderstood or misinterpreted. Indeed, the majority of published nanotoxicological studies which fail to report relevant details are simply uninterpretable, thus their conclusions can be thoroughly erroneous. A list of the relevant items to be reported in nanosafety study is suggested in Table 1.

The need to reduce bias in the publication of findings is a common feature in sciences and has received particular attention in the medical studies [10]. It has a specific importance for nanosafety issues, as nanomaterials have elicited an early consideration of the need and scope for regulation. The novelty of nanomaterials and their innovative properties raise considerable interest, but also significant fears, in the general population. Scientific studies in this field are often picked up by media sources, which can lead to a biased perception of danger of nanotechnology. Safety and risk management for nanomaterials are compounded by the fact that they are not a uniform group of substances [11] and regulatory decisions require data that are simply not available yet. There is a particular paucity of biological and toxicological data covering nanomaterials.

Many researchers do not find significant biological/toxicological effects when studying the activity of nanomaterials for particular endpoints. This is highly valuable information and should be contributed to the evidence-base. The availability of both positive and negative findings helps us to build a more complete and relevant picture of the complex biological effects of a given nanomaterial. It is, nevertheless, important to acknowledge that even if an effect is shown at one organism level in the short term, there may in fact

be no consequences from the use of these materials in the long term and at a different ecological and organism level, and vice-versa.

The need for meta-analysis as tool for interpreting nanosafety studies

Since no studies can concomitantly address all relevant aspects of nanosafety, an important tool to resolve outstanding questions will be to perform meta-studies. Bearing this in mind, research papers should report on materials and methods as extensively as possible to make the data suitable for subsequent re-analysis. In this context, again it is absolutely critical to report "no effect" data in studies, since meta-studies based on biased individual studies cannot avoid being likewise biased. To this end, two actions could be taken. First, we should raise awareness in the editors of scientific journals, and in the scientific community at large, on the absolute importance and practical need of publishing "no effect" data in studies. Second, if publication in the scientific literature of "no effects" studies cannot be ensured, creation and awareness of a (peer-refereed) online repository should be facilitated, to provide a means to disseminate scientific findings of value to future meta-analysis studies. The proposed database should include all scientific work worldwide regardless of language, but with clear data definitions and guidelines. As an incentive, data contributions need to be recognized and citable, with an equivalent standing to a peer-reviewed publication in a journal. We propose such repository could be harmonized and coordinated under European auspices and incorporate results from national and international initiatives. Contributions may also be encouraged through funding policy requirements similar to the NIH public access policy where all NIH

funded peer-reviewed manuscripts have to be accessible to the public on PubMed Central.

Current European initiatives including NapiraHub, Qnano, the Nanosafety Cluster and others emerging such as a publicly-controlled and updated open instrument based on the Wikipedia model [12] may be considered to at least partially fulfill this role. A panel of experts acting as an Editorial Board should oversee the peer review of unpublished data to be included, encouraging contributions of "no effect" studies. For published studies, we should encourage Journal Editors to include in their manuscript guidelines and instructions for authors and peer-reviewers regarding the need to provide such "no effect" data as supplementary information that after review and acceptance would be added to the repository.

The complexity of the tasks and challenges outlined above makes it clear that there is probably no possibility of producing a "perfect" scientific study on nanosafety. Nevertheless, full reporting of all robust studies, with and/or without effects being observed, should not be beyond the realms of possibility to avoid and address publication bias. Where an evidence base is needed by so many, with responsibilities ranging from health protection to responsible technological innovation, reporting on materials and methods should be even more meticulous, since we cannot be sure which information may turn out to be important in the future. The challenges of such a meticulous level of characterization are not underestimated, but this can be assisted by the continuing development and adoption of pre-characterized reference materials for study. By

developing and adhering to suitable guidelines, we will be able to progress further towards a better understanding of the potential risks associated with nanomaterials.

Acknowledgement.

The authors like to thank Andrew Nelson for his valuable input to the manuscript.

REFERENCES

[1] REACH regulation and guidance documents downloadable from: http://guidance.echa.europa.eu/

[2] Schulze C, Kroll A. Lehr C.-M. Schaefer U.F., Becker K, Schnekenburger J, Schulze-Isfort C., Landsiedel R, & Wohlleben W. Nanotoxicology 2, 51-61 (2008).

[3] European Commission, SCENIHR – 2009

[4] Savolainen K, Alenius H, Norppa H, Pylkkänen L, Tuomi T, Kasper G. Toxicology. 2010 Mar 10;269(2-3):92-104.

[5] Boczkowski J, Hoet P. Nanotoxicology. 2010 Mar;4(1):1-14. Review.

[6] Röcker, C. Pötzl, M., Zhang, F., Parak, W.J., & Nienhaus, G.U Nat. Nanotechnol. 4, 577-580 (2009).

[7] De Paoli Lacerda SH, Park JJ, Meuse C, Pristinski D, Becker ML, Karim A, & Douglas JF. ACS Nano, 4, 365-379 (2009).

[8] Casals, E., Pfaller, T., Duschl, A., Oostingh, G.J., & Puntes, V.F. ACS Nano, 4, 3623–3632 (2010).

[9] Monopoli, M.P. Walcyk, D. Campell, A. Elia, G., Lynch I., Baldelli Bombelli F., Dawson. K.A. JACS Feb 2 (2011)

[10] Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR., Lancet Apr 13;337(8746):867-72, 1991

[11] Paul JA Borm, David Robbins, Stephan Haubold, Thomas Kuhlbusch, Heinz Fissan, Ken Donaldson, Roel Schins, Vicki Stone, Wolfgang Kreyling, Jurgen Lademann, Jean Krutmann, David Warheit, Eva Oberdorster. Particle and Fibre Toxicology 3:11 2006

[12] LiquidPublication project. Information downloadable from: http://project.liquidpub.org/

TABLE 1 **. Important items to be reported in nanosafety studies**

- **Characterisation Dataset**
  - **Physical characteristics of nanomaterials**

    Specify quantitative and qualitative information including:

    - size, and size-distribution, surface area, shape, surface charge, as measured in the test media

    - Description of analysis (i.e. time: as-received, as-dosed, after experiment; conditions: as supplied, following aliquot preparation; method: TEM, Z-Potential, DLS, X-ray diffraction, UV-VIS).

  - **Identify potential confounders**.

    In particular, data on contamination by bacteria, spores and their metabolic products (e.g., lipopolysaccharides and other known ligands able to activate immune receptors).

  - **Identify ageing of nanomaterials**.

    Information pertinent to time-dependent changes of the nanomaterial sample should be identified and reported, including storage conditions and observed changes in behaviour and characteristics between experiments.

- **Composition of solvent or carrier**.

  The composition of the test fluids used in the biological tests (cell culture media or physiological buffers) should be reported, e.g. pH, osmolarity, ionic strength, the presence and absence of any ions, proteins, surfactants and other additives which might affect the surface properties and agglomeration/dissolution of nanomaterials dispersed therein.

- **Full description of the (biological) assay**
  - **Protocol description.**

    Sufficient details of the assay protocol, in particular highlighting any adjustments to standard protocols, should be provided to enable i) the experiment to be reproduced, and ii) data from comparable experiments to be identified for meta-analysis.

  - **Assay validity.**

    The basis and validity of the chosen bio-assay for the nanomaterial and endpoint under investigation should be stated, e.g. healthy, acute or chronic disease model, young or old age, genetic background, chronic or cumulative exposure.