Contribution of mesocosm testing to a single-step and exposure-driven environmental risk assessment of engineered nanomaterials

Melanie Auffan, Armand Masion, Catherine Mouneyrac, Camille de Garidel-Thoron, Christine Ogilvie Hendren, Alain Thiery, Catherine Santaella, Laure Giamberini, Jean-Yves Bottero, Mark R Wiesner, et al.

NanoImpact 13 (2019) 66–69

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.elsevier.com/locate/nanoimpact

Research paper

Contribution of mesocosm testing to a single-step and exposure-driven environmental risk assessment of engineered nanomaterials

Mélanie Auffan[a,b,d], Armand Masion[a,b,*], Catherine Mouneyrac[c], Camille de Garidel-Thoron[a,b], Christine Ogilvie Hendren[d], Alain Thiery[b,e], Catherine Santaella[b,f], Laure Giamberini[g], Jean-Yves Bottero[a,b], Mark R. Wiesner[d], Jérôme Rose[a,b,d]

[a] CNRS, Aix-Marseille Univ, IRD, INRA, Coll France, CEREGE, Europole Arbois, BP 80, 13545 Aix en Provence, France
[b] Labex SERENADE, Europole Arbois, BP 80, 13545 Aix en Provence, France
[c] Laboratoire Mer, Molécules, Santé, Université Catholique de l'Ouest, 49000 Angers, France
[d] Center for the Environmental Implications of Nano Technology (CEINT), Duke University, Box 90287, 121 Hudson Hall, Durham, NC 27708, USA
[e] Aix-Marseille Univ, Univ Avignon, CNRS, IRD, IMBE, Marseille, France
[f] Aix Marseille Univ, CEA, CNRS, LEMIRE, 13108 Saint-Paul-lez-Durance, France
[g] Univ Lorraine, CNRS, UMR 7360, LIEC, Campus Bridoux, Rue Gen Delestraint, 57070 Metz, France

ABSTRACT

Environmental risk assessment of nanomaterials generally relies on a decision-tree based strategy which provides guidance and protocols for the determination of a collection of hazard end-points. Mesocosm testing is based on a different approach. This method consists in monitoring the evolution of a re-created miniature ecosystem subsequent to a nanomaterial contamination. The only decision in this risk assessment strategy is the definition of an environmentally relevant exposure scenario (incl. dose), which, given current analytical capabilities, may unfavorably affect the nature and precision of parameters and endpoints to be determined. Despite these limitations, mesocosm testing bears clear advantages for the determination of both exposure and hazard in a single experiment, and for producing dependable and intercomparable data.

1. Knowledge gaps in the risk assessment of nanomaterials

Despite numerous studies over the past two decades examining the effects of nanomaterials, the available data have not yet been translated into a comprehensive risk assessment (RA) strategy covering the entire life cycle. This stems from several issues.

- There is a marked imbalance in risk related research efforts, hazard studies outnumbering by far those determining exposure to nanomaterials. This situation is not specific to nanomaterials; for conventional chemicals, there is also a greater focus on hazard, although the gap with exposure in this case is less pronounced.
- Not all aspects of the life cycle of nanomaterials are addressed equally in studies. Occupational safety being more studied than later parts of the life cycle (viz. consumer and environmental exposure and associated hazard), due in part to the clear exposure pathways posed for workers, and the current state of (non-nano-specific) regulations which puts worker protection at the forefront.
- The current situation regarding the effects of nanomaterials in the use and end-of-life phases of the life cycle shows a lack of data as well as a lack of suitable methods to examine exposure as well as hazard.

In the present communication we address the role of mesocosm testing to reduce this gaps in the case of environmental RA.

2. Addressing post manufacturing life cycle stages

It is only natural that occupational safety looms large in risk assessment, due to the large quantities, and high concentrations of nanomaterials handled early in the lifecycle in the workplace. High concentrations facilitate efficient monitoring, sometimes with personal devices (Asbach et al., 2017; Iavicoli et al., 2018). Nonetheless, nano-specific monitoring is still in its infancy and the current technology is incapable of measuring the full range of nano-sized materials (Kuhlbusch et al., 2011; Kuhlbusch et al., 2018). Despite these drawbacks, nanomaterial measurement in occupational settings, where the target of measurement is usually known a priori, the high stakes (worker's health) associated with a negative outcome, have combined to make the occupational context one of the better documented aspects within the life cycle of nanomaterials.

In contrast, the data regarding the later parts of the life cycle are

* Corresponding author at: CEREGE, Europole de l'Arbois, BP80, 13545 Aix-en-Provence Cedex 04, France.
E-mail address: masion@cerege.fr (A. Masion).

https://doi.org/10.1016/j.impact.2018.12.005
Received 22 October 2018; Received in revised form 18 December 2018; Accepted 21 December 2018
Available online 22 December 2018
2452-0748/

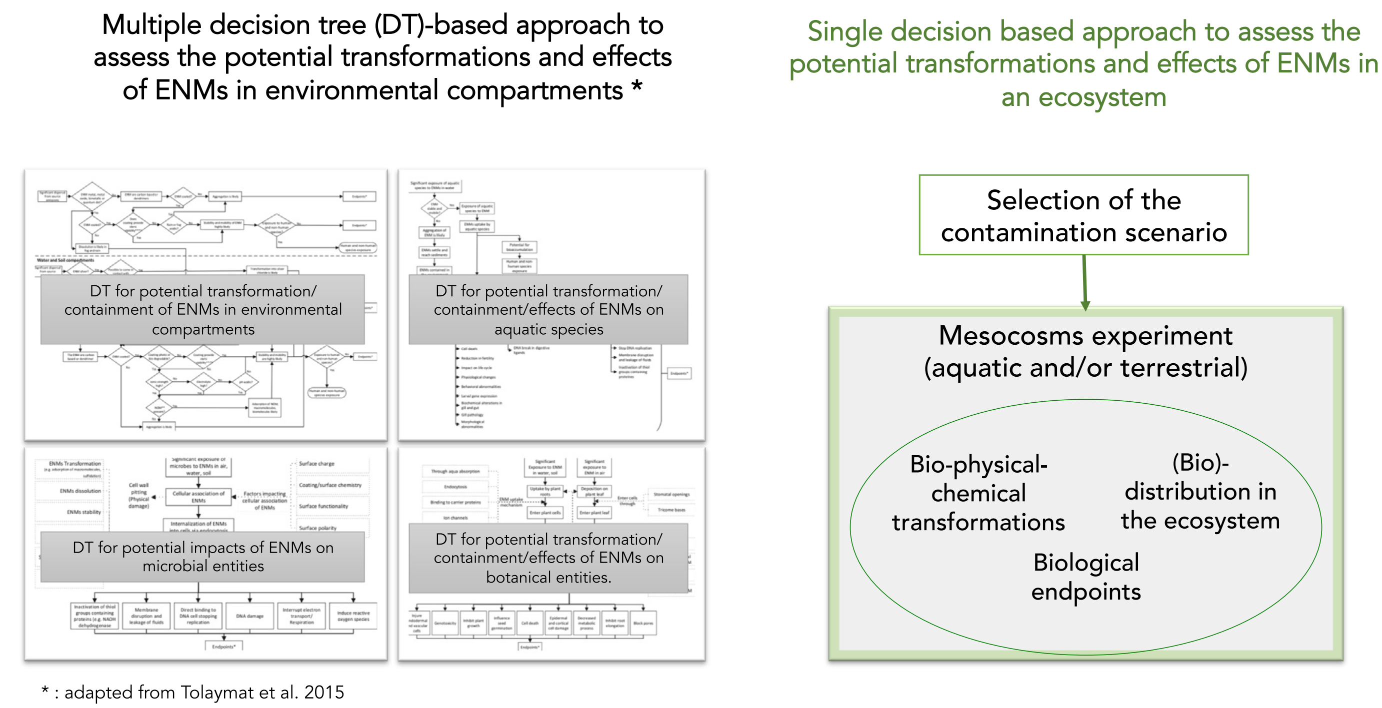

Fig. 1. Decision tree vs. exposure scenario driven risk assessment strategy.

scarce, especially for exposure determinations. Consumer and environmental exposures are typically analytically-challenged by the need to differentiate nanomaterials from complex and potentially large natural backgrounds, lower concentrations, unknown composition(von der Kammer et al., 2012) There are only a few experimental determinations of actual contamination levels, often employing a specialized analytical strategy (Gondikas et al., 2018; Gondikas et al., 2014) (state-of-the-art equipment, expert handling, …) that is unfit for general or standardized use. As a consequence, the current knowledge regarding potential consumer and environmental exposure relies heavily on modeling efforts that entail considerable uncertainty stemming from assumptions on production volumes, product use, human behavior, transformation processes, release rates, and myriad other processes that may determine exposure concentrations (Giese et al., 2018; Gottschalk et al., 2015; Sun et al., 2016).

3. Re-balancing hazard-driven RA and exposure-driven environmental RA

Exposure is not always neglected or of minor concern in the risk assessment (e.g. radiation RA or classical chemical RA). For instance, minimizing the exposure (i.e. the ALARA (viz. as low as reasonably achievable) safety principle) is the only means to mitigate the radiation risk. Regarding chemicals in general, some approaches support an exposure-driven risk assessment (Embry et al., 2014), where hazard and exposure are considered following a two-tiered, interacting process. Using this procedure, hazard and exposure terms in the risk equation can be re-balanced, with the objective of achieving a more efficient RA in terms of cost and time, by avoiding expensive higher-tier investigations when a material is determined to be benign based on lower tier hazard or exposure evaluations (Embry et al., 2014). Nevertheless, this approach has yet to be applied in the context of nanotechnology related risks, and suitable methods remain to be developed for non-occupational safety assessment in a (pre)regulatory and (pre)normative context.

4. A suitable tool: mesocosm testing

The attention paid to hazard characterization has translated into guidelines endorsed (or on track to be endorsed) by several agencies (e.g. OECD, ISO) (OECD, 2006; OECD, 2014; Petersen et al., 2015). These guidelines, which are not always nano-specific, are essentially (pre)regulatory or (pre)normative in nature, and consequently there are precision and reproducibility requirements on specific metrics, as well as for the ease of implementation by a large audience. The tests are meant to generate "standardized" sets of data for a more robust hazard and thus risk characterization. Unfortunately, this focus on a specific parameter/end point in a simplified system causes these tests to be of limited relevance to describe the effects of a nanomaterial during its use phase and its disposal and/or recycling.

Mesocosm testing offers a means of providing meaningful data to inform environmental risk assessment for such complex systems. Among the many definitions for mesocosm, a more general one describes a mesocosm as an enclosed and, essentially self-sufficient (but not necessarily isolated) experimental environment or ecosystem with a number of interdependent system parameters. Mesocosms are surprisingly under-utilized (a library search with keywords "mesocosm" and "nano*" returns less than 200 references since the first publication in this field). Yet, there are several benefits in using this tool (Ferry et al., 2009).

1/ One of the main features is that mesocosm testing provides exposure and hazard data in a single experiment, thereby eliminating not only the need but also all the biases linked to the separate determination of physical-chemical and biological parameters. Indeed, a common risk assessment strategy consists in progressing along a decision tree which requires the measurement/determination of a given parameter at each step with a specific Standard Operating Procedure (SOP). This process usually involves several decision trees (e.g. occupational vs. environmental exposure, in vivo vs. in vitro toxicity), an "exposure" tree usually feeding into a "toxicity" tree (Fig. 1) (Tolaymat et al., 2015). The consequence is a multiplicity of experimental conditions under which these parameters are determined, which makes it complicated to relate them to one another.

Also, these stand-alone SOPs ignore any synergetic or antagonistic effects that do occur in an ecosystem but that are not included in the dichotomy of a decision tree based process. For example, some ligands may accelerate the solubilization of a metal oxide nanomaterial, while other may cause the precipitation of its dissolved forms. A mesocosm experiment does not obey the sequential prioritization of parameters and end-points of a decision tree. The observed situation is the result of the interaction of a number of biotic and abiotic mechanisms which are interdependent in ways that are precluded when these factors are separated and controlled in a laboratory setting. There is no external control over the occurrence of a given phenomenon within the mesocosm system, nor over the time at which it occurs or its amplitude. In essence, a mesocosm can serve as a highly complex and low throughput functional assay (Hendren et al., 2015) whose results represent the combined behavior of a whole system under given conditions.

2/ Precisely because mesocosms typically do not entail a large number of replicates varying isolated parameters across a wide range of values, it is essential that these experiments are conducted using realistic exposure scenarios. The degree of resources required for these studies also requires that relevant combinations of conditions are studied to ensure both feasibility of the study and environmental pertinence. In addition to re-creating a self-sustaining ecosystem (i.e. necessarily a collection of biological targets), this therefore involves applying doses to achieve environmentally relevant concentrations of nanomaterials. Doses should result in exposure conditions for which systems are allowed/expected to evolve, but not to be disrupted, i.e. mesocosm testing should be applied to typical contamination levels rather than addressing unusually high concentrations such as those resulting from an accidental release. Modeling results can serve as guidance to define the experienced concentration. This approach stands in contrast with targeted toxicity tests, which are typically operated at significantly higher concentrations (Holden et al., 2016; Handy et al., 2012). Adhering to environmentally relevant concentrations in mesocosm experiments implicitly represents a paradigm shift, in so far as this is a move towards less precision and less control over the progress of the test. Indeed, with the current state of instrumentation, sub-ppm to ppb concentrations of a given element can be detected routinely (outside of background issues); however the methods for determining the speciation require concentrations at or beyond the upper limit of the predicted levels. As a consequence, some physical-chemical parameters and biological endpoints that are accessible with standardized tests, cannot be determined in a mesocosm experiment. However, this limitation is not a permanent obstacle, as it may be overcome with instrumentation improvements, and mesocosm testing bears many advantages that outweigh current analytical imprecision. It is precisely this lack of control that makes mesocosms an invaluable tool to determine the fate and effect of nanomaterials. Since systems are allowed to evolve without outside influence, the relevance of the collected exposure and hazard data is high since they embody a level of complexity that is beyond what current models are able to predict. For instance, mesocosm testing allows the determination of the (bio)distribution of nanomaterials and the associated kinetics within an ecosystem without a priori assumptions of predominant physical chemical and biological mechanisms. There is no single tool or combination of tools that describes transport/mobility and biological uptake and effects of nanomaterials at a comparable level of confidence.

3/ A generic SOP needs to address ecosystem stabilization criteria, i.e. when a mesocosm is ready for the contamination phase, to define a core set of parameters to be monitored and to provide guidance on how the measurements should be performed. Besides this framework, mesocosms are very versatile. They allow for a variety of exposure scenarios in a variety of ecosystems. This includes for example pulse vs. chronic dosing of the nanomaterial contaminant, introduced in various forms (e.g., suspensions or aerosols) and at different stages of the lifecycle of a nanomaterial. Besides examining the effects of pristine materials, the duration of the experiment can be adjusted to allow for aging of the introduced nanomaterial, which is interesting for nanomaterials from products with a short life cycle (e.g. cosmetics), or the contaminants can be pre-aged for products with a long use phase (e.g. paints and stains). The versatility of mesocosm testing applies also to the ecosystem. It can be aquatic, terrestrial or both, in a continental, coastal or marine setting. Whenever possible, fauna and flora should preferably be taken from a natural site with proper authorization and adherence to ethical guidelines, i.e. avoiding focusing on model organisms such as *Arabidopsis*, *E. coli*, or zebrafish. Organisms should be chosen to be representative of a given environment (e.g. tropical wetland, desert), to which these organisms are already adapted. Depending on what comparisons are sought, some components of the mesocosm may be of off-site origin to allow for comparable replicas and/or stable reference experiments, and the choice of the "control" material may lean on established standards when available. For example, a commercial mineral water might be used instead of a natural source, thereby providing quasi constant composition and bacterial quality. This can be a valuable alternative to on site sampling, especially for long term experiments with indoor mesocosms to compensate for evaporation.

5. Moving forward

5.1. Sound experimentation

Mesocosm testing with its rather crude and limited parameter determination may be viewed as incompatible with the precision of standardized procedures and harmonized data treatment and exchange. However, with a focus on "fit-for-purpose", mesocosms may serve as functional assays to test assumptions and generate new hypotheses. Indeed, previous work (Lowry et al., 2012; Buffet et al., 2013; Tella et al., 2014) paved the way for sound SOPs based on mesocosm methodology and eventual standardization. To do so, the robustness of the procedure needs to be assessed. In the present context, robustness is the ability of a stabilized/equilibrated ecosystem to withstand the introduction of a nanomaterial contamination without any abrupt changes in global physical chemical parameters or population shifts, i.e. a certain buffering capacity. It has been demonstrated in a proof of concept initiative (Auffan et al., 2016; Auffan et al., 2014) that even smaller sized mesocosm set-ups possess the required robustness.

5.2. Needs for guidance

The result is the possibility investigating virtually every ecosystem situation. This multiplicity does not equate with operational complexity. By definition, mesocosms run themselves, the critical points being ensuring an adequate equilibration of the system and a well-designed realistic exposure scenarios. The work is of monitoring and ensuring operation including consistent start conditions and well-controlled dosing protocols, as opposed to the tight control of each parameter. Once set in motion, observations of how system parameters proceed unperturbed can allow us to probe how they might influence one another. As mentioned above, these phases of the experiment should follow criteria to be collated into a (pre)normative guidance document.

The ease of operation and the "hands off" character of mesocosms testing is in sharp contrast with the efforts deployed to monitor as thoroughly as possible the effects of the ecosystem contamination. Again, in an effort to produce useful and intercomparable data, guidance should be provided on the parameters to monitor and on the way to acquire them (type of probes, periodicity of aliquot sampling…).

5.3. Data management

In all cases, mesocosm experiments generate a substantial amount of data. In this context, the key to usefulness is a sound data management (Marchese-Robinson et al., 2016) and a data filtering/reduction strategy. Indeed, monitoring obeys some imperatives (e.g. continuous temperature or pH readings to detect anomalies) that become less pressing once the experiment is completed., i.e. a large amount of data generated solely for surveillance purposes can be discarded at the end of the experiment, but their statistical relevance needs to be kept on record. A step in this direction are the data recording templates developed within several international programs (e.g. US CEINT, FP7 Nanoreg, H2020 NanoFase) (Fadeel et al., 2018). These data are meant to be fed into databases (NIKC, eNanoMapper, NanoCommons) to enable a sound and inter-operable knowledge base, (Karcher et al., 2018) which, given the extraordinary versatility of mesocosm testing, has the eventual objective of providing a comprehensive basis for environmental risk assessment.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This work has received funding from Excellence Initiative of Aix - Marseille University - A*MIDEX, a French “Investissements d'Avenir” program, through its associated Labex SERENADE project. This work is also a contribution to the OSU-Institut Pythéas.

References

Asbach, C., Alexander, C., Clavaguera, S., Dahmann, D., Dozol, H., Faure, B., Fierz, M., Fontana, L., Iavicoli, I., Kaminski, H., MacCalman, L., Meyer-Plath, A., Simonow, B., van Tongeren, M., Todea, A.M., 2017. Review of measurement techniques and methods for assessing personal exposure to airborne nanomaterials in workplaces. Sci. Total Environ. 603, 793–806.

Auffan, M., Mouneyrac, C., Rose, J., Fito, C., Chaurand, P., Masion, A., Perrein, H., Châtel, A., Bruneau, M., 2016. Development of an aquatic Mesocosms Platform allowing the evaluation of kinetics of aggregation. In: Nanoreg Public Deliverable D3.05, vol.. https://www.rivm.nl/en/About_RIVM/Mission_and_strategy/International_Affairs/International_Projects/Completed/NANoREG/deliverables:s_ezJOgJTEaL0lCqO1cAJQ/NANoREG_D3_05_DR_Development_of_an_aquatic_Mesocosms_Platform.org.

Auffan, M., Tella, M., Santaella, C., Brousset, L., Pailles, C., Barakat, M., Espinasse, B., Artells, E., Issartel, J., Masion, A., Rose, J., Wiesner, M.R., Achouak, W., Thiery, A., Bottero, J.-Y., 2014. An adaptable mesocosm platform for performing integrated assessments of nanomaterial risk in complex environmental systems. Sci. Rep. 4 (5608).

Buffet, P.E., Richard, M., Caupos, F., Vergnoux, A., Perrein-Ettajani, H., Luna-Acosta, A., Akcha, F., Amiard, J.C., Amiard-Triquet, C., Guibbolini, M., Risso-De, Faverney C., Thomas-Guyon, H., Reip, P., Dybowska, A., Berhanu, D., Valsami-Jones, E., Mouneyrac, C., 2013. A mesocosm study of fate and effects of CuO nanoparticles on endobenthic species (*Scrobicularia plana*, *Hediste diversicolor*). Environ. Sci. Technol. 47 (3), 1620–1628.

Embry, M.R., Bachman, A.N., Bell, D.R., Boobis, A.R., Cohen, S.M., Dellarco, M., Dewhurst, I.C., Doerrer, N.G., Hines, R.N., Moretto, A., Pastoor, T.P., Phillips, R.D., Rowlands, J.C., Tanir, J.Y., Wolf, D.C., Doe, J.E., 2014. Risk assessment in the 21st century: roadmap and matrix. Crit. Rev. Toxicol. 44, 6–16.

Fadeel, B., Farcal, L., Hardy, B., Vazquez-Campos, S., Hristozov, D., Marcomini, A., Lynch, I., Valsami-Jones, E., Alenius, H., Savolainen, K., 2018. Advanced tools for the safety assessment of nanomaterials. Nat. Nanotechnol. 13 (7), 537–543.

Ferry, J.L., Craig, P., Hexel, C., Sisco, P., Frey, R., Pennington, P.L., Fulton, M.H., Scott, I.G., Decho, A.W., Kashiwada, S., Murphy, C.J., Shaw, T.J., 2009. Transfer of gold nanoparticles from the water column to the estuarine food web. Nat. Nanotechnol. 4, 441.

Giese, B., Klaessig, F., Park, B., Kaegi, R., Steinfeldt, M., Wigger, H., von Gleich, A., Gottschalk, F., 2018. Risks, release and concentrations of engineered nanomaterial in the environment. Sci. Rep. 8.

Gondikas, A., von der Kammer, F., Kaegi, R., Borovinskaya, O., Neubauer, E., Navratilova, J., Praetorius, A., Cornelis, G., Hofmann, T., 2018. Where is the nano? Analytical approaches for the detection and quantification of TiO_2 engineered nanoparticles in surface waters. Environ. Sci. Nano 5 (2), 313–326.

Gondikas, A.P., von der Kammer, F., Reed, R.B., Wagner, S., Ranville, J.F., Hofmann, T., 2014. Release of TiO_2 nanoparticles from sunscreens into surface waters: a one-year survey at the old Danube Recreational Lake. Environ. Sci. Technol. 48 (10), 5415–5422.

Gottschalk, F., Lassen, C., Kjoelholt, J., Christensen, F., Nowack, B., 2015. Modeling flows and concentrations of nine engineered nanomaterials in the Danish environment. Int. J. Environ. Res. Public Health 12 (5), 5581–5602.

Handy, R.D., Cornelis, G., Fernandes, T., Tsyusko, O., Decho, A., Sabo-Attwood, T., Metcalfe, C., Steevens, J.A., Klaine, S.J., Koelmans, A.A., Horne, N., 2012. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ. Toxicol. Chem. 31 (1), 15–31.

Hendren, C.O., Lowry, G.V., Unrine, J.M., Wiesner, M.R., 2015. A functional assay-based strategy for nanomaterial risk forecasting. Sci. Total Environ. 536, 1029–1037.

Holden, P.A., Gardea-Torresdey, J.L., Klaessig, F., Turco, R.F., Mortimer, M., Hund-Rinke, K., Hubal, E.A.C., Avery, D., Barcelo, D., Behra, R., Cohen, Y., Deydier-Stephan, L., Ferguson, P.L., Fernandes, T.F., Harthorn, B.H., Henderson, W.M., Hoke, R.A., Hristozov, D., Johnston, J.M., Kane, A.B., Kapustka, L., Keller, A.A., Lenihan, H.S., Lovell, W., Murphy, C.J., Nisbet, R.M., Petersen, E.J., Salinas, E.R., Scheringer, M., Sharma, M., Speed, D.E., Sultan, Y., Westerhoff, P., White, J.C., Wiesner, M.R., Wong, E.M., Xing, B.S., Horan, M.S., Godwin, H.A., Nel, A.E., 2016. Considerations of environmentally relevant test conditions for improved evaluation of ecological hazards of engineered nanomaterials. Environ. Sci. Technol. 50 (12), 6124–6145.

Iavicoli, I., Fontana, L., Pingue, P., Todea, A.M., Asbach, C., 2018. Assessment of occupational exposure to engineered nanomaterials in research laboratories using personal monitors. Sci. Total Environ. 627, 689–702.

Karcher, S., Willighagen, E.L., Rumble, J., Ehrhart, F., Evelo, C.T., Fritts, M., Gaheen, S., Harper, S.L., Hoover, M.D., Jeliazkova, N., Lewinski, N., Marchese-Robinson, R.L., Mills, K.C., Mustad, A.P., Thomas, D.G., Tsiliki, G., Hendren, C.O., 2018. Integration among databases and data sets to support productive nanotechnology: challenges and recommendations. Nanoimpact 9, 85–101.

Kuhlbusch, T.A.J., Asbach, C., Fissan, H., Göhler, D., Stintz, M., 2011. Nanoparticle exposure at nanotechnology workplaces: a review. Part. Fibre Toxicol. 8, 22.

Kuhlbusch, T.A.J., Wijnhoven, S.W.P., Haase, A., 2018. Nanomaterial exposures for worker, consumer and the general public. Nano 10, 11–25.

Lowry, G.V., Espinasse, B.P., Badireddy, A.R., Richardson, C.J., Reinsch, B.C., Bryant, L.D., Bone, A.J., Deonarine, A., Chae, S., Therezien, M., Colman, B.P., Hsu-Kim, H., Bernhardt, E.S., Matson, C.W., Wiesner, M.R., 2012. Long-term transformation and fate of manufactured Ag nanoparticles in a simulated large scale freshwater emergent wetland. Environ. Sci. Technol. 46 (13), 7027–7036.

Marchese-Robinson, R.L., Lynch, I., Peijnenburg, W., Rumble, J., Klaessig, F., Marquardt, C., Rauscher, H., Puzyn, T., Purian, R., Aberg, C., Karcher, S., Vriens, H., Hoet, P., Hoover, M.D., Hendren, C.O., Harper, S.L., 2016. How should the completeness and quality of curated nanomaterial data be evaluated? Nanoscale 8 (19), 9919–9943.

OECD, 2006. Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms).

OECD, 2014. Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. OECD Publishing, Paris.

Petersen, E.J., Diamond, S.A., Kennedy, A.J., Goss, G.G., Ho, K., Lead, J., Hanna, S.K., Hartmann, N.B., Hund-Rinke, K., Mader, B., Manier, N., Pandard, P., Salinas, E.R., Sayre, P., 2015. Adapting OECD aquatic toxicity tests for use with manufactured nanomaterials: key issues and consensus recommendations. Environ. Sci. Technol. 49 (16), 9532–9547.

Sun, T.Y., Bornhoft, N.A., Hungerbuhler, K., Nowack, B., 2016. Dynamic probabilistic modeling of environmental emissions of engineered nanomaterials. Environ. Sci. Technol. 50 (9), 4701–4711.

Tella, M., Auffan, M., Brousset, L., Issartel, J., Kieffer, I., Pailles, C., Morel, E., Santaella, C., Angeletti, B., Artells, E., Rose, J., Thiery, A., Bottero, J.-Y., 2014. Transfer, transformation, and impacts of ceria nanomaterials in aquatic Mesocosms simulating a pond ecosystem. Environ. Sci. Technol. 48 (16), 9004–9013.

Tolaymat, T., El Badawy, A., Sequeira, R., Genaidy, A., 2015. An integrated science-based methodology to assess potential risks and implications of engineered nanomaterials. J. Hazard. Mater. 298, 270–281.

von der Kammer, F., Ferguson, P.L., Holden, P.A., Masion, A., Rogers, K.R., Klaine, S.J., Koelmans, A.A., Horne, N., Unrine, J.M., 2012. Analysis of engineered nanomaterials in complex matrices (environment and biota): general considerations and conceptual case studies. Environ. Toxicol. Chem. 31 (1), 32–49.