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# Assessing human health and environmental hazards of nanomaterials- Best practice for REACH Registrants

**Second GAARN meeting**
**Helsinki, 21-22 January 2013**

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**Best practices – Second GAARN meeting**

**Reference:** ECHA-13-R-04-EN
**Publ.date:** 19 April 2013
**Language:** EN



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# 1. Objectives

The purpose of the Group Assessing Already Registered Nanomaterials (GAARN) is to build a consensus in an informal setting on *best practices* in assessing and managing the safety of nanomaterials under the REACH Regulation, and thereby increase confidence and mutual understanding among stakeholders so that nanomaterials can be sustainably developed.

The GAARN group consists of several experts from Member States, the European Commission, ECHA and industry. The group has selected three registration dossiers that include nanoforms or nanomaterials, and aims to review and exchange views on how these registration dossiers meet the REACH information requirements in the areas of physicochemical properties and substance identity (SID), human health and environmental hazards, and exposure and risk assessment, specifically for these nanoforms. GAARN aims to discuss best practices for each selected registered nanomaterial and to develop recommendations on how to fill potential information gaps. The GAARN initiative foresees three meetings to discuss the above-mentioned points.

This report summarises the outcomes of the second GAARN meeting. This meeting was held in Helsinki from 21 to 22 January 2013 and focused on discussing the approach and challenges faced by participant registrants when assessing the human health and environmental hazards of their substances while registering them under REACH. The outcomes of this discussion can be viewed as generic recommendations for the hazard assessment of nanomaterials under REACH, while considering the present scientific knowledge on the field of nanotoxicology and practices, as well as challenges from participating registrants.

# 2. Summary

Before the meeting, ECHA and the participating lead registrants (LRs) for the three selected registered substances exchanged a number of questions based on the information provided in their registration dossiers for the hazard endpoints. Only two of the three LRs sent questions to the Agency. The aim of this exchange of questions was to offer a basis for discussion at the meeting so that both parties (ECHA and the LRs) could be aware of their concerns and limitations related to assessing the hazards of nanoforms, and to focus the discussion on how nanoforms have been addressed in the respective dossiers.

The GAARN plenary sessions included presentations by the three LR representatives, followed by ECHA's responses to the questions received from the corresponding LRs.

# 3. Best practices

## 3.1 General considerations

### 3.1.1 Use of non-testing data

The use of non-testing data, such as data generated by read-across, is supported for nanomaterials as for any other substance. When considering reading across to another nanoform or a counterpart bulk material, a solid scientific justification should be provided in the IUCLID dossier of the registered substance. It is insufficient to justify the use of data for read-across based only on the chemical composition of a nanomaterial, and further physicochemical parameters such as aspect ratio, shape, form, solubility, surface area, charge, surface treatment etc. should provide a reliable dataset to support a sound scientific interpretation of the similarities or differences among (nano)forms.

A basis for grouping the nanoforms/nanomaterials of interest (in terms of their similarity) should be established using the similarity rules specified in Annex XI of the

REACH Regulation. The hypothesis, or basis for the grouping, should be used to define what characteristics a nanoform/nanomaterial should have in order to belong to a category. The similarity rules (which could also be called criteria or principles) might be used individually and are case-dependent. However, a category (and similarity) may be justified on more than one basis, as multiple justifications usually increase the confidence in the category. The hypothesis will help to show if the grouping applies to the category members for either environmental or toxicological endpoints or both, and if it is adequate for all routes of exposure and duration of effects (Practical Guide 6, ECHA 2009).

### 3.1.2 *In vitro* testing

Alternative methods such as *in vitro* methods can be relevant for hazard identification, and despite their current limitations, can be useful as a supportive tool for *in vivo* testing. However, when directly using the results obtained from *in vitro* methods for hazard assessment, many of the tests may need adaptation before they are applied (e.g. appropriate sample preparation needs to be performed and adequate controls defined to check possible interferences).

### 3.1.3 Reliability and use of existing data

Peer-reviewed scientific studies should be considered, and included in the specific endpoint section of the IUCLID dossier, for assessing the hazards of a registered nanoform and characterising its toxicological profile as comprehensively as possible. In addition, the available data from emerging peer-reviewed publications can be considered to build multiple lines of evidence as requested when reporting a weight of evidence approach in a selected IUCLID endpoint (Annex XI). For example, a number of (eco)toxicological tests on nanomaterials may have been reported in peer-reviewed academic journals.

Compared to older published hazard data, recent scientific articles include a more detailed description and characterisation of the physicochemical properties of the nanoforms investigated, as well as the physicochemical properties on the biological media used for the studies (e.g. primary particle size, aggregation/agglomeration, half-times, shape, cristalinity, surface area, charge, surface treatment, solubility etc.).

Regardless of the year of publication, it is essential that sufficient and unambiguous information on the physicochemical properties of the nanoform are reported in the peer-reviewed studies to make them useful for registration purposes under REACH. The methodology used for sample preparation and dosimetry of exposure systems should also be well-defined and reported in the specific endpoint section to allow an adequate use and interpretation of the data presented. Extensive literature reviews also provide a good basis for determining the relevance of future *in vivo* studies and should be included where relevant in the REACH registration dossiers.

### 3.1.4 Surface treated nanomaterials

The REACH registration dossier should report information on the surface treatment of nanomaterials. Registrants are encouraged to provide physicochemical information on the hazard properties of each form if the scope of the registration dossier aims to cover these different nanoforms. Information on the coating of nanomaterials is essential as surface modifications may affect the toxicokinetics of nanomaterials. Therefore, coated and uncoated nanomaterials should have separate IUCLID endpoint study records for

the different hazard endpoints. If an adaptation to the REACH information requirements is used, the registrant should ensure that it meets the requirements in Annex XI.

## 3.2. Specific considerations

### 3.2.1 Bioavailability-toxicokinetics

The use of toxicokinetic data is encouraged for grouping substances in relation to read-across. The mechanisms leading to toxic effects of a substance might be better understood with supporting data on the physicochemical properties. However, non-toxic effects cannot be explained only on the basis of physicochemical properties, thus adequate and supportive data on toxicokinetics are crucial. Moreover, use of toxicokinetic data can also be useful when extrapolating from *in vitro* to *in vivo* situations. Where there is evidence of a systemic translocation of nanoparticles, further investigations on absorption, distribution, metabolism and excretion parameters should take special consideration. If data on toxicokinetics are available, it should also be considered for determining the testing strategies for environmental endpoints, as results from mammalian studies produce valuable information for non-mammalian tests designs.

### 3.2.2 Bacterial mutation assays

Bacterial mutation assays should not be used as a single test for nano(particle) mutagenicity but should be used in conjunction with a range of mammalian cell gene mutation tests. The Ames test may not allow a robust evaluation of nano(particle) mutagenicity given that, unlike mammalian cells, bacterial cells lack the uptake of particles via endocytosis (Doak et al., 2012)

### 3.2.3 Sample preparation

It is generally recommended that registrants provide a detailed description of the sample preparation for (eco)toxicological assays in the relevant hazard endpoints of the IUCLID dossier, even if this goes beyond the information required in the standard OECD guidelines. The OECD guidance on sample preparation and dosimetry (OECD, 2012) does not aim to be conclusive due to the diversity of types of nanomaterials. Guidance for nanomaterial testing and characterisation will be further developed as the field advances and more experience is gained.

### 3.2.4 Environmental parameters

Environmental parameters, such as dissolved organic material (DOM and its detailed composition – humic and fulvic acids), ionic strength, pH, etc. play an important role in stabilising nanomaterials, and can thus affect their bioavailability. Most laboratory studies do not take into account the effect of such parameters during the experimental design. Nevertheless, this is not only relevant for nanoforms, as the bioavailability and thus hazard assessment of other chemical substances (e.g. metal oxides) is influenced by many of the above-mentioned parameters. In the best scenario, prior work investigating the effects of these conditions on the stability and behaviour of nanoforms could be used to help select the most adequate experimental design.

### 3.2.5 Dispersing agents

The use of dispersing agents should be avoided for sample preparation for testing purposes. If the use of a dispersing agent is unavoidable to stabilise the dispersion, information on the concentration used and structural formula of the substance has to be provided in the relevant hazard endpoints of the IUCLID dossier. Given that the use of dispersing agents may change the behaviour, fate and bioavailability of the nanomaterial, appropriate controls should be documented in the study report, and a careful interpretation of the test results should be undertaken with special attention given to the potential interaction of dispersing agent.

### 3.2.6 Solubility and dispersion

For *in vivo* and *in vitro* (eco)toxicological studies, organisms and cells should be exposed or dosed with a test medium containing dispersed nanomaterials. Therefore, any toxicity tests using *in vivo* and *in vitro* methods should pay special attention to the agglomeration/aggregation behaviour, and the insoluble/partially-soluble nature of nanomaterials. Solubility studies are relevant for investigating the nano-effect and providing mass comparisons, and should be conducted simulating the test exposure conditions. If such studies are conducted for specific tests, the results should be reported at the study endpoints of the IUCLID dossier. A number of techniques have been used to determine dissolution of nanoforms over time, and these include dialysis, centrifugation and ultracentrifugation, among others. The results of these dissolution experiments offer important supportive information in REACH registration dossiers as they help to understand the overall behaviour of the particles in the test media.

### 3.2.7 Test selection and design

The half-life of nanoforms in suspension is often dependent on the initial loading concentration, with higher concentrations leading to faster precipitation rates. Thus, knowledge on aggregation/sedimentation of the nanoforms in a given medium is relevant for adequate test designs (e.g. flow through, semi-static etc.). The selection of the initial loading concentration should be carefully considered in order to get the best dose-response relationships.

High concentrations of nanoforms may impair the swimming ability of small invertebrates (e.g. daphnids). Testing at these high concentrations should be avoided as this type of physical impairment would not reflect the hazardous properties of the substance. For ecotoxicological endpoints, long-term studies are highly recommended for substances that show low toxicity in acute studies, as the experimental design and lower initial loading rates for sub-chronic studies will help to overcome problems of high agglomeration and sedimentation. Moreover, most hazard assessments derived from available toxicological data from published peer-reviewed studies relate to short-term studies, whereas long-term studies are scarce. Thus, given that the mode of action of nanoforms is yet to be properly characterised, carefully designed long-term studies might be of more relevance for an appropriate hazard identification.

### 3.2.8 Relevant endpoints for ecotoxicity testing

The R.7 ECHA Guidance was recently updated with appendices containing recommendations for nanomaterials based on the work conducted in the scientific community as well as in international organisations (compiled in the RIP oNs 2-3). These recommendations offer advice to registrants on how to identify potential hazards based on the latest scientific developments in the field of nanotoxicology. In principle, the standard biological endpoints used in regulatory hazard assessment remain

appropriate for nanomaterials in terms of supporting data for environmental risk assessment. Moreover, when considering testing data on aquatic pelagic toxicity, provision of data on a number of parameters is recommended: such as fish ventilation rate, gill pathologies, fish mucus secretion, fish brain pathology and enzyme activity (catalase, superoxide dismutase), as further described in the ECHA Guidance Appendix to R.7b.

### 3.2.8 Detection in the solid matrix/porous media

As indicated in the ECHA Guidance Appendix to R.7b, characterisation and concentrations of nanomaterials should be monitored before and if possible during and/or at the end of the test. Detecting and quantifying nanomaterials from porous media (e.g. soil or sediments) is challenging, particularly for those nanomaterials made of chemical constituents that are highly abundant in the natural environment (e.g. many metals and metal oxide nanomaterials, carbon materials etc.). Current scientific techniques address this challenge through labelling of the nanomaterial (e.g. isotopic labelling). Nevertheless, this remains a costly approach. In view of this, it is recommended that well-characterised nanomaterials are delivered to soil and sediment systems in the form of water-based dispersions or mixed as dry material. If the nanomaterial is introduced and homogenised directly in solid or sediment media, care should be taken during homogenisation so that the test material is not unintentionally damaged.

## 4. Conclusions

The Commission, MSCAs and ECHA indicated the importance for the registrant to describe the scope of the registration dossier, in line with the current nanomaterial definition (2011/696/EU). The IUCLID dossier should include a detailed physicochemical description of the substance registered, including any additive/capping agent used following the manufacturing process.

The provisions that apply to the registration of nanomaterials under REACH are the same that need to be fulfilled for any other chemical substance. However, in line with scientific developments, there are specific considerations that the registrant should report in specific endpoint sections, as this information will facilitate the evaluation of the adequacy of the tests performed and data obtained with regard to the safety assessment of nanomaterials (e.g. sample preparation, solubility/dispersion, use of stabilisers etc.).

The registration dossier should contain a comprehensive physicochemical characterisation of the registered nanoform(s) (First GAARN meeting best practices report). Only when well-characterised nanoforms are reported in the dossier, can a read-across approach or use of existing data (e.g. weight of evidence) be considered for the purpose of hazard assessment. Generating data on toxicokinetics might also be considered for grouping substances in relation to read-across approaches, or extrapolating from *in vitro* to *in vivo* situations.

The majority of standard biological endpoints used in regulatory hazard assessment remain appropriate for nanomaterials in the context of supporting data for environmental risk assessment. However, as steady-state systems essential for ecotoxicology testing are difficult to attain when testing nanoforms, changes on sample preparation and dosimetry have been foreseen for most of the tests (OECD, 2012). Parameters such as particle solubility and stability in the test media are essential parameters, among others, to be reported for (eco)toxicological studies, as the information obtained is necessary for exposure considerations. Indeed, mass comparisons of concentrations nanomaterials versus the concentration of the chemical fraction dissolving from the nanomaterials are needed to understand the source of the hazardous effects reported. Therefore, as indicated in the ECHA Guidance Appendix to R.7 a-c, REACH Implementation Projects (2-3), and Guidance on Sample

Preparation and dosimetry for the safety testing of manufactured nanomaterials (OECD, 2012), this information should be reported in the robust study sections of the relevant hazard endpoints.

The lack of short-term toxicity should encourage the registrant to investigate the potential sublethal and long-term effects, as these studies might be of better relevance for an appropriate hazard identification, given the unknown specific mode of action of most nanomaterials, widespread exposure considerations, and difficulties on sample preparation and dosimetry of high concentrated exposure suspensions.

# 5. References

Guidance on information requirements and chemical safety assessment
Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance
http://echa.europa.eu/documents/10162/13632/appendix_r7a_nanomaterials_en.pdf

Guidance on information requirements and chemical safety assessment
Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance
http://echa.europa.eu/documents/10162/13632/appendix_r7b_nanomaterials_en.pdf

Guidance on information requirements and chemical safety assessment
Appendix R7-2 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance
http://echa.europa.eu/documents/10162/13632/appendix_r7c_nanomaterials_en.pdf

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