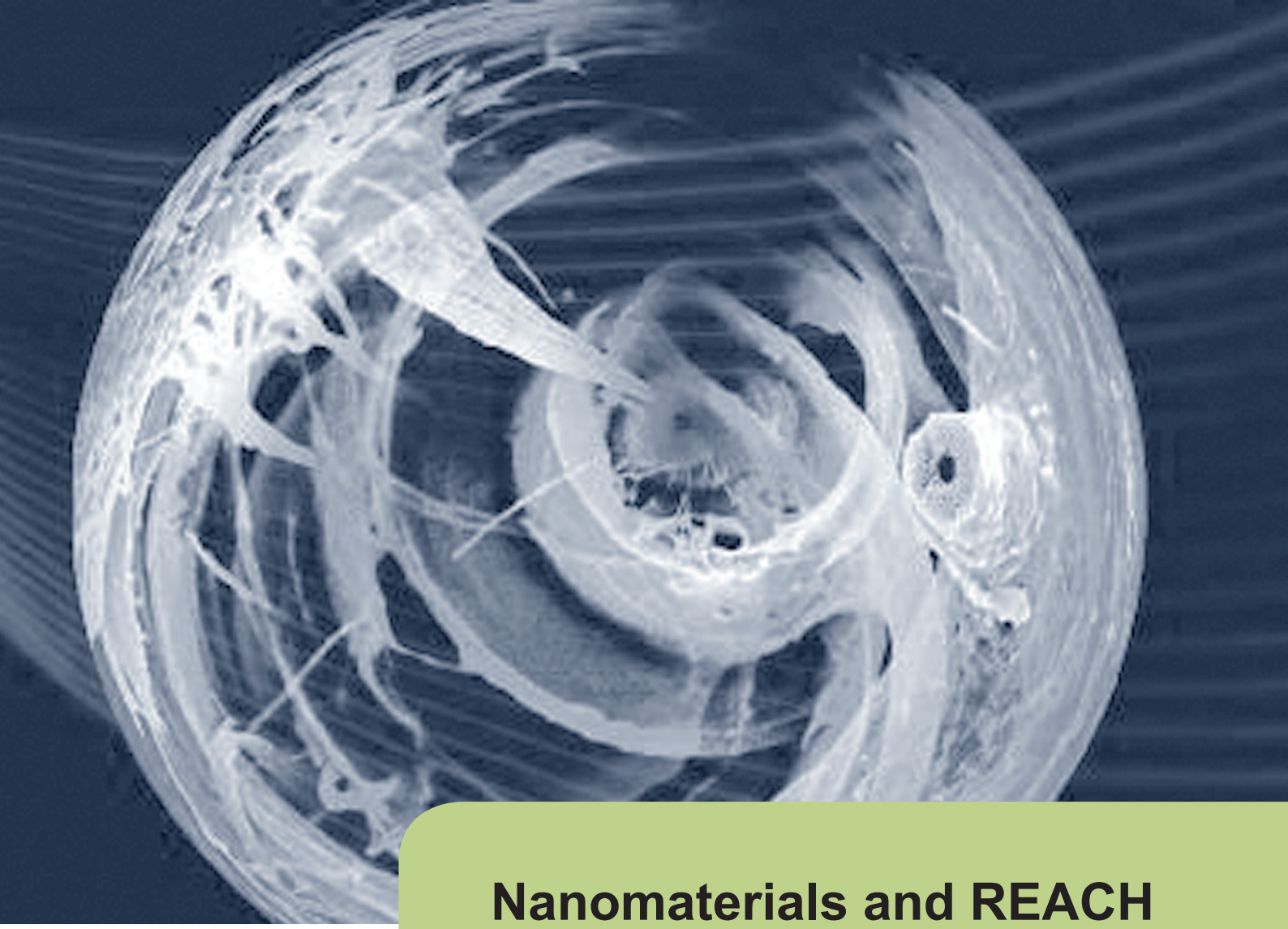


# Nanomaterials and REACH

## Background Paper on the Position of German Competent Authorities

Umwelt Bundes Amt
Für Mensch und Umwelt

BfR
Bundesinstitut für Risikobewertung

baua:
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

# Nanomaterials and REACH – Background Paper on the Position of German Competent Authorities

**Contents**

## Nanomaterials and REACH



## Appendices:

# Nanomaterials and REACH

## 1 Introduction

The present background paper reflects the position of the German federal authorities on the regulation of nanomaterials (NMs) under REACH. It is intended as a basis for preparing decision-making routes for political processes responses to from outsiders (e.g. Bundestag deputies or NGOs). With respect to the imminent negotiations on the regulation of NMs under REACH in the EU it is intended to explain and justify the position of the german competent authorities. This paper also deals with the regulatory need for ultrafine fibres and particles.
If required the document will be adapted to fit the current discussions and knowledge.

## 2 Recommendation on the definition of nanomaterials (NMs)

The recommendation of the European Commission[1] on the definition encompasses natural, incidental or manufactured NMs, including their aggregates and agglomerates with at least 50% of the number-based primary particle size distribution being within the range of 1 - 100 nm. The definition opens up the possibility of using a threshold of between 1 % and 50 % for the number size distribution in statutory regulations if this is justified by environmental, health, safety or competitive considerations.
In deviation from the definition a number of explicitly listed materials (fullerenes, graphene flakes and single-wall carbon nanotubes – SWCNT) whose dimensions are < 1 nm count as NMs. In addition it may be specified in regulations that materials with a volume-based specific surface area of > 60 $m^2/cm^3$ are regarded as NMs.
Furthermore the recommendation – and in particular the limit for the number size distribution – is to be reviewed by December 2014 to establish whether it should be modified in the light of experience accumulated and scientific and technical development.
The proposed definition is welcomed. There are, however, problems regarding the feasibility of the definition in that to date there have been no generally recognised, standardised methods for determining the necessary parameters; this could, for example, lead to problems in enforcement. The BAuA is collaborating with the University of Magdeburg on an automated image recognition and counting procedure for primary particles in workplace samples, and it is intended that a pilot version be available by 2014. If this is successful, this procedure will be suitable for use in combination with devices for determining the dustiness of solids, e.g. the BAuA Shaker procedure[2], for enforcing the above definition, and it can be passed on for standardisation.

[1] COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterials (2011/696/EU) (OJ EU No. L 275 p. 38 of 20.10.2011)
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:DE:PDF

[2] Kuhlbusch et al. Particle and Fibre Toxicology 2011, 8:22,
http://www.particleandfibretoxicology.com/content/8/1/22

## 3 Need for regulation in REACH

### 3.1 Nanomaterials within the meaning of the definition recommendation

The manufacture, import and use of substances are regulated by the European Chemicals Regulation REACH. This regulation is based on the precautionary principle.

NMs are a challenge for chemicals regulation. Even if they, as substances, basically fall under REACH, the existing regulations are not adequate to deal with their specific features. There are a number of those features which have to be considered when it comes to regulation. For NMs surface characteristics probably play a greater role than the volume characteristics of the materials; in addition quantum physical and quantum chemical effects must be taken into account, especially in the lower nanometer range. Their potential to cause damage is due to the fact that they may behave very differently from bulk materials because of their small size and their properties. It is therefore necessary to assume that they can also cause special toxicological and ecotoxicological effects. These effects may also vary considerably within the nano range, depending on certain parameters. For this reason a large number of chemically identical NMs may be allocated to one bulk material in individual cases (see below, chapter 5).

In general, NMs are not regarded as separate substances within the meaning of the current REACH rules, but as substances in a certain form (see below, chapter 4). That is why nanoforms for which a related bulk form exists are registered together with this.
It is generally recognised that REACH in its conception, its tools and methods (testing forhazard assessment, risk estimation and risk management measures) provides the suitable framework of the safe handling of substances in nanoform. Furthermore many experts consider that the testing requirements, test strategies and test methods under REACH to be in principle applicable to nano-scale substances, if subjected to methodological adaptations. The scientific basis for these adaptations, e.g. of testing methods and the Technical Guidance Document for Risk Evaluation, are drawn up by OECD[3,4] and by others.

There is however a lack of clear specifications regarding data requirements and documentation within the registration dossier. In these items the REACH Regulation must be adapted and extended.
Some stakeholders are of the opinion, however, that a change in the existing laws is not necessary. They believe that REACH already covers nano-scale substances adequately in that the use of all substances must be safe. However, a major aspect is not taken into account here: in REACH there is no trigger which renders the notion of the Regulation statutorily mandatory to the full extent for nano-scale substances as well, taking account of nano-specific characteristics.

In the spirit of legal clarity, equality of treatment and fulfilment of the precautionary principle it is essential to clearly lay down the requirements for NMs in REACH. The instruments of the REACH Regulation (dossier evaluation, substance evaluation, authorisation, restriction, safety data sheet etc.) must permit a specific treatment of NMs.
Within the framework of a regulation to be created for NMs it is necessary in particular to clarify what special testing obligations are required for NMs, what tonnage thresholds are to apply for NMs and how surface-treated NMs are to be regarded.

[3] OECD No. 14 – ENV/JM/MONO(2009)20 Guidance Manual for the Testing of Manufactured Nanomaterials: OECD‘s Sponsorship Programme.

[4] OECD No. 15 – ENV/JM/MONO(2009)21 Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials.

### 3.2 Fine particles and fibres

Many forms in which NMs occur exhibit morphological similarities with materials already known and examined, especially fibrous ones. In science consideration is therefore often given to transferring knowledge gained from the field of fibre toxicology to nanotoxicology. Beyond the „nano-specific“ concern mentioned under 3.1, risks for human health and the environment are discussed in this context, which are tied to the feature of particle release, but not necessarily to the size dimensions given in the definition of NMs (< 100 nm).
This includes

1. fibrous materials which may release respirable, bio-resistant fibre particles. According to international convention[5] the term „respirable“ is used to describe so-called WHO fibres which have a diameter of less than 3 µm, a length greater than 5 µm and a length-to-diameter ratio („aspect ratio“) of greater than 3.
2. Materials which may release respirable, bio-resistant granular particles. The term „respirable“ is used to describe particles which meet the convention for the separation characteristic according to EN 481. With an aerodynamic particle diameter of 4.0 µm, 50.0 % of all airborne substances with this particle diameter are separated (equivalent to 55.9 % of the inhalable fraction).
3. Materials which may release explosive particles. According to EN 14034-1 these are normally particles with particle diameters below 500 µm which react exothermally with air on ignition.

These materials also give rise to specific information and testing requirements which have not yet been similarly described in REACH to the necessary extent. The morphological characterisation needed to identify NMs should also apply to fine particles and fibres in order to fill these regulatory gaps and to avoid taking innovations in a direction which is incorrect and, in regulatory terms, avoidable, e.g. in the case of fibrous NMs in the direction of fibres with thicknesses greater than 100 nm. The regulations to protect against risks due to the particles mentioned can currently be found mainly in the non-harmonised regulations governing occupational safety and health (Art. 153 TFEU). The provisions governing the classification of asbestos, mineral wool and ceramic fibres in the CLP Regulation and existing standards on the assessment of the dustiness of solids offer the necessary starting point for a future regulation for the legally binding and implementable identification of corresponding materials under REACH. The standardisation and validation of these procedures are therefore a central component of the German contribution to the call „Regulatory Testing of Nanomaterials“ in the 7$^{th}$ EU Research Framework Programme. With regard to possible toxicological testing requirements for fibrous materials reference can be made to the test for bio-solubility specified in the Technical Rules for Hazardous Substances (TRGS 905) (see Appendix IV). Other necessary testing requirements in this context are also outlined in this appendix.

[5] Justifications of the Committee on Hazardous Substances concerning the evaluation of substances, activities and procedures as carcinogenic, mutagenic or reprotoxic – Inorganic fibres under
http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/TRGS/Begruendungen-905-906.html

## 4 Bulk material and nano

On the EU level there has been a discussion for some time on whether size, shape and design of a nano material can be regarded as a so-called *‚identifier'* or *‚characteriser'*. The difference between the two terms is that, in the former case, the shape, size, design of a substance are regarded as criteria for its definition (*‚identifier'*), while in the latter case they (only) describe the characteristic features of the substance (*‚characteriser'*). Any stipulation of *‚identifier'* would result in the independent fulfilment of the substance term and hence an obligation to register the nanomaterial separately. The proposal to characterise a substance in its bulk or nanoform(s) together in one registration, on the other hand, follows the notion of regarding the form, size and shape as specific features of a manifestation of the substance (*‚characteriser'*).

Usually the substance identity for a well defined substance under REACH is defined solely by the molecular structure and chemical composition. Bulk- and nanomaterial are chemically identical.[6] This means that, for a substance which occurs both as a bulk material and on a nano scale, a joint registration dossier is required, in other words the *‚characteriser'* approach is adopted.

Even if Germany recognises that there are good reasons for treating the aforementioned features as *‚identifiers'* we see clear benefits in treating them as *‚characterisers'* (see Appendix I). However, as a general rule, for the nanoform and the bulkform there should, however, be different information requirements. The information requirements, chemical safety assessment, the Chemical Safety Report, the use conditions etc. must in each case take account of the bulk and nanoform(s) individually. Groupings of nanoforms are conceivable. In this respect there is, however, still a need for further clarification regarding the differentiation between nanoforms within a substance.

Compared with substances in bulk form, for which data is normally available from laboratory tests *in vitro* and *in vivo* and occasionally from case studies in occupational medicine and from epidemiological surveys, data on NMs is mostly scarce. This is even true for NMs which have been relatively well examined since for these studies characterisation of the sample materil is often inadequate or a completely lacking and a suitable preparation of the samples has rarely been conducted. As a result these studies cannot, or only to a limited extent, be compared with data for substances in bulk form and other NMs.

The information requirements under REACH therefore have to be adapted in order to cover possible hazards of NMs and to facilitate subsequent measures of risk reduction. The most important parameters which distinguish nanoforms of substances from the bulk form are morphological properties, water solubility and surface characteristics. This also applies to respirable granular and fibrous particles.

If the properties of a material differ from those of another in a relevant way additional tests may be necessary. This is one of the basic principles of REACH and applies both to conventional and for nanoscale substances. Information requirements which describe these additional tests must be added to the annexes of REACH. For NMs a separate Annex XVIII is proposed for this. The necessary information has to be submitted by the registrant and if tests are waived this must be justified scientifically.

[6] Exceptions are, for example, fullerenes and CNTs.

## 5 Difference between NMs

The differences between the individual nanoforms of a substance must be taken duly into account when defining the obligations regarding NMs. The diversity of the nanoforms of a substance may lead to different testing requirements.
The following parameters are considered to be important as criteria for defining different NMs and for characterising respirable granular and fibrous particles within a substance identity:

- Morphological characterisation:
  - size
  - crystalline structure
  - geometry/shape
  - rigidity
  - durability
- Water solubility
- Surface characteristics:
  - surface charge
  - hydrophobia
  - (photo-)catalytic properties
  - absorption/adsorption or binding of (certain) molecules
  - functional groups
  - agglomeration behaviour
  - volume-specific surface area (also for dry fine particles)

The data for these delimiting features are to be identified in a first step for all nanoforms and for all respirable granular and fibrous particles (> 100 kg/a) in order to subsequently decide whether different nanoforms of the same identity should /must be grouped for the test programme or considered separately through a screening. For example, different forms such as spheres, bars or fibres may lead to different testing requirements. Furthermore a low water solubility (< 100 mg/L) may serve as a trigger for a specific test programme. It still remains to be clarified how these screening parameters – individually or also in certain combinations – are used in detail to distinguish between different nanoforms and which circumstances would trigger a separate test programme for a nano material. A possible model is shown in Appendix II.

## 6 Tonnage quantities threshold

For NMs from 100 kg/a (total production or import quantity of all nanoforms of a substance) reduced registration requirements should be introduced[7]. These should comprise, in addition to details of the substance identity, a basic characterisation of the different nanoforms, as well as details of their uses.
At the same time consideration should be given to the introduction of a minor threshold. Furthermore all the data in the hands of the registrant with regard to the different nanoforms must be documented.

If, for a total quantity of all nanoforms of the substance from 100 kg/a, no single nanoform reaches 100 kg/a, a complete basic characterisation must be undertaken for at least one NM. For the other nanoforms a description of how they differ from this material must be given.

For NMs from 1 t/a (total quantity of all nanoforms of a substance) the data requirements of a new Annex XVIII to be implemented in REACH shall apply (see chapter 8). In addition a chemical safety assessment must be conducted for all nanoforms of the substance. These chemical safety assessments must be documented within one Chemical Safety Report (see chapter 9).

[7] Where a substance is being registered, the data for the simplified registration must be submitted within the framework of this registration

The data requirements must be fulfilled for all nanoforms according to the tonnage band of the total quantity of the respective nanoforms. If none of the nanoforms lies within the tonnage band of the total quantity of all nanoforms of the substance, the data requirement according to the tonnage band of the total quantity of all nanoforms of the substance must be fulfilled for the most relevant nanoform.[8] Criteria for this decision on relevance still have to be developed (e.g. quantitatively most significant form, functionally most important form, form intended with manufacture, form of probably greatest toxicological relevance, etc.).
This would mean, for example: if for a substance four different nanoforms with a total quantity of 200 t/a are available and of all nanoforms less than 100 t/a is manufactured in each case, the data requirements for 100 t/a according to the proposed Annex XVIII must be fulfilled for the most relevant form. The selection of the most relevant form has to be justified by the registrant. For the other three nanoforms the data requirements must be fulfilled according to the respective quantity (> 10 t/a) in accordance with the proposed Annex XVIII.

## 7 Waiving

There is a possibility of waiving tests if there is a justification for doing so. In column 2 of Annexes VII-X and in Annex XI the REACH Regulation provides for various possibilities for waving tests if there is a justification for this. Accordingly, relevant specification must also be laid down for the information requirements regarding NMs. Basically there are three conceivable possibilities for a waiver (see Appendix II):

1. Use of data by referencing between bulk and nanoform of a substance,
2. Use of date by referencing between different nanoforms of a substance,
3. Read-across between substances with different chemical identity (possibly various bulk and nanoforms), (Q)SAR.

The details for applying the above-mentioned waiving possibilities should be described in a REACH Guidance Document. The advantage is that this can be adapted to keep up with scientific progress with less effort than a statutory text.

However, fundamental criteria for delimiting different nanoforms in relation to one another should be included in the REACH Regulation itself. Adherence to these criteria might give rise to profound burdens for companies which require a legally binding basis and, under certain circumstances, may go beyond the scope of a guidance document.

The proposed test programme provides for specific tests on NMs.
On the basis of suitable data (of the bulk material or other nanoforms) and the provisions in the guidance document yet to be formulated it will be possible to waive tests on a case by case decision. At the present time this will rarely be possible since the testing methods and guidance documents for the testing of NMs have to be revised or drawn up.[9] To the extent that knowledge is gained on the toxicokinetics and systemic effects of NMs it will be possible to justify test waivers and the possibility to make use of it will increase.
Basically under Annex XI para. 3 a ii it is the case also for NM that the waiver of a 90-day test is inadmissible, even if there is a 28-day test.[10] Vice versa waiver of a 28-day test is also possible for NMs if there is a 90-day test. Subchronic or chronic toxicity studies (90 or 365 days) are absolutely essential for NMs in order to obtain the necessary knowledge of the systemic availability and toxicokinetics of

[8] The will avoid false incentives to artificially separate into different nanoforms and it ensures that there is a basis of data in relation to the nanoforms of the substance.

[9] OECD WPMN SG4, Working Objectives for 2012: NM in TG403, TG412, TG413 and TG436

various NMs, since nanoparticles (< 100 nm), in contrast to larger particles (300 nm), might accumulate in tissues, act there in an organotoxic way and induce inflammatory reactions.[11,12]

In individual cases it may be possible to waive tests if the bulk material is classified in the highest category and this classification is also applied to the NMs. A general waiving by applying the maximum classification is not possible since sufficient (quantitative) data must be available for a risk assessment of NMs.
Waiving is not possible on the basis of tests conducted on the bulk material which have resulted in a non-classification. It must be assumed that the systemic distribution as well as water solubility of the NM and the bulk material may deviate leading to different and/or more severe adverse effects of the NM.

One particular challenge is the question of handling surface-treated NMs. A possible approach here is given under chapter 10 and in Appendix VI.

## 8 Test programme pursuant to Annex XVIII REACH Regulation

On the basis of the standard data requirements of Annexes VII-X of the REACH Regulation, Annex XVIII describes the quantity-dependent, specific data requirements for NMs (see Appendix VII).

**Physicochemical data:**
The testing requirements are shown in Appendix III. They apply to nanomaterials covered by the definition of the Commission and for all respirable granular and fibrous particles (see chapter 3.2).The requirements may have to be developed further.

**Toxicological data (see Appendix IV):**

- From 1 t/a the standard data requirements pursuant to Annex VII of the REACH Regulation apply with the following adaptations: acute toxicity testing of NMs has to be performed by the inhalation route instead of the oral route usually taken. Beyond the standard test for *in vitro* genotoxicity on bacteria provided for in Annex VII, two tests for genotoxicity with mammalian cells *in vitro* are required in addition. These tests are specified for bulk materials in the next tonnage band (Annex VIII).

- From 10 t/a a 28-day study is foreseen for NMs as for bulk materials according to Annex VIII of the REACH Regulation. This study has to be conducted by the inhalation route. As a deviation from the remarks of OECD TG 412 and the Test Method Regulation B.8 an exposure-free follow-up phase of 28 days and additional examination parameters are necessary for NMs.
  As in Annex VIII of the REACH Regulation, a second test for acute toxicity is necessary. For NMs this test shall normally be conducted by oral administration (instead of inhalation for bulk material).

[10] Regulation (EC) No. 134/2009 of the Commission of 16 February 2009 to amend Regulation (EC) No. 1907/2006 of the European Parliament and the Council on the registration, evaluation, authorisation and restriction of chemical substances (REACH) with respect to Annex XI

[11] Silver nanoparticles (100 nm) circulating in the blood pass the blood-brain barrier and become enriched in the brain, lung, liver and spleen. The activity of *natural killer cells* is severely reduced at 100 nm and 20 nm particle release and indicates immunotoxicity. De Jong 2012, Conference on Nano-Silver February 9th 2012,
http://www.bfr.bund.de/cm/349/toxicokinetics-and-toxicity-of-nanosilver.pdf

[12] Silver particles of 22, 42 and 71nm in size were detected after being administered orally (14d) in the brain, lung, liver, kidneys and testicles, and they had an effect on the ratio of immune cells (CD4+/CD8+), but not particles of 323nm in size. By administering 0.25 mg/kg, 0.5 mg/kg or 1.0 mg/kg of Ag nanoparticles of 42 nm diameter adverse effects arose in the liver and kidneys. Park-EJ et al, Env. Toxicol. Pharmacol 30 (2010) 162-168.

- From 100 t/a a 90-day study is also required for NMs analogously to Annex IX of the REACH Regulation. The study must normally be conducted by inhalation as administration route. As a deviation from the remarks of OECD TG 413 and the Test Method Regulation B.29 (rodents only) an exposure-free follow-up phase of 90 days and additional examination parameters are required for NMs. The provisions from Annex XI para. 3 a ii of the REACH Regulation do not allow for the waiving of a 90-day study on the basis of the results of a 28-day study[13] (see chapter 7 „Waiving"). In this tonnage band the tests on NMs to establish developmental toxicity and toxicity to reproduction must be conducted by the inhalation route.

- At 1000 t/a first the conditions described in Annex X of the REACH Regulation apply with respect to the conduct of studies for chronic toxicity and carcinogenicity. As a deviation from Annex X for bulk material administration by inhalation is to be given preferences for NMs. The need for a follow-up period must be checked and adapted according to the exposure duration and life expectancy of the animal species or strain used.[13] Taking account of the additional examination parameters to be re garded in the 28-day and 90-day studies and all the data available for NMs, consideration must be given to an adaptation of the test design and, where relevant, extended examination parameters for the chronic or carcinogenic studies.

- Additional testing requirements for respirable, bioresistant, fibrous NMs: Where fibrous materials are present it must also be verified whether there are bioresistant nano-scale fibres of asbestos-like dimension (WHO fibres). Corresponding test regulations are available based on experience with asbestos and synthetic mineral fibres, and these can also be applied to fibres of NM (WHO fibre dimension > 5 µm, diameter < 3 µm, length-to-diameter ratio > 3:1). From the results of these tests it is possible to obtain a classification for WHO fibres with respect to carcinogenicity.

**Ecotoxicological data (see Appendix V):**

The following requirements only apply to NMs within the meaning of the definition of the EU Commission (see 3.1).

- From 1 t/a the test programme according to Annexes VII+VIII applies. The acute daphnia test is omitted in favour of the chronic daphnia test from Annex IX. On account of the behaviour of NMs in their tendency to agglomerate and sediment, the sediment is regarded as an especially relevant exposure route. More information is therefore needed in this respect even at low tonnages. A chronic test is to be considered instead of an acute test for the examination of fish (for formulation see column 2).

- The waiving criteria formulated hitherto for the tests in column 2 of Annexes VII-X of the REACH Regulation cannot be applied in this way to NMs. The low water solubility in particular as the sole exclusion criterion for ecotoxicological tests cannot justify a test waiver for NMs. Rather it must be justified in addition that the NM is not absorbed by organisms and is not capable of penetrating biological membranes. Good water solubility in a substance (> 100 mg/L) can, however justify the waiving of tests on the nanoform if there is a test for the bulk form. An explanation for the remark „if there are justified indications that aquatic/microbiological toxicity is unlikely to occur" must be inserted in the preliminary remarks to Annex XVIII or in Annex XI.

- From 10 t/a the test programme of Annex IX of the REACH Regulation additionally applies as does the chronic sediment test from Annex X as a supplement. The identification of degradation products remains at 100 t/a since no NM-specific problem is to be expected here and the degradation (except

[13] see OECD Guidance Document No 116

in special cases of surface treatment) normally does not play a crucial role for NMs.
The short-time test for terrestrial plants remains at 100 t/a. In the case of bioaccumulation a *fish-feeding-study* is to be given preference over the BCF test, because the latter frequently fails to give a realistic picture of the accumulation behaviour of NMs.

- From 100 t/a the test programme from Annex X of the REACH Regulation applies in addition.
  The chronic plant test and the reproduction test for birds remain at 1000 t/a.

- From 1000 t/a a chronic plant test and reproduction test for birds must also be conducted.

## 9 Additional consequences

**Chemical Safety Report from 1 t/a**

The Chemical Safety Report is a central element in REACH. In this the registrant documents the substance safety assessment according to Annex I of the REACH Regulation. It has to be clarified how the Chemical Safety Report is to be structured if both the bulk form and nanoform of a substance are present. It would be conceivable to document this separately in a number of Chemical Safety Reports or to group them in one Chemical Safety Report. A joint Chemical Safety Report for the nanoforms of the substance and the bulk form would appear to be an advantage since it would facilitate direct comparisons between the different forms of the substance. But this is not possible if for NMs and bulk material different tonnage quantity thresholds (1 t/a - 10 t/a) trigger the obligation to prepare a Chemical Safety Report. In any case different nanoforms must be examined individually in a Chemical Safety Report, however, without excluding a joint examination with respect to certain aspects.

**Obligations of downstream users**

It must be noted that downstream users who manufacture/produce the nanoforms from bulk material are subject to extended obligations as compared to a downstream user of bulk materials. There are two possibilities here:

**Alternative 1**: Following from the regulatory system and on the basis of the decision to consider size as a „characteriser“, a „nano producer“ who is not a substance manufacturer himself is to be regarded as a downstream user. The existing regulations, and in particular the obligation of the downstream user to draw up a Chemical Safety Report, are not sufficient, however, to regulate this situation in an appropriate way. They should be supplemented by making a specific „Nano Chemical Safety Report“ of the „nano producer“ necessary. This would have to be submitted – possibly requiring own studies – to ECHA and would also be subject to an evaluation. The corresponding data requirements would have to be compared with that of a substance manufacturer who directly manufactures NMs. Basically, however, there should be the possibility with this alternative – as in general for downstream users – of communicating the use „NM production“ up the supply chain, combined with the possibility for the upstream manufacturer/supplier himself to cover the assessment of the NM production in his dossier and, where relevant, in the Chemical Safety Report.

**Alternative 2:** A facility which obtains NMs through physical processes (grinding of the bulk material), has the same obligations as a manufacturer within the meaning of the REACH Regulation.[14] Depending on the wording of the regulation relating to surface-treated NMs this should also be phrased analogously for chemical modifications. An exemption from this obligation is possible if the supplier of the source material covers the manufacture and result of such a manufacturing process completely in his registration dossier.

**Adaptations in the titles authorisation and restriction**

Basically authorisations and restrictions should cover the substance as a whole. But if a restriction/authorisation requirement is only necessary for selected forms or only for bulk or NMs, this should be possible. This should also apply to all respirable granular and fibrous particles.

This is undoubtedly already possible today for restrictions. For authorisations this may be regarded as possible within the framework of the interpretation of the regulation. However, a clarifying amendment may be necessary here.

**Substance evaluation according to Title VI**

Basically,substance evaluation encompasses the substance on its own. But it should be specified analogously to the notions behind authorisation/restriction that it is also possible to be evaluate only the NM or certain nanoforms, or respirable granular and fibrous particles. Here an adjustment of the regulations would appear to be desirable, in order to identify clearly the party responsible to fulfil the information obligations which might arise from the substance evaluation.

**Adjustments in Annex II/Art. 31**

Annex II (complementary to the corresponding guidance document) should describe what nano-specific details should be included in the safety data sheet. Reference can be made here to the activities of ISO[15], and also to an Australian paper for the ECOSOC Sub-Committee of Experts on the GHSST/SG/AC.10/C.4/2010/19 – (Australia) „Information relating to nanomaterials for inclusion on the guidance on the preparation of Safety Data Sheets (SDS)“ as well as the Swiss report “Safety data sheet: Guide for synthetic nanomaterials” http://www.bag.admin.ch/nanotechnologie/12171/12176/index.html?lang=en.

Essentially it is a matter of transparency stating clearly in the SDS whether a NM or respirable granular and fibrous particles are concerned and which data are available for the NM. The information on the identity must include adequate information to characterise the actual material. Normally a safety data sheet should be drawn up for the nanoform separately from that for the bulk form. This is accordingly true for different nanoforms and for all respirable granular and fibrous particles.

It must be checked whether details of the nanoform should also be specified if the NM is classified as not hazardous. Thereby transparency as desired by many parties could be achieved without the necessity to develop new instruments.

[14] The obligations of a downstream user in accordance with Art. 37 combined with Annex XII do not secure a full evaluation of the material

[15] Das ISO Technical Committee (TC 229) is working on a technical report (ISO/DTR 13329, Nanomaterials - Preparation of Material Safety Data Sheet (MSDS)) concerning the formulation of safety data sheets for NMs.

## 10 Problems regarding surface-treated NMs (coating)

Surface-treated NMs are regarded as a special nanoform of the treated source material. This is only justified if the surface treatment does conflict the basic substance identity of the source material. In terms of substance identity this could basically be assumed if, for example, 80 % (w/w) of the non-modified reagent remains. More specific criteria will have to be developed. The surface-treated NM must be included in the registration of the source material. The procedure described in chapter 5 applies. If a different substance is predominantly present, it must be treated as a new substance. There is a need for further clarification to obtain a precise delimitation.
A possible procedure for dealing with surface-treated NMs is described in Appendix VI.

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

Federal Institut for Ocupational
Safety and Health

**Bundesstelle für Chemikalien**
Friedrich-Henkel-Weg 1–25
44149 Dortmund

Dr. Frauke Schröder
Tel: 0231 9071 2279
Fax: 0231 9071 2679
E-Mail: chemg@baua.bund.de

Dortmund, 23.05.2011

# Preliminary thoughts for an integration of nanomaterials into the REACH Regulation
# Thought-starter by the German CA

It is generally acknowledged, that REACH already provides a suitable overall framework of concepts, procedures and tools for the safe handling of nanomaterials: testing, hazard and risk assessment and risk management measures. In principle, testing requirements, test strategies and most test methods for chemicals under REACH are considered by the majority of experts as being also suitable for nanomaterials. However, nanomaterials exhibit some particular characteristics which need to be taken into account when adapting REACH to nanomaterials.

Potential nano-specific adaptations and additions to REACH are addressed at EU level by the CASG Nano and the three almost finalised RIPoN-Projects.
In particular, RIPoN 1 dealt with the question whether (a) nanomaterial(s) of a substance and the corresponding bulk material are to be considered as the same substance or not. It should also review the guidance document on substance identification in order to identify sections that need adaptations to cover nanomaterials.
Four case studies of different nanomaterials (including different nanoforms) were developed and the applicability of the current guidance on substance identity was investigated. It was noted that in principle the existing guidance could be applied to nanomaterials; however, no consensus could be reached on which parameters would be necessary to determine the identity of a nanomaterial.

Generally, for a well-defined substance under REACH, substance identity is determined by molecular structure and chemical composition alone. For nanomaterials it is agreed among experts that size, shape and surface characteristics do affect the physico-chemical and also hazard properties of the materials. Therefore, it is discussed whether these characteristics should be highlighted as a characteriser or an identifier and in consequence may be the crucial criterion for the deduction of a substance under REACH.

In Germany the same discussion is ongoing and we would like to share our interim thoughts and conclusions as well as still unsolved issues with other MS:

It is clearly acknowledged that the size of a nanomaterial is one determining factor related to the change of properties of the substance. However, this does not necessarily lead to the consequence of creating different new substances.
In the view of the German CA a bulkmaterial and its corresponding nanomaterials have the same chemical composition and are, therefore, chemically identical. Consequentially, this would mean that they have to be covered together in one registration dossier, with size and other nano-specific characteristics as important characterising elements of the nanomaterial, which trigger further examination.

This means different information requirements and, consequently, separate safety assessment and risk management measures should apply for the bulkmaterial and the nanomaterial(s). In consequence, it will be necessary to adapt the different instruments of REACH appropriately. The nanomaterial(s) has(have) to be characterised thoroughly by parameters such as size, morphology, aggregation potential, etc. and, based on this characterisation, relevant nano-specific testing needs to be performed in order to carry out an adequate chemical safety assessment separately for the bulk form and the nanomaterial(s). Read-across between the bulkmaterial and the nanomaterial(s) as well as waiving should in general be possible, as for any substance, provided it can be justified and is thoroughly documented. A nano-specific test programme, still needs to be developed, considering where appropriate different nanoforms. Furthermore, criteria need to be developed for the decision in which cases read-across between different nanomaterials (and the bulk material) is appropriate.

Other issues that are still being discussed on national level:

- lower tonnage threshold for registration of nanomaterials;
- the possible implementation of a threshold below which nanomaterials do not need to be reported at all;
- the development of a nano-specific test programme,
- criteria for the distinction between and grouping of different nanomaterial(s), that may be treated separately or together for testing, respectively;
- the handling of surface treated nanomaterials.

I hope this is a useful contribution for the further discussion on EU level.

On behalf of the German CA

Frauke Schröder

## Appendix II – Considerations regarding Criteria for the Sharing of Data between Different Nanoforms

as at: 22.09.2011

## A. Preliminary thoughts

The German concept to regulate the requirements for NMs under REACH provides to establish a framework where NMs are not substances in their own right, but can be considered separately with respect to various requirements under REACH. The aim is to subject NMs within the joint substance registration to nano-specific testing requirements and risk assessment.

Special characteristics concerning toxicokinetics and environmental fate, together with the existing uncertainties and special features with regard to mode of action, necessitate requirements which go beyond those implemented to date in REACH. For example, compared to bulk materials, NMs have a substantially greater specific surface area which is available for a reaction with other substances or for interaction with biological systems. Dose-response-relationships as usually derived, address this issue insufficiently.

Consequently more data and tests have to be requested for NMs already at lower tonnages. For consistency reasons other obligations under REACH should be adapted also.

The behaviour and (eco-)toxicological effects of nano-scale substances are significantly influenced both by their physico-chemical and their morphological properties. Modification of these parameters should therefore ideally result in independent nano-specific testing requirements for each form. However, in terms of proportionality and practicability this leads to an unacceptable testing effort and should be avoided.

In principle it is assumed that the registrant manufactures only a few different nanoforms of a substance. Yet there may be cases where a large number of different nanoforms are manufactured for different applications. In those cases, approaches for grouping and waiving are particularly important in order to avoid unnecessary animal testing and unnecessary costs while at the same time ensuring an adequate level of protection.

With respect to the testing requirements it has therefore to be considered whether and according to which criteria certain nanoforms or nanoforms and the bulk form of a substance can be regarded as comparable so that data can be shared. This is to be investigated in a screening step. For this purpose criteria must be developed which enable the registrant to check the comparability of the different forms of a substance.

Figure 2 is a schematical diagram showing the registration and screening for the development of the test programme for different nanoforms of a substance.

Afterwards the registrant can additionally resort to the waiving approach. The REACH Regulation states in column 2 of Annexes VII-X and in Annex XI various possibilities for waiving. In the planned Annex XVIII and a conceivable adaptation of Annex XI, it would be possible to describe the special features with respect to nanomaterials.

Basically three groups of cases must be considered:

1. Use of data by referencing between bulk form and nanoform.

2. Use of data by referencing between different nanoforms of a substance.

3. Read-across between different substances (different nanoforms/bulk form).

Annex XI allows for various justifications for waiving:

- Testing is scientifically not necessary [No. 1]
  - Use of existing data
  - Weight of evidence (overall picture of existing data)
  - (Q)SAR
  - In-vitro methods
  - Grouping and read-across approach
- Testing is technically not possible [No. 2]
- Substance-specific, exposure-dependent testing [No. 3]

Compared to conventional chemicals, substantially less knowledge exists for nanomaterials in order to apply these methods. But activities aimed at achieving progress in the area of (Q)SAR[1] and grouping/read-across approaches[2] are increasing.

The key points presented below must be adapted in accordance with scientific progress.

## B. Key points

Characterisation of the nanomaterial

The individual nanoforms of a substance must be adequately characterised. This is the prerequisite for comparing different nanoforms and to draw any conclusions which materials will probably have comparable properties. It must be assumed that in the next few years a large amount of additional knowledge will be acquired in this respect[3].

There is a growing consensus about the characterisation of NMs in the context of the risk assessment or the necessary tests:[4] the MinChar parameter list (http://characterizationmatters.org/parameters/) compiles all the relevant factors. To date, however, not for all parameters routine standardised methods have been developed.

The knowledge obtained from material characterisation should be used to state upon the comparability of different forms or their (eco)-toxicological properties.

As regards the comparability of different forms, two cases can be distinguished:

1. Different nanoforms (and where applicable the bulk form) are comparable in such a way that they overall can be considered together and the tests for one form are representative for another one.
2. A specific endpoint or property or a specific test performed for one nanoform (and where -applicable the bulk form) covers this specific issue for another form. In this respect the

[1] Descriptions of approaches can be found, for example, in Burelloet al. "*QSAR modeling of nanomaterials*; *Nanomed. Nanobiotechnol".* **2011**, Puzyn et al. "*Toward the Development of "Nano-QSAR": Advances and Challenges"*; *small* **2009**, *5*, 2494-2509

[2] In the context of the OECD it is being considered whether, during the further development of the "Guidance on Grouping Chemicals" [ENV/JM/MONO(2007)28], NMs can also be incorporated. See in the paper ENV/JM/HA(2011)4 the item "Applying the concept of chemical categories and analogue approach to manufactured nanomaterials"

[3] Descriptions of approaches can be found, for example, in Burelloet al. "*QSAR modeling of nanomaterials*; *Nanomed. Nanobiotechnol".* **2011**, Puzyn et al. "*Toward the Development of "Nano-QSAR": Advances and Challenges"*; *small* **2009**, *5*, 2494-2509

[4] Oberdörster "Principles *for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy*" Paricle and Fibre Toxicology **2005** 2:8; Stone et al. "*Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisatio"* Science of the Total Environment **2010**, *408*, 1745–1754; Berube et al."C*haracteristics and classification of nanoparticles: Expert Delphi survey*" Nanotoxicology, **2011**; *5*, 236-243

forms can partially be considered together. However, a general referencing is not possible.[5]

To date it is not possible either to make sound assumptions with respect to the selection of the probably most critical material on the effect side or to make predictions of the environmental fate and exposure.

It must be noted here that, regarding cumulative exposure, in many cases the combined exposure to the different nanoforms of a substance must be considered.

Below, it is distinguished between the screening stage in which it may be possible to group the different nanoforms and waiving of individual tests/studies at individual end points.

Screening stage

As a basic principle, forms which do not differ to a relevant degree with regard to the basic parameters can be regarded as comparable.

Materials with relevant differences in physico-chemical (PC) data and reactivity should clearly be regarded as different.

To date no reliable information is available to which variations are acceptable for individual parameters. In many cases it will remain a case by case decision. It is desirable to develop appropriate screening tests where applicable, to gain experience on comparability.

Chemical and morphological parameters shall be used to delimit different nanoforms. The following initial criteria and their suitability for a possible grouping of different nanoforms of a substance must be discussed and supplemented where necessary.

One criterion for the comparability of different nanoforms is the change in the parameters and properties listed below, or their influence on the behaviour of the respective nanoform, as well as environmental effects of the respective nanoform.

## Chemical parameters

The chemical composition influences the substance identity whose results are already incorporated in the determination of the substance identity. It remains to be substantiated as to whether the questions of surface treatment play a role on the level of substance identity or in the screening stage.

### Morphological parameters

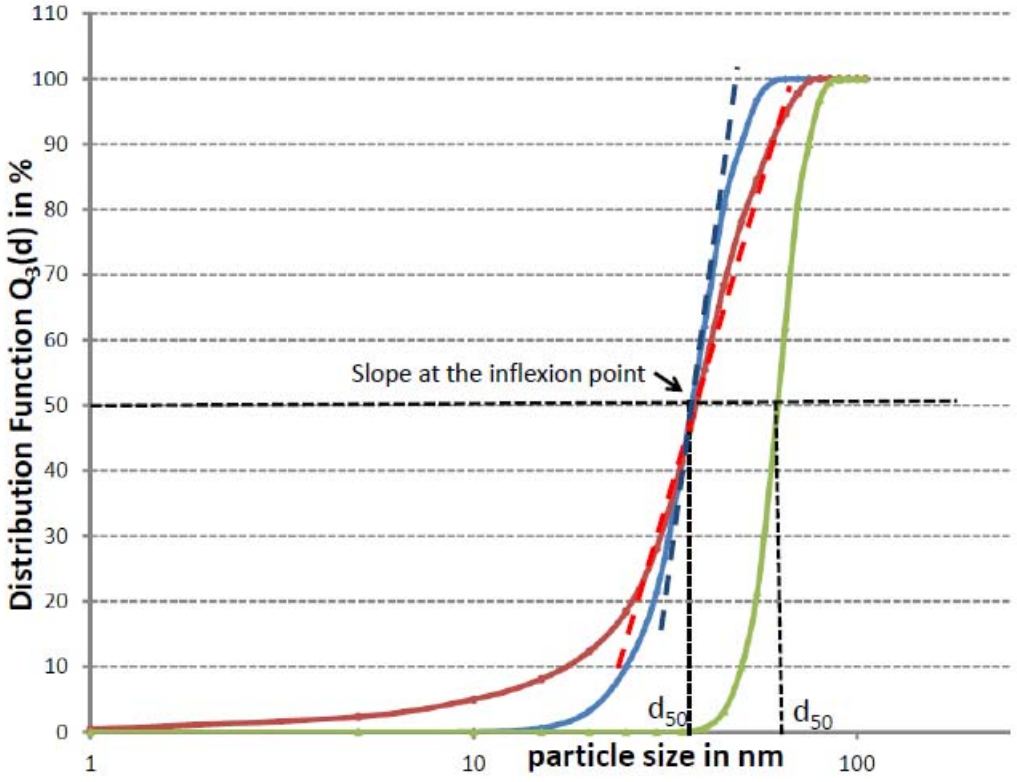
Primary particle size

As the particle size decreases the nano-specific features increase. In addition, the particle size influences the stability and environmental behaviour as well as the possibility for uptake into the organism and into single cells. With very small particle sizes the crystalline character diminishes against an increasing molecular behaviour.

[5] The Classification and labelling Regulation does not foresee over-labelling. If there are clear indications that a material is substantially less critical, this is usually not covered. On the other hand such an approach is basically possible with respect to the risk evaluation and the risk management measures. It must be clarified that classification and labelling are to be dealt with. (Especially on account of Annex XI, which normally demands that the procedures permit classification and labelling.)

If the primary particle size distributions largely match, nanoforms can basically be grouped together. In case of relevant differences, nanoforms are to be considered separately. The subsequent possibilities for grouping and waiving are not affected by this.

The number-based size distribution function ($Q_3(d) = \int q_3(d)*d(d)$) of the primary particles or their inflexion point ($d_{50}$) and the slope in the inflexion point (width of the distribution), respectively could serve as a parameter for the comparison of different nanoforms (Figure 1). As a basis for decision-making the position of $d_{50}$ (e.g.: <100nm, <30nm, <10nm), the width of the distribution or the portion of the fraction below a certain primary particle size (e.g.: <100nm, <30nm, <10nm) of the different nanoforms must be compared.




ms.

ty,

icles.

is.

o

CNTs,
nd

**Water solubility**

If a material exhibits sufficient water solubility different nanoforms can be considered together. In principle, tests can then also be referenced from the bulk form to the nanoform.

When determining water solubility it must be ensured that the concentration achieved in the solubility test is not only attributable to sub-fractions of the substance examined or its forms, respectively. Since the water solubility increases as the primary particle size decreases[7], it is not possible to conclude on the water solubility of larger nanoforms or the bulk form from the water solubility of very small nanoforms. It may also be necessary to pay attention to pH dependence. It must be demonstrated that in fact no nanoparticles remain below the solubility threshold.

[6] Mendive et al. "*Adsorption of oxalate on anatase (100) and rutile (110) surfaces in aqueous systems: experimental results vs. theoretical predictions*" Physical Chemistry Chemical Physics **2009**, 11, 1794–1808

[7] Fan et al. *"Relationship between solubility and solubility product: The role of crystal size and crystallographic direction"* Geochimica et Cosmochemica Acta **2006**; 70, 3820-3829

Under these conditions a water solubility of 100 mg/l[8] can be regarded as sufficient in the above sense.

High water solubility can also lead to a situation where different crystalline structures can be considered together.

It must be noted that surface treatments may influence the stability of the NM in water. In such cases it is therefore not possible to simply refer to the water solubility of the untreated substance.

**Surface properties**

Since NMs have a very large specific surface area, surface properties determine their behaviour to a great extent. The interactions with biological systems and molecules take place on the surface area. Changes in the surface area influence the behaviour. Therefore, surface properties are of central importance in assessing NMs. The surface properties include a whole series of parameters.

Different surface properties lead temporarily to a situation where nanoforms can not be grouped together in the screening stage.

In addition changes to the agglomeration behaviour indicate that the surface properties of the NM have changed. Relevant differences in the agglomeration behaviour mean that nanoforms are to be considered separately. The subsequent possibilities for grouping and waiving are not affected by this.

Important surface properties which should be considered for the comparison of different nanoforms are:

- surface charge
- hydrophobicity
- (photo-)catalytic properties
- absorption/adsorption or bonding of (certain) molecules
- functional groups (e.g. –OH; where relevant by surface treatment)
- agglomeration behaviour (resulting from this).

Qualitative changes (a certain property occurs for the first time) and quantitative changes (a property changes significantly) of the surface properties must be examined. Further considerations concerning surface-treated NMs can be found in Appendix VI.

**Implementation in REACH**

Up to now, no general conclusions with respect to the joint assessment of different nanoforms can be drawn due to a lack of adequate data on the relationship between the physicochemical parameters and the (eco-)toxicological properties. Approaches are currently under discussion in the literature.[9]

The article part of the REACH Regulation should describe that nanoforms which differ to a relevant extent in their chemical and morphological parameters are individually subject to testing requirements according to a new Annex XVIII. In an Annex to the REACH text it

[8] It is conceivable that further knowledge here could permit a subsequent reduction to 10 mg/l.

[9]. 'See, for example. Fubini et al.; *„Physico-chemical features of engineered nanoparticles relevant to their toxicity“ Nanotoxicology* **2010**, *4*, 347-363

would then be possible to list the parameters to be considered, e.g. as Section 2.4 in Annex VI "Description of the nanoform" (or in Annex XVIII).

On the level of guidance documents the differences regarded as relevant should be described. The abstract criterion for this should also be described in a recital of the amendment to the regulation and possibly in Annex XVIII.

Waiving and grouping approaches

Even if it has been concluded in the screening stage that a separate test of the different nanoforms is necessary, this does not mean that the complete test programme has to be performed for each nanoform. Waiving is still possible and can and should be used. In particular it is possible to develop a tailored test programme which brings together the testing requirements for the individual nanoforms or substance groups while making use of waiving.

In order to enable waiving and data sharing, data have to be adequate and usable for risk assessment. This means that possible differences with respect to the environmental fate, toxicokinetics and biological effects have to be considered. As in the screening stage, the chemical parameters, morphological parameters, solubility and surface properties of the nanoforms are therefore crucial. For decision-making QSAR data and *in vitro* test systems can also play a role. The registrant[10] bears the responsibility to decide and plausibly show whether the changed parameters or properties of a nanoform are relevant for individual tests and whether it is possible to waive the test because it is covered by the test of another form. Furthermore it can be checked whether a realistic worst-case consideration of the different nanoforms is possible.

Thereby the remarks in Annex XI and the remarks to be developed in column 2 of the planned Annex XVIII are the basis. These should be elaborated further on the level of guidance documents.

In Annex XI a paragraph on NMs should be inserted in the preliminary remarks with roughly the following wording:

> Annex XVIII includes information requirements for all nanoforms of substances as a function of the quantity in which they are manufactured or imported. According to the special provisions in column 2 of Annex XVIII and according to the general provisions in section 1 of the present Annex the registrant may deviate from the standard test programme. Such deviations can be checked by the Agency within the framework of the evaluation of the dossier.
>
> Where a number of nanoforms of a substance have to be considered during the tests the registrant may test the probably most critical nanoform instead of all nanoforms. The selection must be justified. While adhering to the criteria given under No. 1 (Annex XI) it is also possible to share data between the bulk form and nanoform or between different nanoforms of a substance.

[Further adaptations under Annex XI Nos. 1-3 are to be checked. This must be done in connection with the further wording of Annex XVIII.]

[10] The decision of the registrant must possibly be checked by the ECHA within the framework of the dossier evaluation etc.

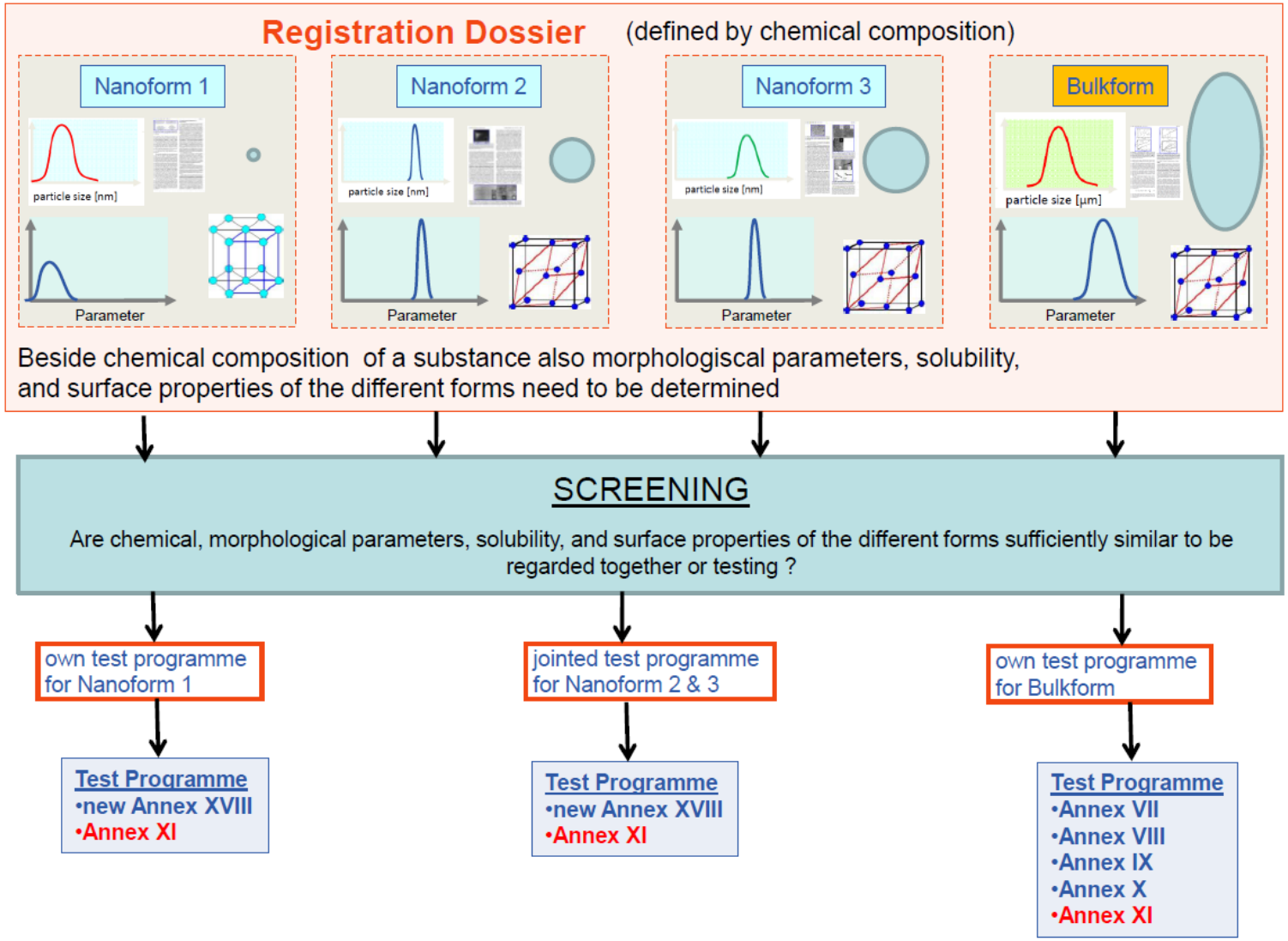



Figure 2: Diagram of the registration and screening for development of the test programme for different nanoforms of a substance

## Appendix III – Characterisation and physicochemical data requirements regarding nanomaterials

as at: 22.08.2011

Prior to characterisation an adequate identification must be made of the nanomaterial (source material, manufacturing route, …).

### Relevant tests to characterise nanomaterials

Below only a few methods are given as examples which can be referred to characterise the nanomaterial. In the end for different nanomaterials a case-by-case decision must always be made, e.g. taking the source material and the individual form into account.

| **Characterisation / Determination** | **Method (by example)** |
|---|---|
| Appearance (form, length to width ratio) | Atomic force microscopy (AFM)<br>Transmission electronic spectroscopy (TEM) /Scanning electron microscopy (SEM)<br>Small-angle x-ray scattering (SAXS)<br>UV-VIS spectrum<br>Raman spectroscopy |
| Aggregation and agglomeration behaviour | Dynamic light scattering (DLS)[1]<br>Brunauer-Emmett-Teller method (BET)<br>Transmission electron microscopy (TEM)<br>Small-angle neutron scattering (SANS) |
| Size distribution | Dynamic light scattering (DLS)[1]<br>Scanning mobility particle sizer (SMPS)[1]<br>Field flux fractioning (FFF)<br>Small-angle X-ray scattering (SAXS)<br>Nanoparticle trace analysis (NTA)<br>Ultracentrifugation |
| Specific surface area | Brunauer-Emmett-Teller method (BET)<br>NMR[2]<br>Small-angle x-ray scattering (SAXS)<br>Ultracentrifugation [3] |
| Surface activity | Auger electron spectroscopy<br>Ultraviolet photoelectron spectroscopy (UPS)<br>FT-IR<br>Chemisorption |
| Surface charge (zeta potential) | Isoelectric point (IEP)<br>Particle charge sizer (PCS)<br>Dynamic light scattering (DLS)[1]<br>Electrophoretic mobility (EPM) |
| UV/VIS spectrum | |
| Crystalline structure or modification | X-ray diffraction (XRD)<br>TEM+FT |

[1] Although suitable primarily for approximately spherical particles, the results obtained in relation to other nanoforms, such as rods, can be flawed.
[2] Depending on the composition of the NM, this may only be possible using special NMR devices
[3] Applicable where the specific surface has a measurable effect on the distribution behaviour of the NM

**Physicochemical tests according to the requirements of Annex VII of the REACH Regulation (EC) No. 1907/2006**

| STANDARD INFORMATION REQUIRED | POSSIBLE PROBLEMS IN ADAPTATION / REMARKS |
|---|---|
| 7.1. State of the substance at 20°C and 101.3 kPa | |
| 7.2. Melting/freezing point | In different publications on the physicochemical testing of NMs it is stated that the melting point is dependent on particle size (the smaller the particle, the lower the melting temperature). |
| 7.3. Boiling point | Not necessary if information is available from the bulk material since the melt, which is already no longer a nanomaterial, passes into the gaseous state. |
| 7.4. Relative density | |
| 7.5. Vapour pressure | Normally not necessary because:<br>• the vapour pressure is not significant with very high melting and boiling points<br>• often the melt, which is no longer a nanomaterial, is evaporated (dynamic method), or the vapour pressure is overestimated due to aerosol formation. |
| 7.6. Surface tension | Only necessary if a sufficiently high water solubility applies and no information is available on the bulk material. Otherwise the value determined for the bulk material can be used because it involves the examination of dissolved material which is no longer a nanomaterial. |
| 7.7. Water solubility | |
| 7.8. Partition coefficient n-octanol/water | Only necessary if a sufficiently high water solubility applies. Here the value determined for the bulk material can be used because it involves examination of dissolved material which is no longer a nanomaterial. |
| Technical characteristics:<br>o dispersibility/stability<br>o dustiness | |
| 7.9. Flash-point | Methods should be adapted (e.g. to smaller quantities of test substance) |
| 7.10. Flammability | |
| 7.11. Explosive properties | |
| 7.12. Self-ignition temperature | |
| 7.13. Oxidising properties | |

## Appendix IV, Test programme Toxicology

In order to identify the intrinsic toxic properties of NMs, specifically tailored tests are required which are quantity-triggered as the tonnage triggered standard information requirements for bulk materials of REACH Annexes VII-X.

It is envisaged to extend the REACH Regulation by adding a further Annex XVIII, which describes the standard information required for NMs.

Annex XVIII will be based on REACH Annexes VII–X for bulk materials. The modifications required are described in Chapter 8 "Test programme" of the background paper and are seen as necessary standard information requirements for NMs.

In the following explanatory remarks on the toxicological testing requirements for NMs mentioned under chapter 8 "Test programme" of the background paper are given. The proposals regarding the toxicological test programme for nanomaterials under REACH are described in Appendix VII to the background paper.

General recommendations:

It is absolutely essential for all toxicological testing that the test material be characterised in accordance with Annex XVIII and that its physical form be monitored during the experiment. This is a prerequisite for a toxicological assessment and risk assessment of NMs as well as for a possible waiving, e.g. by the grouping of NMs (see chapter 7 “Waiving” of the background paper)

Genotoxicity

*Beyond the standard test provided for in Annex VII with respect to in vitro genotoxicity in bacteria, two tests for genotoxicity with mammalian cells in vitro are necessary for NM from 1 t/a.*

The standard test to identify the mutagenic potential (as one aspect of genotoxicity) is the Ames test, which uses bacteria from *salmonella typhimurium* strains. Their bacterial cell wall may interfere with the uptake of NMs into the cell, and so a large number of false negative results can be expected in this usually highly specific test. The OECD[1] recommends that NMs always be examined with a test battery of three in vitro tests for genotoxicity: the bacterial gene mutations assay (Ames), a mammalian cell-based gene mutations assay (HPRT), and a mammalian cell-based cytogenicity test or micronucleus test. With the exception of the Ames test, these tests are required in accordance with Annex VIII for substances in bulk form as from 10 t/a. All three tests are completely validated and not much time-consuming. Positive results can primarily be expected from the test systems with mammalian cells; the Ames test should nevertheless be included in the test battery because a positive result of this highly specific test is a strong indicator of any mutagenic effect.

Standard route of administration

*For the first test to examine acute toxicity and all further tests with repeated administration, the inhalation route is the standard administration route for NMs instead of the oral administration route normally used for bulk material.*

For insoluble NMs exposure by inhalation is the administration route of the highest importance because the majority of NMs contain inhalable or respirable fractions. Oral exposure and dermal exposure towards industrial chemicals in nano form are in principle possible, but in most cases of lower-ranking importance when compared to exposure by inhalation. It is therefore recommended, taking account of the most probable type of exposure, that the standard route of administration be changed from oral to inhalation. This concerns testing for acute toxicity and testing after repeated administration (i.e. subacute

[1] See discussion in [OECD2009]. OECD No. 15 - ENV/JM/MONO(2009)21 Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials

(28 days), subchronic (90 days) and chronic (2 years) studies, as well as studies to investigate reprotoxicity and carcinogenicity).

Extended follow-up phase

*As a deviation from the explanations of the OECD test methods and the Test Method Regulation (EC) No 440/2008 concerning tests with repeated administration, extended exposure-free follow-up phases are necessary for NMs.*

Extended follow-up phases serve to identify the distribution of NMs in organs and in the organism which might exhibit a different or delayed pattern compared to the bulk material; it also serves to identify possible particle persistence. Extended follow-up phases also serve to identify either possible increases in effects or adverse effects, which might occur after a delay (e.g. effects in the respiratory tract or, as a result of translocation and accumulation, in other organs).
Subacute studies on NMs should include an extended follow-up phase of 28 (instead of 14) days; in the case of subchronic tests this should be 90 (instead of 28) days. For chronic studies a follow-up phase has to be included. All available data, as well as the life expectancy of the animal species and animal strain and the preceding exposure time have to be taken into account.

Additional study parameters

*As a deviation from the explanations of the OECD test methods and the Test Method Regulation (EC) No. 440/2008 concerning tests with repeated administration, additional study parameters are required for NMs.*

These consist of additional clinical-chemistry parameters, additional morphological parameters and/or additional functional-morphological examinations.

In addition to the standard requirements of the respective test method, the specifications of the OECD TG412 for examination of the respiratory tract to investigate the broncho.alveolar lavage fluid (BALF) and the possible deposition and translocation of NMs in and into other organs after inhalation must be implemented. Furthermore the histopathology should comprise a larger number of tissue sections per organ for the respiratory tract and for suspected target organisms, in order to improve the statistical power and to cover pre- and neoplastic lesions in repeated dose studies. Additional studies concerning the suspected mode of action may include inflammatory markers or NM-induced cell proliferation.

The extent of the additional study parameters must be in accordance with the actual state of knowledge of the OECD activities concerning the updating of test regulations.[2] On the basis of information available it may in individual cases also be necessary to conduct additional specific examinations (e.g. morphological or functional examinations of the cardiovascular system, immune system and nervous system).

This applies to subacute (28 days), subchronic (90 days) and chronic (2 years) toxicity studies, as well as all tests for reproductive toxicity and carcinogenicity.

Additional testing requirement for respirable, bio-resistant fibrous NMs:

Inflammation and a probable carcinogenic effect after inhalation are regarded as relevant health hazards of respirable, bio-resistant, fibrous NMs.

A carcinogenic effect after inhalation is assumed for bio-resistant, nano-scale fibres of asbestos-like dimension (WHO fibres). Thus, w*here fibrous materials are present it must be demonstrated that bio-*

[2] OECD WPMN SG4, Working Objectives for 2012: NM in TG412, TG413

*resistant nano-scale fibres of respirable dimension (WHO fibre dimension >5 µm, diameter <3 µm, length-to-diameter ratio >3:1) are not present. The corresponding test requirements obtained from the experience with asbestos and man-made mineral fibres can also be applied to fibres of NMs. With the results of these tests it is possible to derive a hazard evaluation concerning carcinogenicity.*

Prospects

The need for nano-specific information requirements of Annex XVIII presented here, possibly in addition to the requirements for bulk materials (Annexes VII to X), copes with the precautionary principle. It is anticipated that further adaptations of the testing requirements will be necessary in the coming years as an increasing amount of knowledge is accumulated, which might increasingly justify and enable waiving. Waiving will be rare in the beginning, but it might increase to the extent that standardised tests of the near future have shown that results from substances in bulk form can be utilized for NMs.

## Appendix V – Key points for the ecotoxicological test requirements for the adaptation of the REACH Regulation to nano-specific requirements

as at: 25.04.2012

### A. Preliminary thoughts

The data catalogue from 1 tonne or more per year must permit the drawing up of a Chemical Safety Report. The data must be sufficient for the purpose of classification and labelling.

The results regarding acute ecotoxicity do not to a particular degree yield any reliable statements on the risk profile with respect to nanomaterials (NMs). Observations from different NMs such as the ability to penetrate membranes and translocate into cells, residence time in cells and interaction with organic and biological molecules indicate a risk potential with regard to chronic effects. Studies show that NMs in biological systems among other things penetrate cell barriers and can reside in cells. It may be assumed that in this way more substance passes directly into the cells and a greater toxic effect will arise in the cell than through conventional absorption mechanisms due to coupled effects (e.g. ion toxicity and particle toxicity), depot effect or the "Trojan horse effect" (ability of nanomaterials to absorb other substances and to enable them in this way to gain access to cells, which they would not have normally). But also NMs which are regarded as inert can cause oxidative stress through the enhanced formation of free radicals. The energy states of NMs can attain values which, in contrast to bulk materials, correspond with biological reactions in the organism and hence influence these. Effects on the biochemical and physiological level as well as histological findings were observed in various organs.[1] The testing requirements for NMs should take account of this knowledge. The considered end points must be adapted if necessary.

To obtain reliable documentation of the risk potential of NMs, primarily chronic studies should therefore be referred to and normally given priority over acute tests.[2]

Differentiated consideration must be given for NMs for instance the fact that the exposure routes provided in the test guidelines cannot always be adhered to. In the aquatic domain, exposure to NMs may no longer be via water alone, but also via the intake of food (fish: feeding on agglomerates on the tank bottom and off the tank walls, Daphnia: feeding off edible algae to which NMs adhere). This does not conform to the existing test regulations at first glance, but reflects a realistic scenario.

To predict the environmental fate of NMs it is assumed that the specifications in the existing standard procedures are not always adequate. It is also evident that standard procedures, such as OECD 106, in their present form are not applicable to NMs. Here adaptations and new developments are necessary. In particular the existing procedures for the biological degradation of substances may not be directly applicable to yield relevant information for most organic NMs or their organic coatings. If suitable testing systems are available corresponding tests must therefore be supplemented.

Furthermore a comprehensive characterisation of the NMs must be conducted in order to obtain information from the material properties with respect to the anticipated environmental effects in future.

---

[1] Frederici "Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss)*: Gill injury, oxidative stress, and other physiological effects" Aquatic Toxicology 84 (2007) 415–430
Ramsden "Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (Oncorhynchus mykiss): no effect on growth, but subtle biochemical disturbances in the brain" Ecotoxicology 18 (2009) 939–951
Navarro "Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi " Ecotoxicology 17 (2008) 372–386
Smith "Toxicity of SWCNT to rainbow trout: Respiratory toxicity, organ pathologies, and other physiological effects" Aquatic Toxicology 82 (2007) 94-109

[2] Because of the 2nd ATP to the CLP Ordinance Regulation the possible lack of acute tests is no longer a serious problem with regard to the classification and labelling of the substance.

## B. Key points

We consider it appropriate to formulate differentiated requirements while retaining the existing tonnage bands for a test programme for NMs.

- From 1 tonne or more per year the test programme applies in accordance with Annexes VII+VIII of the REACH Regulation. The acute Daphnia test no longer applies on account of the chronic Daphnia test from Annex VIII. Because of the tendency of NMs to agglomerate and sediment, the sediment is regarded as a particularly relevant exposure route. Even at low tonnages therefore more information is required. In the fish test a chronic test should be considered instead of an acute one (wording in column 2).

- The waiving criteria formulated to date for the tests in column 2 of Annexes VII-X cannot be applied to NMs. The poor water solubility as the only exclusion criterion for ecotoxicological tests cannot justify test waiving for NMs. Rather it must be justified in addition that the NM is not absorbed and is not capable of penetrating biological membranes. On the other hand very good water solubility may justify the waiving of tests of the nanoform if a test is available for the bulkform of the substance. An explanatory note on the remark "if there are mitigating factors indicating that aquatic/microbiological toxicity is unlikely to occur" must be inserted into the preliminary remarks for Annex XVIII or in Annex XI.

| COLUMN 1<br>STANDARD INFORMATION REQUIREMENTS | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 9.1 Aquatic toxicity | |
| 9.1.2 Growth inhibition study aquatic plants (algae preferred) | The study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur. |
| 9.1.2 Long-term toxicity testing on invertebrates (preferred species Daphnia) | The study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur. |
| 9.1.3 Fish short-term toxicity test: the registrant may consider long-term toxicity testing instead of short-term. | The study does not need to be conducted if:<br><br>— there are mitigating factors indicating that aquatic toxicity is unlikely to occur. ;<br><br>— a long-term aquatic toxicity study on fish is available.<br><br>Long-term aquatic toxicity testing within the meaning of No. 9.1.6 in Annex XVIII shall be considered if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms. The choice of appropriate test(s) will depend on the results of the chemical safety assessment. The long-term aquatic toxicity study on fish (Annex IX Section 9.1.6) shall be considered if the substance is poorly soluble in water. |
| 9.1.4 Activated sludge respiration inhibition testing | The study does not need to be conducted if:<br><br>— there is no emission to a sewage treatment |

| COLUMN 1<br>STANDARD INFORMATION REQUIREMENTS | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | plant;<br><br>— there are mitigating factors indicating that microbial toxicity is unlikely to occur,<br><br>— the substance is found to be readily biodegradable and the applied test concentrations are in the range of concentrations that can be expected in the influent of a sewage treatment plant. The study may be replaced by a nitrification inhibition test if available data show that the substance is likely to be an inhibitor of microbial growth or function, in particular nitrifying bacteria. |
| 9.2 Degradation | To be phrased later. |
| 9.2.1 Biotic | |
| 9.2.1.1 Ready biodegradability | |
| 9.2.2 Abiotic | |
| 9.2.2.1 Hydrolysis as a function of pH | |
| 9.3 Fate and behaviour in the environment | To be phrased later. |
| 9.3.1 Adsorption/desorption screening | |

- From 10 tonnes or more per year the test programme from Annex IX will apply in addition as will the chronic sediment test from Annex X. Identification of the degradation products will remain at 100 tonnes or more per year because no NM-specific problem is to be expected and the degradation does not play a crucial role for NMs (except with respect to surface treatment). The short-time test for terrestrial plants remains at 100 tonnes or more per year. In the case of bioaccumulation a *fish feeding study* is to be preferred to the BCF test because the BCF test often fails to give a realistic picture of the accumulation behaviour of NMs.

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 9.1 Aquatic toxicity | |
| 9.1.6 Long-term toxicity testing on fish | The study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur. |
| 9.2 Degradation | |
| 9.2.1 Biotic | |
| 9.2.1.2 Simulation testing on ultimate degradation in surface water | To be phrased later. |
| 9.2.1.3 Soil simulation testing (for substances with a high potential for adsorption to soil) | To be phrased later. |
| 9.2.1.4 Sediment simulation testing (for substances with a high potential for adsorption to sediment) | To be phrased later. |
| 9.3 Fate and behaviour in the environment | |

| | |
|---|---|
| 9.3.2 Bioaccumulation in aquatic species, preferably fish | To be phrased later. |
| 9.3.3 Further information on adsorption/desorption depending on the results of the study required in Annex XVIIIa | To be phrased later. |
| 9.4 Terrestrial toxicity | A study does not need to be conducted if direct or indirect exposure of the soil compartment is unlikely.<br>The choice of the appropriate tests depends on the outcome of the chemical safety assessment.<br>In particular for substances that have a high potential to adsorb to soil or are very persistent, the registrant shall consider long-term toxicity testing instead of short-term. |
| 9.4.1 Short-term toxicity to invertebrates | |
| 9.4.2 Effects on soil micro-organisms | |
| 9.5.1 Long-term toxicity to sediment organisms | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment indicate the need to investigate further the effects of the substance and/or relevant degradation products on sediment organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. |

- From 100 tonnes or more per year the test programme from Annex X applies in addition. The chronic plant tests and reproduction test for birds remain at 1000 tonnes per year.

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 9.2 Degradation | Further biotic degradation testing shall be proposed if the chemical safety assessment according to Annex I indicates the need to investigate further the degradation of the substance and its degradation products. The choice of the appropriate test(s) depends on the results of the chemical safety assessment and may include simulation testing in appropriate media (e.g. water, sediment or soil). |
| 9.2.1 Identification of the degradation products | |
| 9.3 Fate and behaviour in the environment | |
| 9.3.4. Further information on the environmental fate and behaviour | Further testing shall be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 if the chemical safety assessment according to Annex I indicates the need to investigate further the fate and behaviour of the substance. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. |
| 9.4 Terrestrial toxicity | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment according to Annex I indicates the need to investigate further the effects of the |

| | |
|---|---|
| | substance and/or degradation products on terrestrial organisms. The choice of the appropriate test(s) depends on the outcome of the chemical safety assessment.<br>These studies do not need to be conducted if direct and indirect exposure of the soil compartment is unlikely. |
| 9.4.4 Long-term toxicity testing on invertebrates | |
| 9.4.3 Short-term toxicity to plants | |

- From 1000 tonnes or more per year chronic plant test and reproduction test for birds.

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 9.3 Fate and behaviour in the environment | |
| 9.3.4. Further information an degradation products | |
| 9.4 Terrestrial toxicity | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment according to Annex I indicates the need to investigate further the effects of the substance and/or degradation products on terrestrial organisms. The choice of the appropriate test(s) depends on the outcome of the chemical safety assessment.<br>These studies do not need to be conducted if direct and indirect exposure of the soil compartment is unlikely. |
| 9.4.6 Long-term toxicity testing on plants | |
| 9.6.1 Long-term or reproductive toxicity to birds | Any need for testing should be carefully considered taking into account the large mammalian dataset that is usually available at this tonnage level. |

In column 2 the adaptation possibilities are to be phrased similarly to Annexes VII-X. Clarification of the handling of surface treatments may affect the wording in column 2 and may also affect the test requirements (e.g. handling organic compounds for the surface treatment of inorganic materials).

## Appendix VI – Discussion paper on the handling of surface-treated nanomaterials with respect to the registration obligations of the REACH Regulation

as at: 14.11.2011

### A. Preliminary thoughts

Surface treatment of nanomaterials may lead to substantial changes of their properties. Since the high specific surface area of NMs can generally lead to modified properties when compared to bulk material, the question of properly addressing surface-treated NMs is an important challenge under REACH.

### B. Current situation

Frequently, NMs are subject to surface treatment. The aims of this treatment are to protect the surface from undesirable reactions or degradation, to prevent agglomeration and aggregation, to insert certain functional groups for specific reactions or to modify certain physical properties ( e.g. by suppression of photo-catalytic properties). For the surface treatment use can be made of substances whose nature is either organic or inorganic and which bond with the NM. Furthermore they can be applied hierarchically.

The surface treatment may influence and govern the risk profile of NMs to a crucial degree.

Up to now the legal handling of chemical surface treatments of NMs has not yet been clarified. In the FAQ for REACH[1] (point 6.3.8) there are remarks on surface-treated substances. It is not clear, however, whether this concept (registration obligation only for the precursors, taking account of the surface treatment there) is also applicable to NMs.

A detailed description of the subject of surface treatment can be found in the report RIPoN1 (pp. 27-35), where the different views of the representatives of industry and the member states/ECHA concerning that issue become obvious. In the report it is clearly elaborated that surface treatment can have a highly significant effect on the properties of NMs**.**

For surface-treated NMs there are the following regulatory options:

1. They are regarded as substances on their own.
2. They are regarded as a mixture of the reaction product at the surface with the inner, unmodified part of the NM.
3. Application of FAQ 6.3.8 for NMs.
4. They are regarded as a separate nanoform. Criteria are developed to decide under which conditions specific test requirements must be fulfilled for this particular nanoform.

<u>Option 1 – They are regarded as substances on their own</u>

It would be possible to regard the surface-treated NM as a substance on its own, which could be described as a reaction product of substance A (core material) and substance B (agent for surface treatment). Since with surface treatment basically any conceivable combination of different substances A and B would be possible, the problem of the extreme splitting of similar materials into various substances on their own would arise. The consequence would be that tonnage bands which trigger a registration obligation would not be reached. It is also challenging to develop clear criteria which would allow defining and checking under which conditions surface treatment results in a new substance and how the different surface treatments can be defined in relation to one another. The questions to be clarified here would include, for example, the type of binding which leads to a new substance and how resistant this would have to be to external influences.

Usually, such a heterogeneous material (in the interior of the particle the reagent A and on the surface the reaction product from substance A + substance B) would certainly not be regarded as a substance within the meaning of the substance definition.

[1] ECHA; Frequently Asked Questions about REACH - June 2010 - Version 3.1 [http://echa.europa.eu/doc/reach/reach_faq.pdf].

Option 2 – They are regarded as a mixture of the reaction product on the surface with the inner, unmodified part of the NM

Here the problem arises that the quantity of the reaction product on the surface is very small, but nevertheless has significant influence on the properties. As already mentioned in option 1, this leads to a situation where the necessary tonnage bands are hardly reached, but it also involves methodological difficulties. It will therefore hardly be practicable to gather data for the reaction product bound to the surface. It is suspected that its behaviour/reactivity would clearly differ from the same but unbound reaction products. The regulations for mixtures could cover the special features of such solids only to a very limited extent. The limits provided for in the Dangerous Preparations Directive or the CLP Regulation would not permit a proper classification of these materials. The substance present on the surface of the particles plays a substantially greater role in a homogenous mixture. This means that very small quantities (below the consideration limits of the CLP Regulation) could be decisive for the properties of the whole particle.

Option 3 – Application of FAQ 6.3.8 for NMs

If one were to apply FAQ 6.3.8. to surface-treated NMs as well, the product of the surface treatment would not be subject to registration. In the context of registrations of the precursors the surface treatment would have to be described. The surface treatment itself should be regarded here as a downstream application.

> FAQ 6.3.8. Do I have to register chemically surface-treated substances?
>
> The surface treatment of a substance is a “two dimensional” modification of macroscopic particles. A “two dimensional” modification means a chemical reaction between the functional groups only on the surface of a macroscopic particle with a substance which is called a surface treating substance.
> By this definition it becomes clear that this kind of modification means a reaction of only a minor part (surface) of a macroscopic particle with the surface treating substance, i.e. most of the macroscopic particle is unmodified.
> Therefore a chemically surface treated substance cannot be regarded as a mixture nor be defined by the criteria of the Guidance for identification and naming of substances under REACH. With the same reasoning, a chemically surface-treated substance could not be reported for EINECS nor be notified according to Directive 67/548/EEC because it was covered by the separate EINECS entries of both the basis substance (macroscopic particle) and the surface-treating substance.
>
> Taking this decision up under REACH means a consequent continuation of former decisions. Using the same line of arguments, chemically surface-treated substances should not be registered as such under REACH, but the following requirements should be fulfilled:
>
> 1. Registration of the basis substance (macroscopic particle)
> 2. Registration of the surface treating substance
> 3. Description of the use “surface treatment” in the registration dossier of the surface treating substance and in the registration dossier of the basis substance
> 4. Any specific hazards or risks of the surface treated substance should be appropriately covered by the classification and labelling and by the chemicals safety assessment and resulting exposure scenarios.

In discussions in RiPoN-1 ECHA explained that this FAQ cannot be applied to NMs. For NMs the surface-to-volume ratio increases so rapidly that the surface can no longer be regarded as a minor part of the substance. Modification of the surface therefore has to be regarded as a part of the manufacturing process. With NMs it must be expected that surface treatment will heavily influence desirable and undesirable properties of the NMs.

Without any further adaptations the application of the FAQ does not yield adequate information on the surface-treated material. The manufacturers of such materials would only be subject to the obligations of a downstream user. In particular there are no clear testing obligations for the modified material (but only for the educts).

Option 4 – They are regarded as a special nanoform. Criteria are developed under which conditions specific test requirements must be fulfilled with regard to the particular nanoform.

This option can only be formulated within the framework of the proposed modification of the REACH requirements in accordance with the *'characteriser'* approach.

The surface-treated NMs are regarded as a special nanoform of the treated starting material. This is only justified if the surface treatment does not cast doubt on the basic substance identity of the starting material. In terms of substance identity this could basically be assumed if, for example, 80% of the unmodified educt remains. If the value goes below that limit, a different substance is present. More concrete criteria may possibly have to be developed. The surface-treated NM has to be covered within the framework of the starting material registration. If the surface treatment is not carried out by the registrant of the starting material (and is also not covered by his registration), the one carrying out the surface treatment should be subject to the obligations of a manufacturer (e.g. obligation to register) within the meaning of the REACH Regulation. For the surface-treated NM the data required according to the foreseen Annex XVIII have to be provided. The requirements which also have to be fulfilled for different nanoforms shall apply here.

As for untreated NMs the registrant has to justify which of the nanoforms could be considered together in one registration. Criteria must be developed for this purpose. Basically, reference can be made to the deliberations in Appendix II where reference is already made to important criteria such as surface properties which come into play here in particular. Criteria must be formulated in detail on the guidance document level.

This option avoids any unnecessary splitting of the surface-treated NMs and at the same time ensures an appropriate information requirement. It should be noted that, at present, there is no standardised method for determining the degree of surface treatment.

## Appendix VII – Draft Annex XVIII

## STANDARD INFORMATION REQUIRED FOR NANOMATERIALS MANUFACTURED OR IMPORTED IN QUANTITIES OF ONE TONNE OR MORE

### Table 1: Characterisation

| Characterisation / Determination | Method (by example) |
|---|---|
| Outer form (form, length:width ratio) | Atomic force microscopy (AFM)<br>Transmission electronic spectroscopy (TEM) /Scanning electron microscopy (SEM)<br>Small-angle X-ray scattering (SAXS)<br>UV-VIS spectroscopy<br>Raman spectroscopy |
| Aggregation and agglomeration behaviour | Dynamic light scattering (DLS)[1]<br>Brunauer-Emmett-Teller method (BET)<br>Transmission electron microscopy (TEM)<br>Small-angle neutron scattering (SANS |
| Size distribution | Dynamic light scatter (DLS)[1]<br>Scanning mobility particle sizer (SMPS)[1]<br>Field flux fractioning (FFF)<br>Small-angle X-ray scatter (SAXS)<br>Nanoparticle trace analysis (NTA)<br>Ultracentrifugation |
| Specific surface area | Brunauer-Emmett-Teller method (BET)<br>NMR[2]<br>Small-angle X-ray scatter (SAXS)<br>Ultracentrifugation[3] |
| Surface activity | Auger electron spectroscopy<br>Ultraviolet photoelectron spectroscopy (UPS)<br>FT-IR<br>Chemisorption |
| Surface charge (zeta potential) | Isoelectric point (IEP)<br>Particle charge sizer (PCS)<br>Dynamic light scattering (DLS)[1]<br>Electrophoretic mobility(EPM) |
| UV/VIS Spectrum | |
| Crystalline structure or modification | X-ray diffraction (XRD)<br>TEM+FT |

[1] Although suitable primarily for approximately spherical particles, the result obtained is defective in relation to other nanoforms, such as rods.

[2] Depending on the composition of the NM, may only be possible using special NMR devices

[3] Applicable where the specific surface has a measurable effect on the distribution behaviour of the NM

**Table 2: Nano-specific test programme > 1 t/year**

| **COLUMN 1**<br>**STANDARD INFORMATION REQUIRED** | **COLUMN 2**<br>**SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1** |
|---|---|
| **Physicochemical information** | |
| 7.1. State of the substance at 20°C and 101,3 kPa | |
| 7.2. Melting/freezing point | |
| 7.3. Boiling point | The study does not need to be conducted if there is available information on the bulk material because the melt, which no longer represents a nanomaterial, passes into the gaseous state. |
| 7.4. Relative density | |
| 7.5. Vapour pressure | The study/ies do(es) not generally need to be conducted because:<br>• the vapour pressure is not significant with very high melting and boiling points<br>• often the melt, which no longer represents a nanomaterial, is evaporated (dynamic method), or the vapour pressure is overestimated due to aerosol formation. |
| 7.6. Surface tension | The study only needs to be conducted if there is sufficiently high water solubility and there is no available information on the bulk material. Otherwise the value determined for the bulk material can be taken because it involves the examination of dissolved material which no longer represents a nanomaterial. |
| 7.7. Water solubility | |
| 7.8. Partition coefficient n-octanol/water | The study only needs to be conducted if there is sufficiently high water solubility. The value determined for the bulk material can be taken because it involves consideration of dissolved material which no longer represents a nanomaterial. |
| Technical characteristics:<br>o Dispersibility/stability<br>o Dustiness | |
| 7.9. Flash-point | Methods should be adapted (e.g. to smaller quantities of test substance) |
| 7.10. Flammability | |
| 7.11. Explosive properties | |
| 7.12. Self-ignition temperature | |
| 7.13. Oxidising properties | |
| **Toxicological information** | |
| 8.1. Skin irritation or skin corrosion<br>8.1.1. The assessment of this endpoint shall comprise the following consecutive steps:<br>(1) an assessment of the available human and animal data,<br>(2) an assessment of the acid or alkaline reserve,<br>(3) *in vitro* study for skin corrosion,<br>(4) *in vitro* study for skin irritation.<br>OECD TG 439 | 8.1.1. Steps 3 and 4 do not need to be conduced for the nanomaterial if:<br>— the available information indicates that the criteria are met for classification of the nanomaterial as corrosive to the skin or irritating to eyes, or if classification of the substance in bulk form as corrosive to the skin or irritating to eyes can be transferred, or<br>— referencing of classification as corrosive to the skin or irritating to the eyes from another nanoform can be justified, or |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | — the nanomaterial is flammable in air at room temperature, or<br>— the substance in bulk form or the nanomaterial is classified as very toxic in contact with skin, or<br>— an acute toxicity study of the nanomaterial by the dermal route does not indicate skin irritation up to the limit dose level (2 000 mg/kg body weight). |
| 8.1.2. *In vivo* skin irritation<br>OECD TG 404 | 8.1.2. A study of the nanomaterial does not need to be conducted if:<br>— administration by the dermal route can be exluded, or<br>— the substance in bulk form or the nanomaterial is classified as corrosive or irritating to the skin, or<br>— the nanomaterial is a strong acid (pH < 2,0) or base (pH > 11,5), or<br>— the nanomaterial is flammable in air at room temperature, or<br>— the nanomaterial is classified as very toxic in contact with skin, or<br>— an acute toxicity study of the nanomaterial by the dermal route does not indicate skin irritation up to the limit dose level (2 000 mg/kg body weight).<br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred. |
| 8.2. Eye irritation<br>8.2.1. The assessment of this endpoint shall be performed by three steps.<br>(1) assessment of the available human and animal data,<br>(2) assessment of the acid or alkaline reserve,<br>(3) *in vitro* study for eye irritation.<br>OECD TG 437/438 | 8.2.1. Step 3 does not need to be conducted for the nanomaterial if:<br>— the available information indicates that the criteria are met for classification as corrosive to the skin or irritating to eyes, or<br>— the classification of the substance in bulk form as corrosive to the skin or irritating to eyes can be transferred to the nanomaterial;<br>— the nanomaterial is flammable in air at room temperature.<br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred. |
| 8.2. Eye irritation<br>8.2.2. *in vivo* eye irritation | 8.2.1. A study of the nanomaterial does not need to be conducted if: |

| **COLUMN 1**<br>**STANDARD INFORMATION REQUIRED** | **COLUMN 2**<br>**SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1** |
|---|---|
| OECD TG 405 | — the substance in bulk form or the nanomaterial is classified as irritating to eyes with risk of serious damage to eyes, or<br><br>— the substance in bulk form or the nanomaterial is classified as corrosive to the skin and provided that the registrant classified the substance as eye irritant, or<br><br>— the nanomaterial is a strong acid (pH < 2,0) or base (pH > 11,5), or<br><br>— the nanomaterial is flammable in air at room temperature.<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred. |
| 8.3. Skin sensitisation<br><br>The assessment of this endpoint shall comprise the following two consecutive steps:<br><br>(1) assessment of the available human, animal and other data,<br><br>(2) *in vivo* testing.<br><br>OECD TG 429 | 8.3. Step 2 does not need to be conducted for the nanomaterial if:<br><br>— the substance in bulk form is classified as „high-potency“ sensitising, and classification is transferred to the nanomaterial, or<br><br>— the available information indicates that the nanomaterial should be classified for skin sensitisation or corrosivity, or<br><br>— the nanomaterial is a strong acid (pH < 2,0) or base (pH > 11,5), or<br><br>— the nanomaterial is flammable in air at room temperature.<br><br>The Murine Local Lymph Node Assay (LLNA) is the first-choice method for *in vivo* testing of substances in bulk form. Where there is *lege artis* no available evidence to the contrary, this testing is also recommended for nanomaterials. Another test should be applied in case of inappropriateness (e.g. for metals). Justification for the use of another test shall be provided.<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred. |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 8.4. Mutagenicity[4]<br><br>8.4.1. *In vitro* gene mutation study<br>in bacteria<br>8.4.2. *In vitro* gene mutation study<br>in mammalian cells<br><br>8.4.3 *In vitro* cytogenic study in mammalian cells or *in vitro* micronucleus study | 8.4. Usually,the study does not need to be conducted if:<br>— the substance in bulk form is classified as mutagenic category 1A or 1B, or<br>— the substance in bulk form or the nanomaterial is known to be carcinogenic category 1A or 1B, or mutagenic category 1A or 1B,<br>and classification is transferred to the nanomaterial.<br><br>8.4.2. Usually the study does not need to be conducted if:<br>— adequate data from a reliable *in vivo* mammalian gene mutation test are available.<br><br>8.4.3. Usually the study does not need to be conducted<br>— if adequate data of the nanomaterial from an *in vivo* cytogenetic test are available;<br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred.<br>Further *in vivo* mutagenicitiy studies shall be considered in case of a positive result. |
| 8.5. Acute toxicity | 8.5. The study/ies do(es) not generally need to be conducted if:<br>— the nanomaterial is classified as corrosive to the skin. |
| 8.5.1. By inhalation route | 8.5.1 Alternatively, testing by the oral route is appropriate if exposure of humans via inhalation of aerosols, particles or droplets can be exluded. |
| **Ecotoxicological information** | |
| 9.1 Aquatic toxicity | |
| 9.1.2 Growth inhibition study aquatic plants (algae preferred) | A study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur. |

[4] The OECD Environment Directorate recommends a test of nanomaterials for genotoxicity using an array of the three established *in vitro* studies (TG 471, 474 und 476) because it is being discussed which test leads to non-nano-specific false information despite usability in principle [OECD2009].

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| 9.1.2 Long-term toxicity testing on invertebrates (preferred species Daphnia) | A study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur. |
| 9.1.3 Short-term toxicity testing on fish: the registrant may consider long-term toxicity testing instead of short-term | The study does not need to be conducted if:<br>— there are mitigating factors indicating that aquatic toxicity is unlikely to occur. ;<br>— a long-term aquatic toxicity study on fish is available.<br>Long-term aquatic toxicity testing within the meaning of No. 9.1.6 in Annex XVIII shall be considered if the chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms. The choice of appropriate test(s) will depend on the results of the chemical safety assessment. The long-term aquatic toxicity study on fish (Annex IX Section 9.1.6) shall be considered if the substance is poorly soluble in water. |
| 9.1.4 Activated sludge respiration inhibition testing | A study does not need to be conducted if:<br>— there is no emission to a sewage plant, or<br>— there are mitigating factors indicating that microbial toxicity is unlikely to occur, or<br>— the substance is found to be readily biodegradable and the applied test concentrations are in the range of concentrations that can be expected in the influent of a sewage treatment plant. The study may be replaced by a nitrification inhabitation test if available data show that the substance it likely to be an inhibitor of microbial growth or function, in particular nitrifying bacteria. |
| 9.2 Degradation | To be phrased later. |
| 9.2.1 Biotic | |
| 9.2.1.1 Ready biodegradability | |
| 9.2.2 Abiotic | |
| 9.2.2.1 Hydrolysis as a function of pH | |
| 9.3 Fate and behaviour in the environment | To be phrased later. |
| 9.3.1 Adsorption/desorption screening | |

**Table 3: Nano-specific test programme > 10 t/year**

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| **Toxicological information** | |
| 8.5. Acute toxicity | 8.5. Generally, the study/ies do(es) not need to be conducted if:<br>— the nanomaterial is classified as corrosive to the skin. In addition to the inhalation route (8.5.1), for substances other than gases, the information mentioned under 8.5.2 and 8.5.3 shall be provided for at least one other route. The choice for the |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | second route will depend on the nature of the nanomaterial and the likely route of human exposure. If there is only one route of exposure, information for that route only needs to be provided.<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred. |
| 8.5.2. by oral route<br><br>OECD TGs 420, 423 or 425 [OECD2009]<br><br>8.5.3. by dermal route | 8.5.2. Testing by the oral route is appropriate if exposure of humans via ingestion cannot be excluded.<br><br>8.5.3. Testing by the dermal route is appropriate if<br><br>(1) dermal contact in production and/or use of the nanomaterial is likely and<br><br>(2) physicochemical and toxicological properties of the nanomaterial suggest potential for a significant rate of dermal resorption. |
| 8.6. Repeated dose toxicity<br><br>8.6.1. Short-term repeated dose toxicity study (28 days), one species, male and female, with extended duration of follow-up (≥28d);[5] most appropriate route of administration, having regard to the likely route of human exposure. | 8.6.1. The short-term toxicity study (28 days) for the nanomaterial does not need to be conducted if:<br><br>— a reliable sub-chronic (90 days) or chronic toxicity study of the nanomaterial is available, provided that an appropriate species, dosage, solvent and route of administration were used;; or<br><br>— where the nanomaterial undergoes immediate disintegration and there are sufficient data on the degradation products; or<br><br>— relevant human exposure can be excluded in accordance with Annex XI Section 3.<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred and adequate data to support a robust risk assessment are available.<br><br>The appropriate route shall be chosen on the following basis:<br><br>Testing by the inhalation route is appropriate where human exposure via inhalation cannot be exluded. |

[5] Length of follow-up and study parameters must be adapted to the state of knowledge of relevant international committees (e.g. according to the OECD Test Guidelines or the REACH Test Method Regulation).

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | Testing by the oral route is appropriate if:<br><br>— where exposure via inhalation can be exluded, and<br><br>— where the conditions for the dermal route according to 8.5.3 are not given<br><br>Testing by the dermal route is appropriate if:<br><br>(1) dermal contact in production and/or use of the nanomaterial is likely and<br><br>(2) the physicochemical and toxicological properties of the nanomaterial suggest potential for a significant rate of dermal resorption.<br><br>The sub-chronic toxicity study (90 days) (Annex IX, Section 8.6.2) shall be proposed by the registrant if: the frequency and duration of human exposure indicates that a longer term study is appropriate,<br><br>and one of the following conditions is met:<br><br>— other available data indicate that the nanomaterial may have a dangerous property that cannot be detected in a short-term toxicity study, or<br><br>— appropriately designed toxicokinetic studies reveal accumulation of the nanomaterial, its degradation products or its metabolites in certain tissues or organs which would possibly remain undetected in a short-term toxicity study but which are liable to result in adverse effects after prolonged exposure.<br><br>Further studies regarding the nanomaterial shall be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 in case of:<br><br>— failure to identify a NOAEL in the 28 or the 90 days study, unless the reason for the failure to identify a NOAEL is absence of adverse toxic effects, or<br><br>— particular concern regarding the toxicity of the nanomaterial in the 28 days test (e.g. serious/severe effects), or<br><br>— indications of nano-specific effects or particle deposition in organs for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity, cardiovascular system), or<br><br>— the route of exposure used in the initial repeated dose study was inappropriate in relation to the |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | expected route of human exposure and route-to-route extrapolation cannot be made, or<br><br>— particular concern regarding exposure (e.g. use of the nanomaterial in consumer products leading to exposure levels which are close to the dose levels at which toxicity to humans may be expected), or<br><br>— effects shown in nanomaterials with a clear relationship in molecular structure with the nanomaterial being studied, were not detected in the 28 or the 90 days study. |
| 8.7. Reproductive toxicity<br><br>8.7.1. Screening for reproductive/developmental toxicity, one species (OECD 421 or 422), if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant | 8.7.1. This study does not need to be conducted if:<br><br>— classification of the substance in bulk form as reproductive toxic category 1A or 1B is transferred to the nanomaterial, or<br><br>— where it can be justified, one nanomaterial is referenced to other nanomaterials of the same substance, if the classification (category 1A or 1B) referred to is transferred, or<br><br>— the substance in bulk form or the nanomaterial is known to be a genotoxic carcinogen and appropriate risk management measures are implemented, or<br><br>— the substance in bulk form or the nanomaterial is known to be a germ cell mutagen and appropriate risk management measures are implemented, or<br><br>— relevant human exposure can be excluded in accordance with Annex XI, Section 3, or<br><br>— a pre-natal developmental toxicity study of the nanomaterial (Annex IX, Section 8.7.2) or a two-generation reproductive toxicity study (Annex IX, Section 8.7.3) is available.<br><br>If a nanomaterial is known to have an adverse effect on fertility, meeting the criteria for classification as Repr Cat 1A or B1, and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for developmental toxicity must be considered.<br><br>If a nanomaterial is known to cause developmental toxicity, meeting the criteria for classification as Repr Cat 1A or 1B, and the available data are adequate to support a robust risk assessment, then no further testing for developmental toxicity will be necessary. However, testing for effects on fertility must be considered.<br><br>In cases where there are serious concerns about |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | the potential for adverse effects on fertility or development, either a pre-natal developmental toxicity study (Annex XVIIIc, Section 8.7.2) or a two-generation reproductive toxicity study (Annex XVIIIc, Section 8.7.3) may be proposed by the registrant instead of the screening study.<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred and adequate data to support a robust risk assessment are available. |
| 8.8. Toxicokinetics<br><br>8.8.1. Assessment of the toxicokinetic behaviour of the substance to the extent that can be derived from the relevant available information<br><br>8.8.2 OECD TG 417 study, modified where appropriate | 8.8.2 The study does not need to be conducted if a quantitative assessment can be performed according to 8.8.1 |
| **Ecotoxicological information** | |
| 9.1 Aquatic toxicity | |
| 9.1.6 Long-term toxicity testing on fish | A study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely. |
| 9.2 Degradation | |
| 9.2.1 Biotic | |
| 9.2.1.2 Simulation testing on ultimate degradation in surface water | To be phrased later. |
| 9.2.1.3 Soil simulation testing (for substances with a high potential for adsorption to soil) | To be phrased later. |
| 9.2.1.4 Sediment simulation testing (for substances with a high potential for adsorption to sediment) | To be phrased later. |
| 9.3 Fate and behaviour in the environment | |
| 9.3.2 Bioaccumulation in aquatic species, preferably fish | To be phrased later. |
| 9.3.3 Further information on adsorption/desorption depending on the results of the study required in Anhang XVIIIa | To be phrased later. |
| 9.4 Effects on terrestrial organisms | These studies do not need to be conducted if direct or indirect exposure of the soil compartment is unlikely.<br>The choice of the appropriate test(s) depends on the outcome of the chemical safety assessment. In particular for substances that have a high potential to adsorb to soil and that are very persistent, the registrant shall consider long-term toxicity testing instead of short-term. |
| 9.4.1 Short-term toxicity to invertebrates | |
| 9.4.2 Effects on soil micro-organisms | |
| 9.5.1 Long-term toxicity to sediment organisms | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment indicate the need to investigate further |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | the effects of the substance and/or relevant degradation products on sediment organisms. The choice of the appropriate test(s) depends on the results of the chemical safety assessment. |

**Tabelle 4: Nano-specific test programme > 100 t/year**

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| **Toxicological information** | |
| 8.4. Genotoxicity<br>8.4.1. *In vivo* gene mutation study<br>8.4.2 *In vivo* cytogenetic study or *in vivo* micronucleus study | 8.4. If there is a positive result for the nanomaterial in any of the *in vitro* genotoxicity studies in Annex XVIIIa or XVIIIb and no results of an *in vivo* study are available, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the registrant.<br>If there are positive results for the nanomaterial from an *in vivo* somatic cell study, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.<br>The study does not need to be conducted if<br>— the substance in bulk form is classified as mutagenic category 1A or 1B and the classification is transferred to the nanomaterial. |
| 8.6. Repeated dose toxicity<br>8.6.1. Sub-chronic toxicity study (90 days), one species, rodent, male and female, with extended duration of follow-up (≥90d);[6] most appropriate route of administration, having regard to the likely route of human exposure. | 8.6.2. The sub-chronic toxicity study (90 days) does not need to be conducted if:<br>—the substance in bulk form is classified as STOT RE Cat 1 and the available data of the nanomaterial are adequate to support a robust risk |

[6] Length of follow-up and study parameters must be adapted to the state of knowledge of relevant international committees (e.g. according to the OECD Test Guidelines or the REACH Test Method Regulation).

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | assessment, or<br><br>— a reliable chronic toxicity study of the nanomaterial is available, provided that an appropriate species and route of administration were used, or<br><br>— a nanomaterial undergoes immediate disintegration and there are sufficient data on the degradation products (both for systemic effects and effects at the port of enty).<br><br>Aforementioned exemptions for waiving tests can, where justified, be referenced from one nanomaterial to other nanomaterials of the same substance, if the classification referred to is transferred and adequate data to support a robust risk assessment are available.<br><br>The appropriate route shall be chosen on the following basis:<br><br>Testing by the inhalation route is appropriate if:<br><br>— exposure of humans via inhalation cannot be exluded.<br><br>Testing by the oral route is appropriate if:<br><br>— exposure via inhalation can be exluded, and<br><br>— dermal route is unlikely.<br><br>Testing by the dermal route is appropriate if:<br><br>(1) skin contact in production and/or use of the nanomaterial is likely; and<br><br>(2) the physicochemical properties of the nanomaterial suggest a significant rate of absorption through the skin; and<br><br>(3) one of the following conditions is met:<br><br>— toxicity is observed in the acute dermal toxicity test at lower doses than in the oral toxicity test, or<br><br>— systemic effects or other evidence of absorption of the substance is observed in skin and/or eye irritation studies, or<br><br>— *in vitro* tests indicate significant dermal absorption, or<br><br>— significant acute dermal toxicity or dermal penetration is recognised for structurally-related nanomaterials.<br><br>Further studies of the nanomaterial shall be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 in |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | case of:<br><br>— failure to identify a NOAEL in the 90 days study unless the reason for the failure to identify a NOAEL is absence of adverse toxic effects, or<br><br>— particular concern regarding the toxicitiy of the nanomaterial (e.g. serious/severe effects), or<br><br>— indications of nano-specific toxic effects or particle deposition in organs for which the available evidence is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform nano-specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity, cardiovascular toxicity, nano-specific distribution), or<br><br>— particular concern regarding the exposure (e.g. the use of the nanomaterial in consumer products leading to exposure levels which are close to the dose levels at which toxicity to humans may be expected). |
| 8.7. Reproductive toxicity | 8.7. A study of the nanomaterial does not need to be conducted if:<br><br>— the substance in bulk form is classified as reproductive toxic Cat 1A or 1B and classification is taken over for the nanomaterial, or<br><br>— where it can be justified, one nanomaterial is referenced to other nanomaterials of the same substance when the classification referred to is transferred, or<br><br>— the substance in bulk form or the nanomaterial is known to be genotoxic carcinogen and appropriate risk management measures are implemented, or<br><br>— the nanomaterial is known to be a germ cell mutagen and appropriate risk management measures are implemented.<br><br>If a nanomaterial is known to have an adverse effect on fertility, meeting the criteria for classification as Repr Cat 1A or 1B, and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for developmental toxicity must be considered.<br><br>If a nanomaterial is known to cause developmental disturbances, meeting the criteria for classification as reproductive toxic Cat 1A or 1B, and the available data are adequate to support a robust risk assessment, then no further testing for developmental toxicity will be necessary. However, |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | testing for effects on fertility must be considered.<br><br>8.7.2. The study of the nanomaterial shall be initially performed on one species. A decision on the need to perform a study at this tonnage level or the next on a second species should be based on the outcome of the first test and all other relevant available data.<br><br>The study does not need to be conducted if the substance in bulk form has been classified as reprotox. Cat 1A or 1B on the basis of a former positive screening study and is taken over for the nanomaterial. |
| 8.7.2. Pre-natal developmental toxicity study, one species, most appropriate route of administration, having regard to the likely route of human exposure (B.31 of the Commission Regulation on test methods as specified in Article 13 (3) or OECD 414).<br><br>8.7.3. Two-generation reproductive toxicity study, on species, male and female, most appropriate route of administration, having regard to the likely route of human exposure, if the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues. | 8.7.3. The study of the nanomaterial shall be initially performed on one species. A decision on the need to perform a study at this tonnage level or the next on a second species should be based on the outcome of the first test and all other relevant available data. |
| **Ecotoxicological information** | |
| 9.2 Degradation | Further biotic degradation testing shall be proposed if the chemical safety assessment according to Annex I indicates the need to investigate further the degradation of the substance and its degradation products. The choise of appropriate test(s) depends on the results of the chemical safety assessment and may include simulation testing in appropriate media (e.g. water, sediment or soil). |
| 9.2.1 Identification of degradation products | |
| 9.3 Fate and behaviour in the environment | |
| 9.3.4. Further information on the environmental fate and behaviour | Further testing shall be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 if the chemical safety assessment according to Annex 1 indicates the need to investigate further the environmental fate and behaviour of the substance. The choice of appropriate test(s) depends on the results of the chemical safety assessment. |
| 9.4 Effects on terrestrial organisms | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment according to Annex I indicates the need to investigate further the effects of the substance and/or degradation products on terrestrial organisms. The choice of the appropriate |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | test(s) depends on the outcome of the chemical safety assessment. These studies do not need to be conducted if direct and indirect exposure the the soil compartment is unlikely. |
| 9.4.4 Long-term toxicity to invertebrates | |
| 9.4.3 Short-term toxity to plants | |

**Table 5: Nano-specific test programme > 1000 t/year**

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| **Toxicological information** | |
| 8.4. Genotoxicity | 8.4. If there is a positive result in any of the *in vitro* genotoxicity studies in Annexes VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all available data.<br>If there is a positive result from an *in vivo* somatic cell study available, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered. |
| 8.6.3. Long-term toxicity | 8.6.3. A long-term repeated toxicity study (≥ 12 months) may be proposed by the registrant or required by the Agency in accordance with Articles 40 or 41 if the frequency and duration of human exposure indicates that a longer term study is appropriate and one of the following conditions is met:<br>— serious or severe toxicity effects of the nanomaterial of particular concern were observed in the 28-day or 90-day study for which the available evidence is inadequate for toxicological evaluation or risk charaterisation, or<br>— effects shown in nanomaterials with a clear relationship in molecular structure with the nanomaterial being studied were not detected in the 28-day or 90-day study, or<br>— the nanomaterial may have a dangerous property that cannot be detected in a 90-day study.<br>A case-by-case consideration shall be appropriate. |
| | 8.6.4. Further studies of the nanomaterial shall be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 in case of:<br>— toxicity of particular concern (e.g. |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| | serious/severe effects), or<br><br>— indications of nano-specific effects and particle deposition in organs for which the available evidence is inadequate for toxicological evaluation and/or risk characterisation. In such cases it may be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity, cardiovascular toxicity), or<br><br>— particular concern regarding exposure (e.g. the use of the nanomaterial in consumer products leading to exposure levels which are close to the dose levels at which toxicity is observed). |
| 8.7. Reproductive toxicity | 8.7. The studies need not be conducted if:<br><br>— the nanomaterial is known to be a genotoxic carcinogen and appropriate risk management measures are implemented, or<br><br>— the nanomaterial is known to be a germ cell mutagen and appropriate risk management measures are implemented, or<br><br>— the nanomaterial is of low toxicological acitivity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and there is no or no significant human exposure.<br><br>If a nanomaterial is known to have an adverse effect on fertility, meeting the criteria for classification as Repr Cat 1A or 1B, and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for developmental toxicity must be considered.<br><br>If a nanomaterial is known to cause developmental toxicity, meeting the criteria for classification as Repr Cat 1A or 1B, and the available data are adequate to support a robust risk assessment, then no further testing for developmental toxicity will be necessary. However, testing for effects on fertility must be considered. |
| 8.7.2. Developmental toxicity study, one species, most appropriate route of administration, having regard to the likely route of human exposure (OECD 414). | |
| 8.7.3. Two-generation reproductive toxicity study, | |

| COLUMN 1<br>STANDARD INFORMATION REQUIRED | COLUMN 2<br>SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1 |
|---|---|
| one species, male and female, most appropriate route of administration, having regard to the likely route of human exposure, unless already provided as part of Annex IX requirements | |
| 8.9.1. Carcinogenicity study | 8.9.1. A carcinogenicity study may be proposed by the registrant or may be required by the Agency in accordance with Articles 40 or 41 if:<br>— the nanomaterial has a widespread dispersive use or there is evidence of frequent or long-term human exposure, and<br>— the nanomaterial is classified as mutagen category 2 or there is evidence from the repeated dose study(ies) that the substance is able to induce hyperplasia, pre-neoplastic lesions and/or other modifications, giving evidence of nano-specific organic lesions.<br>— there is evidence of carcinogenic potential gained from knowledge of structurally related nanomaterials.<br>If the nanomaterial is classified as mutagen category 1A or 1B, the default presumption would be that a genotoxic mechanism for carcinogenicity is likely. In these cases, a carcinogenicity test will normally not be required.<br>A case-by-case consideration shall be appropriate. |
| **Ecotoxicological information** | |
| 9.3 Fate and behaviour in the environment | |
| 9.3.4. Further information on degradation products | |
| 9.4 Effects on terrestrial organisms | Long-term toxicity testing shall be proposed by the registrant if the results of the chemical safety assessment according to Annex I indicates the need to investigate further the effects of the substance and/or its degradation products on terrestrial organisms. The choice of the appropriate test(s) depends on the outcome of the chemical safety assessment. These studies do not need to be conducted if direct or indirect exposure of the soil compartment is unlikely. |
| 9.4.6 Long-term toxicity to plants | |
| 9.6.1 Long-term or reproductive toxicity to birds | Any need for testing should be carefully considered taking into accout the large mammalian dataset that is usually available at this tonnage level. |