Characterisation of nanomaterials for REACH dossiers - best practice

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Outline

- Nanomaterials under REACH: reflections from 2010 registrations
- The EC recommendation for nanomaterials definition-implications
- Characterising nanomaterials - methods and key information to report
- Conclusions

Nanomaterials registered by 2010 (1)

- No agreed EC definition of nanomaterials available at the time

- No specific provisions for nanomaterials
  - Nevertheless, REACH applies to nanomaterials

- Discussions on REACH Implementation projects on nanomaterials (RIP-oN) ongoing

- IUCLID provided two tick boxes that allowed registrants to indicate if nanomaterials are included in the dossier

Nanomaterials registered by 2010 (2)

- JRC and ECHA assessed 25 dossiers covering nanomaterials submitted by the 2010 registration deadline
- The project involved an assessment of the information included in nanomaterial registration dossiers, and their adequacy
- Results can be found at:

http://ec.europa.eu/environment/chemicals/nanotech/pdf/jrc_report.pdf

Nanomaterials registered by 2010 (3)

- Key shortcomings noted:

  - Insufficient description of scope of registration in terms of nanoforms

  - Lack of identification/characterisation for each nanoform for each registrant (lead/member registrant).

  - Different forms not addressed transparently throughout dossier (including endpoints, manufacturing process, classification and labelling, uses, as well as possible exposure assessment and risk characterisation).

Nanomaterials - looking forward

- Significant advancement has taken place since 2010 registrations

  - EC recommendation for the definition of a nanomaterial
  - Publication of RIP-oN results
  - Updated guidance documents for nanomaterials

- ECHA aims to provide registrants with best practices that can be used to improve the quality and transparency of nanomaterial registration dossiers

Nanomaterial characterisation

- This webinar focuses specifically on characterisation of nanomaterials, however, other aspects play a significant role in quality of dossier

- Two key issues:
  - Does my dossier cover nanomaterials?
  - How should I characterise my nanomaterial?

- Substance identification: sufficient information on scope of nanomaterials covered in dossier needed.

Nanomaterial definition

- A substance is considered a nanomaterial if:
  - 50% of particles by <u>number</u> 1-100 nm in one or more dimensions
  - Volume specific surface area >60 m^2/cm^3

- The definition also includes particles in agglomerates or aggregates whenever the <u>constituent</u> particles are in the size range 1 nm-100 nm

- In specific cases, the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

Nanomaterial definition - implications (1)

- The definition is based solely on size, not on hazard or risk
  - Nanomaterial does not automatically imply the substance is hazardous
- The definition itself does not create new information requirements on REACH registration dossiers
- However, it provides clarity on what is considered a nanomaterial
- Registrants should consider how they comply with the REACH information requirement if they have a nanomaterial

Nanomaterial definition - implications (2)

- Key question: does my dossier cover nanomaterial(s)?
  - Need sufficient information on particle size distribution and/or
  - Need sufficient information on surface area

Nanomaterials: characterising size

- REACH information requirement: granulometry: Annex VII, 7.14
- Granulometry can cover different information on particle size:
  - Particle size vs. particle size distribution
  - Number based vs. mass/volume based distribution
  - Constituent (primary) particle size
  - Aggregates: particles bound by strong forces
  - Agglomerates: particles bound by weak forces

Nanomaterials: characterising size (2)

- Information on constituent particle size distribution by number needed
- Other information is also useful, and should be included:
  - e.g. information on aggregation/agglomeration may be useful for exposure assessment
  - Information on dustiness may be useful
- Particle size may vary significantly depending on manufacturing method/between different registrants
  - Some registrants may manufacture a nanomaterial, while others might not. Which size should be submitted?

Nanomaterials: characterising size (3)

- General recommendations:

  - Include different information on particle size distribution (primary particle size by number, agglomeration, aggregation), as this information is complimentary. Different types of particle size may serve different functions

  - As particle size may vary significantly by manufacturer, include information from different manufacturers (in case of joint submissions)

  - Other properties may also vary significantly - registrants should consider if the available information on other forms is sufficient for their substance

Particle size: challenges (1)

- The EC recommendation for the definition of nanomaterials does not refer to any measurement method:
  - **Question:** which method should I use?
  - **Answer:** no single method can cover all size ranges-not unique to nanomaterials
- Particle size distribution is method specific
- Each method has advantages and pitfalls

Particle size: challenges (2)

- Registrants should tailor their particle size characterisation for their particular substance
  - In-house methods/industry developed methods can be used for characterisation

  - e.g. data that are generated for QC purposes for manufacturing of the NM

  - Use a variety of methods to characterise different aspects of particle size

Examples of commonly used methods for characterising nanomaterials: advantages and disadvantages

Measuring particle size: electron microscopy

- Optical microscopy:
  - Limited resolution: $d = \lambda/2NA$. With visible light $\lambda = 550nm$, $NA = 1.5$ maximum resolution 0.2 nm
- Electron microcopy overcomes this limit as electrons have a much shorter wavelength (100 keV electron beam 3.7 pm)
- Can achieve very high magnifications/high resolutions
- SEM: Scanning Electron Microscope
- TEM: Transmission electron microscope
- SEM can be combined with EDX to give information on chemical composition

Electron microscopy: challenges (1)

- Air will interact with electron beam, therefore need a high vacuum environment.
  - This can have an impact on samples: vacuum/drying samples can affect size
- Need for a conductive sample:
  - Non-conductive samples can be imaged, but may require a thin layer of a conductive material
- Equipment can be expensive
- Specialist training needed

Electron microscopy: challenges (2)

- Counting: in order to achieve a reliable narrow mean particle size or particle size distribution, a large number of particles need to be measured
- Need of a representative sample

Electron microscopy in a REACH dossier

- What should be included in a study summary (minimum):
  - A representative image (or more than one, preferably at different magnifications)
  - A description of the method used, number of particles counted
  - Any sample preparation (e.g. sputter coating)
  - Aggregate/agglomerate/primary particle?
  - Particle size distribution, a mean value is not sufficient

Dynamic Light Scattering (DLS) (1)

- Principle:
  - Particles in a solution subjected to a light source (laser) resulting in scattering
  - Time dependent fluctuation in scattering due to Brownian motion of particles
  - Fluctuation in intensity converted to size/size distribution using mathematical equations (Stokes-Einstein relationship), gives a polydispersity index

Dynamic Light Scattering (DLS) (2)

- Advantages
  - Simple, fast, easy to use, cheaper compared to electron microscopy
- Disadvantages
  - Assumes spherical particles (high aspect ratio substances should not be used)
  - Dispersion is a problem
  - Agglomeration is a problem
  - Reports volume based distributions
  - Some instruments also can give number distributions, but large errors possible
- A laser scattering method (ISO 13320:2009)available, but not specific to nanomaterials

DLS in a REACH dossier

- What should be included in a study summary (minimum):
  - Description of sample preparation is important for DLS:
    - Details of any methods used for dispersion (e.g. sonication time)
    - Solvent used - solvent refractive index should be entered when doing the analysis
    - Any dispersing agent (identity and concentration used)
    - Concentration

Other possible methods (1)

- Small Angle Xray Scattering (SAXS):
  - Size range: 1-300 nm
  - Cannot distinguish pores from particles
  - Assumes spherical particles, not suitable for non-spherical particles
  - Cannot be used for powders consisting of porous particles

Other possible methods (2)

- Differential mobility analyser (for aerosols)
- Scanning mobility Particle Sizer (aerosols)
- Nanoparticle Tracking Analysis (in suspensions)
- Aerosol Time of Flight Mass Spectroscopy (aerosols)
- Aerosol Particle Mass Analyzer (APM)

A word on aggregation/agglomeration

- Aggregate:

  a particle comprising of strongly bound or fused particles

- Agglomerate:

  collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components

- Stability/evolution of particles (high vs. low energy)

- It is possible to use surfactants, physical measures to disperse particles

Conclusion on particle size

- Different methods, each with its own advantages/ disadvantages

- It is best to use more than one method to characterise size

- Standardised methods for measuring number based particle size distributions are not yet available

- Further information on usefulness of various methods can be found at:

http://publications.jrc.ec.europa.eu/repository/ bitstream/111111111/26399/1/irmm_nanomaterials %20%28online%29.pdf

Surface area (1)

- Surface area: a proxy for size
- Relation to toxicity (mechanisms): dose response curves normalised for surface area give different results compared to per mass basis
  - Measured using a gas adsorption isotherm
  - Expose substance to a gas (usually nitrogen, but others such as carbon dioxide, argon, krypton used) at different pressures, measure adsorption
  - Calculate surface area based on Brunauer, Emmett, Teller (BET) theory (computer)

Surface area (2)

- Advantages:
  - Relatively easy to perform and obtain results
  - Can be used to substitute for number based particle size distribution

- Disadvantages:
  - Only possible for solids
  - Need to dry samples and vacuum and heat
  - Some substance have a high surface area, but are not necessarily nanomaterials, instead they have highly porous structures (e.g. activated carbon, MOFs)

Surface area in a REACH dossier

- Preferably expressed as volume specific surface area
- Should include the following information:
  - Method description (note: ISO 9277:2010-Determination of the specific surface area of solids by gas adsorption-BET method)
  - Any sample preparation (e.g. degassing, temperature, vacuum used)
  - Identity of gas used
  - Adsorption isotherm

Shape

- Relationship between shape and chemical reactivity, and potentially (eco)toxicity (carbon nanotubes)
- Shape shown to influence uptake by cells
- Large number of structures (spheres, triangles, prisms, rods, tubes, onions)
- Usually requires microscopy

General issues with nanomaterial characterisation

- Sample preparation: can affect results significantly:
  - Physical methods (e.g. sonication)
  - Chemical environment (solvent, dispersing agents)
- Powder, aerosol, solution/dispersion
- Size is method dependent: no single method is perfect, and each method has some disadvantages. Use of multiple methods preferable (but adds to testing costs)

Issues (2)

- Characterisation: as supplied vs. as tested-storage and stability
- As supplied: most direct to measure, however may not adequately describe tested material, as substance characteristics can change with time
- As tested: may be more challenging. Complications (e.g. interaction with media, proteins, etc, agglomeration/dissolution)

Particle size: recommendations

- As particle size is method dependent, detailed information should be included on the used method
- Different information available on particle size (e.g. primary particle, aggregate, agglomerate), and different metrics possible (e.g. weight based vs. number based distributions). Each piece of information is valuable.
- More detailed information allows for better evaluation

Further information

- Further information can be found on ECHA's new web section on nanomaterials:

  http://echa.europa.eu/en/chemicals-in-our-life/nanomaterials

Thank you

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http://echa.europa.eu/en/web/guest/
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