

Electronic Supplementary Material 2

Table 2S. Overview of the information gathered about the availability of methods, protocols and guidelines for the additional information requirements and criteria for additional information and category of the highest-ranked category of identified methods needed to acquire/fulfill information requirements set by the European chemicals legislation REACH for nanomaterials (NMs). TGS: Internationally accepted test guideline or standard; TGSUD: Internationally accepted test guideline or standard under development; SCI: Established as standard method in scientific literature; O: Other method and/or more research needed; N: No method identified. Other abbreviations: TR: technical report; GD: guidance document; Organization for Economic Co-operation and Development (OECD); ECHA: the European Chemicals Agency.

Criteria for additional information requirement	Additional information requirement	Methods, protocols and guidance	Method category
(7.7)The substance appears “insoluble” in water		<ul style="list-style-type: none"> • OECD WNT Project 1.5 and 3.10¹; OECD GD 318.² 	TGSUD
	(7.7) Limit test up to the detection limit of the analytical method shall be performed. For nanoforms the potential confounding effect of dispersion shall be assessed when conducting the study.	<ul style="list-style-type: none"> • OECD TG 318³ addresses dispersion stability of nanomaterials in simulated environmental media using multi-parameter matrix and analysis by DLS and ICP-MS/OES. 	TGS
(9.1.1) The substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.		<ul style="list-style-type: none"> • OECD WNT Project 1.5 and 3.10¹; OECD GD 318.² 	TGSUD

(9.1.1) The long-term aquatic toxicity study on Daphnia (Annex IX, section 9.1.5.) shall be considered

(7.14ter) There is an indication that specific additional particle properties significantly influence the hazard of or the exposure to those nanoforms

- Essential to determine the kinetics of changes in surface reactivity/composition, article stability/mobility in solution etc
- Surface speciation and chemical speciation prediction in solution is essential to assess.⁴

(7.14ter) Further testing for nanoforms covered by the registration shall be considered by the registrant or may be required by the Agency in accordance with Article 41

(9.1.3) The chemical safety assessment according to Annex I indicates the need to investigate further effects on aquatic organisms.

(9.1.3) Long-term aquatic toxicity testing as described in Annex IX shall be considered.
The choice of the appropriate test(s) will depend on the results of the chemical safety assessment.

(9.1.3) The substance is poorly water soluble, or for nanoforms if they have low dissolution rate in the relevant test media.

OECD WNT Project 1.5 and 3.10¹; OECD GD 318²; Dissolution rate in physiological fluids to be further developed and predictivity for biodurability in vivo and in vitro to be examined.⁵

TGSUD

(9.1.3) The long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6) shall be considered

(8.8.1) For nanoforms without high dissolution rate in biological media

- OECD WNT Project 1.5 and 3.10¹; OECD GD 318²; Dissolution rate in physiological fluids to be further developed and predictivity for biodurability in vivo and in vitro to be examined.⁵

TGSUD

(8.8.1) A toxicokinetics study shall be proposed by the registrant or may be required by the Agency in accordance with Article 40

(9.2) Nanoforms that are not soluble, nor have high dissolution rate

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(9.2) Morphological transformation (e.g. irreversible changes in particle size, shape and surface properties, loss of coating)

- Oxidation: Morphological transformation - oxidation: Band gap measured by UV-vis-NIR absorption measurements of the dry powder of the material; probe force microscopy; soft X-ray methods.⁵
- Electrochemical characterization methods are available.^{6,7}

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(9.2) Chemical transformation (e.g. oxidation, reduction)

- Combining TEM with energy-dispersive X-ray spectroscopy (EDX) makes it possible to confirm the elemental composition of the nanoparticles.^{6,8,9} Attenuated Total Reflection Fourier transform infrared Spectroscopy (ATR-FTIR) can monitor adsorbed molecules (e.g. loss of coating) and oxide changes.^{6,10}
- For metals, surface oxide characteristics can be assessed with electrochemical methods. It was used in Hedberg et al.,⁶ with a technique based on a graphite paste electrode to perform cyclic voltammetry in buffer solutions and open circuit potential measurements in DMEM+. The electrochemical surface oxide characterization complemented and verified by Raman spectroscopy, X-ray diffraction, and X-ray photoelectron spectroscopy.
- Zeta potential: : Lowry et al.¹¹ presents guidance on overcoming main challenges associated with zeta-potential measurements and on the parameters that should be provided together with the measurements to enhance interpretability. Guidance on how to accurately conduct and report zeta potential measurements for metal nanomaterials, specifically in Skoglund et al.¹²
- Atomic force microscopy (AFM) – changes in particle morphology and size.^{e.g. 13}
- X-ray Absorption Spectroscopy (including EXAFS/XANES/NEXAFS).¹⁴

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(9.2) Other abiotic degradation (e.g. photolysis)

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(9.4) For substances that have a high potential to adsorb to soil

- Spectroscopic tools like FTIR¹⁰ and EXAFS.¹⁵ (XAS – TGSUD including both XANES/NEXAFS/EXAFS).
- Equilibrium partitioning does not apply since thermodynamic equilibrium does not occur for nanomaterials.^{5,16}
- OECD TG 312 Leaching in Soil Columns¹⁸ is applicable for nanomaterials.^{17,19,20} Draft OECD guidance document on testing nanomaterial behaviour in soils using OECD test guideline 312 under development.²¹ OECD Test Guideline 303A on Particle attachment and removal from wastewater can be used as alternative approach to predict sorption of nanomaterials.
- Method for determination of retention of silver and cerium oxide nanomaterials in soils.²²
- Draft OECD Test Guideline on Agglomeration Behaviour of Nanomaterials in Different aquatic Media,²³ for measurement of (Hetero)Agglomeration, aggregation, particle attachment and removal.

(9.4) The registrant shall consider long- term toxicity testing instead of short-term.

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(9.4) For substances that are very persistent

- A wide range of transformations occur (agglomeration, aggregation, dissolution, etc.) and a clear definition of persistence is needed (i.e. pristine/parent materials).
- Persistence: general issue with persistence and not only in soil. Persistence is arbitrary due to constant transformations of NMs in environment.
- For nano consider morphological, chemical other and changes on shape size, etc. OECD WNT 3.16 project: Guidance document environmental abiotic transformation of nanomaterials¹ under

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development. This guidance aims to cover abiotic core transformation and coating degradation. Both documents likely to be available in 2023 or 2024. It may be possible to conduct a qualitative assessment in the meantime.²¹

9.4) The registrant shall consider long- term toxicity testing instead of short-term.

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