UMASS LOWELL

ALTERNATIVES ASSESSMENT CONSIDERATIONS FOR THE ANALYSIS OF ENGINEERED NANOMATERIALS

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of the Lowell Center for Sustainable Production at UMass Lowell.

www.sustainablechemistrycatalyst.org

AUTHORS

Aude Bechu and Molly Jacobs from the Sustainable Chemistry Catalyst at UMass Lowell.

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ABOUT

The Sustainable Chemistry Catalyst is an independent research and strategy initiative, based at the Lowell Center for Sustainable Production within UMass Lowell, that is focused on accelerating the transition to safer, more sustainable chemistry through research and analysis and stakeholder engagement with scientists, policymakers, and commercial actors.

Sustainable Chemistry Catalyst
University of Massachusetts Lowell
Lowell Center for Sustainable Production
600 Suffolk Street, Lowell, MA 01854
(978) 934-2997
www.sustainablechemistrycatalyst.org | www.uml.edu

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LIST OF ABBREVIATIONS

CAS RNs	Chemical Abstract Service Registry Numbers
CBI	Classified Business Information
CNTs	Carbon nanotubes
ECHA	European Chemical Agency
EFSA	European Food Safety Authority
ENM	Engineered nanomaterials
EPA	U.S. Environmental Protection Agency
EUSES	European Union System for the Evaluation of Substances
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good laboratory practices
IC2 Guide	Interstate Chemicals Clearinghouse's (IC2) Alternatives Assessment Guide
ISO	International Standards Organization
JRC	Joint Research Council
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCT	Life cycle thinking
MWCNT	Multiwalled carbon nanotubes
NAMs	New Approach Methods
OECD	Organization for Economic Cooperation and Development
PFAS	Per- and polyfluoroalkyl substances
QSAR	Quantitative Structure Activity Relationships
REACH	Registration, Evaluation and Authorization of Chemicals
RSL	Restricted Substances List
SCCS	European Commission's Scientific Committee on Consumer Safety
SbD	Safe by Design
SDS	Safety data sheet
TiO_2	Titanium Dioxide
TSCA	Toxic Substances Control Act
WHO	World Health Organization

INTRODUCTION

Mitigating the impact of toxic chemicals on human health and the environment requires an informed transition to safer alternative chemicals, materials, and technologies. Alternatives assessment is a multi-disciplinary approach to support informed substitution and is defined as a "*process for identifying and comparing potential chemical and non-chemical alternatives that could replace chemicals of concern on the basis of their hazards, performance, and economic viability*" (National Research Council, 2014). To date, these approaches have been primarily used to support the transition to safer chemicals and processes. However, they were not designed to fully capture emerging chemicals/materials of concern, namely engineered nanomaterials (ENMs).

Because ENMs offer superior functionality in a range of applications, they are now emerging (in development or commercially available) as replacements for functions currently being served by chemicals of concern. Examples include:

- Multi-walled carbon nanotubes (MWCNTs) as replacements for halogenated flame retardants (Araby et al., 2021)
- Nanowhiskers as a replacement for per- and polyfluroralkyl substances (PFAS) in stain resistant textiles and carpets (NanoTex, n.d.)
- Organosilicates as replacements for PFAS surfactants in aqueous film forming foams (Davis, 2022)
- Polymer nanocomposites as replacements for phthalate plasticizers (TDA Research, 2017)

ENMs are demonstrating beneficial performance attributes, but a lack of guidance regarding how these materials should be assessed regarding their hazards and intrinsic exposure potential across their life cycles has stymied evaluations in alternatives assessment.

The Sustainable Chemistry Catalyst at UMass Lowell was asked by the Washington State Department of Ecology to explore considerations for evaluating ENMs and microplastics in the context of alternatives assessment processes. The first section of this White Paper provides an overview of key background concepts for thinking about ENMs, especially in comparison to conventional chemicals. The second section outlines considerations for addressing ENMs in alternatives assessments. These considerations were developed using the Interstate Chemicals Clearinghouse's Alternatives Assessment Guide (Version 1.1) (IC2 Guide) as a alternatives assessment framework model using those modules focused on: (a) identifying potential alternatives, (b) hazard assessment, (c) comparative exposure assessment, and (c) life cycle impacts. As such, these considerations for the evaluation of ENMs in alternatives assessment are also applicable to other guidance documents. The last section of this White Paper, **APPENDIX I,** provides a detailed review of the literature pertaining to the evaluation of ENMs. Considerations for the evaluation of alternatives assessment were derived from this comprehensive literature review. Although placed in an appendix, this literature review outlines key methodological developments regarding the assessment of potential human and environmental impacts associated with ENMs. The review focuses on methodologies that are aligned with approaches used for alternatives assessments, including risk assessment and life cycle assessment. The review is recommended for any reader needing additional background to better understand the rationale for the considerations outlined.

BACKGROUND: ENGINEERED NANOMATERIALS – WHAT ARE THEY?

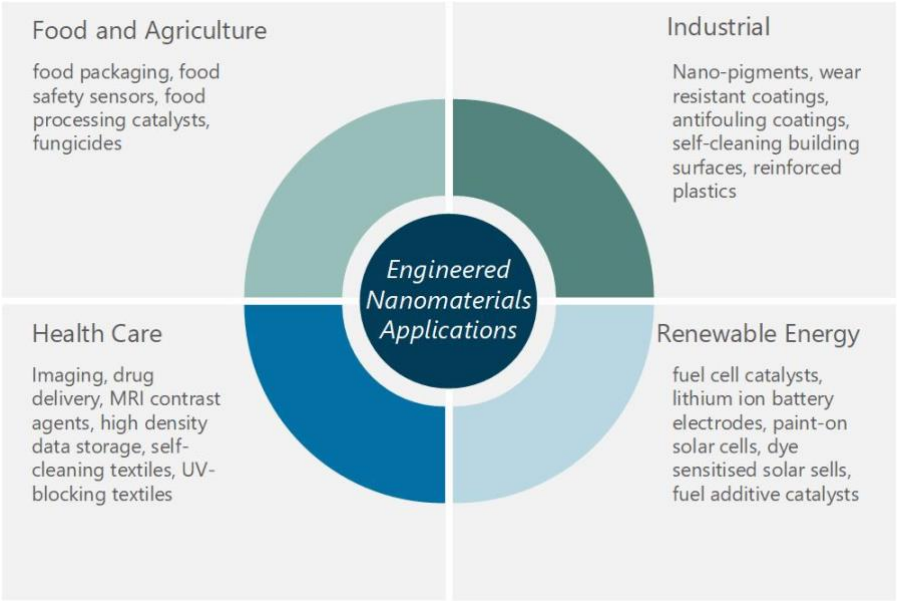
Over the past two decades, ENMs have garnered significant attention for their potentially beneficial applications in medicine, industrial processes, and consumer products due to their advantageous physicochemical properties. ENMs can be solid, liquid, or gaseous substances and are defined by ISO as *materials in any external dimension in the nanoscale or with an internal surface structure at the nanoscale, which is 1 to 100 nanometers* (ISO, 2008). Although nanomaterials can occur naturally (such as carbon nanotubes emitted from volcanic eruptions), nanomaterials are being engineered to maximize functional advantages associated with their unique physicochemical properties.

At the nanoscale, fundamental mechanical, electrical, optical, and other properties of ENMs can significantly differ from their bulk material counterparts. ENMs composition can vary - from quantum dots, fullerenes, carbon nanotubes, oxides (e.g., titanium dioxide, iron oxide), metals (e.g., gold, silver), to organic structures like dendrimers and cellulose (Janković & Plata, 2019).

ENMs syntheses can be tuned to produce materials with specific surface areas, surface reactivities, chemical coatings, shapes, and sizes. These novel physicochemical properties have provided the basis for ENMs to offer a range of benefits in numerous industrial applications. The global market for ENMs is expected to reach $38 billion by 2029 after a decade of robust growth at a compound annual growth rate (CAGR) of 17.8% (ECHA, 2022c). As shown in **FIGURE 1**, ENMs are being developed and deployed in a variety of industry sectors and applications.

Depending on the regulatory context, assessment of the safety of ENMs may or may not be treated different than bulk chemicals. For example, the European Chemicals Agency (ECHA's) REACH regulation (Registration, Evaluation and Authorization of Chemicals) outlines specific requirements for ENMs, while U.S. EPA's Toxic Substance Control Act (TSCA) does not outline ENM-specific obligations for manufacturers.

FIGURE 1: Applications of Engineered Nanomaterials



Inherent Hazard and Exposure Concerns of ENMs

Concern regarding the health and safety of ENMs emerged in tandem with the growth of the technology. Many of the same physicochemical properties that make ENMs technologically and commercially desirable are the same properties that can make them more toxic than the same substance of a larger size.

Decades of particle toxicology research have established that particle size influences hazard. The intrinsic exposure potential of unbound, un-agglomerated ENMs via inhalation, ingestion, and dermal exposure is significant given their extremely small size.

- Particles of less than 10μm can be inhaled and cause exposure to the lung (SCCS, 2023a).
- Particles can be tuned to enter the surface layers of skin through hair, sweat, and other glands (Krishnan & Mitragotri, 2020).
- Ingested particles that have a slow dissolution rate may maintain their nano-specific activity (e.g., antimicrobial) that leads to adverse impacts in the GI tract or other organs (Di Cristo et al., 2021).
- Once inside the body ENMs can continue to redistribute, including having the potential to cross the blood brain barrier (Guo et al., 2021).
- ENMs also distribute in the environment due to their ability to remain suspended in air and water (Hochella Jr et al., 2019).

BOX 1: According to the European Commission's Scientific Committee on Consumer Safety (SCCS), certain physicochemical and toxicological properties as well as uses of ENMs are linked to a higher degree of safety concern (Bernauer et al., 2021). These properties include (Bernauer et al., 2021):

- "The ENM has constituent particles that have sizes in the lower range of the nanoscale (1–100 nm).
- The ENM is insoluble, or only partially soluble.
- The chemical nature of the ENM suggests the potential for a toxicological hazard.
- The ENM has certain physical/morphological features (e.g., needle shape, rigid long fibres) that point to potential for harmful effects.
- The ENM has surface reactivity in terms of catalytic (including photocatalytic) activity, potential for radical formation, or other surface properties (e.g., that can enhance cellular uptake, or confer allergenicity due to proteinaceous surface).
- The ENM has a different biokinetic behavior than the conventional equivalent. For example, on the surface a modification/coating (e.g., hydrophobic coatings, encapsulation) has been applied to the core nanoparticle to alter their ADME (absorption, distribution, metabolism, elimination) properties and as a result make them more accessible systemically or enable them to reach different tissues compared to the neat nanoparticles and/ or their conventional chemical forms.
- The ENM is used as vehicle to carry other substances - which have not been assessed for safety as individual components, or together in the nano-scale entity,
- There is evidence for persistence/accumulation of the ENM in the body.
- Nanoparticles have other distinctive properties not present in conventional form of the same material or a new activity/function (e.g., a smart/functional nanomaterial).
- The ENM is so novel that it does not have a conventional comparator to allow assessment of changes in properties, behavior, or effects.
- The assessment of genotoxicity is inadequate, e.g., in vitro studies are without information on stability of the test suspension, or evidence of cell exposure (internalization)."

The following exposure considerations were also mentioned as certain uses of ENMs can lead to increased safety concerns (Bernauer et al., 2021):

- "There is a likelihood of systemic exposure of the consumer to nanoparticles through the use of final products, especially those that may alter surface characteristics of the nanoparticles and thereby enhance their absorption in the skin (skin penetration).
- The frequency of use, and/or the amounts of the consumer product are relatively high.
- The ENM is used in a product is inhalable and the particles are respirable (can reach respiratory epithelium/ alveoli)."

CONSIDERATIONS FOR THE EVALUATION OF ENGINEERED NANOMATERIALS IN ALTERNATIVES ASSESSMENT

This section outlines specific considerations to advance assessment of ENMs in alternatives assessments based on applying current knowledge, tools, and resources that have emerged over the last decade as reviewed in **APPENDIX I**. Recommendations were developed using the IC2 Guide as a model framework although are generalizable to other frameworks given direct overlap in the assessment steps/mode. Recommendations address four assessment modules:

A. Identification of alternatives
B. Hazard assessment
C. Exposure assessment
D. Life cycle consideration

These recommendations reflect differences in assessment methods and practices that will be needed for ENMs in the modules as compared to conventional chemicals. Other primary modules of an alternatives assessment, including assessments of performance and cost/availability were not determined to have specific adaptation needs for ENM-based alternatives.

Although this document utilizes the format and content of the IC2 Guide, the following recommended adaptations are generalizable to other frameworks given direct overlap in the assessment steps/modules.

The recommendations below focus only where adaptation in alternatives assessment methods is needed for the evaluation of ENMs. Specific challenges envisioned in the operationalizing of these considerations are noted where applicable. Also noted where applicable is the need for expert judgment for the assessment of ENMs as certain considerations require a high level of technical expertise. However, this expertise is no different than what is already needed when assessing hazards and exposure potential in alternatives assessments other than the additional requirement that assessors should have a working knowledge of ENMs.

As progress continues to be made in the validation of methods for ENMs, the following considerations for alternatives assessment will need to be continually adapted.

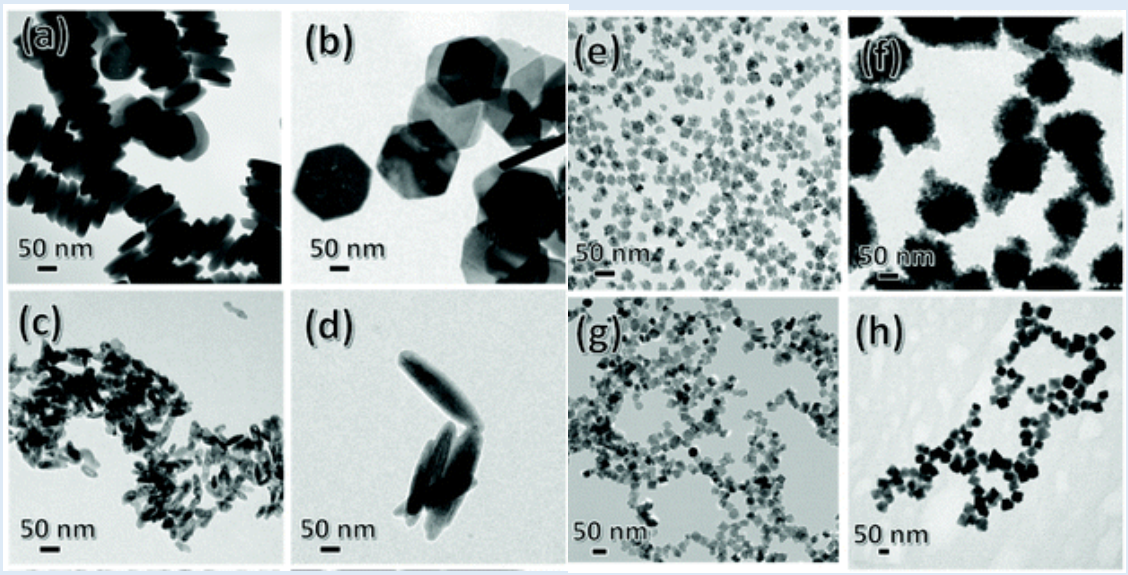
A. Identification of Alternatives

Identifying functionally equivalent alternatives for consideration in an alternatives assessment is one of the first steps of the approach. The IC2 Guide states, *"Alternatives may include chemical substitutions, alternative materials, and changes to the product process or product redesign to eliminate a particular chemical. The widest range of possible alternatives should be researched, including emerging technologies."* Considerations for ENMs associated with this step in the assessment include:

1. *Identify physicochemical properties of ENM alternative, including CAS registry number and Trade Name if available.*

Alternatives assessment relies on the use of chemical abstract service registry numbers (CAS RNs) to identify the specific chemical alternative under evaluation. No analogous system exists to uniquely identify an ENM based on its specific physicochemical properties. Although some ENMs have CAS RNs (e.g., fullerenes and carbon nanotubes), many do not and thus cannot be differentiated from bulk form chemicals (e.g., nano silica, nano gold, cadmium dots, etc.,).

EXAMPLE: The tuning of the synthesis of iron oxide ENM can give rise of many different shapes and sizes, despite the chemical composition being constant (Gavilán et al., 2021). It is important to consider physicochemical properties beyond chemical composition.



Some ENMs have trade names and can support uniquely identifying the ENMs under assessment. These trade name have accompanying set of physicochemical properties as listed on product specification documentation.

Each ENM differs based on specific physicochemical properties and these characteristics are used to identify the specific material. **TABLE 1** proposes a set of recommended physicochemical characteristics of the ENM alternative to support identifying the ENM under review. These physicochemical characteristics are used as the basis for registration ENMs under REACH. The European Chemical Agency's (ECHA) has established research methods to determine these physicochemical properties (ECHA, 2022a). Most of these physicochemical characteristics are also used later to help inform the intrinsic exposure potential of an ENM.

TABLE 1: Recommended physicochemical properties of ENMs that should be defined when identifying ENMs as potential alternatives, based on SCCS's Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS, 2023a) and ECHA's Guidance on Registration and Substance Identification and Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint Specific Guidance (ECHA, 2022a, 2022b).

Property Type	Physicochemical Property	Definition (SCCS, 2023a)
Chemical	**Chemical identity**	Information on structural formula(e)/molecular structure(s) of the constituents of NM along with chemical and common names, and CAS RNs (where available).
	Chemical composition	Information on full chemical composition of the NM including purity, nature of impurities, coatings, or surface moieties, doping material, encapsulating materials, processing chemicals, dispersing agents, and other additives or formulants e.g., stabilizers.
Physical	**Number-based size distribution**	Data and graphical representation of the size distribution of primary and secondary particles in terms of mean (±SD) and median sizes in nm should be provided.
	Shape	Shapes include, but at not limited to, particle-, tube-, rod- or fibre.
	Crystallinity	Description of crystalline form (amorphous, polycrystalline, crystalline including specification of phase and volumetric fraction as well as spatial distribution).
	Surface area	Information on Brunauer, Emmett and Teller (BET) specific surface area of the NM, and volume specific surface area (VSSA).
Physico-chemical	**Solubility and dissolution rate**	Dissolution is the process by which a nanomaterial in an aqueous medium or biological environment is dissolving into their constituent ions or molecules (ECHA, 2022b). Dissolution rates in relevant solvent(s) for soluble and partially soluble ENMs (solubility should not be confused with dispersibility of insoluble ENMs). For insoluble dispersible ENMs: information on dispersibility in terms of a relative amount of the particles that can be dispersed in a suspending medium.
	Dustiness	Dustiness may be defined as the propensity of a powder to form airborne dust by a prescribed mechanical stimulus. It depends on a number of factors such as physicochemical properties of the particles (e.g. size, shape, relevant density, type of coating), the environment (e.g. moisture, temperature), the type of process (e.g. energy applied), the interaction between particles during agitation (e.g. friction shearing) and the sampling and measurement configuration (ECHA, 2022b).
	Zeta potential	Electrophoretic mobility can also be used as an indicator of particle (in)stability in solution (ECHA, 2022b).

Use of CAS RNs in embedded in the current IC2 Guide because CAS RNs are often used to classify a chemical in hazard assessment data sources such as authoritative lists or GHS listings. Updating language in IC2 Guide is needed to account for the limits of CAS RNs in the assessment of ENMs and to stress the importance of outlining specific physicochemical properties.

Challenge: *Although specific physicochemical characteristics are list on product specification documentation, it is unclear what physicochemical properties will be deemed Classified Business Information (CBI). Moving forward information in Table 1 should be made available on regulatory*

dossiers for ENMs submitted under REACH. Safety Data Sheets (SDSs) are not required to report all physicochemical properties (EU Commission, 2020).

B. Hazard

The assessment of hazard is central and fundamental to an alternatives assessment. The IC2 Guide includes multiple levels of assessment, with greater expertise and investments of time and resources required for the higher levels. Additional considerations for the evaluation of ENMs in alternatives assessments include:

1. ***Deprioritize or eliminate ENMs if the material or one of its components appears on authoritative lists of existing hazard data or classifications.***

Screening based on the use of authoritative lists is the first step in all hazards assessment levels (e.g., level 1, 2, and 3). If the assessor finds that EMNs contain hazardous chemicals, this provides justification that a given ENM is not a safer alternative. As stated by the EU SCCS in its Scientific Advice on the Safety of Nanomaterials in Cosmetics, "As a general rule, where chemical component(s) are toxic, as such or when put together in the form of a nanomaterial, they should constitute a trigger for concern over safety to the consumer health" (Bernauer et al., 2021).

EXAMPLE: Cadmium may be a constituent of an ENM used in electronics. The ENM should be assigned a "high" score for carcinogenicity based on clear evidence for the bulk chemical (Group 1 carcinogen classified by the International Agency for Research on Cancer (IARC), an authoritative list).

ENMs are beginning to be added to authoritative lists, such as the inclusion of TiO_2 in California's Proposition 65 (OEHHA, 2018). However, there is limited mention of EMNs on authoritative lists that were built centered on chemicals. Therefore, the absence of ENMs or their chemical components on screening lists should not be the only prerequisite to considering ENMs as safer alternatives. A deeper analysis is needed.

Additional research on ENMs that were not granted regulatory approval for use (especially relevant in the EU context) will be needed to supplement use of authoritative lists to screen out undesirable ENM alternatives in the assessment. As there is no authoritative list that gathers ENMs that have not been authorized for use, the assessor should search for decisions related to application of the ENM under assessment. For example, the authorization or restriction of ENMs for use in cosmetics in the EU is outlined in the annexes of the Cosmetics Products Regulation (ECHA, 2023; SCCS, n.d.).

There may be opportunities to engineer ENMs so that inherently hazardous chemical constituents are rendered non-biologically active, such as by using additional surface treatments or coatings that bind the chemical of concern in matrix. These considerations should be addressed in the exposure assessment module, which focuses on considering additional physicochemical properties of the ENM relevant to its intrinsic exposure potential.

Challenge: *The feasibility of gaining public access to information about ENMs that have been*

denied regulatory approvals for market entry is unclear. Research into the transparency of different regulatory approvals processes will support a better understanding of the data sources that can be used to adapt existing authoritative list screening steps during a hazard assessment when ENMs are being considered.

2. Identify likely physical and chemical transformation products of ENMs.

Alternatives assessments address the hazards of an alternative across its life cycle. The alternative, once released to the environment, may undergo chemical reactions (e.g., hydrolysis and oxidation) leading to the formation of transformation products. Considering transformation products and their hazards is not new; it is standard practice in hazard assessment methodologies such as the GreenScreen® approach – an approved methodology for use with the IC2 Guide.

ENMs usually transform in the environment both physically and chemically from their original form. ENM transformations in the environment are highly dependent on physicochemical properties (Baun et al., 2017; OECD, 2019). Distinct from chemical transformations, ENMs can also change size or shape (physical transformations) which may also impact their intrinsic hazard.

ENM transformations (chemical and physical) can be addressed through an evaluation of physicochemical properties, the likely environmental conditions during each life cycle stage, and known transformation products. These expected transformation products can be then assessed for hazard. In the assessment of ENM transformation products, data from pristine ENMs may not be suitable for read across to ENM transformation products due to the latter's differences in physicochemical properties.

EXAMPLE: Scientists at the US National Labs examined silver nanoparticles in consumer products as well as their simulated transformations during ingestion followed by processing in a wastewater treatment plant. They tracked the chemical transformations of silver in its elemental state (Ag^0) to silver ions, silver sulfide and silver chloride. They found that ingestion changes both the chemical speciation and the morphology of silver nanomaterials (Potter et al., 2019).

The OECD outlined many possible transformation of ENMs in their "Decision Framework for Physico-chemical Characterisation" (OECD, 2019). In addition, various standardized tests can be employed to probe the potential transformation of EMNs (Nielsen et al., 2021).

Challenge: *Consideration of transformation products significantly complicates the hazard assessments of ENMs as the dominant transformation products may be difficult to determine. Assessors should decide which transformation products to consider and document justification.*

3. Utilize guidelines on data quality when assessing toxicological studies of ENMs and, if applicable, their transformation product(s).

Hazard assessments routinely rely on existing toxicological studies. In the case of ENMs, toxicological studies available in the literature may be of varying quality because efforts to standardize toxicological studies of ENMs are ongoing.

Specific chemical hazard assessments, such as Cradle to Cradle, ChemForward and GreenScreen, will often indicate whether the substance assessed is in nano form. The chemical hazard assessment should also indicate exposure routes for each of its hazard endpoints and assess the quality of the ENM-specific studies.

Existing test guidelines for chemicals have been evaluated for their applicability to ENMs and, where necessary, additional test guidelines through the Organization for Economic Cooperation and Development (OECD) have been established. The best practices for testing ENMs are continuously updated under REACH and through the OECD's Series on the Safety of Manufactured Nanomaterials.

Several methods have emerged to assess the data quality of toxicological studies of ENMs (SCCS, 2023a). For example, the World Health Organization (WHO), which focuses on the use of Good Laboratory Practice (GLP), adheres to OECD testing guidelines, and peer review (Lee et al., 2017). Another approach by the GUIDENano project relies on both the rigor of the toxicological test and the physicochemical properties of ENMs reported (Fernández-Cruz et al., 2018). NanoCRED examines the relevance and reliability of ecotoxicity data (Hartmann et al., 2017). Such approaches can be adapted by the alternatives assessor to justify whether a study is of high enough quality for inclusion.

EXAMPLE: NanoCRED assigns reliability scores of the study depending on its fulfilment of critical and important criteria. Examples of critical criteria (which are filled by the most reliable studies) include:

- Use of appropriate controls such as metal ions, and dispersants.
- Using test organisms that are appropriate for the nanomaterial and test conditions.
- Definition of exposure duration.
- Consideration of the stability of ENMs in data collection and interpretation.

Challenge: *Assessing the quality of data from ENM toxicological studies requires chemistry and toxicological expertise and this will not be appropriate for Level 1 hazard assessments under the IC2 Guide.*

4. When insufficient toxicological data on the ENM being assessed as an alternative exists, employ grouping methods to assist with using available data on "similar" materials.

When experimental data are unavailable or of limited quality for the ENM being assessed, hazard data on similar ENMs and/or bulk chemicals can be used for grouping and read-across approaches. Grouping gathers evidence from similar ENMs (with known toxicological effects) to justify the proposed connection between physicochemical properties and biological effects. This connection can then be used to predict the toxicological effects of an ENM being assessed.

Guidance for grouping ENMs together to support toxicity assessments has been outlined by ECHA to support the registration of ENMs under REACH (ECHA, 2019; ECHA, 2016). These grouping techniques require justifying the inclusion of a specific set of similar ENMs based on specific physicochemical properties. The list of specific physicochemical properties to consider for

grouping are a subset of those used to identify a given ENM in the identification of the alternatives module (**TABLE 1**). Grouping rules are also relevant for the assessment of transformation products given that the availability of hazard data on a specific ENM that has been transformed in the environment will be unlikely.

According to ECHA, certain physicochemical properties are required for understanding hazardous properties as a basis for grouping (see **APPENDIX II** for descriptions):

- Chemical composition, including impurities.
- Particle size distribution
- Shape and crystallinity
- Surface functionalization and treatment
- Surface area (by volume or mass or both)

ECHA does not outline steadfast rules for determining which ENMs can be considered as "similar enough" – it is up to the assessor to determine and justify based on the best available information, including for the following additional physicochemical properties, as deemed useful:

- Solubility
- Dispersion stability
- Dissolution rate
- Dustiness
- Biological or surface reactivity

When using read-across methods, it is important to explore the boundaries of the group of ENMs to which the read-across hypothesis applies to ensure that the physicochemical properties used to justifying the group do not underestimate the potential hazard of the ENM under assessment.

EXAMPLE: The EU Joint Research Center has published grouping studies on titanium dioxide (TiO2) and multi-walled carbon nanotubes (Aschberger et al., 2019; Lamon et al., 2018). The following hypothesis from six TiO_2 samples was used for read-across of two TiO_2 samples of unknown genotoxicity:

"Nano-TiO2 in its uncoated form has the potential to damage DNA, but this can be masked by the presence of coating or by the large amounts of impurities on the surface of the NM [nanomaterial]" (Lamon et al., 2018).

Challenge: *Establishing grouping rules requires chemistry and toxicological expertise and this will not be appropriate for Level 1 hazard assessments under the IC2 Guide. Examples of grouping ENMs according to ECHA guidance are currently scarce.*

5. In the case of data gaps in toxicological data, assess whether ENM-specific Quantitative Structure Activity Relationship (QSAR) models are validated and available.

In hazard assessments of bulk chemicals, physicochemical properties are often used to model or predict some endpoints when data gaps exist. However, for some hazard endpoints, the physicochemical properties of ENMs should not be used in existing modeling/prediction tools (ECHA, 2022b). These include:

a) Use of octanol-water partition coefficient to predict bioaccumulation. Equilibrium concentrations of ENMs in different media is not achieved as quickly as chemicals.
b) Use of water solubility parameters to predict bioavailability and mobility. The small size of ENMs can increase their mobility and therefore bioavailability.
c) Use of granularity to predict inhalation potential. Methods that measure granularity often do not have a wide enough range of measurement to account for ENMs.
d) Use of bulk data to assume low hazard in ENM endpoints. For example, low flammability in bulk silver did not translate to low flammability for nanosilver (Sass et al., 2016).

Due to the complexity of ENMs, no one physicochemical property of ENMs has been linked to a hazard endpoint in the same style as chemicals (e.g., chemical's octanol-water partition coefficient predicts bioaccumulation). In practice, multiple physicochemical properties of ENMs may be linked to specific hazard endpoints. For example, the concern regarding high aspect ratio nanomaterials' (HARN) ability to cause mesothelioma has been linked to a combination of size, shape, and chemical identity of ENMs (Tran et al., 2008).

QSARs are widely used tools to help fill data gaps in physicochemical properties, environmental fate, and biological effects. Currently, QSARs commonly used for chemicals cannot be readily adapted to ENMs due to the complex composition, size, and shape of nanomaterials. Although QSARs have been developed specifically for ENMs, particularly for metals, metal oxides, fullerenes and carbon nanotubes, these predictive models specific for ENMs are not yet widely adopted in the regulatory communities (OECD, 2022b). However, the lack of robust QSARs may be addressed in the future, therefore the assessor is encouraged to survey more recent developments.

Challenge: *Using QSAR requires chemistry and toxicological expertise and this will not be appropriate for Level 1 hazard assessments under the IC2 Guide. The quality and applicability of QSARs should be assessed specific to each ENM as well – for example a QSAR may be applicable for non-coated metal oxide ENMs but not for coated carbon-based ENMs.*

6. Review and reconsider decision rules for the treatment of ENM alternatives; remaining data gaps and uncertainties on key hazard endpoints should be treated with the utmost caution given the high potential for exposure.

Within the IC2 Guide, approved hazard assessment methodologies to be used often have specific decision rules embedded in the method regarding the treatment of data gaps and scoring/benchmarking methods that are used to support decision-making. Although the data to support the consideration of ENMs in an alternatives assessment has grown significantly in the last decade, a key challenge remains the associated data uncertainties.

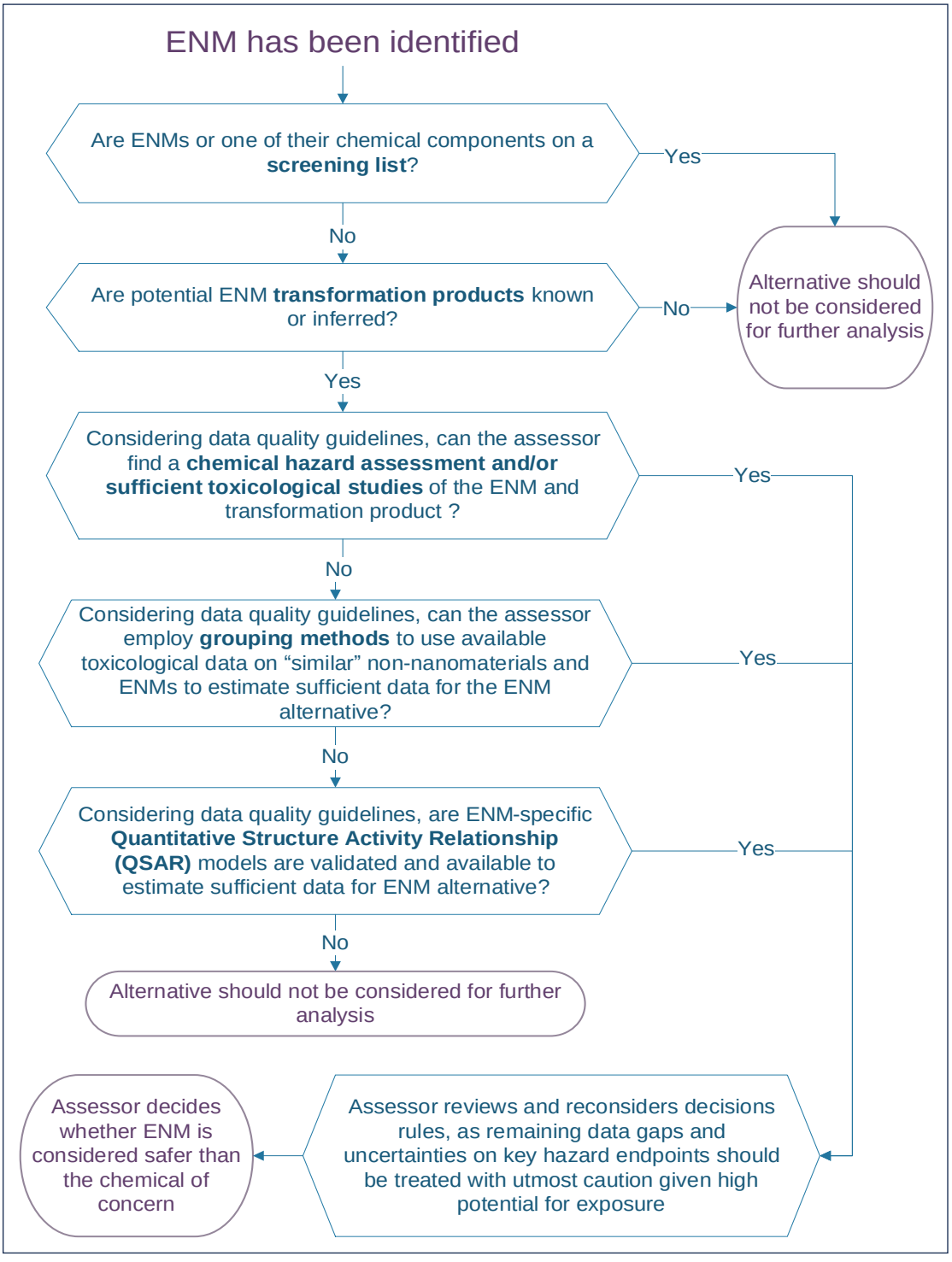
The need to use predictive hazard tools rather than directly measured data will be a consistent challenge encountered when assessing ENMs. For example, if a type of nano-silica is being considered as an alternative, it is highly unlikely that the *exact* nano-silica has been experimentally tested given variations in physicochemical properties. The assessor should decide on the limits of grouping use in their decision making.

Key hazard endpoints, such as carcinogenicity, reproductive/developmental toxicity may not have any direct measurement data for ENMs of a specific class. The assessor should decide whether these data gaps (and other) justify discounting an alternative from further assessment. Although there are no established rules for deeming ENMs as safe(r), attributes that increase the level of concern are listed by the European Commissions' Scientific Committee on Consumer Safety (SCCS, 2023b). Minimum criteria for safer have been developed for chemicals (OECD, 2021c), which the assessor may choose to adopt for ENMs.

Summary

FIGURE 1 (below) abbreviates the hazard assessment considerations reviewed above and suggests specific decision rules where determinations about whether to proceed with the assessment or screen out an ENM alternative.

FIGURE 1: Considering Engineered Nanomaterials in the Hazard Module of Alternatives Assessment
An Overview of Recommendations



C. Exposure Assessment

The purpose of an exposure assessment in an alternatives assessment is to determine the differences in the intrinsic exposure potential of alternatives relative to the chemical of concern for humans and ecosystems, regardless of external exposure controls in place (i.e., filtered ventilation or use of gloves, etc.). Three considerations for the evaluation of ENMs in the exposure assessments module are outlined below.

1. Use ENM physicochemical properties to inform the inherent exposure potential of ENMs.

The IC2 Guide's Level 1 and 2 exposure assessments includes an evaluation of physicochemical properties that support an understanding of the intrinsic exposure potential of a substance. Several physicochemical properties, outlined in **TABLE 1**, are important for understanding hazard as well as the intrinsic exposure potential of ENMs. Additional ENM physicochemical properties that influence exposure parameters are outlined in ECHA's chemical safety assessment guidance for registration under REACH (ECHA, 2019) (see **APPENDIX II**).

Throughout their life cycle, ENMs, unlike chemicals, undergo physical changes that may mediate exposure. The assessor should consider whether ENM transformation products influence exposure at relevant life cycle stages.

EXAMPLE: How physicochemical properties influencing exposure potential

CONSIDERING THE SOLUBILITY/DISSOLUTION RATE:

If an ENM is highly soluble in water and has a high dissolution rate in relevant biological (or environmental media, then it is likely that the nanoform is rapidly, increasingly, and finally completely present in its molecular or ionic form. The ENM can therefore be expected to behave similarly and elicit the same response as the non-nanoform of the substance. If, however, the ENM under investigation is poorly or only partially soluble with a low dissolution rate in biologically or environmentally relevant test media, then it will likely be present in the test system in a particulate form.

EXAMPLE: An alternatives assessment of ENMs in ceramics considers the potential for occupation exposure. In the manufacturing stage of ceramics, exposure to EMNs may occur through mixing of raw materials (Bessa et al., 2020). Ribalta et al (2019) found a high degree of correlation between dustiness and exposure concentrations in the handling of powder materials for ceramics (powders had a diameter of 3.4–120 µm). Therefore, an alternatives assessor may choose to use dustiness as an indicator of occupational exposure.

Challenge: *Although test guidelines are available for these physicochemical properties, it is unclear how readily available data will be for use in an alternatives assessment. Such data should be associated with registration dossiers under REACH, although not available as of this writing.*

2. ***Consider adopting a decision rule: ENMs absence in biomonitoring and environmental monitoring is a minor data gap (rather than a major data gap) because biomonitoring studies have historically been focused on chemicals.***

The IC2 Guide's Level 1 Exposure Assessment addresses whether the alternative has been found in either biomonitoring or environmental monitoring studies. Currently, lack of bio-environmental studies on an alternative in the IC2 guidance is suggestive of a "serious data gap that may affect the alternative's viability as a safer alternative." Bio- and environmental monitoring of ENMs remains in its infancy. As of this writing, ENMs are not on any standardized analyte list for human biomonitoring programs nor for any environmental monitoring programs, such as those required by regulatory authorities.

3. ***Consider adopting a decision rule: Exposure scenarios that demonstrate a likelihood of inhalation exposure should be of high concern for those ENMs where hazard data are unable to demonstrate reduced concern for toxicity, including where no data is available.***

Where inhalation exposure is likely, and where there is no evidence of "low" concern for key hazard endpoints such as such as carcinogenicity, reproductive/developmental toxicity, mutagenicity, persistence, etc., using measured or modeled hazard data (including the use of predictive physicochemical properties), such ENM alternatives could be screened out from further consideration.

In addition, the assessor may also consider hot spots of exposure to EMNs that may be relevant to vulnerable populations (e.g., oral exposure because children mouth on toys) where hazard data cannot demonstrate a reduced concern for toxicity.

EXAMPLE: The SCCS states that tris-biphenyl triazine, TiO2 and ZnO nanomaterials are "not to be used in applications that may lead to exposure of end-users lungs by inhalation" (ECHA, 2023). In EU cosmetics regulations, there is no nanomaterial that is approved for use in sprays.

D. Life Cycle Considerations

In both hazard and exposure assessment components in an alternatives assessment, life cycle considerations are embedded throughout. If an assessor chooses to execute the life cycle module, hazard, and exposures for the ENM alternative have already been considered across all life cycle stages (manufacturing, product manufacturing, use, disposal). In the IC2 Guide, the life cycle module is focused on addressing additional environmental impacts not considered in other modules (e.g., energy use, climate impacts, eutrophication, etc.).

1. ***If conducting a quantitative life cycle assessment (LCA), use nano-specific models to inform endpoints.***

If the assessor chooses to execute a full LCA of the ENM alternative, they should consider the LCA tools developed to assess ENM fate and transformation (Salieri et al., 2018). The use of such

tools for cradle-to-grave life cycle assessment of an ENM is recommended because existing LCA tools were developed with chemicals in mind. The assessor should be aware of the limitations of such tools during the interpretation of the LCA results for ENMs.

Conclusion

As stated earlier, the considerations offered above are based on a review of current environmental and human health assessment practices for ENMs (**APPENDIX I**). This is a fast-evolving landscape, and assessors should take stock of continued developments for the evaluating ENMs in alternatives assessments.

APPENDIX 1: LITERATURE REVIEW OF ENGINEERED NANOMATERIAL CONSIDERATIONS IN SAFETY AND SUSTAINABILITY ASSESSMENTS

To support evaluations of engineered nanomaterials (ENMs) in alternatives assessment, a review of the existing literature was conducted. The review focused on the status of assessment methodologies for ENMs to inform specific components of an alternatives assessment, including: (a) the identification of alternatives, (b) hazard assessment, (c) comparative exposure assessment, and (d) life cycle considerations. These four components comprise specific modules of the Interstate Chemicals Clearinghouse's (IC2) Alternatives Assessment Guide (2017) (IC2 Guide) – a model alternatives assessment framework used to guide this project. These modules are most relevant to the question of what adaptations are needed to current assessment methods and practices when considering ENMs as possible alternatives. Literature reviewed includes key reports from governmental and quasi-governmental sources, websites, and peer-reviewed research articles. Each subsection starts with a brief overview of the purpose of the given alternatives assessment module as context for adapting/applying emerging best practices for the health and safety evaluation of nanomaterials. This is not a comprehensive review of the ENM literature, but rather, extracts notable and seminal sources that provide key insights for alternatives assessments.

The published literature to date on alternatives assessment methods related to the evaluation of ENMs is sparse. However, alternatives assessment methods are aligned with other fields that have received much greater attention related to the adaptation of assessment methods for ENMs, including risk assessment and life cycle assessment (LCA). Experts agree that the existing risk assessment paradigm is applicable to the evaluation of ENMs but adaptations are needed to account for the specific properties of ENMs (OECD, 2022b). The literature reviewed captures the needs and considerations for nano-specific risk assessments given limited data availability, as well as the progress made over the last decade in validated methods and updated regulatory requirements, particularly in the EU. Existing methods for LCA have also been determined to be broadly applicable for ENMs (OECD, 2015). However, given that lack of adequate data inputs for LCAs on ENMs is an overarching limitation to current use of the method, this review focuses mostly on aspects of life cycle thinking as informed by this literature.

A. Identification of Alternatives

The identification of a functionally equivalent alternative in an alternatives assessment involves identifying "chemical substitutions, alternative materials, and changes to the product process or product redesign to eliminate a particular chemical" (Interstate Chemicals Clearinghouse, 2013).

Commonly, chemical substitutes can be identified with a Chemical Abstract Service registry number (CAS RN). However, many ENMs cannot be identified uniquely by CAS RN. A CAS RN reflects the molecular composition of the substance and does not differentiate substances based on their size or other structural characteristics (Lynch et al., 2020). In addition, multicomponent ENMs (e.g., a core with a coating) can contain more than one chemical,

which the CAS system is not designed to consider. Although some ENMs appear to have a CAS RN, such as fullerenes, other ENMs are commonly grouped under the CAS RN of the bulk material. For example, there is no unique CAS RN for nano silica or nano silver; instead, each use the CAS RN of the bulk substance.

The precise design of ENMs has led to a proliferation of materials of different sizes, shapes, compositions, and other properties. In addition, industrial-scale synthesis routes of ENMs can also produce mixtures of particles with a wide range of sizes. Therefore, identifying a specific ENM necessitates more than just listing a CAS RN and requires characterizing a range of physicochemical properties. **TABLE 1** describes the minimum set of property information needed to differentiate the ENM from other materials as outlined by specific government authorities.

The listing of properties for regulatory registration and reporting is meant to ensure that many possible factors are considered for ENMs and their intended uses. However, when conducting risk assessments of specific nanomaterials, knowledge of all these properties may not be necessary. The EU Joint Research Center (JRC) proposed a set of key physicochemical characteristics of ENMs based on their intended uses, which was later adopted in the REACH regulation (ECHA, 2019). The guidance acknowledges that not all characteristics may be relevant for all ENMs. For example, physical hazards of nanomaterials (e.g., flammability or explosiveness) may not apply to inert titanium dioxide nanomaterials in paints. However, particle composition, size and shape are almost always relevant to ENM use registrations.

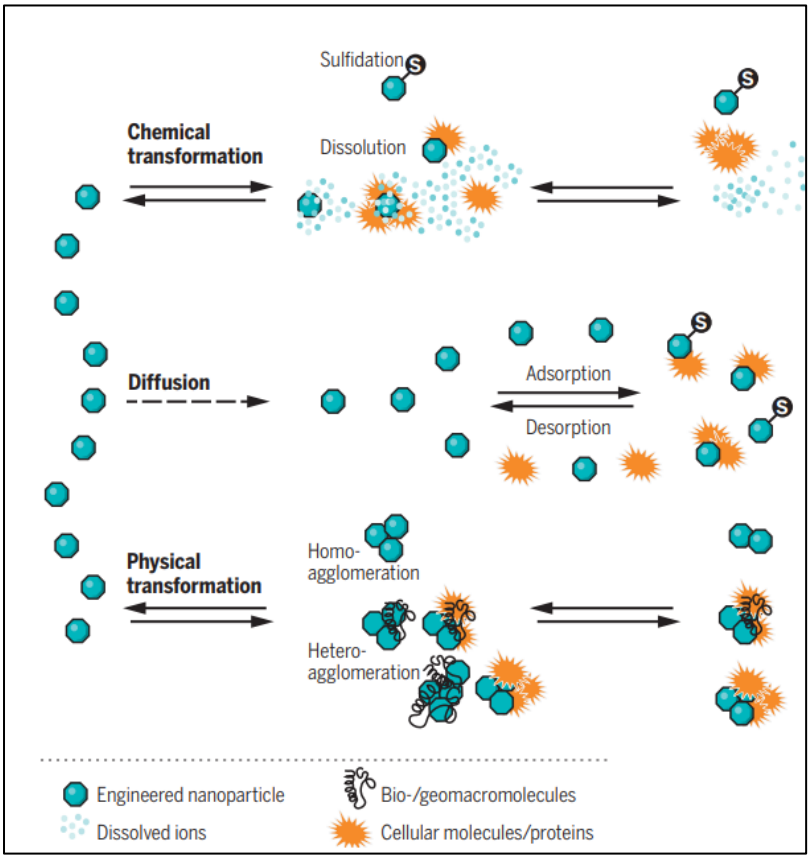
TABLE 1: Physicochemical properties used to report the identity of an engineered nanomaterial by specific authorities (ECHA, 2022a; EPA, 2017)

Property	REACH*	EPA
Size	x	x
Shape	x	x
Size Distribution	x	x
Chemical composition	x	x
Specific Surface Area	x	x
Surface Chemistry	x	x
Crystal Structure	x	
Dustiness	x	
Crystallite Size	x	
Functionalization method	x	
Batch-to-batch variabilities or impurities	x	
Solubility	x	

*Note: These physicochemical properties are necessary for registration requirements under REACH. However, data from these registrations are not currently publicly available.

The physicochemical characteristics of ENMs often change after exposure to the environment or biological media (**FIGURE 1**). This includes physical transformations (such as aggregation) and chemical transformations (such as dissolution and photochemistry reactions). These transformations modify physicochemical properties and produce "aged" nanomaterials. Transformations are especially relevant for reactive nanomaterials, such as silver, which may dissolve or sulfidize.

FIGURE 1: Illustration of possible nanomaterial transformations, which can occur in the environment or after contact with biological media (Valsami-Jones & Lynch, 2015)



The changes in ENM physicochemical properties in different environments have the potential to impact the study of ENM toxicity. Guidelines have been developed by the Organization for Economic Cooperation and Development (OECD) to encourage the reporting of ENM changes in different media in toxicity or environmental fate and transport studies (OECD, 2017); (OECD, 2019). EU guidance for the risk assessment of ENMs for food and animal feed applications require that the material be characterized at different stages, including the pristine state as manufactured, the material as prepared for testing, and on the material as present in products and applications (EFSA, 2021).

As ENMs become more complex in both synthesis and use (i.e., advanced materials or metastable materials), the potential for more complex transformations increases. For example, high-entropy-alloy nanoparticles (composed of 8 elements) are being studied as catalysts and have recently been demonstrated as effective for the hydrogen evolution reaction (Wu et al., 2022).

B. Hazard Assessment

A hazard assessment is key to the selection of safer alternatives. Hazard assessments focus on evaluating adverse effects to humans and other species as driven by the inherent properties of a given substance, mixture, or process. In alternatives assessment using the IC2 Guide, hazard assessments consist of first using authoritative lists of chemicals of concern to screen out problematic alternatives and then evaluating alternatives based on toxicity data for an array of hazard endpoints using comparative hazard assessment methods.

In the absence of *in vivo* and *in vitro* data, the IC2 Guide also relies on quantitative structure activity relationship (QSAR) data and grouping to overcome a lack of experimental data. Emerging methods and practices for the identification and assessment of ENM hazards are reviewed below.

Use of Authoritative Lists

To date, only a few ENMs appear on authoritative lists. This includes multi-walled carbon nanotubes-7 (MWCNT-7) which was classified by IARC as "possibly carcinogenic" (Group 2B) (IARC, 2017). Titanium dioxide is also considered by IARC as "possibly carcinogenic to humans" (Group 2B) and is included on California's Prop 65 list (airborne, unbound particles of respirable size) based on evaluation of ultrafine particles, which are in the nano-size range (OEHHA, 2018). In addition, multiple NGOs and companies are including ENMs on their restricted substance lists (RSLs). Examples include carbon nanotubes (CNTs) on ChemSec's SINList, which is for both single- and multi-walled carbon nanotubes, irrespective of size and chemical modifications (Hansen & Lennquist, 2020). GreenScreen® Certified has also included 7 specific ENMs on their RSL for Food Service wear. Nike has restricted nanomaterials without stringent hazard assessments in all their footwear, apparel, and equipment product lines (CEH & CPA, 2022; Nike, 2022).

In addition to authoritative lists, regulatory approvals are relevant to screening ENMs for certain applications. In the EU, many regulatory directives require an authorization process to obtain approvals for the use of ENMs. For example, the EU Cosmetics Directive requires authorization of ENMs used as colorants, preservatives, and UV filters in cosmetics. To date, only 4 ENMs have received such authorization (SCCS, n.d.). This authorization process is an attempt to be proactive in keeping substances of concern off the market, rather than reactive, where problematic chemicals are elevated in the market because of a failure in chemicals management legislation regarding pre-market reviews. ENMs that are not authorized present a significant risk to human health and the environment – a similar justification for placing a substance on chemicals of concern/authoritative list.

Use of Bulk Form Hazard Information

If a substance does not appear on authoritative lists, the IC2 Guide indicates the assessor should gather and compare hazard information. For ENMs, there may be a completed hazard assessment available for the bulk form, but the assessor should use caution when applying this bulk hazard information to an ENM. There are various methods to determine whether nanomaterials have specific "nano-relevant" properties, such as the Swiss Precautionary Matrix (Höck et al., 2018). There are two scenarios where it may be appropriate to use the bulk hazard information for an ENM; either when an ENM is composed of a restricted chemical or when an ENM is readily soluble.

Guidance from the European Commission outlines that hazard data for specific substances comprising an ENM (e.g., toxicity data on silver for nanosilver, or data on silica for nanosilica) should be used to inform toxicity. Current risk assessment guidance issued by the European Food Safety Authority (EFSA) includes considerations about the carcinogenic, mutagenic, and reproductive toxicity properties of one or more of the chemical components of an ENM (EFSA, 2021). This guidance is aligned with design rules emerging from the green nano/Safe by Design (SbD) literature recommending the avoidance of ENMs that contain known toxic elements in which the ions or small molecules pose recognized hazards to human health and the environment until direct test data is available to demonstrate safety (Hutchison, 2008; Schwirn et al., 2021). As stated by the European Commission's Scientific Committee on Consumer Safety (SCCS) in its Scientific Advice on the Safety of Nanomaterials in Cosmetics, "As a general rule, where chemical component(s) are toxic, as such or when put together in the form of a nanomaterial, they should constitute a trigger for concern over safety to the consumer health" (Bernauer et al., 2021).

Another possible scenario for nanomaterials hazards to be "grouped" with their corresponding bulk materials occurs when the nanomaterial is soluble (i.e., readily dissociates into its molecular or ionic components in water) (EFSA, 2021). In other words, ENMs that lose their nano-specific properties when added to water resemble the bulk form of their substance in solution. For example, an ENM composed of sodium chloride dissolves after contact with water, creating sodium and chloride ions in solution – exactly as bulk sodium chloride behaves. Therefore, the toxicological assessment of sodium chloride is applicable to nanoscale sodium chloride. According to the European Chemicals Agency (ECHA), an ENM with a solubility of > 100 mg/L in water within 24 hours is exempt from ENM-specific guidance for environmental toxicological testing. For human health endpoints, the nanomaterial should be greater than > 33.3 g/L and have a half-life of water dissolution of ≤ 10 minutes. As the latter values are not yet based on OECD guidelines or equivalent validated procedures in biological media, these values may be updated when more information is available (ECHA, 2022b). With expert judgement, OECD proposes that nanomaterials that aggregate irreversibly in realistic conditions may also be considered bulk materials (OECD, 2019). However, this may be applicable to nanomaterials only at certain stages in their life cycle, such as after an integration into a product.

Use of GHS to Classify Hazards of Nanomaterials

In the case where an ENM's hazard assessment should not be derived directly from the bulk hazard assessment, the assessor can leverage ENM-specific hazard data. Roughly a decade ago, there was a sheer absence of health and safety data on ENMs; that is not the case today. Over the last decade, there has been a tremendous growth in the toxicity assessments of ENMs.

Use of GHS (Globally Harmonized System of Classification and Labelling of Chemicals) hazard categories underlies nearly all alternatives assessment hazard assessment approaches. The OECD Guidelines for the testing of chemicals are the foundation of the GHS system (UNECE, 2019). However, because physicochemical properties of ENMs differ from conventional chemicals, several efforts have reviewed the applicability of GHS to ENMs. One such effort was sponsored by the Nordic Council of Ministers, which examined 4 ENMs and the applicability of existing test methods and GHS criteria for 5 hazard classes (Larsen et al., 2019). In general, current GHS classification criteria were found to be applicable although important caveats were noted. The

assessment revealed that classification differences were observed for the same type of ENM given differences in purity. The assessment also revealed that testing at high doses for ENMs with high surface areas and low density may not be technically achievable, which was especially relevant for testing via inhalation route. Insufficient data/data gaps were also apparent for most ENMs assessed.

A second effort sponsored by the World Health Organization similarly evaluated 11 ENMs and the applicability of existing GHS criteria for 9 hazard classes (Lee et al., 2017). This review did not offer substantive insights about the applicability of specific test methods and related criteria. However, similar to the review above, data gaps were noted across all ENMs reviewed.

The OECD Testing Program of the OECD Working Party on Manufactured Nanomaterials has spurred by the development of standardized test methods and associated toxicity research efforts (OECD, 2023b). The OECD continues to develop testing guidelines that consider ENMs, such as the recently released "Guidance Document on Aquatic and Sediment Toxicological Testing of Nanomaterials" which modifies the Growth Inhibition Test for Freshwater Alga and Cyanobacteria (TG 201) (OECD, 2022a). Characterization of nanomaterials is also incorporated into the OECD Test Guidelines for Chemicals in Section 1: Physical Chemical Properties (2023a). Therefore, as these methods and guidelines are operationalized, GHS testing will contain ENM-specific considerations. Assessors, however, should use caution when examining past studies that inform GHS but do not consider ENM properties.

It is important to review specific OECD test guidelines as some for bulk chemical substances should not be used or have been significantly adapted for ENMs. One important example of this is the use of the Ames test for mutagenicity. The scientific community has concluded that the standard bacterial Ames assay test is usually not adequate to be part of the battery of mutagenicity tests for "poorly soluble" nano particles (ECHA, 2022b).

Extensive dossiers on 11 commonly used (or soon to be used) ENMs are available based on research sponsored or reviewed by the OECD Test Program (OECD, 2023b). Extensive research conducted through US EPA's Office of Research and Development on ENMs has been organized in a relational data repository, NaKnowBase. The NaKNowBase connects physicochemical properties of researched ENMs with their toxicity impacts (human, ecological, environmental fate impacts) – an important feature given the broad variation of physicochemical properties within the same type of ENMs that may result in varied impacts (Boyes et al., 2022). ENanoMapper, funded by the EU, also gathers nanomaterial hazard information, with an emphasis on the physicochemical properties reported by each study (FP7, 2023).

Data from ENMs in their pristine form can be different than if the ENM was tested in specific environmental media or in various biological/environmental compartments of their life cycle. Thus, the form of the ENM tested may not always best represent the form to which cells and organisms are exposed. The OECD recommends that assessors ensure that the ENM being examined in a toxicological study: (a) has been adequately characterized, (b) represents a realistic conservative form of the material to which an individual or organism has been exposed, and (c) is adequately representative of the test material used in the effects testing (OECD, 2022b).

Data Gaps in *In Vitro* and *In Vivo* Data

Despite the growth in research and subsequent publications on the health and safety of ENMs, data gaps still abound. Data is often lacking for ENMs on important endpoints for alternatives assessments, such as carcinogenicity and reproductive toxicity (OECD, 2023b).

To fill these data gaps for a specific ENM under assessment, assessors may choose to examine toxicological studies of similar EMNs. Methods such as grouping, read-across and QSARs can support hazard assessments where the physicochemical properties of an ENM are different than for other similar ENMs where hazard and physicochemical data have been published. However, similar ENMs may have different physicochemical properties, such as shape and coating. In addition, the agglomeration/aggregation properties of ENMs present additional challenges.

Predictive Hazard Methods – Grouping

The sheer variety of ENMs and their potential to transform complicates assessment efforts for safety and sustainability. To decrease this complexity, there is growing practice in the use grouping based on specific physicochemical characteristics. This enables the use of read-across from nanomaterials of known toxicity and environmental transformations to those with untested impacts. Justifying a grouping rationale for ENMs supports the use of available data on "surrogate" materials, as it is unlikely that safety/sustainability assessment data will be available for the exact ENM material under investigation.

In the early 2000s, one of the first approaches to grouping nanomaterials was developed based on scientists' experience with asbestos' physicochemical properties. High aspect ratio nanoparticles (HARN) have 3 key characteristics which correlate to asbestos and therefore a high risk of mesothelioma: (a) a diameter small enough to reach sensitive areas of the lung; (b) a length long enough to cause certain inflammatory pathways in the lung; and (c) bio-persistence such that the impact is maintained (Tran et al., 2008). This approach has continued to be employed in many nanomaterials grouping models such as NanoRiskCat and the GRACIOUS Framework (Hansen et al., 2014; Stone et al., 2020). If more data is available regarding the HARN, a recent integrated approach to testing and assessment has been developed which includes predictive toxicology tests and *in vitro* methods to inform assessors of the potential for the same adverse impact pathway that leads to mesothelioma (Murphy et al., 2021).

Data gaps in the characterization of physicochemical properties and lack of sufficient toxicological testing of nanomaterials are two reasons that read across is carefully used by regulators, often with substantial justification (ECHA, 2022d). Recent amendments to the EU REACH regulation have set guidelines for justifying a nanomaterial's grouping into a "set of nanoforms" (ECHA, 2019). This grouping is based on extensive physicochemical characterization of both the group and the candidate nanomaterial.

The starting point for grouping nanomaterials is "what they are" (chemical/physical characteristics, see **FIGURE 2**) followed by a consideration of the relevant properties of "what they do" and "where they go". An example "set of nanoforms" across different endpoints does not appear to be publicly available from ECHA. The OECD and Joint Research Council (JRC) recently tested these ECHA grouping parameters and determined that grouping is possible and useful, but the hazard endpoint should first be tentatively linked to the ENM's physicochemical property (OECD, 2018). A potential hypothesis for the grouping of certain ENMs are the use of HARNs

(FIGURE 3) which are generally accepted as hazardous.

Alternatives assessments considering ENMs have employed a range of grouping approaches by physicochemical property. When analyzing silver nanomaterials with GreenScreen®, Sass and colleagues grouped according to core composition (silver) as well as coating function that allowed for the use of silver ENM as an antimicrobial and size distribution (1-100 nm) (Sass et al., 2016). This assessment also benefited from the existence of a standardized particle; AGS-20. A recent GreenScreen® on titanium dioxide grouped nano-sized titanium dioxide particles with non-nano pigment grade and respirable size particles (≤ 10 µm) (WAP, 2021). Although specific studies were cited in the assessment, specific physicochemical considerations outside of particle size were not reported.

FIGURE 2: Key properties of nanomaterials, grouped by either physical identity or intended use. This information was identified by ECHA as the basic information needed to implement read across for hazard assessments of nanomaterials. Properties that are requirements for REACH registration are in bold (ECHA, 2019).

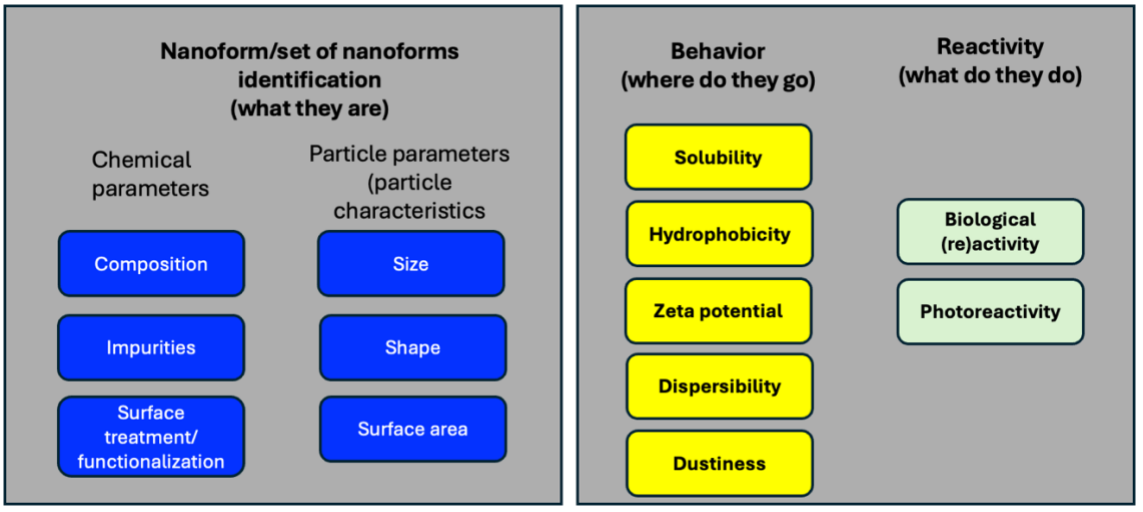


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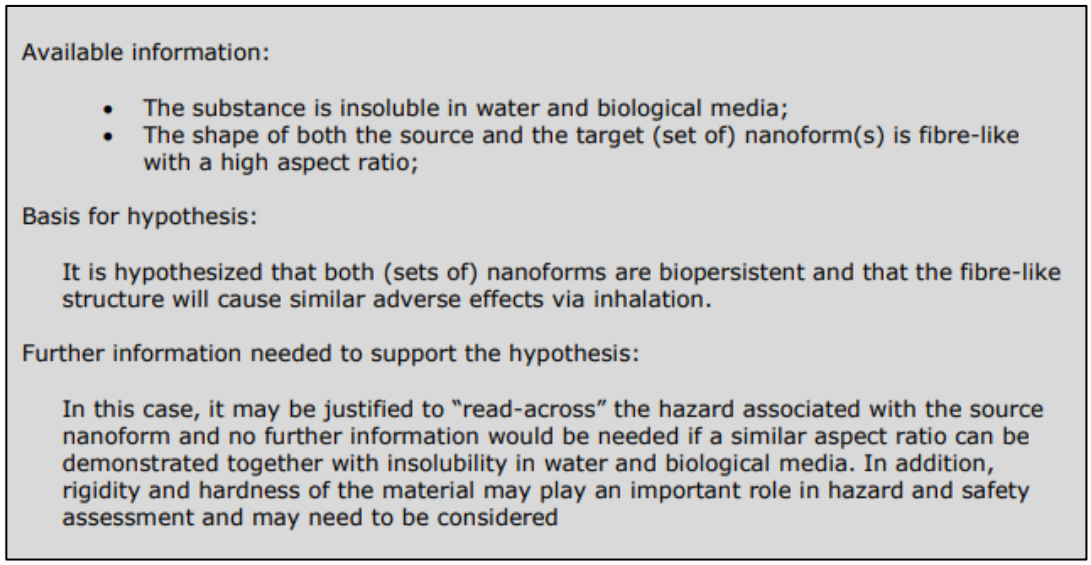
FIGURE 3: Example of grouping hypothesis based on high aspect ratio for inhalation effects (ECHA 2019)



Predictive Hazard Methods: Read-Across and QSAR methods

Read-across and QSAR methods adapted for ENMs are available but are generally not yet accepted as stand-alone hazard results due to a lack of standardization of the input data (test methodologies and nanomaterial properties) and a lack of robustness (ECHA, 2019). Research and refinement of these modeling methods is an active field of study, with both the EU (through REACH and research efforts) and OECD test methodology standardization efforts.

Nanomaterial-specific predictive hazard modelling methods are necessary because specific physicochemical properties indicative of potential hazards used for conventional chemicals are not applicable to ENMs. Such is the case for use of the octanol water coefficient, solubility, and granularity. For instance, the octanol water coefficient, which predicts the bioaccumulation potential for chemicals, should not be applied to ENMs because the thermodynamic equilibrium of nanomaterials cannot be reached at the same rate as small chemicals (ECHA, 2022b). The dissolution and aggregation of nanomaterials can also skew the results of the bioaccumulation test. Guidance for the registration of ENMs under REACH recommends alternative fate descriptors which are currently under development by the OECD (ECHA, 2022b).

Solubility (as a proxy for mobility) should also not be readily applied to ENMs. For chemicals, a lack of solubility generally decreases the mobility of a chemical and decreases its chances for access to humans or other organisms. For ENMs, a lack of solubility does not necessarily mean that the material cannot be dispersed in solution. A stable ENM dispersion could lead to high mobility. Therefore, guidance for the registration of ENMs under REACH does not recommend waiving certain toxicological endpoints for nanomaterials solely based on insolubility (ECHA, 2022b). Granularity is commonly used to consider whether inhalation of a material is possible. However, due to the small size of ENMs and their ability to agglomerate, the characterization used for granularity must be fit for a wide range of particle measurements. Therefore, ECHA recommends multiple methods to effectively capture the large size range of particle measurements (ECHA, 2022b).

Safe by Design (SbD)

Awareness of the inherent health and safety concerns of ENMs has spurred the growth of the SbD concept in both the EU and the US. The concept aims to reduce adverse effects on human health and the environment by altering the design of nanomaterials to ensure safety across their life cycle. The concept envisions tuning specific physicochemical properties to reduce the toxicity of ENMs, such as altering the chemical functionalization or shape of the material (Jiménez et al., 2022).

In general, SbD involves an iterative assessment process that divides the innovation process into a predefined set of stages and activities. The progression from one stage to the next is regulated by a gate, where the project is evaluated according to a set of adaptable safety criteria, leading to a decision regarding whether the project should continue or not. SbD incorporates predictive toxicology testing using New Approach Methods (NAMs) early in the innovation phase to better discern safer options to advance towards commercialization (Jiménez et al., 2022).

At the time of writing, the lack of robust QSAR and read-across methods calls for the use of grouping or toxicological studies to determine whether an ENM that is safe by design is, indeed, safe by design.

C. Exposure Assessment

Alternatives assessment focuses on the selection of safer alternatives rather than controlling exposure as the key to decreasing harmful impacts on people and the environment. This component of the assessment answers the question: *Is the alternative preferable, equivalent to, or potentially worse than the priority chemical of concern given the potential for exposure.*

Qualitative exposure assessments are recommended for alternatives assessment, and focus on how the possible exposure concerns are different depending on the alternative at each stage in the chemical or product life cycle (from manufacturing to end-of-life) (Interstate Chemicals Clearinghouse, 2017). Assessments primarily consider the potential for exposure based on inherent or intrinsic chemical and physical properties as well as expected use scenarios and do not, necessarily, attempt to quantify those exposures, except where necessary to understand potential trade-offs.

To date, alternatives assessments examining ENMs have considered ENM physicochemical properties in the exposure assessment using a qualitative manner. For example, Gilbertson and Ng (2016), when assessing ENM alternatives to chemical flame retardants considered the possibility for volatilization of the ENM alternative during use. The authors also acknowledged the opportunity to configure the ENM with specific physicochemical properties to minimize exposure during manufacturing but did not state what those should be. Hjorth et al. (2017) recommended the use of control banding, such as the NanoRiskCat approach, to estimate exposure potential in alternatives assessment. The NanoRiskCat approach establishes that ENMs in the air or suspended in liquids have high exposure potential, ENMs that are surface bound have a medium exposure potential, and ENMs embedded inside of solids have a low exposure potential.

Inhalation as an Inherent Concern for ENM exposures

The intrinsic inhalation exposure potential of unbound, un-agglomerated ENMs is significant given their extremely small size (Braakhuis et al., 2021). In addition, because ENMs (particularly nanoparticles) have a slow rate of settling, some can remain suspended in air for longer periods of times and become more broadly dispersed over wider geographic areas than larger particles of the same size. ENMs that are in the form of powders, sprays or aerosols may result in a high potential for exposure in either manufacturing or consumer product use settings. Exposure could also happen in later life cycle stages due to mechanical or physical processes, such as sanding cured paint or weathering processes. In such situations, there is a high potential for inhalation exposure prior to any controls. Consequently, the default assumption is that for any unbound ENM, inhalation exposure is highly likely.

The high concern for nanomaterials' impacts via inhalation has been noted by ECHA and the OECD (OECD, 2022b), several academics, and other regulators:

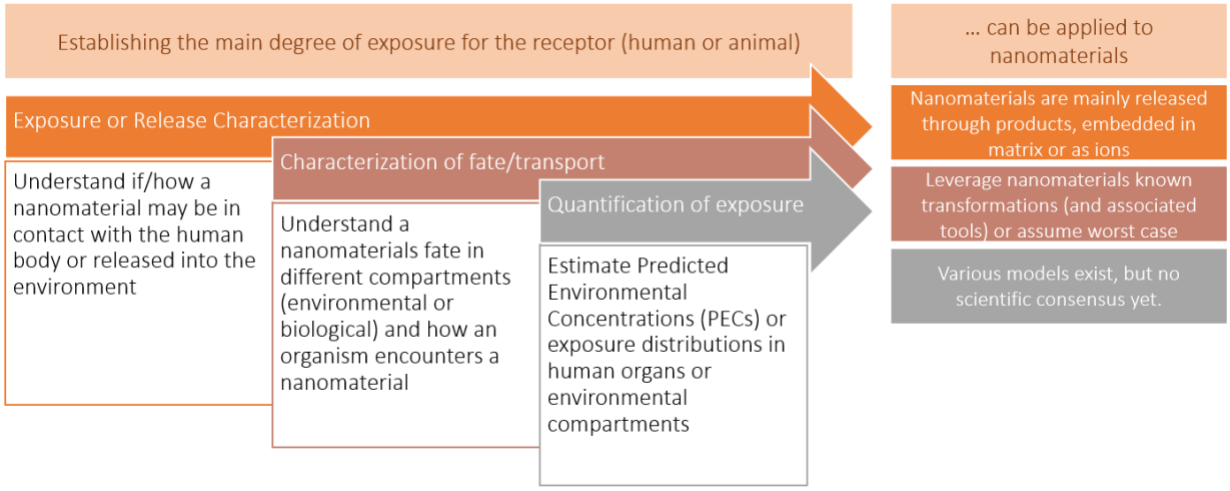
- ENMs that are poorly soluble can induce pulmonary effects if inhaled at high concentrations because lung clearance mechanisms are unable to remove the particles given rate of exposures (Braakhuis et al., 2021).
- Nanoparticles deposited in the lung can translocate to distal sites (Aragon et al., 2017; Erdely et al., 2009; Mercer et al., 2013).
- Pulmonary exposure to nanoparticles can cause cardiovascular effects and some may be carcinogenic (Kuempel et al., 2017; Li et al., 2007; Nurkiewicz et al., 2008; Rittinghausen et al., 2014; Sargent et al., 2014).
- High aspect ratio nanomaterials (HARNs) are considered an inhalation hazard and therefore have extremely low exposure limits that are often based on fiber numbers (Visser et al., 2022).
- Recent decisions by the EU SCCS have informed restrictions in the EU for ENMs such as the use of nano-zinc oxide and nano-titanium oxide in spray-based cosmetics, including sunscreens because of the high exposure potential and concern for impacts on the pulmonary system (SCCS, n.d.).

ENM Exposure Considerations in Risk Assessment

Risk assessment of ENMs has led to the development of various exposure assessment frameworks, the majority of which are qualitative in nature. This is partly due to the difficulty in assessing the transformations of nanomaterials under environmentally relevant conditions. One such example in the difficultly of setting quantitative metrics for exposure is the agglomeration of nanomaterials that may lead to decreased uptake of nanomaterials in toxicological studies, complicating studies that set a high dose for the no observed effect concentration (NOEC). Therefore, exposure assessments should consider possible changes to the form of ENMs across the life cycle, which are often dictated by physicochemical properties (Oomen et al., 2018). This is echoed in nanomaterial safety guidance developed by the EU SCCS, which emphasizes that information on size distribution of ENMs in products is needed due to the link between ENM size and uptake (SCCS, 2023a).

Advances in exposure assessment techniques for ENMs have been made through the development of various tools and standardization practices, as recently reviewed by the OECD considering adaptations needed in existing methods (OECD, 2022b; **FIGURE 4**).

FIGURE 4: Exposure considerations for chemicals and their development in reference to nanomaterials.



As previously mentioned, inhalation of ENMs is considered a route of significant concern. When ENMs are embedded into products, recent reviews have pointed out that the release of nanomaterials often involves fragmentation of the matrix or dissolution of the nanomaterial into ions (Svendsen et al., 2020). ENMs that are released as fragments are often associated with other components of the product, such as plastic fragments. For assessment purposes, it is therefore key to understand the product, its use, and how (and how strongly) the ENM is embedded/bonded in the matrix.

Once released from a product (or as free nanomaterials due to emissions/releases during manufacturing), ENMs are transformed and transported in the environment. Their many physicochemical characteristics have presented a challenge in determining their environmental fate and behavior (Hansen et al., 2011). Due to the relatively low concentration of ENMs in comparison to other chemicals, materials, or biological matter and their inherent reactivity, it is very common for nanomaterials to form hetero-aggregates. ENMs may also dissolve over time, and this dissolution may be accelerated in certain environments (such as the acidic digestive tract of certain organisms) (OECD, 2019).

Commonly used exposure assessment models, such as European Union System for the Evaluation of Substances (EUSES) have been determined to be insufficient for ENMs given that (a) the extent/rate of dissolution is unknown or not included in current models and the same goes for (b) the extent/rate of aggregation/settling and (c) the extent of association with sediment. These factors, in addition to lack of specific knowledge about transformation processes, renders significant gaps in current understanding of fate and transport of ENMs (Baun et al., 2017). Based on a review of environmental fate literature to date on ENMs, Baun et al. (2017) do suggest however that surface affinity is a specific physicochemical property that may provide significant insight into the environmental fate and behavior of ENMs. Surface affinity is a crucial parameter in determining the attachment of nanoparticles in environmental matrices and their tendency to heteroaggregate.

The OECD recently analyzed exposure models developed for quantification of consumer and occupational exposure of nanomaterials. The qualitative models Stoffenmanager Nano v1.0 and Swiss Precautionary Matrix v3.1 were identified as appropriate to prioritize nanomaterials exposure to consumers whereas quantitative methods tended to overestimate exposure (OECD, 2021a).

In alternatives assessment, quantification of exposure is not typically necessary, except when exposures from the alternatives are likely to be the same or higher than the chemical of concern, but if there are significant uncertainties, quantitative assessments could be informative (Greggs et al., 2019). There are also tools developed for ENM exposure in specific scenarios, such as the ConsExpo tool for ENM inhalation exposure due to spray products. Quantitative tools used for the modelling of nanomaterials in the environment have also recently been evaluated by the OECD (OECD, 2021b). One such model is SimpleBox4Nano which adds nanomaterial considerations through different physicochemical factors, with recently proposed critical limits (Meesters et al., 2019). These models simplify the environmental transformations of ENMs into three categories: free ENMs, bioavailable ENMs (< 450 nm, may be aggregated with natural colloids), and nonbioavailable ENMs (> 450 nm).

Data gaps in ENM exposure knowledge

Like predictive hazard assessments, certain tools applicable for measuring exposure to chemicals are not applicable to ENMs. For example, OECD TG 417 (which delves into toxicokinetics) is not applicable to ENMs because it does not account for particulates and instead relies on diffusion and perfusion over a relatively short time frame (Rasmussen et al., 2016). This has led to the development of ENM-specific standards such as ISO/TR 22019:2019 "Nanotechnologies-Considerations for performing toxicokinetic studies with NMs" and may lead to updated OECD guidance (ISO, 2019).

There is also limited data concerning ENMs in bio- and environmental monitoring studies. Small-scale studies testing ENMs in workers or in ENMs in environmental media, such as ambient air or soil, do exist, but no large-scale bio- or environmental monitoring studies of ENMs were found (Bergamaschi et al., 2022; Bocca et al., 2023).

In the absence of experimental data or estimations, various regulatory agencies have adopted the stance that ENM exposure is more likely than when handling bulk materials. The SCCS assumes that dermal absorption of ENMs is either equivalent or higher than conventional substances (Bernauer et al., 2021). NIOSH guidance on inhalation recommended exposure limits (RELs) for 2 ENMs (Ag and TiO_2) are roughly 10x lower than that of larger particle sizes (NIOSH et al., 2022). WHO recommends an occupational exposure limit (OEL) that is equivalent to or lower than the bulk material (WHO, 2017).

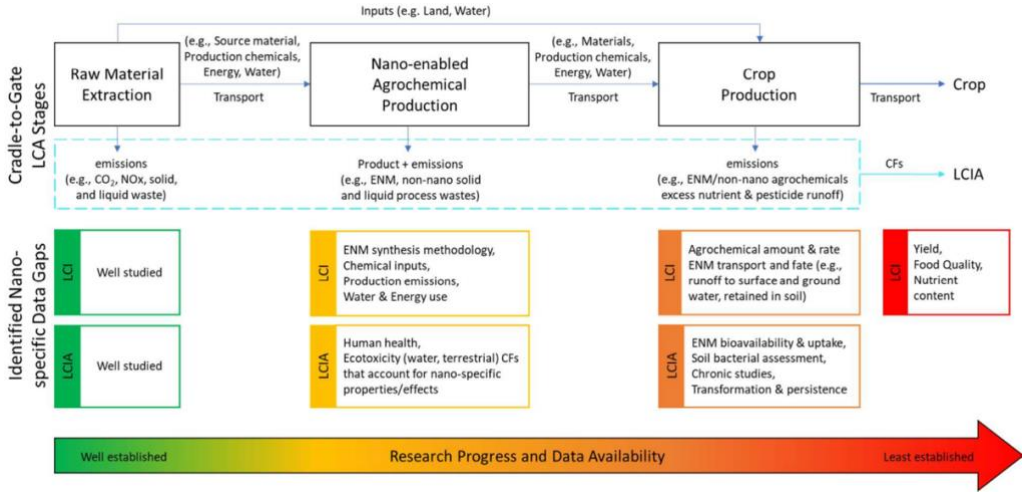
D. Life Cycle Assessment

Life cycle assessment refers to the quantification of impacts in various categories (from climate change to eutrophication) throughout the life cycle of a product. This method is standardized through ISO 14040. While less standardized, LCT also considers the life cycle of a product, avoids burden-shifting from one stage to another, and embraces the many social, environmental, and economic categories similar to LCA. With LCT, only stages where the alternative will differ significantly from the incumbent product should be considered.

The process for creating a life cycle inventory (LCI) can benefit alternatives assessment by mapping the life cycle of a nanomaterial and its use in specific products. This could allow the assessor to compare the impacts of different manufacturing routes of nanomaterials, including the selection of raw materials. For example, when considering the production of silver

nanomaterials, a recent review of LCAs pointed out that the silver precursor acquisition (including mining and refining) was the main contributor to greenhouse gas emissions (Temizel-Sekeryan & Hicks, 2020). Therefore, certain hot spots in a nanomaterial's production in alternatives assessment can be effectively identified through analysis of a corresponding LCA (**FIGURE 5**).

FIGURE 5: Degree of confidence in nanomaterials life cycle analysis, from most established (left, green) to least established (right, red) (Pourzahedi et al., 2018).



Since end-of-life impacts are quantified in an LCA, these methods were analyzed for their adaptation to ENMs and LCT for alternatives assessment. Just as in the hazard and exposure assessments, a lack of nanomaterial-specific data to feed into an LCI is commonly cited as a barrier (OECD, 2015). This lack of foundational data also impacts important intermediate data points in LCA called characterization factors. These characterization factors are a combination of chemical – or nanomaterial – persistence, exposure, and probability of adverse effects to a certain population. Characterization factors are especially key for human health endpoints and ecotoxicity. Certain groups have hypothesized characterization factors for individual nanomaterials (and thus effectively brought together many studies on nanomaterial hazard and exposure), but there is not yet consensus in the field for the specific values. Alternatives assessment practitioners can use the data behind characterization factor determinations as a foundation for determining a potential "hot spot" in the ENM life cycle.

One model that supports the data behind LCA's characterization factor is the USEtox model, with proposed adaptations for the exposure to consider the various forms of nanomaterials present (Salieri et al., 2018). These adaptations include a consideration of two fractions for ENMs – a soluble and insoluble fraction. In this context, the soluble fraction indicates ionic components, while the insoluble fraction is intact ENMs (either free or hetero aggregated to colloids). In the absence of data, the model recommends the assumption of complete dissolution of the ENM, a precautionary approach as ions are typically more hazardous than bulk elemental forms. Such adaptations to the USEtox model have not yet been integrated into the Product Environmental Footprint (PEF) methodology, which is in development in the EU to bring LCA into sustainability-related policies.

The integration of ENM-specific considerations for endpoints in LCA have not yet become

standard in the field. For example, models such as eutrophication and greenhouse gas emissions have not yet been adapted to consider ENM properties – if needed at all.

APPENDIX II: PHYSICOCHEMICAL PROPERTY DESCRIPTIONS (REPRODUCED FROM ECHA 2019)

Properties Influence Hazard Traits	
Size and size distribution	The size of the particles of the nanoform affects other physicochemical parameters, such as crystallinity, zeta potential and specific surface area, and may determine exposure, and whether the nanoparticle can be internalized into an organism. Once internalized, particle size may also affect the distribution within the body, and the toxicity at both the point of entry and distally. Size distribution is not a static parameter; it may also change during the course of (environmental) toxicity testing (as well as during the lifecycle of the material) due to e.g., partial dissolution, interaction with test media or preferential absorption of smaller particles
Shape and crystallinity	Particle shape may affect the internalization of a nanoform (e.g., the ability of a nanoform to penetrate into a cell) and its (environmental) toxicity. In inhalation studies, particle shape may influence nanoform deposition within the lungs and may also influence its persistence in the lungs and probably in other sites. Particle shape may also influence other parameters, such as zeta potential. Crystal structure may for some nanoforms influence other properties of the material (e.g., reactivity, zeta potential, Hamaker constant) in a way that affects human and environmental toxicity. Decreasing size of particles may introduce crystallographic changes in the material (contraction of the crystal lattice or deformation).
Surface area, including porosity	The increase of relative surface area with decreasing particle size may increase the reactivity of a nanoform relative to its mass and/or volume. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure.
Chemical composition	Detailed information on chemical composition is fundamental for determining human health and environmental effects of nanoforms, as is the case for non-nanoforms. However, size, shape, and surface characteristics of a nanoform may cause the nanoform to exhibit a different behavior compared to the non-nanoform of a material with the same composition.
Impurities	As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.
Surface treatment/ functionalization	The term surface chemistry indicates the chemical composition at the surface of the particles as a result of chemical coating and/or surface treatment of the particle. Surface chemistry influences dissolution behavior and agglomeration behavior of nanoforms. Considering hazard endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine which biomolecules adhere to the nanoform, its distribution and cellular uptake, and its toxic effects. In the environment, surface chemistry will influence sorption to environmental or biological media and the reactivity of a nanoform.

Properties Influencing Intrinsic Exposure Potential	
Solubility	The rate of dissolution depends on factors including, but not limited to, the chemical composition, particle size, coating, surface treatment, stability, manufacturing process, and biological environment. The rate of dissolution gives information on how many ions/molecules are released from the particle over time. The ion(s)/ molecules released may also dictate the toxicity of the nanoforms, which will be an important aspect of the evaluation. "Water solubility" is an intrinsic material property, but in most cases the system-dependent property "dissolution rate in relevant biological media" will be more relevant as this fundamentally affects the bioavailability of substances in the (biological) environment. The relevance of the different media depends on the actual route of exposure and/or the environmental compartment under evaluation.
Hydrophobicity	Hydrophobicity for nanoforms is dependent on e.g., van der Waals energy (as represented by the Hamaker constant) and surface charge. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g., sessile drop contact angle, dye adsorption. While these parameters can influence agglomeration and sorption, as well as "dispersibility in biological media" and dustiness, currently the exact relationships between them are not clear. Hydrophobicity is influenced by the surface treatment/functionalization of the particles. Thus, knowledge on the surface chemistry can give qualitative information about the hydrophobicity of the nanoforms.
Zeta potential	Zeta potential can be used as a proxy for surface charge and may provide information in dispersion stability, degree of agglomeration/de-agglomeration of particles in relevant media. Surface charge may influence systemic distribution and cellular uptake of a nanoform, and ultimately its toxicity. There is additional evidence linking zeta potential to the inflammation potential of nanoscale particles of metals and minerals.
Dispersibility	This parameter can influence the degree of environmental transport and (environmental) exposure. Furthermore, this parameter may influence the degree of internal exposure (particularly by the oral route; however, particle dispersibility also affects nanomaterial mobility within the lung and hence its potential for systemic uptake).
Dustiness	This parameter is mainly relevant for exposure via air (particularly by inhalation) and transport through air. In the environment, this parameter is not relevant to aquatic/sediment exposures and only to a limited extent for soil exposures. Dustiness depends on a number of factors such as physicochemical properties of the particles (e.g., size, shape, relevant density, type of coating), the environment (e.g. moisture, temperature), the type of process (e.g. energy applied), the interaction between particles during agitation (e.g. friction shearing) and the sampling and measurement configuration.

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