

**Framework for the Risk Assessment of Manufactured Nanomaterials under the
*Canadian Environmental Protection Act, 1999***

DRAFT

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Executive summary

Nanotechnology, which can be described as the manipulation of matter at the nanoscale (about 1 to 100 nm), is an emerging technology with enormous innovation potential. Manufactured nanomaterials (NMs) developed using this technology are rapidly entering the Canadian market across a wide range of applications and industries. Chemical substances, including nanomaterials, are regulated under the *Canadian Environmental Protection Act (CEPA)*, which provides the authority to collect information and to assess and manage risks to the environment and human health.

The principles used for chemicals assessment are appropriate for the assessment of nanomaterials, with necessary modifications to address the specificities of nanomaterials. In 2013, the Organization for Economic Co-operation and Development (OECD) recommended its Member Countries apply existing international and national chemical regulatory frameworks to manage the risks association with manufactured nanomaterials:

“to manage the risks of manufactured nanomaterials, [country members should] apply the existing international and national regulatory frameworks or other management systems, adapted to take into account the specific properties of manufactured nanomaterials”
(OECD, 2013)

This framework provides guidance on adapting the existing practices for risk assessment to account for the novel properties exhibited by substances at the nanoscale in accordance with the OECD recommendation.

The framework is divided into three sections. Section 1 introduces the context, the scope and the purpose of the document. It also contains a summary of the policies that support the risk assessment of NMs under CEPA. Section 2 provides an overview of substance risk assessment under CEPA. Although Section 2 is not specific to NMs, it provides context on practices and processes generally used under CEPA to assess substances, considering that many of the principles used for chemicals assessment are appropriate for the assessment of NMs. Section 3 presents modifications to those general practices and process for risk assessment to address the specificities of NMs. Notably, the nanomaterial-specific considerations for risk assessment include discussions on the physical and chemical properties specific for NM identification and characterization, their behaviour and their potential effects on human health and the environment. Furthermore, Section 3 explains how the overall risk characterization of NMs is conducted under CEPA and how uncertainties are weighted into risk characterization.

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List of abbreviations

AF	Assessment Factor
CAS RN	Chemical Abstracts Service Registry Numbers
CCME	Canadian Council of Ministers of the Environment
CEPA	<i>Canadian Environmental Protection Act, 1999</i>
CMP	Chemicals Management Plan
CTV	Chronic Toxicity Value
DSL	Domestic Substances List
ECCC	Environment and Climate Change Canada
GD	(OECD) Guidance Document
GRACIOUS	Grouping, Read-Across, Characterisation and classification framework for regulatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products
HC	Health Canada
IATA	Integrated Approaches to Testing and Assessment
MOE	Margin of Exposure
NMs	Nanomaterials (manufactured nanomaterials)
NSNR (C&P)	<i>New Substances Notification Regulations (Chemicals and Polymers)</i>
OECD	Organization for Economic Co-operation and Development
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
QSARs	Quantitative Structure-Activity Relationships
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (of the European Union)
RCC	(United States-Canada) Regulatory Cooperation Council
RQ	Risk Quotients
SSD	Species Sensitivity Distribution
TG	(OECD) Test Guideline
TMF	Toxicity Modifying Factor
WoE	Weight of Evidence
WPMN	(OECD) Working Party on Manufactured Nanomaterials

1 Introduction

Part 5 of the *Canadian Environmental Protection Act, 1999* (CEPA) provides the Minister of Environment and Climate Change and the Minister of Health (the Ministers) with the authority to assess and manage environmental and human health risks from chemical substances. The *Domestic Substances List* (DSL), as specified in Part 5 of CEPA, is an inventory of approximately 23,000 substances deemed to have been in Canadian commerce, or manufactured or imported into Canada between 1984 and 1986, and described as “existing substances”. Part 5 of CEPA also specifies that substances not on the DSL, described as “new substances”, must be notified to the New Substances Program for assessment prior to import or manufacture in Canada under the [New Substances Notification Regulations \(Chemicals and Polymers\)](#) (NSNR(C&P)).

Under Part 5 of CEPA, the main decisions arising from the risk assessment of substances are the conclusions pertaining to whether or not they meet the criteria under section 64 of CEPA; notably, a substance is entering or may enter the environment in a quantity or concentration or under conditions that:

- a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity
- b) constitute or may constitute a danger to the environment on which life depends; or
- c) constitute or may constitute a danger in Canada to human life or health.

Different from traditional chemical substances, substances manufactured at the nanoscale can be designed to exhibit unique attributes (for example, mechanical, catalytic, electrical, and/or optical). Nanomaterials (NMs) have physical-chemical properties that often cannot be predicted from non-nanoscale substances with the same chemical composition, also referred to as their bulk form. Engineered characteristics of NMs, or variations of different nanoscale forms of the same chemical substances, can result in different physical-chemical properties, such as size, shape, surface chemistry (for example, identity of surface treated groups), and dissolution rates. These characteristics may alter the effects, fate, and exposure profiles of a substance, and may therefore change its potential to harm human health and the environment. Substances are listed on the DSL with their Chemical Abstracts Service Registry Numbers (CAS RN), which specify a chemical composition but do not differentiate among varying physical forms. Compared to their bulk form, NMs may exhibit highly divergent properties relevant to their potential risk to human health and the environment (OECD 2012a; Dekkers *et al.*, 2016; EU 2018). Industry continues to explore NMs for innovations in chemical and material applications. As a result, the global market for NMs continues to see rapid growth, underscoring the need for appropriate regulatory oversight.

Although there are no provisions in CEPA referring specifically to NMs, CEPA applies to substances, defined as “any distinguishable kind of organic or inorganic matter” in whatever size, shape, or physical state. Therefore, CEPA and its provisions apply to substances at the nanoscale, authorizing the Ministers to assess NMs. This does not necessarily imply that all NMs represent an increased risk to human health or the environment; each NM needs to be assessed in consideration of its unique hazard and exposure profile.

1.1 Purpose

The purpose of this document is to establish a framework for the risk assessment of NMs. The framework describes the human health and environmental risk assessment considerations that are modified from risk assessment methods traditionally used for chemicals, and are not necessarily limited to those used under the Chemicals Management Plan (CMP). This framework provides guidance on how NMs are to be assessed for their risk to human health and the environment under CEPA. Additionally, the publication of this document serves to inform stakeholders about the approaches and considerations the Government of Canada often uses for assessing NMs under CEPA.

1.2 Scope

This risk assessment framework outlines approaches and considerations for informing the risk assessment of NMs under CEPA, including both existing NMs on the DSL, and new NMs notified under the NSNR(C&P). This framework takes into consideration the unique properties of manufactured or engineered NMs, and excludes naturally occurring nano-sized particles¹. A substance is assessed as an NM if it meets the criteria described in the [working definition of a nanomaterial \(GoC, 2011\)](#) and particle size distribution threshold (number or mass-based), as stated in section 3.1.

1.3 Policy statement summary

This risk assessment framework is not a regulatory document; rather it reflects the guidance and policies used for the risk assessment of nanomaterials under CEPA.

Policy statements raised within this document are as follows:

- The risk assessment of NMs follows the principles of chemical risk assessment, including the application of weight of evidence (WoE) and precaution;
- In the absence of an international consensus for a system of nomenclature for NMs, both the Chemical Abstracts Service Registry Number (CAS RN²) and information on physical-chemical properties (for example, size, shape, surface chemistry) are used to identify and characterize the range of nanoscale forms;
- Size distribution, shape, and surface chemistry are generally required to assess substances produced at the nanoscale;
- A substance is evaluated as an NM if 10% or more (by number) of its primary particles have at least one internal or external dimension at or within the nanoscale of 1 to 100 nm. If a particle size

¹ Materials that either naturally exist within the nanoscale size range, or exhibit nanoscale properties/phenomena in nature will not automatically be re-classified as NMs (for example, naturally occurring chemical or biological molecules like nucleic acids/DNA/proteins, micro-organisms or cell structures like flagella or ribosomes).

² The CAS RN is the property of the American Chemical Society and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the Government of Canada when the information and the reports are required by law or administrative policy, is not permitted without the prior, written permission of the American Chemical Society.

distribution by number is not available, a substance is evaluated as an NM if at least 1% (by mass) of the primary particles has at least one internal or external dimension at or within the nanoscale;

- Read-across methodologies for NMs are under development. At this time, read-across for NMs is considered case-by-case and as part of the WoE approach to risk assessment. Guidance on read-across methodologies for NMs available from other jurisdictions/organizations (for example, European Union, OECD) is also considered. Grouping strategies employed for ecological and human health risk evaluation of NMs may differ;
- It is possible that a conclusion drawn from a risk assessment is applicable to a range of known NM variants of the CAS RN assessed, or that different nanoforms with the same CAS RN have different conclusions; and
- A conclusion under section 64 of CEPA is not relevant to, nor does it preclude an assessment against the hazard criteria for Workplace Hazardous Materials Information System specified in the *Hazardous Products Regulations* for products intended for the workplace.

2 Overview of regulatory risk assessment under CEPA

2.1 Existing and new nanomaterial legislative and regulatory frameworks

The [CMP](#) is a Government of Canada initiative to reduce the risks posed by substances to Canadians and their environment. In Canada, the existing legislative and regulatory frameworks for traditional (bulk) chemicals are used to assess and manage the potential risks of NMs to human health and the environment. Adaptations of these existing frameworks to both new and existing NM assessments are necessary in some cases to account for the specific characteristics of NMs (for example, physical-chemical properties, and life cycle/transformation product(s)). The general practices used under the CMP, such as for information gathering and prioritization for existing substances and the application of regulations for new substances, are applicable to the assessment and management of NMs.

The CMP employs various approaches to address chemicals on the DSL, including the [Risk Assessment Toolbox](#). To date, risk assessments of existing substances carried out under the CMP have not specifically considered the potential risks from existing NMs. The programs responsible for the assessment of NMs under CEPA at Environment and Climate Change Canada (ECCC) and Health Canada (HC) have [collected data to establish a list of existing NMs that are in-commerce in Canada](#). With the data collected from this initiative, exercises were undertaken to [prioritize the risk assessment of these in-commerce substances](#). Information and data obtained from these prioritization approaches inform the assessment of existing NMs in Canada. Strategies are explored to address data gaps that have been identified.

For new NMs notified under NSNR(C&P), ECCC and HC assess the risk of NMs to Canadians and their environment with the information that importers or domestic manufacturers are obligated to provide. ECCC and HC use this information to assess the notified NM to determine if it meets the criteria under section 64 of CEPA. The type of information required, as well as the length of the assessment period

primarily depends upon the import/manufacture quantity and the appropriate Schedule set out in the NSNR(C&P). Additional data on particle characterization (for example, size, shape, surface area, and/or agglomeration/aggregation state) to inform the risk assessment of NMs are recommended in the updated [New Substances Notification Form for Chemicals, Biochemicals, Polymers and Biopolymers \(including nanomaterials\)](#).

2.2 Chemical risk assessment approaches

Chemical risk assessment is the evaluation of the potential for adverse outcomes from a substance, and considers both the hazard of and exposure to a substance. The hazard associated with a substance is the inherent ability of the substance to cause adverse effects such as toxicity to aquatic organisms or cancer in humans. Exposure is the concentration or amount of the substance that reaches the organism, system, or population in a specific intensity, frequency, and duration (IPCS, 2004; ECCC and HC, 2013; GoC, 2020a). Risk assessment is based on [WoE and precaution](#), and can be qualitative or quantitative. This section describes, at a high level, the risk assessment approach used to assess bulk chemicals under the CMP. NM-specific adaptations of this basic approach are discussed in detail in Section 3.

A tiered approach is often used for regulatory risk assessments of chemicals. As a first tier, assumptions can be made to estimate any of the assessment components including solubility, toxicity, concentrations in products, or concentrations in the environment. Assumptions provide an indication of risk, and can be used as a screening approach. When an unacceptable level of risk is identified using these scenarios, refinements based on more exact information can be considered. Further refinements can be made as more information becomes available. Of the three levels (or tiers) presented in Figure 1 (conceptual representation), the highest tier (tier 3) is based on relatively more complete empirical or mechanistic datasets with a high degree of confidence or certainty in assessment outcomes.

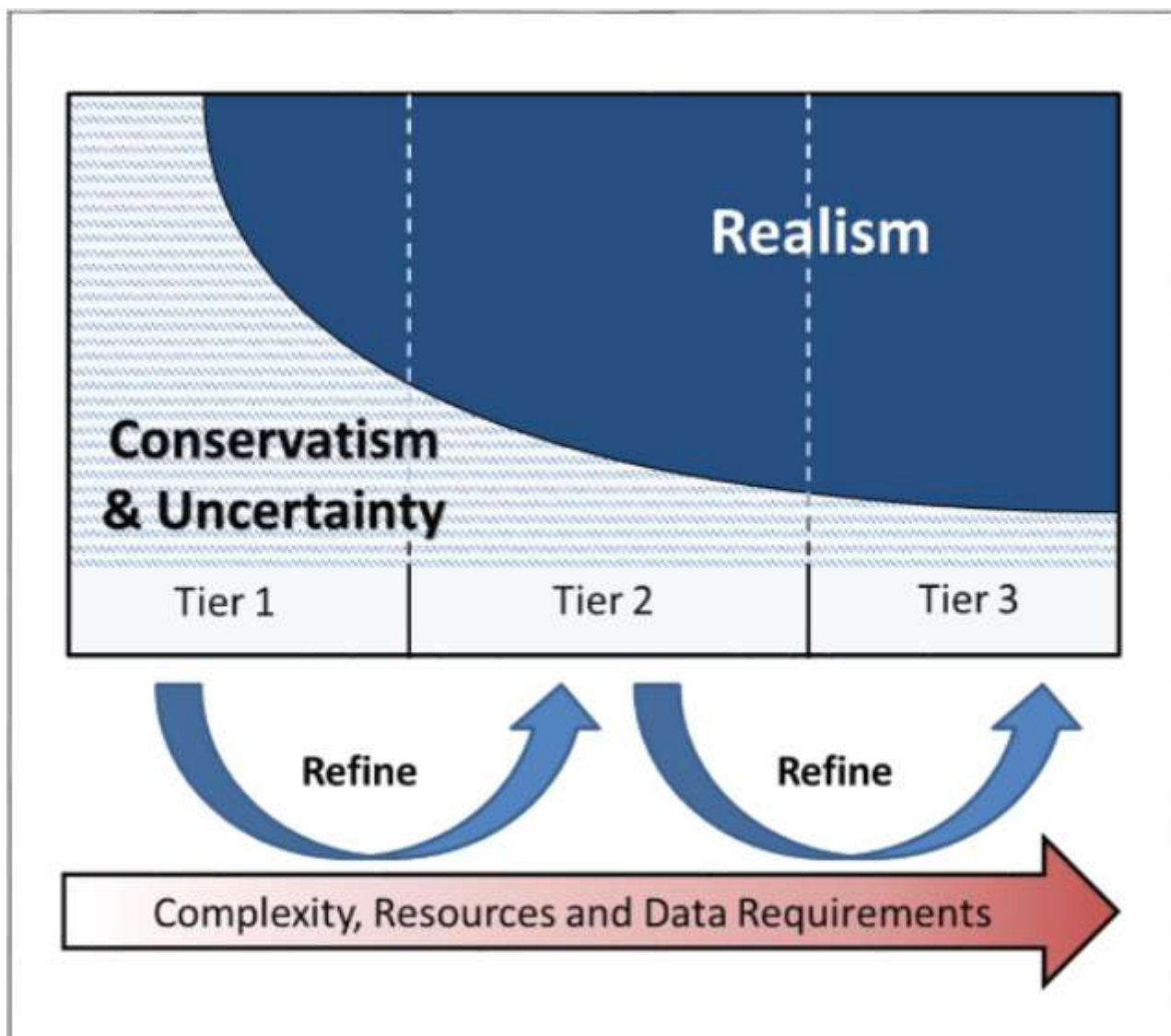


Figure 1. Conceptual representation for tiered approach for chemicals risk assessment

Regulatory risk assessments rely on multiple sources of information and lines of evidence that include, but are not limited to:

- Publicly available information, such as databases and peer-reviewed scientific journals;
- Information gathered or generated by or in collaboration with industry associations and stakeholders;
- Government of Canada-directed research, monitoring and surveillance, and;

- Information or assessments from other federal programs or regulatory jurisdictions (including provincial/territorial and international governments).

These sources contribute to information on the physical-chemical properties, use patterns in Canada, concentrations in environmental media and in products available to consumers, health effects and mechanistic data, as well as the route(s), duration, and frequency of exposure. Data relatable to the Canadian context (for example, products used in Canada, Canadian species or environmental conditions representative of the Canadian environment) are preferred when choosing data sources.

There is often insufficient experimental, monitoring, or measurement data to characterize exposure and hazard with certainty. In some cases, experimental data may be generated and empirical testing (for example, health effect data) can be conducted by industry stakeholders, or by internal or external researchers. When it is not possible to generate the data for risk assessment of a specific substance or a group of substances, predictive tools are used, such as read-across, grouping approaches, exposure modelling, and Quantitative Structure-Activity Relationships (QSARs). These serve as important alternatives to experimental data, providing estimates to fill data gaps. Predictive tools generally assume that substances with similar chemical structures have similar physical-chemical properties, and therefore will have similar environmental fate characteristics, and health effects.

Furthermore, default values or professional judgement may be used to generate assumptions and values that are protective of the environment or human health. Uncertainty in the data, relevance of data to the assessment, and the level of confidence in the data are considered when determining the most appropriate values for use in the risk assessment.

Risk characterization can be described qualitatively or quantitatively. In quantitative risk characterization, quantitative exposure levels and critical effect levels are identified to calculate [Risk Quotients \(RQ\) for environmental risk assessment, and a margin of exposure \(MOE\) for human health risk assessment](#). Refinements to improve estimates of exposure, and uncertainty factors may be applied to account for data gaps or uncertainties in the dataset. The use of RQs and MOEs introduces an important quantitative component in the WoE approach for determining toxicity under CEPA. The RQ is the ratio of the predicted environmental concentration (PEC) over the predicted no effects concentration (PNEC) (the concentration of a substance in an environmental medium below which adverse effects are unlikely to occur (see section 3.7.1.2)). An RQ greater than or equal to 1 suggests that a substance may cause harm to the environment, whereas an RQ less than 1 suggests that a substance is unlikely to cause harm to the environment under the specified exposure scenario. Similarly, the MOE is the ratio between the critical effect level (often, the no-observed-adverse-effect level (NOAEL)) and the exposure level for a given duration and route. A target MOE is determined from the identified critical effect and the uncertainties associated with the health effects and exposure databases. A derived MOE smaller than the target MOE indicates a potential for harm to human health. This MOE approach is not intended for non-threshold genotoxic carcinogens where the risk is associated with any level of exposure.

2.3 Uncertainties and the application of weight of evidence and precaution

CEPA (administrative duties) commits the Government of Canada to implement the precautionary principle so that:

“where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation[...]" (Canada, 1999)

The Ministers apply a WoE approach (that is, a method for decision-making that involves consideration of multiple sources of information and lines of evidence) and the precautionary principle when conducting and interpreting the results of assessments of particular substances, including NMs (ECCC, 2017). GoC (2017a) further elaborates the importance of application of WoE and precaution in assessing chemical substances, as well as consideration of uncertainty in the evaluation process.

Risk estimates always contain some level of uncertainty from a variety of sources ranging from instrument uncertainty in scientific measurements, to the challenge of extrapolating from available information to real-life situations. Uncertainty exists at each step of the risk assessment and influences the risk conclusion. An important step during the course of a risk assessment is the evaluation of the lines of evidence, their associated uncertainties and their strengths, to support a conclusion. Risk assessment reports should include summaries that capture and communicate the uncertainties identified. Uncertainties may also result from data gaps and, where appropriate, an indication of whether more robust data would reduce the uncertainties and enhance the overall confidence in the assessment conclusion are presented in the risk assessment report. Assumptions used in the absence of data and the basis for those assumptions are described in the risk assessment report (GoC, 2014 and 2020a). The application of precaution in risk assessment means using conservative but realistic assumptions to account for uncertainties, while the degree of precaution applied is in proportion with the degree of uncertainty. For example, a conservative assumption may be made about the percent of a substance that is absorbed through the skin of an organism or human in the absence of data. In another example, a conservative assumption on the efficiency of wastewater treatment practices may be used in estimating exposure concentrations in a receiving water (GoC, 2017a).

3 Nanomaterial-specific considerations for risk assessment

The unique characteristics of NMs may alter their effects, fate, and exposure; therefore, nanoscale forms are assessed separately from their bulk form. This section describes the modifications to testing and assessment approaches developed for traditional chemicals that may be required to account for NM-specific properties.

3.1 Definition of a nanomaterial for Canadian regulation

In lieu of a Canadian regulatory definition of NMs, ECCC and HC regulators have been using the [Health Canada working definition](#) (GoC, 2011). Should a regulatory definition come into effect, it will take

precedence. According to the working definition the term “nanomaterial” includes any manufactured substance or product and any component material, ingredient, device or structure if:

- i. It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or;
- ii. It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.

For the purposes of this definition:

- i. The term "nanoscale" means 1 to 100 nanometers, inclusive;
- ii. The term "nanoscale properties/phenomena" means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material; and,
- iii. The term "manufactured" includes engineering processes and the control of matter.

All substances in particulate form have a distribution of particle sizes, where the primary particle size is defined as the dimension(s) of a single discrete particle that is non-aggregated and/or non-agglomerated. A substance is evaluated as an NM if 10% or more by number of its primary particles have at least one internal or external dimension at or within the nanoscale. Using a combination of different measurement methods is recommended. Alternatively, if a particle size distribution by number is not available, a substance is evaluated as an NM if at least 1% by mass of the primary particles has at least one internal or external dimension at or within the nanoscale.

Agglomerated³ or aggregated⁴ (European Commission, 2011; [ISO/TR 18401:2017](#)) nanoscale particles, otherwise referred to as secondary particles, may exhibit the same properties as unbound (primary) particles at the nanoscale and may disintegrate or release nanoparticles during their lifecycle. Thus, agglomerates and aggregates, regardless of their external dimensions, are considered NMs whenever their constituent (primary) particles meet the definition of an NM.

3.2 Grouping strategies for nanomaterials

In 2014, the United States-Canada Regulatory Cooperation Council (RCC) defined seven classes of NMs for grouping according to chemical composition: carbon nanotubes; inorganic carbon; metal and metalloid oxides; metals, metal salts and metalloids; semiconductor quantum dots; and organics and other classes (RCC, n.d.). This classification can provide information on similarities in chemical composition of NMs, but it cannot completely address the complexity of nanoforms (for example, modifications to the size, shape (morphologies) and/or surface modifications to target different behaviours at the nanoscale) that may exist across the breadth of all NMs. Several international bodies such as the OECD Working Party for Manufactured Nanomaterials (WPMN) and GRACIOUS (Grouping, Read-Across, Characterisation and classification framework for regulatory risk assessment of manufactured nanomaterials and Safer design

³ “Agglomerate” means a collection of weakly bound particles or aggregates (for example, held together by van der Waals forces or simple physical entanglement), where the resulting external surface area is similar to the sum of the surface areas of the individual components.

⁴ “Aggregate” means a particle comprising of strongly bonded or fused particles (for example, held together by covalent or ionic bonds, or those resulting from sintering or complex physical entanglement), or otherwise combined former particles, where the resulting external surface area is less than the sum of the surface areas of the individual components.

of nano-enabled products) have indicated that the risk assessment of NMs cannot be completed by only grouping NMs of similar chemical composition. Recent grouping concepts for NMs go beyond chemical composition to consider other properties, such as agglomeration and particle size distribution (OECD, 2016a; ECHA, 2017; GRACIOUS, 2017). In Canada, grouping strategies for NM assessments under CEPA use a combination of the RCC approach and other available approaches.

3.3 Identity and physical-chemical considerations for nanomaterials

Chemical composition and sometimes manufacturing method details are used to identify and assign the CAS RN of a substance. As a result, a CAS RN does not differentiate the bulk and nanoscale forms of a substance. Additionally, it does not consider the variability of nanoform(s) (for example, shapes or size distributions) that could be manufactured for a given substance under that CAS RN.

While there is some work being conducted at the international level (for example, ISO/TS 80004 series to develop terminology and definitions for core terms used in nanotechnology), there is currently no set of nomenclature rules adopted by regulatory jurisdictions for naming and differentiating nanoforms of the same chemical composition. However, the multiplicity of unique nanoscale forms of a substance must be identified and considered during risk assessments. In the absence of an international consensus for a system of nomenclature for NMs, both the CAS RN and information on physical-chemical properties are used to identify and characterize the range of nanoforms. In CEPA risk assessments, all physical-chemical variations identified in the open literature for a given NM are considered when there is an absence of specific information about various nanoforms.

Although several properties, such as chemical composition and impurities are used in the characterization of bulk chemicals, others are unique to the characterization of NMs. Under the new Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) annexes (EU 2018), size distribution, shape, and surface chemistry are the minimum properties required for proper NM identification. These properties also form the basis for NM identification in Canadian risk assessments.

3.4 Environmental fate of nanomaterials

Environmental media (for example, air, water, soil, and/or sediment) are complex matrices of dissolved ions, natural organic matter, micro- and larger particles, etc. Characterization of the relevant environmental medium is required to make accurate environmental persistence and fate predictions, since the behaviour of NMs is partially dependent on external environmental factors, such as pH, and the ionic strength of the surrounding medium (Lin and Tian, 2010). The variability in the environmental media may result in a wide range of possible states for the NM (for example, agglomerated and/or chemically transformed), which could also change over time under varying environmental conditions.

Following the release of NMs to the environment or incorporation into a product, chemical, physical, or biologically mediated transformation of NMs will readily occur (Lowry *et al.*, 2012; Lead *et al.*, 2018). The behavior of the transformed form(s) of NMs, including fate and persistence, may alter considerably relative to their pristine (as manufactured) form. Transformations depend both on the nature of the pristine NMs and environmental conditions (Lead *et al.*, 2018). As a result, risk assessments consider the effects of the transformed form(s) based on environmental, biological, or use conditions over the lifecycle

(for example, manufacture, transport, incorporation into a product(s), use(s), recycling, disposal) of NMs, when sufficient information is available. This approach provides more realistic ecological and human exposure scenarios and a more complete understanding of the environmental or human health risks posed by NMs. The pristine form of an NM is easier to characterize and, for practical reasons, information available for a risk assessment may only consider this form.

Methods to detect manufactured NMs in the presence of complex matrices, or to distinguish naturally occurring and bulk substances sourced from manufactured NMs, are under development and are currently not readily available for all matrices. As a result, our risk assessments rely on currently available models to support environmental persistence and fate predictions, along with generalizations, approximations, and expert judgment, making conservative assumptions as appropriate to account for uncertainties and data quality (see Figure 1).

Environmental fate models based on physical-chemical properties, such as equilibrium partitioning models, traditionally used to estimate the ecological media partitioning and the fate of organic chemicals within the environment are not specifically applicable to NMs. These models do not account for kinetically controlled processes such as colloidal behavior, kinetics of degradation and aggregation behavior that are specific to NMs. Despite a general lack of empirical input data in support of environmental fate models for NMs, models to determine NM fate and exposure are being developed by building conceptually on models developed for bulk chemicals (Di Guardo *et al.*, 2018). For instance, there have been successful efforts to adapt fugacity models for the prediction of NM environmental behavior using such parameters as size, charge, and agglomeration (Utembe *et al.*, 2018). Investigations into these models for their reliability and use in environmental risk assessments have been conducted (OECD 2021a).

The environmental fate and persistence of some NMs follow general trends that can be predicted. For instance, metal-containing NMs that dissociate are likely to release metal ions that dissolve in the aquatic environment (see section 3.8.1). The metal ion is considered persistent under the *Persistence and Bioaccumulation Regulations* of CEPA because it cannot degrade any further, although it may partition among environmental compartments (ECCC and HC, 2017). Sparingly soluble nanoscale forms may be considered persistent and are evaluated on a case-by-case basis. Organic NMs, polymers, and cellulosic NMs tend to undergo degradation in the environment. Their degradation products may be further assessed for their environmental fate and persistence. By using available tools and applying expert judgement, the use of models can provide a foundational understanding of the environmental fate and persistence of some NMs.

3.5 Data considerations for the risk assessment of nanomaterials

When gathering and weighing the relevance and reliability of data to characterize the risk of an NM, the same principles as those developed for chemicals apply (GoC, 2017b and 2020b). When examining NM-specific data, available information is collected and compared, and qualitative and quantitative lines of evidence are considered. For the risk assessment of new NMs, prescribed information required under NSNR(C&P) and submitted by notifiers serve as a primary data resource. For risk assessment of existing NMs, information received from stakeholders (mandatory or voluntary), and from other federal programs, is useful for identifying Canada-specific use patterns and quantities of NMs in commerce.

Available databases for NMs, models, read-across, and QSARs are used to fill data gaps when appropriate. Data gaps for assessing NMs include, but are not limited to, absence of measured concentration data for a given NM in environmental media or in products available to consumers, insufficient information on how NMs transform throughout the product lifecycle, uncertainty about appropriate metrics for exposure quantification, and the lack of fully validated exposure and toxicokinetic models. Additionally, the health effect data for each nanoform with the same chemical composition (under the same CAS RN) is often not available. Dependence on experimental data to fill the data gaps for each nanoform imposes significant limitations in terms of time and cost, and number of animals required for health effect testing. Therefore, to develop robust risk assessments of NMs, strategies are explored to fill data gaps, such as using existing databases, models, grouping, read-across and QSARs, and also applying WoE approaches and precaution in addressing these data gaps and challenges, as described in Section 2.3.

3.5.1 Databases and models

International organizations (for example, European Commission) are developing NM databases which are described in a technical report by the Joint Research Centre of the European Commission (JRC, 2017). These databases provide information on the physical-chemical properties (for example, size, shape, and/or composition), use of and exposure to certain NMs, including measured data available in the public domain for given exposure scenarios. These databases enable the identification of key factors affecting the release of NMs, inform assessment of the environmental and human exposure to a given product, and help refine exposure estimates in environmental and human health risk assessment.

Nano-specific human exposure and environmental exposure models can be used to estimate human exposure to NMs, concentrations of NMs in various environmental media, and environmental fate (Kuhlbusch *et al.*, 2018). For example, as noted in section 3.4, key physical-chemical properties (for example, particle size, dissolution, dispersion rate, and/or agglomeration) are used to make informed predictions about the persistence and fate of NMs in the environment. The OECD WPMN have established an inventory of models for assessing human and environmental exposure to manufactured NMs in risk assessments (OECD 2021a,b,c,d). Depending on its intended purpose(s), each model developed can have a different scope and application domain; therefore, it is essential that the models provide a good match to the relevant scenario(s) in the risk assessment.

3.5.2 Read-across approach for nanomaterials

Read-across is a strategy used for chemical risk assessment that extrapolates data from similar substances (based on one or more of: chemical structure, physical-chemical properties, function, toxicological mode of action, etc.), to fill information gaps for a target substance undergoing risk assessment. For hazard identification, read-across is endpoint specific; therefore, for each endpoint, different source substance(s) may be required. In addition, studies used to support read-across should provide information on relevant endpoints or effects (GoC, 2020c). Because the interactions and behaviours of NMs are governed primarily by their physical-chemical properties, read-across strategies for NMs emphasize the importance of the physical-chemical properties that are unique to NMs, taking into consideration structure, composition, and toxicokinetics of NMs (ECHA, 2017).

Grouping can facilitate a read-across approach. NMs that have similar structure, physical-chemical properties, or toxicokinetics are likely to share similar toxicological characteristics and may be considered as a group. A read-across approach using a grouping allows physical-chemical properties, human health effects and environmental effects and/or environmental fate of the target substance(s) to be predicted from data for reference substance(s) within the same group (Oomen *et al.*, 2015; ECHA, 2016; Lamon *et al.*, 2019). Even though grouping strategies for NMs emphasize the importance of physical-chemical similarities, the properties of NMs most important for environmental and human health effects may differ, and as such, grouping and read-across strategies employed for assessments of NMs may differ between the environmental and human health risk assessments.

For NMs, read-across allows data gaps on potential hazards of a particular nanoform (target) to be filled using existing information about other nanoforms (that is, variants of the same NM) or the corresponding non-nanoscale form (bulk material). The approach for read-across between nanoforms aims at increasing the available information for a target nanoform by using information from other variants (source) of the same NM, provided there is a clear demonstration of similarities in their physical-chemical properties especially key parameters such as primary particle size distribution, shape, and surface chemistry. In addition, read-across between nanoforms requires that exposure, distribution (fate/toxicokinetics) and hazard of the target nanoform are similar to, or less than, the source nanoforms (ECHA, 2016). The recommended approach for read-across between nanoforms requires data on the physical-chemical parameters of each nanoform. This is an important starting point to obtain a better understanding of the behaviour, fate, toxicokinetics, and effect of NMs, which is key in developing a scientific justification for grouping and the use of data for read-across. On the other hand, read-across from the bulk material to the nanoscale form based on the same chemical composition may also be used to inform the risk assessment of NMs for specific endpoints. Relatively large amounts of physical-chemical properties, hazard, and exposure data may exist on the bulk material for a given NM and can be used to understand the potential behaviour of the target NM. To account for uncertainties associated with read-across for NMs, additional uncertainty factors may be applied during the risk characterization.

3.5.3 Quantitative Structure-Activity Relationship for nanomaterials

A QSAR is a statistical model for predicting effects using chemical descriptors such as structural similarity, presence of structural fragments, and physical-chemical properties. QSARs quantitatively relate chemical or biological descriptors to another measured property or characteristic. At present, there are challenges in the development of QSAR models for NMs due to insufficient experimental data on the physical-chemical properties and effects of these materials, as well as the difficulty in defining appropriate NM descriptors (chemical, morphological, or otherwise) (Tantra *et al.*, 2015; Burello 2017). At this time, no QSARs have been developed for wide applicability to NMs. When acceptable QSARs are developed and verified for use in regulatory risk assessments, they will be considered for incorporation into the Canadian risk assessment approach for NMs.

3.5.4 Choice of metrics for nanomaterial risk assessment

Traditionally, risk assessments of chemicals use simple units (for example, mass or mass per unit volume) to quantify the amount of substance in exposure and effects characterizations. For NMs, such units may not always be the most appropriate metric. For instance, the surface area, particle number, or particle

volume tend to be more indicative of the health or environmental effects of NMs (Braakhuis *et al.* 2016; Delmaar *et al.*, 2015; Simkó *et al.*, 2014; Verschoor *et al.*, 2019). Due to the diversity of physical-chemical properties of a nanomaterial that can alter its effects, there is no international consensus on the most appropriate metric for the risk assessment of NMs (ECHA, 2017). However, some metrics have been demonstrated to be appropriate for certain effects and NM types (Delmaar *et al.*, 2015). For example, surface area has been proposed as a metric for expressing concentrations in air for local effects (Braakhuis *et al.*, 2016). Other studies on coated metal and metal oxide nanoparticles report that particle volume is the most appropriate dose metric to describe their effects on various aquatic organisms and on mammalian and fish cell lines (Simkó *et al.*, 2014; Verschoor *et al.*, 2019). Studies that report nano-specific metrics (that is, based on particle surface area, number or volume), in addition to traditional metrics, are particularly useful in exposure and risk characterizations of NMs. Unfortunately, NM studies published to date have been inconsistent in the use of metrics for exposure or health and environmental effects, making comparison among studies difficult. For risk assessment of NMs under CEPA, metrics for both exposure, and health or environmental effects must be in the same or interconvertible units, and thus appropriateness of the metrics used is considered on a case-by-case basis.

3.6 Ecological and human health approaches to characterize exposure to nanomaterials

Exposure characterization describes the predicted or actual concentration (amount) of a substance that reaches a biological system. The aim of exposure assessment is to determine the source, type, magnitude, frequency, and duration of contact with the substance(s) of interest. How a substance is manufactured and used in Canada is critical for characterizing environmental and human exposures. The uses can include industrial, commercial, or consumer applications, which can inform direct exposure assessment (for example, from use of product or consumption of food) and indirect exposure assessment (for example, from the environment through disposal of consumer products). The end-of-life disposal of products also informs the exposure assessment. If applicable (for example, a substance is found in various personal care products and food), combined or aggregated exposure (exposure to the same substance through multiple exposure routes) are considered.

For NMs, it is especially important to characterize the form of the product (for example, spray, cream, gel, article) and the way the NM exists within the product matrix (for example, surface bound vs suspended in the product) to determine its potential for release and availability for exposure. As a first step, the NM may be assumed to be 100% in the free state and available for exposure, but for NMs incorporated into solid materials and cases where there is demonstrated bonding within or to the surface of the matrix, refinement of release rates are considered. It is generally expected that releases of NMs from liquid or powdered products are higher than releases of NMs embedded in solid products (Dekkers *et al.*, 2016). The release rate of a given NM from a product matrix could be further explored by an exposure read-across approach, namely, using available release data of another NM from the same or similar product matrix under similar use conditions. Additional considerations should be given to factors controlling the release of NMs, the criteria for defining similarity, and any extra uncertainties associated with this exposure read-across approach. Such an approach can be complementary to the exposure modeling for exposure assessment on NMs.

3.6.1 Environmental exposure to nanomaterials

3.6.1.1 Ecological exposure scenario identification and considerations

When appropriate and possible, exposure assessment of NMs under this framework includes quantitative exposure estimates for relevant routes of exposure. For example, environmental conditions could favour either suspension, dissociation or aggregation, influencing the fate then exposure pathways. The exposure scenarios most likely used for the determination of risk are identified early in the risk assessment process for further development. Quantification of the exposure is based on the highest release potential, or following an environmental fate characterization, that suggests potential concern for an exposure. Exposure characterization focuses on the most likely and most relevant exposure scenarios for the environment.

For many commercial and industrial activities, ECCC has compiled accepted default parameters for exposure modelling for certain chemical use scenarios (for example, days of operation and emission factors). In addition, typical Canadian characteristics, such as frequency of environmental release and duration of potential exposure, may be applied in determining the environmental exposure. When available, specific use data are preferred, but otherwise the default or previously established values may be used.

3.6.1.2 Predicted environmental concentrations

An important value used to characterize the risk of an NM is the environmental concentration. The input parameters used to characterize exposure may include NM-specific metrics based on surface area, particle number, etc. Calculations are performed for NMs in different media (water, soil, air, and/or sediment) to predict the concentration, which is represented as a PEC.

The aquatic medium is the most commonly characterized for chemicals and is used to evaluate exposure to NMs for Tier 1 exposure characterization (see Figure 1), provided there is an entry pathway to aquatic environments. In these cases, the aquatic PEC (PEC_{aq}) is calculated from the total quantity of the NM used per year (Q), the fraction of loss of the substance into wastewater (L), the wastewater removal rate of the substance at the relevant wastewater treatment system (R), the total duration of release (N), the effluent flow out of the wastewater treatment system (F), and the dilution factor for the receiving water (D).

$$PEC_{aq} = \frac{[Q \times L(1 - R)]}{N \times F \times D}$$

The results derived from the key scenarios, in the form of a PEC, are expressed as single values or distributions, or a range for each scenario. These PECs are compared to the ecological effects data to derive RQs for an NM. PECs can also be calculated, using similar methodology, for degradation products of the NM, or fragments of it.

There is a vast range of PECs that can be estimated from the manufacture and compounding of an NM, and PECs can also be derived for use and disposal of that product and the potential subsequent release of the NM once incorporated into a product.

3.6.2 Human health exposure to nanomaterials

Human exposure assessment of NMs under this framework considers the potential for exposure of the general Canadian population from use of products available to consumers, via food, and via environmental media (for example, ambient and indoor air, soil, dust, water), as well as the potential for exposure of vulnerable populations (for example, children and pregnant women).

Direct exposure to NMs via the use of products available to consumers is a critical source of human exposure when assessing NMs under this framework. All identified uses of NMs in Canada are considered to develop a representative picture of all (potential) products and processes throughout the lifecycle of NMs. Commercial use data received from CEPA section 71 surveys and from HC program partners are particularly informative in understanding uses in Canada relevant to human exposure. Public sources, including repositories of safety data sheets or international government agency reports or databases, can also be helpful in characterizing potential commercial and consumer uses in Canada. Information on product-specific use is helpful to refine exposure estimates. HC has recently published [exposure factors used in human health risk assessments](#) under the CMP, including age group, body weight, body surface area, inhalation rates, etc. All these factors, as appropriate, are considered in the human health exposure assessment on NMs. Additionally, for some product types, such as personal care products, HC has established exposure parameters for consumer exposure models, including frequency and duration of use and the amount of product applied.

For each relevant route of exposure, human exposure assessment of NMs includes consideration of NM concentrations in products, food and environmental media, use amounts, physical forms (for example, size, shape), characteristics (for example, rigidity, durability), fate and transformation in environmental media (relating to indirect exposure), the potential effect of gastrointestinal transformation of NMs on toxicity (if available), and potential for release from products during different lifecycle stages.

Exposure estimates may be refined by applying appropriate empirical, read-across, or modelled absorption fractions for the relevant routes of exposure. Refinements for absorption should also take into consideration the potential for dissolution in relevant biological fluids or media (for example, lung fluid, artificial sweat, and gastrointestinal tract simulation fluid).

3.7 Ecological and human health approaches to characterize effects of nanomaterials

3.7.1 Ecological effects characterization

The main goal of ecological effects characterization is to determine or estimate the potential hazard threshold concentration of a substance expected to be associated with an unacceptable level of adverse effects in sensitive organisms in the Canadian environment (Environment Canada, 2007). There are two main drivers to ecological effects characterization. First, the substance characteristics, which have the most influence on bioavailability⁵, mode of toxic action, and on the toxic response. Sections 3.7.1.1 to

⁵ Bioavailability is the amount of a nanomaterial that is taken up from the environment and is available to cause a biological response in an organism. Uptake may include binding to or diffusion through cell membranes, noting

3.7.1.3 address the ecological effects characterization through substance characteristics. Bioaccessibility⁶ is also defined in relation to bioavailability in section 3.7.1.1. The second driver consists of external factors influencing toxicity and includes environmental mitigation factors. These are discussed in section 3.7.1.4 in relation to environmental characteristics. Finally, section 3.7.1.5 summarizes the approach used to assess the ecological effects of NMs.

3.7.1.1 Ecological bioaccessibility and bioavailability

Characterizing an NM using its bioavailable fraction(s) can account for its effects more accurately (Juganson *et al.*, 2015; Keller *et al.*, 2010; OECD, 2012b). A released NM should be evaluated considering the fractioning of each form, their partitioning in the environment and their respective solubility. Many factors may influence the bioavailability of NMs present in the environment. The released form of the NM (as a primary particle, the dissolved ion, or as an agglomerated (secondary) particle), upon entry into the environment, influences its bioavailability to exposed organisms. Additionally, water solubility of an NM plays a major role in its bioavailability to aquatic species. The bioaccessibility of an NM, or bioaccessible fraction of the NM, corresponds to its potential to come in contact and interact with ecological receptors, and is dependent on the environmental characteristics. Bioaccessibility is important to determine for NMs, for example, a high density NM may sink in the water column making the NM more bioaccessible to benthic organisms. Additionally, an NM may not be bioaccessible if it is sequestered. Bioaccessibility is a factor that influences the bioavailability of an NM, by identifying the fraction of the substance that can be bioavailable under specific environmental characteristics. In summary, the bioaccessible fraction is an estimation of the impacts of the environmental medium (considering its specific characteristics) on the bioavailability of a substance.

The bioavailable fraction(s) of the NM represent the fraction in the environment that has a greater likelihood of uptake by ecological receptors. It is also possible that more bioavailable forms of the NM are generated during manufacture, or at other stages of the life cycle of NMs (for example, degradation of a coating, or following physical or chemical transformation) (Oomen *et al.*, 2018). For instance, the transformed form of an NM subsequent to its release can also affect the NMs' bioavailability. Bioaccessibility and bioavailability can be closely linked together, for example, environmental factors such as pH and ionic strength may also influence NM agglomeration, which can affect 'active' vs 'passive' uptake of NMs by organisms. In a streamlined (lower tier) approach, the most bioavailable form of an NM may be considered as the only form present. More detail about the streamlined approach for ecological risk assessment, based on solubility and bioavailability fractions can be found in section 3.8.1.

that uptake processes include bioaccumulation (McLaughlin, Lanno, 2014). Bioavailability is the combination of the intrinsic property of a substance (dependant of the substances properties) and its bioaccessibility.

⁶ Bioaccessibility represents the nanomaterial, in a soluble form, that has the potential to be bioavailable, but is not necessarily bioavailable, and is usually determined by desorption or dissolution of a contaminant (from a solid phase) by chemical extraction of the environmental medium (usually soils, wastes, or sediments)) (McLaughlin, Lanno, 2014). According to this definition, the magnitude of the bioaccessible pool of the chemical is never less than the magnitude of the bioavailable pool and could also be termed the "potentially bioavailable" pool given no spatial or temporal constraints. Bioaccessibility is directly linked to the environmental characteristics.

3.7.1.2 Determination of the ecological toxicity values

There are two main ways to approach the evaluation of ecotoxicity for a given substance. Both methodologies have the same aim: to estimate the low toxicity threshold for which adverse effects are anticipated in the most sensitive organisms. When the dataset for ecotoxicity is sufficient for a given substance, there may be a possibility for the development of a species sensitivity distribution⁷ (SSD). When the dataset is sparse, a critical toxicity value (CTV) is extracted from the available data. The CTV expresses the lowest concentration of a substance (or degradation or transformation product of a substance) at which an adverse effect is observed in the most sensitive species in a given medium within a set of relevant and reliable data (Environment Canada, 2007). The SSD or CTV is determined for an NM for each of the environmental media where potential exposure is foreseeable. To select the appropriate value representing low toxicity of the NM to aquatic organisms, ECCC follows the methodologies and recommendations from the Canadian Council of Ministers of the Environment (CCME 2007).

For each environmental medium, all of the ecological effects data collected for a specific NM are compared based on the most bioavailable forms, and subject to any standardizations of the dataset necessary to compare toxicity endpoints for duration, type of effects and the magnitude of effects to the endpoints reported (Okonski *et al.*, 2021). The selection of the critical endpoints should be related to and representative of anticipated exposure conditions, to the extent possible. Long-term toxicity data are more likely to be used directly in the comparative dataset as they more realistically represent the environmental exposure of persistent substances such as metal-based NMs (Environment Canada, 2007).

3.7.1.3 Ecological predicted no effects concentrations

The predicted no-effect concentration (PNEC) represents the concentration of a substance in an environmental medium below which adverse effects are unlikely to occur, typically following chronic or long-term exposure in a population (Okonski *et al.*, 2021). The PNEC is determined to be used during the risk characterization process. When the representative toxicity value is determined using a SSD, the 5th percentile of the SSD corresponds to the PNEC, without requiring an assessment factor (AF). Otherwise, a PNEC value is calculated by dividing the CTV by an AF.

$$PNEC = \frac{CTV}{AF}$$

The methodology to estimate an AF relies on the use of extrapolations for intra/inter-species variations and short- to long-term exposure extrapolation (Okonski *et al.*, 2021). The methodology excludes considerations of intra/inter-laboratory variation and lab-to-field variation, but includes quantification of exposure duration (short-term to long-term), extrapolation from lethal to sub-lethal effects, extrapolation from median to low-/no-effect concentrations and consideration of the mode of toxic action. The guidance provided by Okonski *et al.*, (2021) for quantifying the mode of action is not applicable for inorganic NMs, and professional judgement may be applied to estimate this factor. PNECs may also be

⁷ An SSD is a statistical distribution describing the variation among a set of species in toxicity of a certain compound or mixture, Posthuma *et al.* (2002). From this distribution of species sensitivities, a concentration can be selected to identify a lower tolerance level (for example, 95%) to ensure a specified level of species protection is achieved.

expressed as functions of the environmental factors, or as site-specific PNECs, when sufficient data are available to derive these relationships (see next section). The bioavailable fraction of a substance being of prime interest for comparability to the exposure estimate, PNECs may be calculated to represent the bioavailable fractions ($PNEC_{\text{bioavailable}}$) rather than the total concentration of the NM ($PNEC_{\text{total}}$).

3.7.1.4 *Environmental toxicity modifying factors*

Characteristics of the receiving environment such as pH, dissolved organic matter, water hardness, cation exchange capacity and temperature can vary and consequently modify the toxicity of an NM to exposed organisms (Liu *et al.*, 2017). For instance, different environments can influence the fate of the surface coatings on NMs, resulting in significant implications for the assessment and/or prediction of bioaccumulation and the potential toxicity of an NM (Utembe *et al.*, 2018). If possible, the overall risk characterization of an NM needs to account for this impact of the surrounding environment on NM toxicity. Following the compilation and the evaluation of the ecological effects of an NM in various matrices (water, soil, and/or sediment), a Toxicity Modifying Factor (TMF) may be applied. A TMF accounts for the variations from the potential differences in its effects based on these external factors (details can be found in methodologies and recommendations from the CCME (CCME 2007)). The use of TMFs provides a better concordance between the ecological effect estimates with the exposure estimates for risk characterization.

3.7.1.5 *Nanoscale properties impacting the ecological effects characterization*

Analyses of dose and effect concentrations are an important component of the risk assessment of NMs. The small size and larger relative surface area of NMs may allow them to cross biological barriers and interact more directly within the cellular structure when compared to the same substance in its bulk form. There are currently no harmonized practices to evaluate the impact on nanoscale properties (surface chemistry, shape and size effects) on ecological receptors. Some OECD test guidelines (TGs) and guidance documents (GDs) pertinent to ecological endpoints have been proposed or are under preparation to address these gaps in the regulatory testing regime for NMs (for more information see [Nanomet - OECD](#)). It has also been suggested to adapt existing TGs and GDs for bulk substances to consider the nanoscale properties of NMs. When available, harmonized practices, TGs and GDs are adopted and incorporated into the Canadian approach.

In the absence of published OECD test guidelines or guidance specific to NMs for a specific ecological endpoint, ECCC uses alternative strategies to consider the nanoscale properties of NMs. Although the physical effects (effects generated by particular shape and size, and effect of high surface area) of NMs on ecological organisms are largely unknown, for some NMs there appears to be a correlation between the size of particles and the toxicity expressed by exposure to NMs of the same chemical composition (Seitz *et al.*, 2014, Van Hoecke *et al.*, 2009). When warranted, an NM with the closest morphological similarity may be selected for read-across on bioavailability and toxicity. Alternatively, the bioavailability and toxicity may be more influenced by functional groups present on the surface of the NM (Bundschuh *et al.*, 2018), and surface functional groups may be assessed as standalone substances for their toxicity to ecological receptors.

3.7.2 Human health effects characterization approach for nanomaterials

Physical-chemical characteristics of certain NMs may influence their overall hazard to human health. As noted for ecological endpoints, the small size of these substances may allow them to cross biological barriers, and interact more directly with cellular machinery compared to the bulk form of the same substance. The discrete particle nature of NMs allows for effects to be exerted through various direct or indirect processes outside of a cell, but also may allow for novel mechanisms of toxicity when particles are internalized by cells (Sabella *et al.*, 2014). In general, physical-chemical properties at the nanoscale are expected to be relevant for determining deposition and agglomeration (relevant for exposure), transport across biological barriers such as gut epithelium/blood-brain-barrier/skin (relevant for absorption/distribution characterization), accumulation, and dose response relationships (Dekkers *et al.*, 2016). As indicated, many of the effects expected from NMs may be elicited by the bulk form of the substances as well; however, properties emergent at the nanoscale may lead to differences in critical dose levels or localization/concentration of effect leading to higher doses at a target site. The increase in surface area as particle size decreases may lead to higher toxicity at a mass-based dose compared to the bulk form of a substance, particularly when harmful effects are caused by interaction with the surface of a substance (Dekkers *et al.*, 2016).

Similar to chemicals, human health endpoints for hazard characterization of NMs are commonly assessed in standardized mammalian toxicity tests. Analyses of dose and effect concentrations for key endpoints allow derivation of points of departure (that is, in general, the lowest dose at which a biological response or health effect is observed in a toxicological study) for hazard characterization. OECD guidelines for chemical testing offer internationally accepted methods for hazard identification and characterization, as well as physical-chemical property testing. Such testing guidelines are often used as a standard tool for regulatory evaluation of chemicals. According to the Preliminary Review of the OECD Test Guidelines (TG) for their Applicability to Manufactured Nanomaterials, published by the OECD WPMN, the OECD TGs for chemicals are generally considered applicable to manufactured NMs, particularly with regard to investigating their health effects (OECD, 2009). Some of these test guidelines may, however, require further modifications or adaptations to take into account the nanoscale properties of the test materials, such as size, shape, surface area, surface charge, surface chemistry, agglomeration/aggregation, dissolution, etc. (EFSA 2018). To date, a few OECD TGs have been modified to address nanoscale issues and published, for example, the 28-day repeated dose toxicity study-OECD TG 412, the 90-day repeated dose toxicity study-OECD TG 413, and guidance document (GD) on acute inhalation toxicity testing (OECD, 2018; see also [Nanomet - OECD](#)). Further work to update several other OECD GDs or draft new GDs is underway to address the regulatory testing of NMs (Rasmussen *et al.*, 2019).

Ideally, for a comprehensive human health effects assessment of manufactured NMs, endpoints from mammalian toxicity tests would include acute systemic toxicity (oral, dermal, and/or inhalation), skin sensitization, skin/eye irritation, repeated dose toxicity (dermal, oral or inhalation), genotoxicity, reproductive toxicity, developmental toxicity, toxicokinetics, and others. The relevance of human health endpoints to risk assessment depends on the use patterns of NMs and possible routes of human exposure.

In the selection of appropriate toxicological studies and endpoints for use in the risk characterization, several attributes specific to NMs should be taken into account. These include physical-chemical

characteristics of the NM, dosimetry, animal species, and route of exposure (OECD, 2012b). Physical-chemical properties such as size, shape, composition, agglomeration/aggregation state, surface charge, surface activity, and dissolution rate have been considered as key determinants of NM effects (OECD 2012b; EU 2018). Characterization of NMs prior to effects testing is essential for sample preparation and dispersion to ensure that the results are related to the NMs intended for the testing. This information can be used to correlate the nanoparticle physical-chemical properties with any measured biological/toxicological responses, as well as to provide an adequate reference point for comparing toxicity results with the hazard-based findings from other studies (OECD, 2012c). In addition, data on physical-chemical properties are helpful in selecting critical effect endpoints. For example, certain fiber-shaped NMs with a high aspect ratio may represent a unique inhalation hazard due to their length, rigidity, and biopersistence, and therefore, their pulmonary toxicity as an endpoint should be evaluated as a priority.

The appropriateness of testing platforms and species used in toxicity testing is another consideration for assessing NM hazard endpoints. Some test models used for bulk chemicals may not be suitable for NMs. For example, with regard to mutagenicity, the majority of the test methods are applicable for testing the effects of NMs. However, the bacterial reverse mutation test (Ames test) is not considered reliable for the assessment of NMs and should not be used as a single test for mutagenicity. This is because NMs may not be readily taken up by the bacteria used in the assay, resulting in low intracellular bioavailability and potentially leading to false negative results (OECD 2014).

It has been generally accepted that inhalation may be the route with the highest potential for concern for exposure to NMs because toxicity towards the respiratory tract, and translocation of NMs elsewhere via this route of exposure have been identified (Oberdörster *et al.*, 2005; OECD 2012b; ECHA 2016). When performing acute inhalation toxicity and repeated dose inhalation toxicity studies with NMs, it is important to consider lung overload (impaired particle clearance and increased particle retention in the lungs) in the interpreting the study results (OECD 2016b, ECHA 2016).

One of the current challenges in the risk assessment of NMs is the lack of toxicity data for the multiplicity of nanoscale forms with the same chemical identity (under the same CAS RN), but with differing physical-chemical properties. Addressing these data gaps using animal studies may be undesirable from an animal welfare perspective and such an approach poses significant limitations in terms of time and cost. Newer approaches such as alternative test methods (as discussed below), read-across (discussed in section 3.5.2) and *in silico* methods such as QSARs (discussed in section 3.5.3) can facilitate hazard predictions for various nanoscale forms.

3.7.2.1 Alternative test methods and guidelines

In vitro and *ex vivo* studies allow specific toxicological endpoints to be tested under controlled conditions, and at scales that may not be feasible with *in vivo* studies (toxicity tests performed in or on a whole living organism). *In vitro* studies normally use cultured cells (isolated primary cell cultures and immortalized cell lines) or subcellular fractions exposed to a substance under investigation and measured for elicitation of a response, while *ex vivo* studies are performed with tissues or organs collected from organisms with structure and viability maintained. The data derived from these studies may provide important

information about potential mechanisms of toxicity and possible drivers of effects for NMs. High throughput alternative testing is an innovative technique that allows simultaneous testing of a large number of chemicals and/or biological compounds for a specific health effect. It applies alternative testing such as *in vitro* or *ex-vivo* assays in a robust and mechanistic platform, and has been used in pharmaceuticals evaluation and toxicology for many years (Macarron *et al.*, 2011). This technique may enable the ranking of toxicity potential for many different NMs and nanoscale forms and provide a useful basis for selecting appropriate doses for mammalian toxicity studies. In addition, *ex vivo* studies which enable complex and realistic conditions and allow greater control over experimental parameters, can provide more results from the same number of organisms than would be possible with *in vivo* methods as more endpoints can be assayed on a single tissue or organ. It is generally accepted that no stand-alone *in vitro* or *ex vivo* test can replace a standardised *in vivo* method; however, a combination of these methods as part of an integrated approach to hazard characterization will allow for identification of potentially relevant health effects.

There are many *in vitro* and *ex vivo* assays based on a range of cell types/models and endpoints. Most often, these are designed to give insight into the mechanisms of toxicity, including damage to the plasma membrane, mitochondria, lysosomes, or DNA through binding and interaction with intracellular proteins. Some of these assays allow for the study of cellular uptake, attachment, and interaction in the *in vitro* or *ex vivo* system. Common endpoints of interest from *in vitro* and *ex vivo* studies include cytotoxicity and effects on cell viability, reactive oxidative species generation, cytokine induction, genotoxicity, endocrine disruption and reproductive effects.

Recent efforts have also been focused on using Integrated Approaches to Testing and Assessment (IATA) for risk assessment of chemicals (OECD, 2020). Overall, IATA incorporate new methodologies, besides traditional *in vitro* and *in vivo* tests, to make predictions based on existing data from multiple sources (OECD, 2016c). These approaches help to speed up the risk assessment process, while at the same time reducing testing costs and animal use (OECD, 2016c). At present, adverse outcome pathways and read-across are the approaches proposed for developing and using IATA for regulatory risk assessment of NMs (OECD, 2016c).

3.8 Risk characterization of nanomaterials

Similar to the paradigm outlined for bulk chemicals, the risk characterization of NMs is an integrated decision supported by information from both effects and exposure characterization. If an NM is determined to meet the criteria as defined in Part 5, section 64 of CEPA, then risk management measures are considered to prevent or control risks identified. Follow-up activities may be undertaken for those NMs recognized for their potential effects of concern if exposure was to increase or different nanoforms were to be produced. Risk characterization under CEPA applies precaution and WoE approaches. If available, results of any international assessments on the same NM are used to inform Canadian risk assessments.

As done in chemical assessments, the risk characterization of NMs can be qualitative or quantitative. A quantitative risk characterization is supported by the data collected from experimental studies, monitoring, and read-across approaches (as well as modeling when approaches are validated). However,

in some cases, for example, when exposure to an NM of interest is expected to be insignificant, a qualitative risk characterization may be warranted.

A conclusion under CEPA generally applies to its identifier described by a CAS RN. However, in the case of NMs, a group of different nanoscale forms (for example, with differences in size distribution, shape, and/or surface chemistry) of the same substance sharing the same CAS RN may have different hazard or risk potential. Therefore, an assessment approach and the conclusion(s) drawn from that risk assessment may or may not be applicable to a range of known NM variants of the CAS RN. Also, a conclusion under CEPA for a CAS RN may differ between different nanoscale forms of the same CAS RN. Similarly, a conclusion under CEPA for a CAS RN may differ between the bulk form of a substance and the NM form under the same CAS RN. For NMs assessed under CEPA, the results of risk assessments provide critical information for any effective risk management actions subsequently developed.

Additionally, uncertainty associated with each step of the risk assessment should be recognized and integrated into the evaluation report and the conclusion of the risk characterization as a means to maintain transparency through the assessment process.

3.8.1 Ecological risk characterization approach for nanomaterials

Reliable and relevant physical-chemical data pertinent to the characterization of ecological risk form a foundation for the risk assessment of NMs. Physical-chemical data are key to the assessment of persistence, fate and exposure, and inform the assessment of ecological effects. Additionally, knowledge of the transformed form(s) of an NM leads to a more accurate prediction of environmental persistence and fate. The effects data considered in the risk characterization of bulk and ionic forms can be relevant to the risk characterization of some NMs. Knowledge of the factors affecting the type and magnitude of ecological effects caused by a particular NM and its transformation products is crucial to characterizing its ecological effects. The aquatic environment is where substances tend to concentrate and is the easiest compartment to characterize. In this section, an approach is presented that corresponds to a Tier 1 assessment (see Figure 1), focusing on the aquatic environment. If possible, characterization of other compartments is considered in higher tier risk assessments. Essential to determining ecological risk, the bioavailability of NMs is integrated into the risk characterization, and is a function of their degradability in water.

The composition of many NMs includes one or more metals. The flow-chart in Figure 2 represents a proposed streamlined ecological screening approach for metal-based NMs by characterizing bioavailability as established by their degradability in water. Similar screening approaches for non-metallic NMs may be developed in the future, as strategies for risk assessment are being developed for these. The metal-based flow-chart is an important first step that guides problem formulation and the initial characterization of ecological hazards from metal-based NMs. The framing for this approach draws from the guidance for selecting an assessment strategy for organometallic and organic-metal salts (OECD, 2015).

Following the left side of Figure 2, metallic NMs that dissolve or degrade to individual ions or molecules within 28 days (OECD 2001) contribute to the broader source apportionment of these metals to the

Canadian environment. Therefore, if the NM dissolves or degrades, and the assessment conclusion is non-toxic for the bulk CAS RN for the corresponding metal(s), the NM form of that metal is deemed of low concern. However, if risk management is warranted for the corresponding metal(s), source NMs undergo an assessment of their dissolved metal component. Their contribution to the metal moiety(ies) is assessed with procedures established for the bulk metal(s), including, as appropriate, incorporation of TMF relationships and natural background concentrations.

Metallic NMs that do not readily dissolve or degrade to ions undergo an evaluation to understand their transformation(s) and potential for dissolution, and to determine if their effects are physical or attributable to the dissolved NM or its metallic components. Physical effects include effects attributable to the particle shape, size, surface area, morphology, etc., and can elicit responses such as physical obstructions or tissue inflammation in organisms. The right side path of Figure 2 depicts the logic applied for NMs that do not readily dissolve or degrade. First, it is determined if the NM is surface-coated or surface-modified. If the NM does have a surface coating or modification, a secondary determination is made to confirm if the coating readily dissolves or degrades in water or not. OECD (2001) provides guidance on short-term dissolution potential of the coating, solubilized metal-based compounds and transformed products, conducted over a period of 24 hours. The PNEC for physical effects observed from the intact NM, coated, or for which the coating dissolves/degrades quickly, is named the $PNEC_{\text{physical}}$. It is likely that $PNEC_{\text{physical}}$ would consider effects that are relatively independent of the chemical composition. These steps help to determine whether physical or dissolved effects from the NM can be expected and guide the assessment towards the use of either the $PNEC_{\text{dissolved}}$ or $PNEC_{\text{physical}}$ for risk characterization.

This assessment strategy also applies to NMs that partially dissolve or transform, or solubilize in 28 days. It can also apply to NMs with a $PNEC_{\text{dissolved}}$ value that is very low (that also shows adverse effects higher than the $PNEC_{\text{physical}}$ effects). NMs that either partially dissolve or degrade, or where this is unknown or with similar values for $PNEC_{\text{dissolved}}$ and $PNEC_{\text{physical}}$, undergo risk assessment considering both possible resulting forms (dissolved and physical).

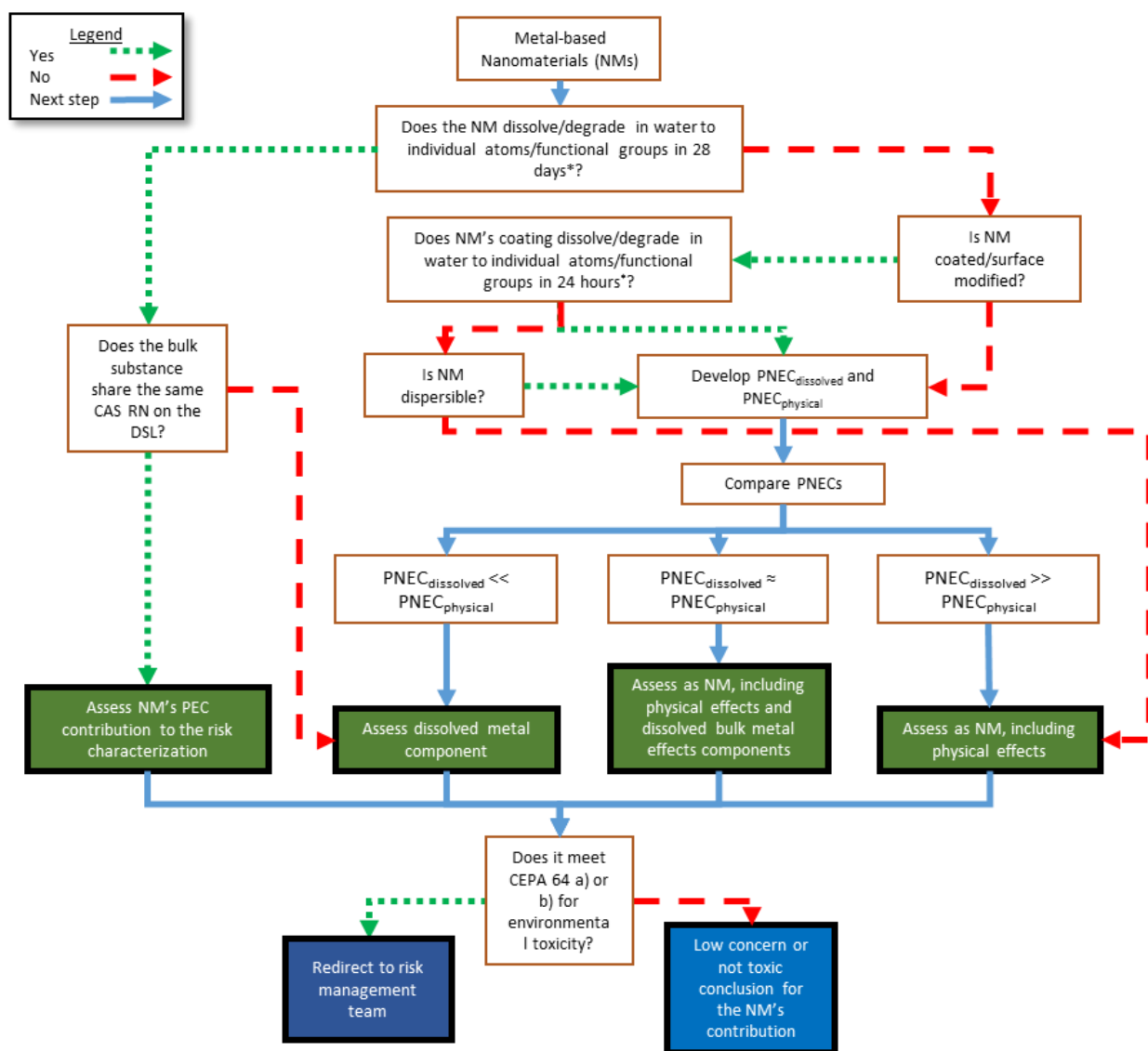


Figure 2. Ecological evaluation screening approach for metallic NMs: problem formulation and initial characterization. * Guidance documents available from OECD (2001)

An environmental exposure assessment considers the routes by which an NM is most likely to be released to the environment. These become the scenarios for development of PECs, relevant PNECs and where possible, a distribution of RQs. Risk characterization integrates the exposure and effects considerations from all relevant ecological processes, including transport and fate, biotic and abiotic chemical transformations, bioavailability fractions, and persistence. A WoE approach is used in the risk characterization section to combine the multiple lines of evidence and their uncertainties (see section 2.3).

3.8.2 Human health risk characterization approach for nanomaterials

Similar to the traditional risk assessment paradigm, human health risk characterization of NMs is an integrated decision supported by information about both human exposure (section 3.6.2) and health effects (section 3.7.2), to inform the conclusion of any potential risk of a given NM under CEPA (Figure 3).

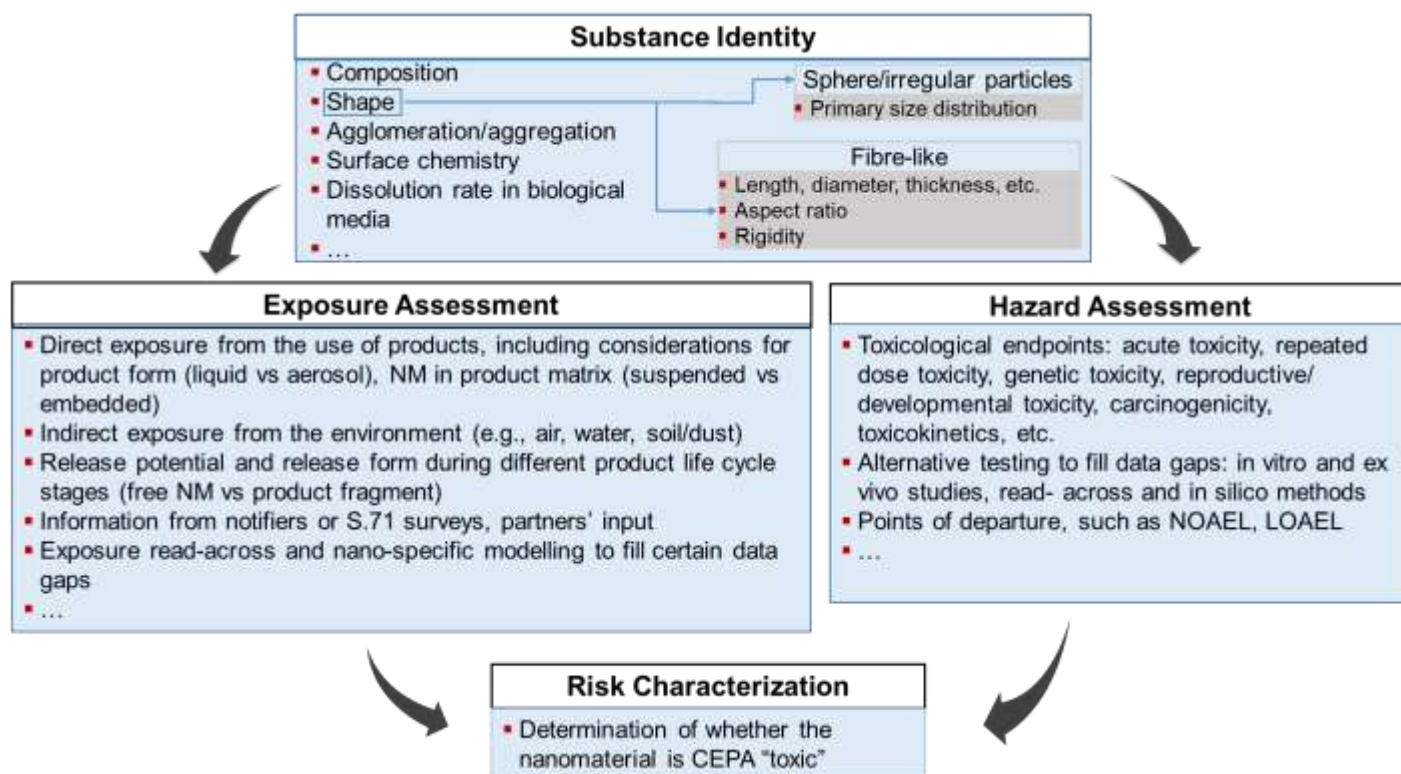


Figure 3. Human health assessment approach for NMs

Human health risks of NMs are characterized based on NM-specific hazards and exposures for relevant routes of exposure. Risk to Canadians are characterized based on, but not limited to, use of products available to consumers, exposure via food, drinking water and environmental media, and with special consideration given to the potential risks to vulnerable populations (for example, children, pregnant women). Sentinel scenarios (that is, those potentially resulting in the highest level of exposure by a given route), including those with the highest potential for exposure based on realistically conservative assumptions, are used for risk characterization. Particular attention is given to the inhalation route for human health risk characterization due to the higher potential for effects, especially for fiber-shaped/high aspect ratio NMs. Exposure and hazard may be expressed in units of mass, particle number, surface area or volume, the choice of which is determined on a case-by-case basis.

For quantification of risk to human health under this framework, a MOE approach is used where the critical toxicity effect level is compared against the estimated exposure for that specified duration and route, and an additional uncertainty factor is also taken into account. More descriptions of MOE are outlined in section 2.2.

4 Conclusions

This document describes a framework and key considerations for risk assessment of NMs, including those in commerce in Canada (that is, DSL NMs) and those new to Canada (new NMs). The risk assessment process is a scientific evaluation that determines the risk posed by a given NM and its known nanoforms

based on both its hazardous properties and the nature and extent of the exposure to Canadians and/or the environment. This process incorporates the WoE approach and precaution, along with consideration of uncertainties associated with hazard and exposure analysis. Depending on the outcome of an NM risk assessment, a determination is made whether the substance meets any of the criteria as set out under paragraphs 64(a), (b), or (c) of CEPA. This allows the Government of Canada to determine whether risk management measures are needed, and if so, what appropriate risk management actions would be required to reduce or prevent risks to the environment and human health.

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