Government of Canada | Gouvernement du Canada

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

Environment and Climate Change Canada
Health Canada

March 2026

Canada

Executive summary

Nanotechnology, which can be described as the manipulation of matter at the nanoscale (about 1 to 100 nanometres), is a technology with enormous innovation potential. Manufactured nanomaterials (NMs) developed using this technology enter the Canadian market across a wide range of applications and industries. Substances, including nanomaterials, are regulated under the *Canadian Environmental Protection Act, 1999* (CEPA), which provides the authority to collect information and to assess and manage risks of substances to the environment and human health.

In 2013, the Organisation for Economic Co-operation and Development (OECD) recommended its Member Countries apply existing international and national chemical regulatory frameworks to manage the risks associated with manufactured NMs:

> *"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).

As such, the principles used for the assessment of substances in Canada are appropriate for the assessment of NMs with necessary modifications to address the specificities of NMs. 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 the assessment of substances are appropriate for the assessment of NMs. Section 3 presents modifications to those general practices and processes 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.

This document was prepared by staff in the Chemicals Management Program at Health Canada and Environment and Climate Change Canada and incorporates input from other programs within these departments. The draft of this document (published June 17, 2022) was subject to a 60-day public comment period. While external comments were taken into consideration, the final content of the document remains the responsibility of Health Canada and Environment and Climate Change Canada.

Table of Contents

List of abbreviations

AF	Assessment Factor
CAS RN	Chemical Abstracts Service Registry Number
CCME	Canadian Council of Ministers of the Environment
CEPA	*Canadian Environmental Protection Act, 1999*
CMP	Chemicals Management Plan
CTV	Critical 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)	*New Substances Notification Regulations (Chemicals and Polymers)*
OECD	Organisation 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 Quotient
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

The *Canadian Environmental Protection Act, 1999* (CEPA) provides the Minister of Environment and the Minister of Health (the Ministers) with the authority to assess and manage environmental and human health risks from substances. The *Domestic Substances List* (DSL) is an inventory of more than 28,792 substances[1] (namely, chemicals, polymers, and organisms) in the Canadian marketplace. These substances are described as "existing substances". A substance not on this list is considered new to Canada and is described as a "new substance", which must be notified to the New Substances Program for assessment prior to import into or manufacture in Canada under the *New Substances Notification Regulations (Chemicals and Polymers)* (NSNR(C&P) (Canada 2018a) or the *New Substances Notification Regulations (Organisms)* (Canada 2018b).

Under Part 5 of CEPA, risk assessments of substances are conducted to determine whether they meet the criteria set out in section 64 of CEPA -- that is, if 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 bulk substances, chemicals and polymers 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. The engineered characteristics of NMs, or variations of different nanoscale forms of the same 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 (OECD 2012a; Dekkers et al. 2016; EU 2018). Substances are listed on the DSL with their Chemical Abstracts Service Registry Numbers (CAS RNs), which specify a chemical composition but do not differentiate among varying physical forms. 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, and if necessary, manage the risk that an NM may pose. This does not imply that all NMs

[1] The DSL is an inventory of substances manufactured in or imported into Canada on a commercial scale. It was originally published in the *Canada Gazette*, Part II on May 4, 1994, and included approximately 23,000 substances deemed to have been in Canadian commerce between January 1984 and December 1986. The DSL is amended, on average, 12 times per year to add, update or delete substances. As of January 2025, the DSL contains more than 28,792 substances and can be accessed through Substances Search.

represent an increased risk to human health or the environment; an NM's unique hazard and exposure profile must be considered to determine whether there is a concern.

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 approaches and considerations that are modified from risk assessment methods traditionally used for chemical substances 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, based on NM-specific hazards and relevant routes of exposure when possible.

1.2 Scope

This risk assessment framework applies to 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, excluding naturally occurring nano-sized particles[2]. A substance is considered as an NM under CEPA 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. This document does not impose any regulatory requirements but rather serves to inform and communicate to stakeholders the approaches and considerations the Government of Canada often uses for assessing NMs under CEPA.[3]

1.3 Policy statement summary

The policy statements raised within this document are as follows:

- The risk assessment of NMs follows the principles of bulk chemical and polymer 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 RN[4]) and information on physical-chemical properties (for example, size, shape, and 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.

[2] 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).

[3] While this framework provides guidance on how NMs are to be assessed under CEPA, it is not meant to capture every possible approach which could be used. Other approaches and considerations may be applied to address NMs on a case-by-case basis.

[4] 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.

- 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 nanometre. 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.

- Read-across methodologies for NMs are under development. Currently, read-across for NMs is considered on a case-by-case basis and as part of the WoE approach to risk assessment. Guidance on read-across methodologies for NMs available from other jurisdictions/organizations (for example, the European Union and the OECD (OECD 2012b; ECHA 2017, 2021)) are also considered. Grouping strategies employed for ecological and human health risk evaluation of NMs may differ.

- It is possible that a finding of potential harm to the environment or human health drawn from a risk assessment is applicable to a range of known variants of the NM and its CAS RN identified in the assessment, or that different nanoforms with the same CAS RN have a different potential to cause harm to the environment or human health.

- An assessment conclusion as set out in section 64 of CEPA is not relevant to, nor does it preclude an assessment against the hazard criteria for the Workplace Hazardous Materials Information System specified in the *Hazardous Products Regulations* for products intended for the workplace. Similarly, a conclusion based on the criteria set out in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

2 Overview of regulatory risk assessment under CEPA

2.1 Existing and new nanomaterial legislative and regulatory frameworks

In Canada, the existing legislative and regulatory frameworks for bulk chemicals and polymers under CEPA 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 CEPA, such as for information gathering and prioritization for existing substances and the application of regulations for new substances, are appropriate for the assessment and management of NMs. Additional information on risk assessment approaches for bulk chemicals and polymers is outlined in the CMP Risk Assessment Toolbox (GoC 2016a).

To date, risk assessments of existing substances carried out under CEPA 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 (Canada 2015). With the data collected from this initiative, exercises were undertaken to develop an approach to address nanoscale forms of substances on the DSL (GoC 2016b). This work is ongoing.

For new NMs notified under the NSNR(C&P), ECCC and HC assess the risk of NMs to people living in Canada and the environment with the information that importers or domestic manufacturers are obligated to provide according to the regulations. ECCC and HC use this information, along with other available information, to assess the notified NM to determine if it meets the criteria set out in 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 to be submitted upon notification, as described in the New Substances Notification Form for Chemicals, Biochemicals, Polymers and Biopolymers (including nanomaterials) (GoC 2023a) and the Guidance document for the New Substances Notification Regulations (Chemicals and Polymers) (GoC 2023b).

2.2 Chemical and polymer risk assessment approaches

This section briefly summarizes the risk assessment approaches used to assess bulk substances under CEPA. Detailed, up-to-date information on these approaches are maintained by the Government of Canada in a series of fact sheets (GoC 2023b). These approaches are generally also applicable to NMs. NM-specific adaptations of risk assessment approaches are discussed in detail in section 3 of this document.

Chemical and polymer risk assessment is the evaluation of the potential for adverse outcomes from a substance and considers both hazard and exposure to determine the risk. The hazard associated with a substance is the inherent ability of the substance to cause adverse effects such as reduced survival, growth or reproduction in 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 2023c).

An essential step in risk assessment is the collection of information on each substance or substance group. 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, ecological and health effects and mechanistic data, as well as the route(s), duration, and frequency of exposure.

When insufficient experimental, monitoring, or measurement data are available, 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 to support risk assessment. Another option includes the use of predictive tools, 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 ecological or 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 the 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 and when applying a WoE approach to reach conclusions (see section 2.3).

Risk characterization can be described qualitatively and quantitatively. In quantitative risk characterization, quantitative exposure levels and critical effect levels are identified to calculate risk quotients (RQs) for environmental risk assessment, and a margin of exposure (MOE) for human health risk assessment (GoC 2022b). Refinements to improve estimates of exposure and uncertainty factors (also known as assessment 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-effect concentration (PNEC) (the concentration of a substance in an environmental medium below which adverse effects are unlikely to occur (see section 3.4.1.3)). 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 risk is associated with any level of exposure.

2.3 Uncertainties and the application of weight of evidence and precaution

Risk assessment under CEPA relies on the application of WoE and precaution to account for uncertainties (GoC 2022a). The Ministers apply a WoE approach (a method for decision-making that involves the consideration of multiple sources of information and lines of evidence) and precaution when conducting and interpreting the results of substance assessments, including NMs (ECCC 2017). Uncertainty in an assessment may result from data gaps and exists at each step of the risk assessment, influencing the risk conclusion. During a risk assessment, the lines of evidence and their associated uncertainties are evaluated in support of a risk conclusion. 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.

3 Nanomaterial-specific considerations for risk assessment

The unique characteristics of NMs may impact their effects, fate, and exposure; therefore, NMs may need to be assessed separately from bulk form substances. This section describes the modifications to testing

and assessment approaches developed for bulk substances that may be required to account for NM-specific properties.

3.1 Definition of a nanomaterial for the purposes of risk assessment under CEPA

ECCC and HC regulators are using the HC working definition (GoC 2011). According to this 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 and 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 (GoC 2023a).

Agglomerated[5] or aggregated[6] (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 life cycle. 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 Identity and physical-chemical property considerations for nanomaterials

A CAS RN is commonly used in identifying a substance and is based on its composition and sometimes manufacturing details; however, it does not differentiate the bulk and nanoscale forms of a substance.

[5] "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.

[6] "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.

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 for a given NM. For CEPA risk assessments on existing NMs, 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 in commerce in Canada. For CEPA risk assessments on new NMs, information related to identity and physical-chemical properties is submitted by notifiers through the NSNR(C&P).

The identification of NMs requires the consideration of properties that are common with bulk substances such as chemical composition and impurities, as well as properties which are unique to NMs such as size and shape. Under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) annexes (EU 2018), size distribution, shape, and surface chemistry are the minimum additional properties required for proper NM identification. These additional properties also form the basis for NM identification in Canadian risk assessments under CEPA. Guidance on measurement methods for these properties are available, including OECD test guidelines (TGs) on determination of volume specific surface area (TG 124), particle size and size distribution (TG 125) for nanomaterials (OECD 2016a, 2022, 2023; Rasmussen et al. 2018).

3.2.1 Substance identity at the point of exposure – aggregates/agglomerates and transformation

Following the release of NMs to the environment or their incorporation into a product, chemical, physical, or biologically mediated transformations of NMs may readily occur (Lowry et al. 2012; Lead et al. 2018). At the point of exposure, the behaviour of the transformed form(s) of NMs may differ considerably relative to their pristine (as manufactured) form. As a result, risk assessments consider the effects of the transformed form(s) based on environmental, biological, or use conditions over the life cycle, when sufficient information is available. This may include consideration of the NM at potential exposure points such as during its manufacture, transport, incorporation into a product(s), use(s), recycling and final disposal. 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.

Transformations depend both on the nature of the pristine NMs and environmental conditions (Lead et al. 2018). Environmental and biological media (for example, air, water, soil, sediment, lung) are complex matrices composed of dissolved ions, natural organic matter, micro- and larger particles and biological molecules, which can interact with and transform NMs. The variability in such media may result in a wide range of possible states for a given NM, such as aggregated, agglomerated and/or chemically transformed particles. For example, NMs may undergo dissolution or aggregation processes in response to variations

in pH, affecting their stability and reactivity (Tran et al. 2024). These transformations can play a role in determining the mobility, bioaccessibility, bioavailability, and toxicity of the NMs at the point of exposure.

In the case where information on the characteristics of transformed NMs in the environmental or biological media is not available, information on the pristine form may be considered in the risk assessment.

3.3 Data considerations for the risk assessment of nanomaterials

When gathering data and weighing their relevance and reliability to characterize the risk of an NM, the same principles as those developed for bulk substance assessment apply (GoC 2022c, 2022d). When examining NM-specific data, available information is collected and analyzed, and qualitative and quantitative lines of evidence are considered. For the risk assessment of new NMs, prescribed information required under the NSNR(C&P) and submitted by notifiers serve as a primary data resource. For the risk assessment of existing NMs, information regarding Canada-specific use patterns and quantities of NMs in commerce is obtained through mandatory (i.e., section 71 of CEPA) or voluntary information gathering activities, as well as from other federal programs and open literature.

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 life cycle, uncertainty about appropriate metrics for exposure quantification, and a lack of fully validated exposure and toxicokinetic models. Additionally, hazard 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 results in significant limitations in terms of time and cost, and number of animals required for hazard testing. Therefore, to develop robust risk assessments of NMs, multiple strategies are explored to fill data gaps, such as using existing databases, models, grouping, read-across and QSARs, and also applying precaution in addressing these data gaps and challenges, as described in section 2.3.

3.3.1 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 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

2016b; GRACIOUS 2017; ECHA 2021). In Canada, grouping strategies for NM assessments under CEPA use a combination of the RCC approach and other available approaches.

3.3.2 Databases and models

International organizations (for example, the 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, 2021b, 2021c, 2021d). 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.

3.3.3 Read-across approach for nanomaterials

Read-across is a strategy used in the risk assessment of substances to extrapolate data from similar substances (based on chemical structure, physical-chemical properties, function, toxicological mode of action), 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 2022e). 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 2021).

Grouping can facilitate a read-across approach. NMs that have similar structure, physical-chemical properties, or kinetics 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 2017, 2021; 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) if applicable. 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 2017, 2021). 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. When there are data gaps on the nanoform and the bulk form is determined to be a reasonable analogue for specific endpoints, 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 those 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 human health or environmental effects of the target NM. To account for uncertainties associated with read-across for NMs, additional uncertainty factors may be applied during the risk characterization.

3.3.4 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 or validated 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 risk assessment approach for NMs under CEPA.

3.3.5 Choice of metrics for nanomaterial risk assessment

Traditionally, risk assessments of chemicals and polymers 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 an NM that can alter its effects, there is no international consensus on the most appropriate metric for the risk assessment of NMs (ECHA 2021). 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.

3.4 Ecological and human health approaches to characterize fate and 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. Examining the life cycle of an NM provides the foundation to support the characterization of environmental and human exposures. A typical life cycle of an NM starts with the manufacture and/or import of the substance in Canada and progresses to use and final disposal. The uses include industrial, commercial, or consumer applications, which informs direct exposure assessment (for example, from use of product or consumption of food) and indirect exposure assessment (for example, exposure of the NM through environmental media such as air or water). The end-of-life disposal of products also informs the exposure assessment. If applicable (for example, a substance is found in various 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 is 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.

3.4.1 Environmental fate and exposure to nanomaterials

3.4.1.1 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 and 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 parameter values for exposure modelling for certain substance use scenarios (for example, days of operation and emission factors). These accepted parameter values may include guideline values from Canadian sources for specific activities, values from accepted models such as from the OECD, those used by other jurisdictions, or values obtained by comparing the exposure of similar substances used in similar activities. 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.

3.4.1.2 Environmental fate and behaviour

The environmental fate of NMs, including their distribution into different environmental compartments and persistence, depends on factors including particle shape, size, agglomeration behaviour, and composition. Predicting the environmental fate of NMs requires knowledge of the substance's behaviour in the environment (throughout its lifecycle) such as aggregation and/or dissolution and other transformations that NM may undergo in environmental media. When information is available, characterization of the relevant environmental media supports accurate environmental persistence and fate predictions of NMs since the behaviour of NMs is partially dependent on external environmental factors, such as pH, and the ionic strength of the surrounding medium. For example, environmental factors can influence the homoaggregation (an NM aggregating with itself) or the heteroaggregation (an NM aggregating with other mineral or natural colloids in the environment) of NMs, which can dramatically impact the fate of NMs (Batley et al. 2012) such as whether the NM remains suspended in the aquatic environment or settles to sediment. Additionally, factors such as organic surface coatings that can form around NMs in the aquatic environment may also have an aggregating or dispersing effect (Thio et al. 2011; Yang and Cui 2013), making it necessary to consider different types of organic matter in solution. Environmentally mediated transformations such as these are considered in an assessment for their influence on the persistence and fate of NMs.

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. The metal ion is considered persistent under the *Persistence and Bioaccumulation Regulations* of CEPA (Canada 2000) because it cannot degrade any further and 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 persistence and fate. 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.

Environmental fate models based on physical-chemical properties, such as equilibrium partitioning models, traditionally used to estimate partitioning and fate of organic chemicals within environmental media, are not specifically applicable to NMs. These models do not account for kinetically controlled processes such as colloidal behaviour, kinetics of degradation and aggregation behaviour 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 substances (Di Guardo et al. 2018). For instance, there have been successful efforts to adapt fugacity models for the prediction of NM environmental behaviour using such parameters as size, charge, and agglomeration (Utembe et al. 2018). As well, multimedia fate models that consider nano-specific processes such as aggregation, attachment, and dissolution have been used successfully for some nanomaterials (Meesters et al. 2014) and are being used in regulatory risk assessment. Investigations into these models for their reliability and use in environmental risk assessments have been conducted (OECD 2021a).

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, risk assessments may rely on currently available models modified to consider NMs 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 section 2.2).

3.4.1.3 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, and others. 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 substances and is used to evaluate exposure to NMs for an initial exposure characterization, provided there is an entry pathway to aquatic environments. In these cases, the aquatic PEC (PEC_{aq}) may be 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 can be 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.

3.4.2 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 populations who may be disproportionately impacted (for example, children and during pregnancy).

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 life cycle of NMs. Commercial use data received in response to CEPA section 71 notices 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 (GoC 2022f) under the CMP, including age group, body weight, body surface area, inhalation rates, and others. All these factors, as appropriate, are considered in the human health exposure assessment on NMs. Additionally, for some product types, such as self-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 life cycle 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).

3.5 Ecological and human health approaches to characterize the effects of nanomaterials

3.5.1 Ecological effects characterization

The main goal of ecological effects characterization is to determine or estimate the potential hazard threshold concentration of a substance that is unlikely to cause adverse effects to the structure or function of an exposed ecosystem (GoC 2022b). There are two main drivers to ecological effects characterization.

First, the substance characteristics, which have the most influence on bioavailability[7], mode of toxic action, and on the toxic response. Sections 3.5.1.1 to 3.5.1.3 address the ecological effects characterization on NMs through substance characteristics. Bioaccessibility[8] is also defined in relation to bioavailability in section 3.5.1.1. The second driver consists of external factors influencing toxicity and includes environmental mitigation factors. These are discussed in section 3.5.1.4 in relation to environmental characteristics. Finally, section 3.5.1.5 summarizes the approach used to assess the ecological effects of NMs.

3.5.1.1 Ecological bioavailability and bioaccessibility

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; however, the same NM may not be bioaccessible to other organisms if it is sequestered in sediment. 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.

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 after its release to the environment 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 risk assessment approach (see Chemicals Management Plan Risk Assessment toolbox - Canada.ca), the most bioavailable form of an NM may be considered as the only form present.

[7] Bioavailability is the amount of a nanomaterial that is taken up by an organism from the environment and is available to cause a biological response (McLaughlin and Lanno 2014). Bioavailability should include measurement of the substance in the environmental media and within the organism (internal concentration).

[8] Bioaccessibility is a surrogate measurement for bioavailability when measurements using organisms are limited. Bioaccessibility represents the soluble form of the nanomaterial and so it is directly linked to the environmental characteristics (McLaughlin and Lanno, 2014). This provides information about which substances are anticipated to be relatively more or less bioavailable. However, some nanomaterials may be taken up as particles and bioaccessibility would only be an approximation of the bioavailability of the substance.

3.5.1.2 Determination of the ecological toxicity values

There are various ways to quantitatively and qualitatively approach the evaluation of ecotoxicity for a given substance. Two methodologies commonly used in CEPA assessments have the same aim: to estimate the threshold concentration below which adverse effects are unlikely in the most sensitive organisms. When the dataset for ecotoxicity is sufficient for a given substance, it may be possible to develop a species sensitivity distribution[9] (SSD) (GoC 2023d). When the dataset is sparse or the dataset does not allow for appropriate curve-fitting required for an SSD, a critical toxicity value (CTV) may be 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). An SSD or CTV may be 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 using an SSD approach, ECCC typically follows the methodologies and recommendations from the Canadian Council of Ministers of the Environment (CCME 2007).

For each environmental medium, all the ecological effects data collected for a specific NM are compared based on the most bioavailable forms. The dataset may be standardized to compare toxicity endpoints for duration, type, and magnitude of effects (Okonski et al. 2021). The selection of the critical endpoints should be related to and representative of any 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).

Qualitative ecological toxicity information on NMs may also be used to support the WoE for the assessment of NMs. The approach involves assessing the impact of NMs using a broader set of observations including behavioural changes, community structure alterations, reduction in survival, and habitat changes among others.

3.5.1.3 Ecological predicted no-effect concentrations

The 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 during the risk characterization process. When the representative toxicity value is determined using an SSD, the 5th percentile of the SSD corresponds to the PNEC and typically does not require an assessment factor (AF). Otherwise, a PNEC value is calculated by dividing the CTV by an AF.

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

[9] 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, a 5th percentile concentration is a typical selection used to be protective of at least 95% of species) to ensure a specified level of species protection is achieved.

The methodology to estimate an AF relies on the use of extrapolations for intra/inter-species variations, endpoint standardization, and mode of action (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, consideration of the number of species and taxonomic groups included in the effects dataset, 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 expressed as functions of the environmental factors, or as site-specific PNECs, when sufficient data are available to derive these relationships (see section 3.5.1.4). 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 ($PNEC_{bioavailable}$) rather than the total concentration of the NM ($PNEC_{total}$).

3.5.1.4 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.

3.5.1.5 Nanoscale properties impacting the ecological effects characterization

Analyses of the environmental exposure 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 of nanoscale properties (surface chemistry, shape and size effects) on ecological receptors. Some OECD 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 TGs or GDs 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.

3.5.2 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 components 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/aggregation, transport across biological barriers such as gut epithelium/blood-brain-barrier/skin (relevant for absorption/distribution characterization), accumulation, elimination, 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 effects 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 bulk chemicals and polymers, 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 (for example, 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 TGs 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, and dissolution (EFSA 2018). To date, a few OECD TGs have been modified to address nanoscale issues and published, including the 28-day repeated dose toxicity study-OECD TG 412, the 90-day repeated dose toxicity study-OECD TG 413, and a GD on acute inhalation toxicity testing (OECD 2018; see also Nanomet - OECD). Further work to update several other OECD TGs and GDs or draft new GDs is underway to address the regulatory testing of NMs (Rasmussen et al. 2019).

Ideally, for a more in-depth 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, carcinogenicity, 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 considered. 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). A proper sample preparation including dispersion protocols (for example, dispersant, sonication) is also critical for NM quantification and dosimetry. 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 fibre-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 substances 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 2017, 2021). 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 interpreting the study results (OECD 2018a). Lung overload is usually associated with poorly soluble particles (at nanoscale or non-nanoscale), which can lead to adverse pulmonary effects that must be distinguished from the effects induced by the intrinsic toxic potential of the particles themselves (ECHA 2021).

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 substance 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 (section 3.3.3) and *in silico* methods such as QSARs (section 3.3.4) can facilitate hazard predictions for various nanoscale forms.

3.5.2.1 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 many 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 standardized *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. As some *in vitro* methods may not be suitable for testing NMs, the OECD has performed a detailed evaluation of the applicability of *in vitro* toxicity testing methods used to determine the health effects of NMs (OECD 2018b).

Recent efforts have also been focused on using Integrated Approaches to Testing and Assessment (IATA) for risk assessment of substances (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 2017). These approaches may help to speed up the risk assessment process, while at the same time reducing testing costs and animal use (OECD 2017). At present, adverse outcome pathways and read-across are the approaches proposed for developing and using IATA for regulatory risk assessment of NMs (OECD 2017).

3.6 Risk characterization of nanomaterials

Similar to the paradigm outlined for bulk substances, the risk characterization of NMs is an integrated decision supported by information from both effects and exposure characterization. The risk characterization of NMs accounts for uncertainty associated with each step of the risk assessment; these uncertainties are recognized and integrated into the assessment to maintain transparency throughout the assessment process. Accordingly, the risk characterization of NMs under CEPA applies precaution and WoE approaches.

An assessment 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. Equally, for an NM assessed under CEPA the potential for a substance to cause harm to the environment or to human health may differ between the bulk form and its NM forms. The results of risk assessments provide critical information for any effective risk management actions subsequently developed.

If an NM is determined to meet the criteria as set out in Part 5, section 64 of CEPA, then risk management measures are considered to prevent or control the risks identified. Follow-up activities may be undertaken for those NMs which did not meet the criteria set out in section 64 of CEPA but are recognized for their potential effects of concern. These substances could meet the criteria set out in section 64, if exposures were to increase or if different nanoforms were to be produced.

3.6.1 Ecological risk characterization approach for nanomaterials

Along with an understanding of the volumes and uses of a nanomaterial, 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 lead to a more accurate prediction of environmental persistence and fate. 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. If possible, the characterization of relevant environmental media should be considered to provide greater precision to the assessment. Essential to determining ecological risk, the bioavailability of NMs is integrated into the risk characterization.

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), and ultimately to reach a conclusion on whether the NM poses a harm to the environment.

3.6.2 Human health risk characterization approach for nanomaterials

Human health risk characterization of NMs is an integrated decision supported by information about both human exposure (section 3.4.2) and health effects (section 3.5.2) to inform the conclusion of any potential risk of a given NM under CEPA (Figure 1).

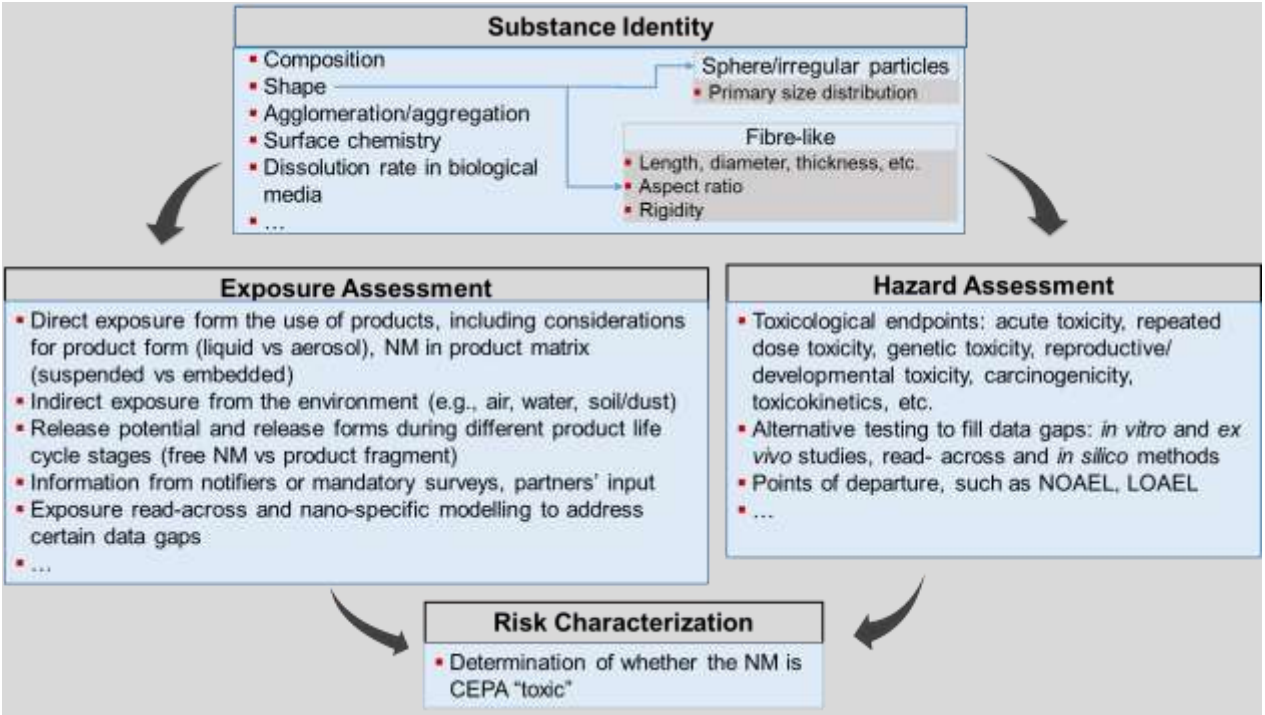

Figure 1. Human health assessment approach for NMs

A chart with four boxes displaying the human health assessment approach for manufactured NMs. The four boxes are titled "Substance Identity", "Exposure Assessment", "Hazard Assessment", and "Risk Characterization". The box "Substance Identity" contains examples of traits of substance identity, such as composition, agglomeration/aggregation, surface chemistry, dissolution rate in biological media, and shape. Shape is further broken down into sphere and irregular particles (where the primary size distribution must be known) and fibre-like particles, where length, diameter, thickness, aspect ratio, and rigidity must be known.

The "Substance Identity" box has two arrows leading from it, one to the "Exposure Assessment" box and one to the "Hazard Assessment" box. Under "Exposure Assessment", key considerations are listed: direct exposure from the use of products, including consideration for product form (liquid vs. aerosol), NM in product matrix (suspended vs. embedded); indirect exposure from the environment (e.g., air, water, soil/dust); release potential and release form during product life cycle stages (free NM vs product fragment); information from notifiers or mandatory surveys, partners' input; and exposure read-across and nano-specific modelling to address certain data gaps.

Under "Hazard Assessment", key considerations listed include: toxicological endpoints: acute toxicity, repeated dose toxicity, genetic toxicity, reproductive/developmental toxicity, carcinogenicity, toxicokinetics, etc.; alternative testing to fill data gaps: in vitro and ex vivo studies, read-across and in silico methods; and points of departure, such as NOAEL (no-observed-adverse-effect level), LOAEL (lowest observed adverse effect level).

From the exposure and hazard assessment boxes, two arrows lead to a box titled "Risk Characterization", where "whether the NM is CEPA "toxic" is determined.

Overall, the chart describes 4 steps in a human health assessment to reach a conclusion under CEPA.

Human health risks of NMs are characterized based on NM-specific hazards and relevant routes of exposure. Risk to people living in Canada is 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 populations who may be disproportionately impacted (for example, children, pregnant people). 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 fibre-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, an MOE approach is used where the critical toxicity effect level is compared against the estimated exposure for that specified duration and route, with additional uncertainty factors considered. 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 potential for harm posed by a given NM and its known nanoforms based on both its hazardous properties and the nature and extent of the exposure to people living in Canada and/or the environment. This process incorporates the WoE approach and precaution, along with consideration of uncertainties. Depending on the outcome of an NM risk assessment, a determination is made whether the substance meets any of the criteria as set out in 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, help inform what appropriate risk management actions would be required to reduce or prevent risks to the environment and human health.

5 References

Batley G, Kirby J, McLaughlin M. 2012. Fate and risks of nanomaterials in aquatic and terrestrial environments. Accounts of Chemical Research. 46.

Braakhuis HM, Cassee FR, Fokkens PH, de la Fonteyne LJ, Oomen AG, Krystek P, de Jong WH, van Loveren H, Park MV. 2016. Identification of the appropriate dose metric for pulmonary inflammation of silver nanoparticles in an inhalation toxicity study. Nanotoxicology. 10(1):63-73. https://doi.org/10.3109/17435390.2015.1012184.

Burello E. 2017. Review of (Q)SAR models for regulatory assessment of nanomaterials risks. NanoImpact. 8: 45-58.

Canada. 1999. Canadian Environmental Protection Act, 1999. S.C. 2023, c.26. Canada Gazette Part III, vol. 22, no. 3 [PDF].

Canada. 2000. Persistence and Bioaccumulation Regulations, SOR/2000-107.

Canada. 2015. Notice with respect to certain nanomaterials in Canadian commerce. Canada Gazette Part I, vol. 149, no. 30: GOVERNMENT NOTICES.

Canada. 2018a. New Substances Notification Regulations (Chemicals and Polymers), SOR/2005-247.

Canada. 2018b. New Substances Notification Regulations (Organisms), SOR/2005-247.

[CCME] Canadian Council of Ministers of the Environment. 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007 [PDF]. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, 1999, Winnipeg.

Dekkers S, Oomen AG, Bleeker EAJ, Vandebriel RJ, Micheletti C, Cabellos J, Janer G, Fuentes N, Prina-Mello A, Movia D, Nesslany F, Ribeiro AR, Leite PE, Groenewold M, Cassee FR, Sips AJAM, Dijkzeul A, van Teunenbroek T, Wijnhoven SWP. 2016. Towards a nanospecific approach for risk assessment. Regul. Toxicol. Pharmacol., 80:46-59.

Delmaar CJ, Peijnenburg WJ, Oomen AG, Chen J, de Jong WH, Sips AJ, Wang Z, Park MV. 2015. A practical approach to determine dose metrics for nanomaterials. Environ Toxicol Chem. 34(5):1015-22.

Di Guardo A, Gouin T, MacLeod M, Scheringer M. 2018. Environmental fate and exposure models: Advances and challenges in 21st century chemical risk assessment. Environ. Sci.: Processes Impacts, 2018, 20, 58-71.

[ECCC] Environment and Climate Change Canada. 2017. Overview of the Existing Substances Program – Chapter 2 CEPA 1999 guiding principles and other policies. Government of Canada, Ottawa (ON).

[ECCC and HC] Environment and Climate Change Canada and Health Canada. 2013. Framework for science-based risk assessment of micro-organisms regulated under the Canadian Environmental Protection Act, 1999. Government of Canada, Ottawa (ON).

[ECCC and HC] Environment and Climate Change Canada and Health Canada. 2017. Screening assessment cobalt and cobalt-containing substances. Government of Canada, Ottawa (ON). (Accessed 2020-01-08).

[ECHA] European Chemicals Agency. 2017. Guidance on information requirements and chemical safety assessment Appendix R7-2 for nanomaterials applicable to Chapter R7c Endpoint specific guidance. ECHA, Helsinki, Finland.

[ECHA] European Chemicals Agency. 2021. Guidance on information requirements and chemical safety assessment Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance. Version 3.0 – Oct. 2021. ECHA, Helsinki, Finland.

[EFSA] European Food Safety Authority. 2018. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. (Accessed 2021-02-09).

Environment Canada. 2007. Overview of the ecological assessment of substances under the Canadian Environmental Protection Act, 1999. Government of Canada, Ottawa (ON).

European Commission. 2011. Commission recommendation of 18 October 2011 on the definition of nanomaterial (text with EEA relevance) [PDF]. 2011/696/EU. Official Journal of the European Union, L 275/38, 20.10.2011. (Accessed 2020-01-08).

[EU] European Union. 2018. Commission regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III, VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances.

[GoC] Government of Canada. 2011. Policy statement on Health Canada's working definition for nanomaterials. Ottawa (ON). Date modified: May 26, 2011. (Accessed 2020-01-08).

[GoC] Government of Canada. 2016a. Chemicals Management Plan risk assessment toolbox. Ottawa (ON). Date modified: May 30, 2016.

[GoC] Government of Canada. 2016b. Consultation document: prioritization approach for nanoscale forms of substances on the Domestic Substances List. Ottawa (ON). Date modified: June 7, 2017.

[GoC] Government of Canada. 2022a. Application of weight of evidence and precaution in risk assessment. Ottawa (ON). Date modified: February 11, 2022. (Accessed 2023-09-06).

[GoC] Government of Canada. 2022b. Use of margins of exposure and risk quotients in risk assessment. Ottawa (ON). Date modified: February 11, 2022. (Accessed 2023-12-28).

[GoC] Government of Canada. 2022c. Canada's approach on chemicals: information gathering. Ottawa (ON). Date modified: March 11, 2022.

[GoC] Government of Canada. 2022d. Approaches for addressing data needs in risk assessments. Ottawa (ON). Date modified: February 9, 2022.

[GoC] Government of Canada. 2022e. Use of analogues and read-across in risk assessment. Ottawa (ON). Date modified: February 9, 2022.

[GoC] Government of Canada. 2022f. Canadian exposure factors used in human health risk assessments. Ottawa (ON). Date modified: February 11, 2022.

[GoC] Government of Canada. 2023a. New substances: Notification forms. Ottawa (ON). Date modified: December 14, 2023. (Accessed 2024-01-09).

[GoC] Government of Canada. 2023b. Guidance document for the *New Substances Notification Regulations (Chemicals and Polymers)*. Ottawa (ON). Date modified: January 16, 2023. (Accessed 2024-01-09).

[GoC] Government of Canada. 2023c. Risk assessment of chemical substances. Ottawa (ON). Date modified: May 19, 2023. Ottawa (ON).

[GoC] Government of Canada. 2023d. Species sensitivity distributions for water quality guidelines and ecological risk assessment. Ottawa (ON). Date modified: May 19, 2023. Ottawa (ON). (Accessed 2023-10-28).

[GoC] Government of Canada. 2023e. The rapid screening approach. Ottawa (ON). Date modified: June 28, 2023. (Accessed 2023-09-06).

GRACIOUS. 2017. Draft GRACIOUS framework for grouping and read-across of nanomaterials for regulatory risk assessment and safe-by-design [PDF]. H2020-NMBP-2017. GRACIOUS – Deliverable D1.2. (Accessed 2020-01-08).

[IPCS] International Programme on Chemical Safety. 2004. IPCS Risk Assessment Terminology. World Health Organization, Geneva. (Accessed 2020-02-05).

ISO/TR 18401: 2017. International Organization for Standardization technical report on: Nanotechnologies – plain language explanation of selected terms from the ISO/IEC 80004 series. Online browsing platform.

[JRC] Joint Research Centre. 2017. JRC Technical reports. Evaluation of the availability and applicability of computational approaches in the safety assessment of nanomaterials [PDF].

Juganson K, Ivask A, Blinova I, Mortimer M, Karhu A. 2015. NanoE-Tox: New and in-depth database concerning ecotoxicity of nanomaterials. Beilstein J. of Nanotechnol. 6:1788-1804.

Keller AA, Wang H, Zhou D, Lenihan HS, Cherr G, Cardinale BJ, Miller R, Ji Z. 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ. Sci. Technol. 44: 1962-1967. https://doi.org/10.1021/es902987d.

Kuhlbusch TAJ, Wijnhoven S WP, Haase A. 2018. Nanomaterial exposures for worker, consumer and the general pubic. NanoImpact. 10: 11-25.

Lamon L, Aschberger K, Asturiol D, Richarz A, Worth A. 2019. Grouping of nanomaterials to read-across hazard endpoints: a review. Nanotoxicology. 13:1, 100-118.

Lead JR, Batley GE, Alvarez PJJ, Croteau MN, Handy RD, McLaughlin MJ, Judy JD, Schirmer K. 2018. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects—An updated review. Environ. Toxicol. Chem. 37: 2029-2063.

Lowry GV, Gregory KB, Apte SC, Lead JR. 2012. Transformations of nanomaterials in the environment. Environ. Sci. & Tech. 46 (13): 6893-6899.

Macarron R, Banks M, Bojanic D, Burns DJ., Cirovic DA., Garyantes T, Green DVS, Hertzberg RP., Janzen WP, Paslay JW, Schopfer U. & Sittampalam SG. 2011. Impact of high-throughput screening in biomedical research. Nat. Rev. Drug Discov. 10: 188–195.

McLaughlin MJ, Lanno R. 2014. Use of "Bioavailability" as a term in ecotoxicology. Integr. Environ. Assess. Manag. 10: 138-140.

Meesters J, Koelmans A, Quik J, Hendriks J, van de Meent D. 2014. Multimedia modeling of engineered nanoparticles with SimpleBox4nano: Model definition and evaluation. Environ. Sci. & Tech. 48.

Oberdörster G, Oberdörster E, Oberdörster J. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 113: 823–839.

[OECD] Organisation for Economic Co-operation and Development. 2012a. Six years of OECD work on the safety of manufactured nanomaterials: Achievements and future opportunities. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2012b. Important issues on risk assessment of manufactured nanomaterials [PDF]. Series on the Safety of Manufactured Nanomaterials No. 33. ENV/JM/MONO(2012)8. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2013. Draft recommendation of the council on the safety testing and assessment of manufactured nanomaterials [PDF]. C(2013)107. OECD, Paris. (Accessed 2020-01-08)

[OECD] Organisation for Economic Co-operation and Development. 2014. Genotoxicity of manufactured nanomaterials: Report of the OECD expert meeting [PDF]. ENV/JM/MONO(2014)34. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2016a. Physical-chemical properties of nanomaterials: Evaluation of methods applied in the OECD-WPMN testing programme [PDF]. Series on the Safety of Manufactured Nanomaterials No. 65 ENV/JM/MONO(2016)7. OECD, Paris. (Accessed 2022-12-06).

[OECD] Organisation for Economic Co-operation and Development. 2016b. Series on the Safety of Manufactured Nanomaterials. No. 76: Grouping and read-across for the hazard assessment of manufactured nanomaterials: report from the expert meeting [PDF]. ENV/JM/MONO(2016)59. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2017. Guidance document on the use of adverse outcome pathways in developing Integrated Approaches to Testing and Assessment (IATA). [PDF]. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2018a. Guidance document on inhalation toxicity studies [PDF]. OECD, Paris. (Accessed 2020-01-08).

[OECD] Organisation for Economic Co-operation and Development. 2018b. Evaluation of in vitro methods for human hazard assessment applied in the OECD [PDF]. OECD, Paris. (Accessed 2022-12-06).

[OECD] Organisation for Economic Co-operation and Development. 2020. Overview of concepts and available guidance related to Integrated Approaches to Testing and Assessment (IATA), OECD Series on Testing and Assessment, No. 329 [PDF]. Environment, Health and Safety, Environment Directorate. OECD, Paris (Accessed 2022-12-06).

[OECD] Organisation for Economic Co-operation and Development. 2021a. Evaluation of tools and models used for assessing environmental exposure to manufactured nanomaterials: Functional assessment and statistical analysis of nano-specific environmental exposure tools and models. OECD Series on the Safety of Manufactured Nanomaterials No. 98, Series on Testing and Assessment, No. 345 [PDF]. OECD, Paris. (Accessed 2021-12-13).

[OECD] Organisation for Economic Co-operation and Development. 2021b. Evaluation of Tools and Models for Assessing Occupational and Consumer Exposure to Manufactured Nanomaterials – Part I: Compilation of tools/models and analysis for further evaluation Series on the Safety of Manufactured Nanomaterials No. 99 Series on Testing and Assessment, No. 346 [PDF]. OECD, Paris. (Accessed 2021-12-13).

[OECD] Organisation for Economic Co-operation and Development. 2021c. Evaluation of tools and models for assessing occupational and consumer exposure to manufactured nanomaterials – Part II: Performance testing results of tools/models for occupational exposure. Project: Assessing the Global Readiness of Regulatory and Non-regulatory Models for Assessing Occupational Exposure to Manufactured Nanomaterials Series on Testing and Assessment, No. 347 [PDF]. OECD, Paris. (Accessed 2021-12-13).

[OECD] Organisation for Economic Co-operation and Development. 2021d. Evaluation of tools and models for assessing occupational and consumer exposure to manufactured nanomaterials – Part III: Performance testing results of tools/models for consumer exposure. Project: Compilation of Available Tools and Models

Used for Assessing Consumer Exposure to Manufactured Nanomaterials and Evaluation of their Applicability in Exposure Assessments Series on the Safety of Manufactured Nanomaterials No. 101 Series on Testing and Assessment, No. 348 [PDF]. OECD, Paris. (Accessed 2021-12-13).

[OECD] Organisation for Economic Co-operation and Development. 2022. Section 1. Physical-chemical properties. Test No. 124: Determination of the volume specific surface area of manufactured nanomaterials [PDF]. OECD, Paris. (Accessed 2022-12-06].

[OECD] Organisation for Economic Co-operation and Development. 2023. Section 1. Physical-chemical properties. Test Guideline No. 125 nanomaterial particle size and size distribution of nanomaterials [PDF]. OECD, Paris. (Accessed 2022-12-06).

Okonski AI, MacDonald DB, Potter K, Bonnell M. 2021. Deriving predicted no-effect concentrations (PNECs) using a novel assessment factor method, Human and Ecological Risk Assessment: An International J.

Oomen AG, Bleeker EAJ, Bos PMJ, van Broekhuizen F, Gottardo S, Groenewold M, Hristozov D, Hund-Rinke K, Irfan MA, Marcomini A, Peijnenburg W JGM, Rasmussen K, Jiménez AS, Scott-Fordsmand JJ, van Tongeren M, Wiench K, Wohlleben W, Landsiedel R. 2015. Grouping and read-across approaches for risk assessment of nanomaterial. Int. J. Environmental Res. Public Health. 12: 13415-13434.

Oomen AG, Steinhäuser KG, Bleeker EAJ, van Broekhuizen F, Sip A, Dekkers S, Wijnhoven S, Sayre PG. 2018. Risk assessment frameworks for nanomaterials: Scope, link to regulations, applicability, and outline for future directions in view of needed increase in efficiency. NanoImpact. 9: 1-13.

Posthuma L, Suter II GW, Traas T (eds.). 2002. Species sensitivity distribution in ecotoxicology. CRC Press LLC, Lewis Publishers, Boca Raton, FL.

Rasmussen K, Rauscher H, Mech A, Sintes JR, Gilliland D, González M, Kearns P, Moss K, Visser M, Groenewold M, Bleeker EAJ. 2018. Physico-chemical properties of manufactured nanomaterials – characterisation and relevant methods. An outlook based on the OECD Testing Programme. Regul. Toxicol. Pharmacol. 92: 8-28.

Rasmussen K, Rauscher H, Kearns P, González M, Riego Sintes J. 2019. Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data. Regul. Toxicol. Pharmacol. 104:74-83.

[RCC] Regulatory Cooperation Council. No date (n.d.). Nanotechnology Initiative final report work element 2, Priority setting: Development of a joint nanomaterials classification scheme.

Sabella S, Carney RP, Brunetti V, Malvindi MA, Al-Juffali N, Vecchio G, Janes SM, Bakr OM, Cingolani R, Stellacci F, Pompa PP. 2014. A general mechanism for intracellular toxicity of metal-containing nanoparticles. Nanoscale. 6:7052-7061.

Simkó M, Nosske D, Kreyling WG. 2014. Metrics, dose, and dose concept: The need for a proper dose concept in the risk assessment of nanoparticles. Int. J. Environ. Res. Public Health. 11 (4): 4026-4048.

Tantra R, Oksel C, Puzyn T, Wang J, Robinson KN, Wang XZ, Ma CY, Wilkins T. 2015. Nano(Q)SAR: challenges, pitfalls and perspectives. Nanotoxicology. 9(5): 636-642.

Thio BJR, Zhou D, Keller AA. 2011. Influence of natural organic matter on the aggregation and deposition of titanium dioxide nanoparticles. J Hazard Mater. 189(1): 556–563.

Tran TK, Nguyen MK, Lin C, Hoang TD, Nguyen TC, Lone AM, Khedulkar AP, Gaballah MS, Singh J, Chung WJ, Nguyen DD. 2024. Review on fate, transport, toxicity and health risk of nanoparticles in natural ecosystems: Emerging challenges in the modern age and solutions toward a sustainable environment, Sci. Total Environ., Vol. 912.

Utembe W, Wepener V, Yu IJ, Gulumian M. 2018. An assessment of applicability of existing approaches to predicting the bioaccumulation of conventional substances in nanomaterials. Environ. Toxicol. Chem. 37: 2972-2988.
Verschoor AJ, Harper S, Delmaar CJE, Park MVDZ, Sips AJAM, Vijver MG, Peijnenburg WJGM. 2019. Systematic selection of a dose metric for metal-based nanoparticles. NanoImpact 13:70-75.

Yang XN, Cui FY. 2013. Stability of nano-sized titanium dioxide in an aqueous environment: Effects of pH, dissolved organic matter and divalent cations. Water Sci. Technol. 68(2): 276–282.