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Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms†

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Quantification of the uptake and elimination of nanomaterials (NMs) by organisms is key in assessing the environmental risks of NMs. For this, uptake models for conventional solutes may be used, although no consensus exists on their applicability for NMs. In this critical review therefore, conventional modelling approaches are scrutinised for their applicability for NMs. Statically derived accumulation factors, like BCF or BAF based on measured concentrations, are considered to be flawed because NMs are thermodynamically not stable, an important assumption for this approach. Dynamically derived accumulation factors, based on kinetic exposure experiments, may be applicable because no equilibrium between the organism and exposure medium is needed. Currently there is no full understanding of the passive uptake of NMs, which hampers assessment of the applicability of biotic ligand models. Passive uptake, however, is generally considered to be very limited, which would imply a limited applicability of BLMs for NMs. Physiologically based pharmacokinetic (PBPK) models, or biodynamic models, have successfully been applied in uptake studies with NMs. Their underlying assumptions can be met in experiments addressing NMs and case studies presented in this review demonstrate their applicability to model NM-form specific kinetics, integrated with environmental fate models, including relevant physiological processes. Their application requires the *a priori* definition of the major mechanisms driving the uptake kinetics and the quantification of the associated kinetic rate constants. This limits their application to those mechanisms for which the kinetic rate constants can actually be quantified. Within these limitations, PBPK models have been shown to be applicable and provide a promising general approach to improve modelling of NM-accumulation in organisms.

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Environmental significance

In this paper, we critically review approaches in modelling the uptake of nanomaterials by biota under environmentally relevant conditions. Existing modelling approaches, developed for the uptake of solutes by organisms, are evaluated within the context of the underlying assumptions and applicability to nanomaterials. Guidance for the selection of modelling approaches is provided, which will enhance the applicability of uptake modelling in environmental risk assessment of nanomaterials.

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Introduction

Environmental risk assessment requires information both on the level of exposure and the hazards that chemicals pose to organisms. The environmental risk assessment paradigm can be applied to nanomaterials (NMs), but the challenges include uncertainty about actual forms and environmental concentrations of NMs and exposure modelling, as well as the difficulty of determining which facets of the physico-chemical properties of NMs are the most important to the hazard.¹ To date, most of the research on environmental risks of NMs has focussed on the characterisation



and quantification of their hazards, using standard toxicity assays or slightly adapted procedures to cope with the special properties of NMs.^{2,3} Dose–response relationships may be derived from nominal exposure concentrations, although the use of measured concentrations is preferred in ecotoxicity testing with NMs. Generally, studies characterise NM-properties in the spiking medium^{4–7} to at least confirm that the expected dose was present, even if the precise form of the NMs in the exposure could have changed. Quantification of the actual form of a NM taken up by organisms is still in its infancy, and the biological matrices present many challenges to NM detection inside organisms. Most uptake or accumulation studies report either qualitative or semi-quantitative data to infer the presence of NMs in tissues, such as electron microscopy observations⁸ and synchrotron X-ray fluorescence analysis for fluorescent NMs,⁹ or use total concentrations, e.g. in the case of metal-based NMs.^{10–12} Only recently, some studies have appeared in which different forms of NMs were reported in tissues of organisms, providing insights into form-specific uptake of NMs.^{13–16} Methods to quantify form-specific concentrations of NMs such as single particle inductively coupled plasma mass spectroscopy (sp-ICP-MS) for metal-based NMs are becoming more available. These developments in analytical methods have enabled modelling approaches that could reveal patterns of form-specific uptake and accumulation of NMs in organisms, such as aspects of absorption, distribution, metabolism and excretion (ADME). This also provides the possibility to predict form-specific uptake of NMs in organisms under environmentally relevant conditions.^{17,18}

Several authors have applied modelling approaches that are commonly used in studies of conventional chemicals to model NM uptake in different terrestrial and aquatic organisms.^{19–22} The models used for conventional chemicals were developed specifically to address the uptake of solutes such as metals or lipophilic organic compounds and were not intended for NMs. The solute chemistry assumptions underlying those models may therefore not be met when applying them to NMs.²³ For instance, fugacity models, used to quantify the accumulation kinetics of non-polar organic compounds, assume equilibrium partitioning which is disputed for NMs.²⁴ Currently, however, there is no consensus on the pros and cons of different accumulation models and whether or not they are applicable to NMs, or on how to select the most appropriate model for specific cases of NMs.

In light of the demands from the perspective of the environmental risk assessment of NMs, this knowledge gap needs to be addressed, hence, in this review we critically review modelling approaches used for conventional chemicals, which may be applicable for describing the uptake and accumulation of NMs in soil and aquatic invertebrates. Invertebrates were selected because they comprise more than 99% of all animals; they are the most diverse group of organisms present on Earth and are integral to several ecological functions (e.g. soil structure and maintenance, nutrient cycling) which link to ecosystem services.^{25–27} The review

will be restricted to whole body accumulation in invertebrate species and not focus on tissue-specific uptake patterns and internal distribution. This is because the literature on form- and tissue-specific uptake of NM and their internal distribution in environmental organisms is currently too limited for a proper review. First, we will provide an overview of different routes of uptake of NMs and their fate in different invertebrate species. Based on this, different approaches used for modelling of the uptake of conventional chemicals will be discussed with respect to their application to NMs. We will address basic assumptions underlying the different modelling approaches that need to be met and will critically discuss the literature using such approaches for NM accumulation in selected species. Finally, we will provide an outlook for future research on modelling approaches and guidance on the applicability of existing modelling approaches to NMs.

Routes of uptake and elimination of NMs in invertebrates

Invertebrates can potentially accumulate NMs actively *via* ingestion and consecutive uptake across the epithelium in the body and to a lesser extent by anal uptake, or passively *via* uptake through body surfaces or body openings (Fig. 1). Feeding strategies of invertebrates vary considerably, but still they can be classified into different functional feeding groups, *i.e.* shredders (chew conditioned food e.g. litter, plants or wood), filter feeders (suspension feeding), gathering collectors (deposit feeders: they ingest sediment and/or soil), scrapers (graze food attached to surfaces), and predators (engulf prey, ingest body fluids).^{28–30}

Depending on the environmental fate of NMs, feeding groups may be differentially exposed to NMs. In aquatic environments, suspension feeders for instance will be exposed predominantly to waterborne NMs while deposit feeders will be exposed mainly to NMs following sedimentation, although the potential for waterborne exposure cannot be excluded. Shredders, scrapers and predators can also be exposed to both, depending on whether they are pelagic or benthic invertebrates. In terrestrial environments, deposit feeders and shredders are probably in first contact with NMs followed by predators, although in cases NMs are available to other soil invertebrates these may also directly be exposed. Aging of NMs in the environment, including transformation processes such as aggregation, sulfidation and dissolution into ions, influences the environmental fate of NMs, and as such their availability for the different groups of organisms. For instance, aggregation of NMs will increase sedimentation in aquatic systems, likely increasing the exposure of deposit feeders. There is some evidence that uptake of metals from NMs (e.g. ZnO) is through the dissolution of ions from the NMs to the pore water of soil which is then available for uptake by organisms.^{21,31} Laycock *et al.* (2016) found that the dermal uptake rates of ZnO NMs based on pore water were comparable to those based on oral and dermal uptake from soil, and it was concluded that the dominant uptake route of



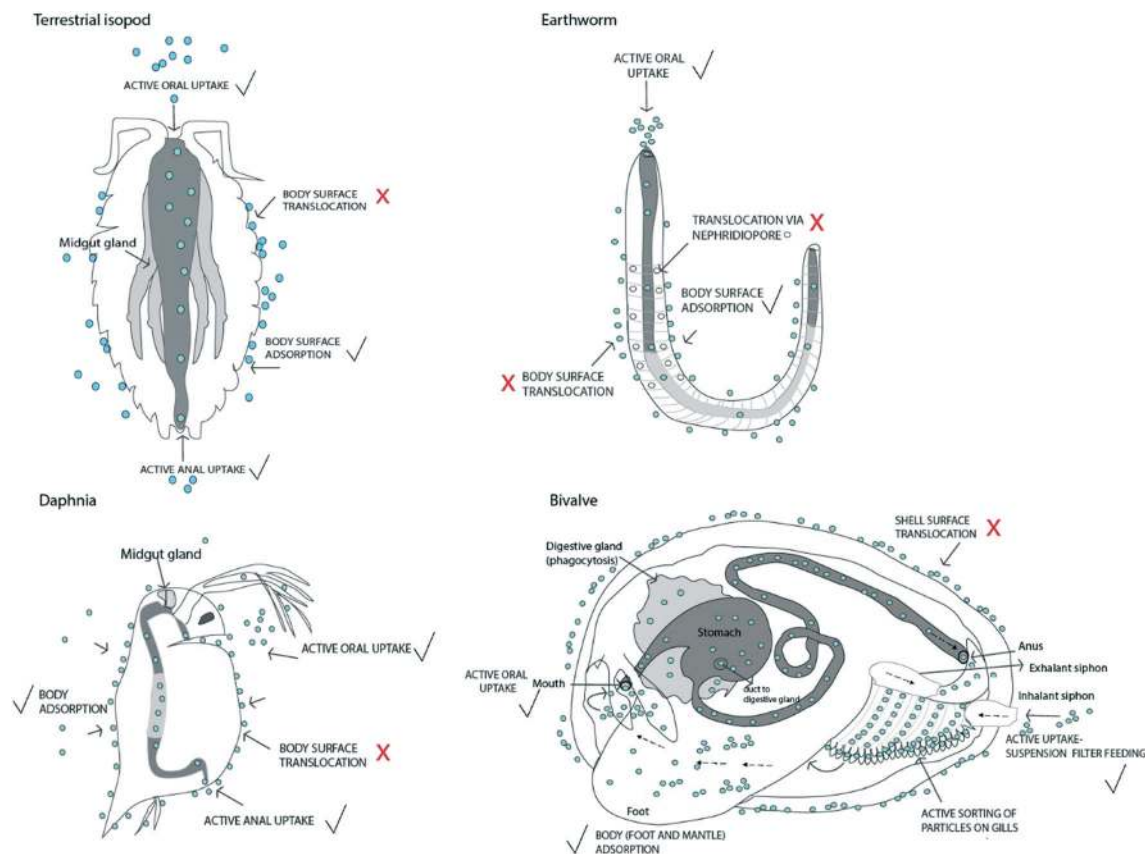


Fig. 1 Active and passive uptake of nanomaterials in different representative invertebrate species commonly used in environmental research: terrestrial isopod (*Crustacea*), earthworm (*Annelida*), water flea (*Crustacea*), and bivalve (*Mollusca*; e.g. *Mytilus* sp.; only one valve with a removed mantle is shown). The organisms are presented schematically, as extracted from Brusca *et al.* (2016)³⁰ and Ruppert *et al.* (2004).²⁵ The gut is shown as a grey tube. Light grey represents the midgut, while the foregut and hindgut regions are dark grey. The position and length of each gut region are not shown at realistic scales. Nanomaterials are shown as blue circles. Routes where NM passage is questionable are marked with a question mark and a red cross. Probable uptake routes are marked with a black tick symbol.

Zn was through the dermis from pore water exposure.³¹ Other transformations, such as sulfidation (e.g. Ag to Ag₂S) usually making NMs less reactive, have also started to be considered in bioavailability kinetic studies in order to reflect more environmentally realistic exposure scenarios.^{14,32} These studies have concluded that the uptake of Ag from Ag₂S NMs is slower compared to pristine Ag NMs which supports the assertion that uptake is largely driven by dissolution of ions from NMs.¹⁴ Uptake of ions from metal based NMs may be *via* the usual uptake pathways for solutes (*i.e.* facilitated uptake through ion channels or active transporters or even electroneutral diffusion,³³ or by endocytosis^{34,35}). However, particulate materials from NMs may also be accumulated, as was shown for coelomocytes from earthworm³⁶ and in snails.³⁴ This endocytosis was shown to be (partly) active, as was indicated by the application of inhibitors of different receptor mediated endocytosis pathways (including clathrin and caveolin mediated uptake). The corona of NMs may change during ageing, depending on the environment they are in, which may affect the interactions of NMs with organisms,³⁷ including the potential receptor mediated endocytosis of the NMs.

A second potential route of active NM uptake is through anal intake of water. This was shown for terrestrial isopods that have a water-conducting system on the dorsal outside part of their body surface that collects the excreted urine that is reabsorbed by the gut to regain the lost water.^{38–40} Anal uptake of water has also been demonstrated for some other crustaceans, such as *Daphnia magna*, commonly used in ecotoxicity testing³⁹ and is common in sea cucumbers.³⁰

Some NMs that are lipid soluble, such as pristine C60, have been demonstrated to move across biological membranes by diffusion, similar to some organic chemicals. However, this does not mean such NMs would also cross the integument of invertebrates by passive uptake processes. The integument is a considerable barrier, often with several anatomical layers and many biological ligands. The structure of such invertebrate integuments, however, varies considerably,³⁰ ranging from lacking a cuticle (*Platyhelminthes*) to being lined with different types of cuticles. Such a lining can be composed of sclerotized proteins without chitin (*Annelida*, *Polychaeta*, *Mollusca*), highly cross-linked collagens and specialised insoluble proteins (“cuticulins”), glycoproteins or lipids (*Nematoda*), or of multi-layered chitin (*Arthropoda*).^{25,30}



The cuticle enables the invasion of hostile environments by the organisms, such as dry terrestrial soils and digestive tracts of hosts, because it drastically reduces the permeability of the body wall, also for NMs. Passive translocation of NMs through any of the invertebrate integuments is therefore very unlikely if the barrier is intact. Paracellular diffusion of NMs in between epithelial cells is also unlikely for most species, either because the epithelium is electrically tight (*i.e.* high resistance, low permeability) or because the presence of bivalent ions (especially Ca^{2+} and Mg^{2+}) and the protein extracellular matrix in the paracellular space would promote rapid aggregation of NMs. However, in a few cases of soft-bodied invertebrates, dermal paracellular uptake of Ag-NMs has been suggested *e.g.* in earthworms.¹⁰ Regardless, in the case of a damaged integument or erosion of the external cuticle when present, NMs may be able to penetrate the tissues. Additionally, dermal uptake of solutes like metal ions resulting from metal NMs may be important in soft bodied species like for instance earthworms.⁴¹

NMs may also enter the body passively *via* openings. Besides the mouth and anus, involved in active uptake, probable body openings that could act as an entrance point for NMs are those related to excretion and gas exchange. Most of the invertebrates possess gills that enable gas exchange. For example, in bivalves, the water effluent runs *via* the inhalant siphon and the particles are directed to the mouth *via* active sorting on the gills (Fig. 1). In the case of terrestrial species, such entrance routes do not exist because gas exchange surfaces are internalised to prevent water loss and are connected to the external environment *via* openings that may have a special mechanism of closure. However, the respiratory system is regarded as very tight to solutes and much less permeable than the gut of animals. In some insects, such as collembolans, a potential entry point of NMs could be the ventral tube, which is the main site of water and salt exchange. This organ enables the absorption of water from surfaces under relatively dry conditions if no water is available for drinking.^{42,43}

Besides actual uptake (*i.e.* body internalisation), NMs may also be adsorbed onto the body surfaces of organisms, which has been shown for some invertebrate species, for example the brine shrimp *Artemia salina*⁴⁴ and the water flea *D. magna*.^{45,46} Whether the structure of the invertebrate body surfaces affects the extent of NM adsorption remains to be investigated. Although surface adsorbed NMs have not crossed epithelia tissues, and are thus not internalised by organisms, they should be considered in case of potential transfer of NMs to higher trophic levels.⁴⁷ This also accounts for NMs in the gut, which are not crossing the gut epithelium, as has been observed often *e.g.* for daphnid species.^{48,49}

Storage and elimination

Once taken up by organisms, NMs can be retained in the body or excreted.⁵⁰ The processes that a species may adopt will depend on the exposure route and concentration, as well

as the detoxification strategies available to the animal. The fate of NMs in the body will depend on the material NMs are made of and the transformations they may have undergone while ageing. For metal-containing NMs that dissolve, it is possible for the free metal ion to be taken up and then subsequently incorporated into a metal storage granule inside the organism. However, whether or not internalised particulate NMs can be added to those storage pathways or act as an initiating 'seed' to form a new storage granule is unclear. Several studies have reported intracellular compartmentalization as an important mechanism to minimise the toxicity of NMs in aquatic and terrestrial invertebrates. NMs and derived materials may accumulate in phagocytic macrophages, *e.g.* in digestive tissues (*i.e.*, gut epithelia, digestive glands) and are thus not distributed in the whole body.^{9,50–52} Numerous electron-dense granules and vacuoles have been observed in the TEM images of the midgut cells of the cutworm *Spodoptera litura* exposed to ionic zinc.⁵³ The number of dense granules and vacuoles and their size correlated with the accumulation of Zn in the midgut. In isopods, the digestive gland or hepatopancreas has been reported as the storage compartments for the bioaccumulation of Ag from Ag-NMs and AgNO_3 (ref. 9) or of Co from CoFe_2O_4 NMs.⁵⁴ Earthworms have the ability to store metals in the chloragogenous tissue, which contains phosphate, calcium and sulphur.⁵⁵ In a toxicokinetics study with silver exposure (as nanoparticles and as ions) of the earthworm *Lumbricus rubellus*, it was indicated that Ag-NMs may have a specific pathway for uptake, detoxification, and excretion *via* the gut wall, liver-like chloragogenous tissue, and nephridia.²¹

The rate of elimination of metals and NMs from organisms has been demonstrated to be predominantly influenced by the organism's physiology, while other parameters such as medium concentrations and characteristics of the NMs⁵⁶ or the exposure route^{9,21} are assumed not to have major impacts in this regard. Therefore, physiological pathways, such as carrier systems (including the ability to exocytose material by *e.g.* macrophages), may assist to depurate NMs. Several different processes may be involved in the elimination strategies of nanomaterials among aquatic and terrestrial invertebrates.⁵⁷ Excretion *via* faeces may help to discharge and detoxify metal-based nanomaterials by invertebrates, although it is likely that most of the NMs in faeces are transient materials not taken up by the organism. The ability of digestive cell vesicles to store metals and then release them either into the lumen of the alimentary canal or into the midgut gland is another process that chemical compounds can undergo to be finally discharged by faeces.⁵⁸ However, coprophagic organisms like terrestrial isopods may re-ingest these discharged materials through the uptake of faeces.

The kinetics of uptake and elimination of metal-based NMs, or derived metal ions, vary among organisms and determine their accumulation patterns.⁵⁹ Several processes of accumulation can be described for ionic forms of trace metals in aquatic invertebrates. For instance, the Zn body concentration is generally levelled by matching the Zn



excretion rate with the uptake rate. Barnacles, however, showed a different strategy, as they were able to store Zn in a detoxified form in granules, which is hardly eliminated because these granules do not have access to ducts leading to excretion. Some invertebrates detoxify Cd by binding to metallothionein.⁶⁰ Other organisms can excrete metals from the metabolic available pool, such as *P. elegans* which can excrete Cu,⁶¹ while other species may excrete metals from the detoxified store. For instance, the amphipod crustacean *Orchestia gammarellus* has the ability to store Cu taken up from food in Cu-rich detoxified granules of ventral caeca cells and release them into the gut lumen. It should be noted, however, that most of the mentioned studies are based on ionic forms of the metals involved. Little information is available on the exact kinetic processes of NMs in invertebrates, which points to an important gap in understanding these phenomena for NMs.

The uptake and elimination kinetics of metal NMs may also be form-dependent, meaning that the same organism can use different uptake and depuration pathways for *e.g.* NMs and ions.⁵⁷ For example, different uptake and elimination strategies have been established for Ag-NMs and ionic Ag in the estuarine polychaete *Nereis diversicolor*.⁸ Ag-NMs were mainly associated with inorganic granules, organelles, and heat denatured proteins, while ionic Ag was associated predominantly with metallothionein. *N. diversicolor* phagocytoses particles in the digestive system; therefore, the fate of particulate materials differs from that of the ionic form. Different accumulation patterns of Ag-NMs and ionic Ag in the estuarine snail *Peringia ulvae* have also been reported, mainly due to the lower Ag-NM uptake and different effluxes of Ag-NMs and ionic Ag.⁶² In that study, the elimination of Ag-NMs occurred in two phases: faster efflux of Ag-NMs followed by slower efflux of dissolved Ag. In the earthworm *L. rubellus*, however, no differences were detected in the fraction to which Ag was associated, the metal-rich granules being the most important for both ionic and particulate forms.¹³ In *Daphnia magna*, CuO-NMs were localized in the gut lumen, with no indication of being internalized in the cells, and the NMs were quickly eliminated from the body.⁶³ In another study, Au-NMs were also retained in the gut lumen of *D. magna* and also no internalization in cells was observed.⁶⁴ The authors used a two-compartment model to describe Au-NM elimination, since it was revealed to be bi-phasic, with a fast elimination rate in the first hour followed by a slower elimination rate. *D. magna* exposed to both Ag-NMs and AgNO₃ through different exposure routes (contamination through water only, food only and both water and food) showed a generally lower elimination rate for Ag-NMs compared to AgNO₃, indicating that Ag from Ag-NM exposure was possibly more difficult to depurate than Ag from AgNO₃.⁶⁵ Additionally, the inert fraction, not excreted from the organism, obtained through kinetics modelling showed that for Ag-NMs this fraction was higher than for ionic Ag.

Biom mineralization is the process by which organisms use minerals to support existing tissues, which can work as a se-

questration strategy for metals, for instance by incorporating them into exoskeletons and relatively inert shells.⁵⁷ Zn, Cd and Cu excretion during the larval development of the midge *Chironomus riparius* was associated with moulting and metamorphosis (exuviae).⁶⁶ There is, however, a need for more studies on the possibility of elimination of metals in NMs and materials from NMs through biomineralization and storage in shells or *via* moults of organisms, and on the potential recirculation of materials in organisms feeding on exuviae. Another strategy for excretion of materials was found in *Mytilus galloprovincialis* exposed to metal oxide NMs, showing the ability to repackage CeO₂-NMs in pseudo faeces and excrete them.⁶⁷

Modelling of uptake and elimination

Here we will review those modelling approaches most widely used to describe and analyse the uptake and accumulation patterns of conventional chemicals in organisms, *i.e.* biotic ligand models (BLMs), accumulation factors and physiologically based pharmacokinetic models (PBPK models) or biodynamic models. Their applicability for NMs will be scrutinised, after which some case studies will be presented which were successful in applying accumulation models to NMs.

Biotic ligand models

Biotic ligand models (BLMs) predict the bioavailability and toxicity for metal exposure *via* direct, passive uptake, assuming reversible, equilibrium binding between metal ions and receptor sites in the organism (the biotic ligands) (Di Toro *et al.*, 2001 (ref. 81)). Metal cations compete with other cations for binding to biotic ligands, while interactions with solution ligands (such as natural organic matter) result in complexation, decreasing the bioavailability of the metals. Several studies have applied BLM approaches to predict the accumulation patterns and effects of metals in the ionic form, including cadmium, nickel and copper in soil organisms and plants.^{68,69}

In assessing whether BLM-type approaches are suitable and/or necessary for modelling passive uptake of NMs, an understanding of why such an approach is needed for ionic metals is useful. The need for a BLM-type approach for ionic metals is derived from the facts that (i) uptake and toxicity are not simple functions of the total concentration of the metal to which the organism is exposed, but rather are functions of the activities of one or more specific metal species (usually the free metal ion), and (ii) uptake and toxicity are not functions of the potential free ion only, but also of competing ions in the exposure medium. Furthermore, the uptake mechanism must meet the mechanistic requirements of the BLM, *i.e.* rapid, reversible association with receptor sites on the organism as the first step in internalisation leading to toxic effects. Some studies^{22,70} have applied the Michaelis-Menten approach to quantify the uptake rates of NMs to organisms in short-term exposures. Such modelling derives the receptor concentration and binding affinity parameters and



could thus be argued to be a BLM-type approach. A more recent work concluded that BLM approaches only predict the acute toxicity of Ag in case the uptake was predominantly *via* the ionic dissolved form of Ag rather than the NM forms.²⁰ The potential applicability of the BLM is further confounded by the relative roles of dietary uptake *versus* direct uptake, as Khan *et al.*, 2014 (ref. 64) showed for *D. magna*. Overall, the results indicate that BLM approaches currently seem less applicable to model the uptake and toxicity of NMs compared to ionic metals, especially over longer periods of time. The BLM will remain of importance in modelling the uptake and toxicity of metals dissolving from NMs during exposure. More research is needed to understand the mechanisms by which NMs are passively taken up by invertebrates and the extent to which this uptake satisfies the requirements of BLM theory, before making more definitive statements on the applicability of the BLM for NMs. This includes the potential for differential uptake of different NM 'species' (*e.g.* with different extents of a particular coating type) and whether components of the exposure medium can compete with NMs for uptake.

Accumulation factors and equilibrium partitioning models

Accumulation factors are the simplest way of describing the uptake of chemicals in organisms. Accumulation factors are defined as ratios between concentrations in the organism and in the surrounding water in the case of aquatic organisms (bioconcentration factor; BCF), in the surrounding soil/sediment in the case of soil/sediment organisms (biota to soil/sediment accumulation factor; BSAF), or in the food (biomagnification factor; BMF). When calculating BCFs and BSAFs from measured concentrations in different matrices, it is assumed that the concentrations in the organisms and the surrounding media are in equilibrium with each other. In a study on the aquatic uptake of TiO₂ NMs in nematodes, it was shown that the ratio between titanium concentrations in organisms and water was clearly dependent on water concentrations.⁶ For the BCF/BSAF concept to be suitable for risk assessment, BCF/BSAF values should be independent of the exposure concentration. However, also for other metals, it has been shown that BCFs and BSAFs tended to be highest at lower exposure concentrations and decrease with increasing exposure level for different metal-salts,⁷¹ hence it was concluded that the BCF/BSAF concept may not always be applicable for metal uptake. Similarly, Praetorius *et al.* (2014) discussed the misconception of using BCFs based on measured concentrations of NMs in organisms and in water/soil, stating that due to the fact that NMs in the environment are in thermodynamically unstable forms they cannot be in equilibrium between two compartments.²⁴ Based on this, it was concluded that the equilibrium partitioning concept was not valid for NMs and that bioaccumulation factors (BCF or BSAF) for NMs cannot be derived from measured concentrations in organisms and other media. With respect to accumulation and BCFs/BSAFs, it needs to be assured that nanoparticles are actually internalised in the tissues of the

organisms. For BMFs, no equilibrium is assumed, since the flow of material is one way from the diet item to the consumer, and feeding may occur in discrete events. Nevertheless, a steady state is assumed, which will only be the case under static test conditions.

Accumulation factors (BCF/BSAF) can also be derived dynamically, based on kinetic experiments that aim at quantifying uptake and elimination rate constants (for details see later discussion on PBPK/biodynamic models). Different types of toxicokinetics models or biodynamic models⁷² can be developed, which incorporate specific processes with respect to accumulation, distribution, metabolism and excretion (ADME) of chemicals,⁷³ which may also be applicable to nanomaterials.⁷⁰ The simplest model only considers uptake and elimination in a one compartment model. According to this model, the uptake phase can be modelled by eqn (1) including both uptake and elimination when exposed.

$$C_{\text{org},t} = C_{\text{org},t=0} + C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(1 - e^{(-k_2 \times t)} \right) \quad (1)$$

$C_{\text{org},t}$: concentration in the organism at time t (mg kg⁻¹);
 $C_{\text{org},t=0}$: concentration in the organism at $t = 0$ (mg kg⁻¹);
 C_{exposure} : concentration in the exposure medium (mg kg⁻¹ or mg L⁻¹);
 k_1 : uptake rate constant (kg_{medium} kg_{organism}⁻¹ per day or L_{medium} kg_{organism}⁻¹ per day);
 k_2 : elimination rate constant (day⁻¹);
 t : time (day).

This model assumes a constant exposure concentration. A steady state under stable conditions will be reached after a certain, usually fairly long period of time, resulting in a BCF/BSAF that equals k_1/k_2 (at longer time periods the parameter $e^{(-k_2 \times t)}$ will reach 0). In this way the BCF/BSAF can be derived dynamically.²⁴ Such dynamic assessment of accumulation factors does not require the establishment of a steady state between compartments (k_1 and k_2 can be derived before the steady state has been reached), and may provide insight into the potential of NMs to be taken up by organisms. However, dynamically derived accumulation factors can vary quite a lot between studies, although this variation is not necessarily related to exposure concentrations.⁶

Based on this discussion and underlying references it can be concluded that accumulation factors should be avoided when based on ratios between organisms and media, due to the fact that equilibrium partitioning theory does not apply to chemicals that are not thermodynamically stable, such as nanomaterials. Furthermore, they should be used with care when derived dynamically in time resolved experiments, because ADME processes related to the fate of nanomaterials in organisms may not always be resolved completely. Nanomaterials can also occur in different forms which may interact and as such affect each other's accumulation patterns. This may also hamper the application of simple accumulation or concentrations factors.



Physiologically based pharmacokinetic (PBPK) or biodynamic models

Accumulation of chemicals in organisms, including nanomaterials, depends on their availability in the exposure medium and on the physiological traits of the species involved, driving accumulation, distribution, metabolism and excretion (ADME). Physiologically based pharmacokinetic (PBPK) or biodynamic models can be used to model these processes dynamically.^{72,73} Different approaches can be applied, depending on routes of uptake and the ADME processes that need to be included (Fig. 2).

The simplest approach considers the organism as a single compartment, with just uptake and elimination as kinetic processes (only include processes 1_a and 1_b combined for uptake; 3_a, 3_b and 3_c combined for elimination, Fig. 2). Uptake under these assumptions is described by eqn (1). When the test organisms are transferred to a clean medium, they will eliminate the NMs. The process of elimination can be described by eqn (2):

$$C_{\text{org}t} = C_{\text{org}t=0} + C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(e^{(-k_2 \times (t-t_e))} - e^{(-k_2 \times t)} \right) \quad (2)$$

$$C_{\text{org}t} = C_{\text{org}t=0} + \text{SF} \times C_{\text{exposure}} \times k_1 \times t + (1 - \text{SF}) \times C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(1 - e^{(-k_2 \times t)} \right) \quad (3a)$$

$$C_{\text{org}t} = C_{\text{org}t=0} + \text{SF} \times C_{\text{exposure}} \times k_1 \times t_e + (1 - \text{SF}) \times C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(1 - e^{(-k_2 \times t_e)} \right) \times e^{-k_2 \times (t-t_e)} \quad (3b)$$

$C_{\text{org}t}$: concentration in the organism at time t (mg kg^{-1}); $C_{\text{org}t=0}$: concentration in the organism at $t = 0$ (mg kg^{-1}); C_{exposure} : concentration in the exposure medium (mg kg^{-1} or mg L^{-1}); k_1 : uptake rate constant ($\text{kg}_{\text{medium}} \text{kg}_{\text{organism}}^{-1}$ per day or $\text{L}_{\text{medium}} \text{kg}_{\text{organism}}^{-1}$ per day), k_2 : elimination rate constant (day^{-1}); t : time (day); t_e is the time at which the test organisms are transferred from a contaminated to a clean medium (day).

In the case of metals, such a simple one-compartment model may not be fully adequate, as storage in a stored fraction may occur in specialized tissues of organisms (process 2C, Fig. 2)⁷³ as was shown in earthworms in which metals were sequestered in stored forms that were not biologically active.⁷⁴ Such storage may also be applicable to nanomaterials or the metal ions released from NMs, which was illustrated in studies on the uptake of Ag-NMs in *D. magna*⁶⁵ and in isopods.⁹ In such cases, the model may be extended with a stored fraction (SF no dimension). In other studies an inert fraction has been used in order to account for the fraction stored in organisms, however, that approach only accounted for storage in the elimination phase of their experiments but was not included in the first experimental phase when organisms were exposed.^{9,65,75} To overcome this, the extended model with a SF can be defined using eqn (3a) and (3b):

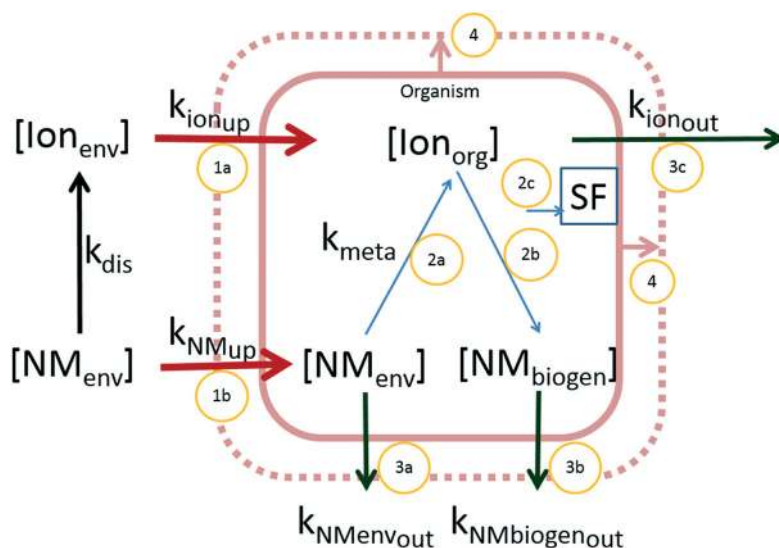


Fig. 2 Conceptual overview of different forms of metal-based NMs and kinetic pathways of uptake and elimination. Forms of materials: NM_{env} : nanomaterial in the original form (can be both in the environment and in the organism), Ion_{env} : ionic form of the NM-material in the environmental compartment (dissolved), Ion_{org} : ionic form in the organism (may originate from both Ion_{env} as well as dissolved from NM_{env} in the organism), $\text{NM}_{\text{biogen}}$: biogenic particulate form, F_i : inert fraction; kinetic processes displayed by arrows. Red arrows: uptake. 1_a: ionic uptake, 1_b: particulate uptake; blue arrows: within organism kinetics. 2_a: dissolution of NMs, 2_b: biogenic formation of particulate materials, 2_c: transport to the inert fraction; dark green arrows: elimination: 3_a elimination of original NM, 3_b: elimination of the biogenic particulate material, 3_c: elimination of the ionic form. Pink arrows: growth dilution (4).



where SF is the stored fraction, having a value between 0 and 1 (for other parameters and units see eqn (1) and (2)).

When applying the modelling with a SF to the work of Ribeiro *et al.* 2017 (ref. 65) using the total Ag-concentrations in *Daphnia magna* after exposure to Ag-NMs¹⁴ based on eqn (3a) and (3b), and comparing this with the outcomes using the models without a SF (eqn (1) and (2)), both approaches were highly significant ($P < 0.001$), but the percentage explained var-

where $C_{\text{org-1}}$ and $C_{\text{org-2}}$ represent the concentrations in the two different compartments within the organism. $C_{\text{org-1}}$ is the compartment with the loosely bound material, $C_{\text{org-2}}$ is the storage compartment, and k_i is the rate constant for the transfer from $C_{\text{org-1}}$ to $C_{\text{org-2}}$. For elimination, the following equations apply:

$$C_{\text{org-1}'} = C_{\text{exposure}} \times \left(\frac{k_1}{(k_2 + k_i)} \right) \times \left(1 - e^{-(k_2 + k_i) \times t} \right) \times e^{-(k_2 + k_i) \times (t - t_c)} \quad (5a)$$

$$C_{\text{org-2}'} = C_{\text{exposure}} \times \left(\frac{k_1}{(k_2 + k_i)} \right) \times \left(\frac{k_1}{(k_2 + k_i)} \right) \times \left(1 - e^{-(k_2 + k_i) \times t} \right) \times \left(1 - e^{-(k_2 + k_i) \times (t - t_c)} \right) + C_{\text{exposure}} \times k_i \times \left(\frac{k_1}{(k_2 + k_i)^2} \right) \times \left((k_2 + k_i) \times t + e^{-(k_2 + k_i) \times t} - 1 \right) \quad (5b)$$

iance increased from 61 to 71% of the total variance with the inclusion of the SF. A SF of 0.099 ± 0.033 was derived (average \pm standard error). The k_1 value increased from 0.190 ± 0.025 to 0.363 ± 0.121 , while the k_2 value increased from 0.031 ± 0.007 to 0.124 ± 0.059 . The SF of 0.099 indicates that 9.9% of all Ag that was internalised by the daphnias during exposure (equals $k_1 \times \text{Conc}_{\text{Ag-medium}} \times \text{day}$) is in the stored fraction. This implies that the percentage stored in the organism is actually higher than, in this case, 9.9% of the accumulated Ag (internalised Ag minus excreted Ag), while this percentage increases over time. This example illustrates that inclusion of potentially relevant storage may affect the quantification of the other rate constants considerably which implies that selection of ADME processes in the modelling is extremely important, and even more that comparison of kinetic parameters between studies can only be done with the total model in mind, including all other kinetic rate constants. This approach to model the stored fraction assumes that there is no limit to the storage capacity of the organisms, be it in *e.g.* metallothioneins for metal-NMs or fat deposits for organic NMs, and secondly that the stored fraction is not eliminated over time. When including such an stored fraction (SF), the ratio between k_1 and k_2 no longer yields a correct estimate of accumulation factors (BCF/BSAF). The assumption that the stored fraction is not eliminated over time may be difficult to meet from a physiological point of view. An alternative to meet this is to assume the organism to be composed of two compartments, one that does eliminate the compound fast and one that accumulates the compound but eliminates it (very) slowly. By doing so, the model describing the uptake rate of the compound separately describes uptake in the two different compartments and includes a rate constant for the transfer of the compound from the first to the second compartment. The models describing the uptake phase are shown in eqn (4a) and (4b) (taken from ref. 73).

$$C_{\text{org-1}'} = C_{\text{org-1}^{t=0}} + C_{\text{exposure}} \times \left(\frac{k_1}{(k_2 + k_i)} \right) \times \left(1 - e^{-(k_2 + k_i) \times t} \right) \quad (4a)$$

$$C_{\text{org-2}'} = C_{\text{org-2}^{t=0}} + C_{\text{exposure}} \times k_i \times \left(\frac{k_1}{(k_2 + k_i)^2} \right) \times \left((k_2 + k_i) \times t + e^{-(k_2 + k_i) \times t} - 1 \right) \quad (4b)$$

Quantification of the rate constants k_1 , k_2 , and k_i for a two compartment model requires measurements of the NM in the storage tissue, which for invertebrates may be difficult and sometimes even impossible because the material may be stored throughout the body. In such a case, application of the model with the stored fraction may be more feasible.

Depending on the complexity of the toxicokinetics of the nanomaterials in the organisms, different model formulations can be integrated in the models, including different excretion pathways with different elimination rate constants, growth dilutions, changes over time of the bioavailable fraction of the chemical in the exposure medium or inclusion of storage in a stored fraction using two compartment models.⁷³ Several studies have used PBPK or biodynamic models to quantify the bioaccumulation of different nanomaterials, with model formulations.^{5,9,21,22,76} Studies on metal-based nanomaterials are generally based on the total metal content and do not take the different forms of the materials into account, although recent studies have focussed on this.¹⁴ A modelling example on the uptake of different forms of Ag in earthworms illustrated the importance of form-specific approaches, and uptake levels and forms of Ag (as particulate or ionic uptake) were found to depend on *i.e.* the rate of dissolution of the silver nanoparticles and ADME processes.¹⁷ These processes thus need to be included in the modelling, using available model formulations and equations.⁷³

Due to transformation and ageing of nanomaterials, stable exposure concentrations as assumed by the simple one-compartment model will hardly be the case. This is best illustrated by a metal-based NM, like Ag NMs. Such NMs are prone to release Ag^+ ions into the environment, so organisms exposed to a medium spiked with Ag NMs in fact will be exposed to a mixture of NMs and free Ag ions (although Ag^+ may also complex with Cl^- or S^{2-}), which might even change in composition over time. In the most simple case of a mixture of Ag NMs and Ag^+ ions, the Ag uptake rate in the test organisms might be modelled with a model that includes two



uptake rate constants to account for the contribution of both Ag forms. The model could take the form

$$C_{\text{org}'} = C_{\text{org}'=0} + \left(\frac{k_{11} \times C_{\text{exp}} \text{Ag-NM} + k_{12} \times C_{\text{exp}} \text{Ag}_{\text{ion}}}{k_2} \right) \times (1 - e^{-(k_2 \times t)}) \quad (6)$$

where k_{11} is the uptake rate of Ag from the Ag-NMs and $C_{\text{exp}} \text{Ag-NM}$ is the Ag-NM concentration in the exposure medium; k_{12} is the uptake rate of Ag from the ionic Ag^+ and $C_{\text{exp}} \text{Ag}_{\text{ion}}$ is the concentration of Ag^+ ions in the exposure medium. Elimination in this situation can be modelled according to eqn (2) or using eqn (3b) in the case of a stored fraction. Also, in this case, it will be hard to derive kinetics-based bioaccumulation factors, as they are dependent on the contribution of each chemical form to the total uptake. However, at stable concentrations the bioaccumulation factor can be calculated as in eqn (7) in which the different uptake rate constants are included according to the relative concentrations of the different forms they apply to:

$$\text{BCF of BSAF} = \left(\frac{k_{11} \times C_{\text{exp}} \text{Ag-NM} / (C_{\text{exp}} \text{Ag-NM} + C_{\text{exp}} \text{Ag}_{\text{ion}}) + k_{12} \times C_{\text{exp}} \text{Ag}_{\text{ion}} / (C_{\text{exp}} \text{Ag-NM} + C_{\text{exp}} \text{Ag}_{\text{ion}})}{k_2} \right) \quad (7)$$

Excretion rates may also be dependent on the form in which the material is present in the organism. In such a case, a different k_2 value may be applied, a fast and a slow elimination rate constant ($k_{2\text{fast}}$, $k_{2\text{slow}}$). The fast elimination stops after the fast eliminating pool is depleted ($t_{\text{end}k_{2\text{fast}}}$). The uptake can be modelled according to eqn (8):

$$C_{\text{org}'} = C_{\text{org}'=0} + C_{\text{exp}} \times \left(\frac{k_1}{(k_{2\text{fast}} + k_{2\text{slow}})} \right) \times (1 - e^{-(k_{2\text{fast}} + k_{2\text{slow}}) \times t}) \quad (8)$$

In combination with eqn (6), different uptake rates can also be included for the different forms. Elimination in the phase that fast elimination still takes place ($t < t_{\text{end}k_{2\text{fast}}}$) can be modelled as in eqn (9a), and after this, according to eqn (9b). When fitting the model to the data, $t_{\text{end}k_{2\text{fast}}}$ can be assessed iteratively together with k_1 , $k_{2\text{fast}}$ and $k_{2\text{slow}}$.

$$C_{\text{org}'} = C_{\text{org}'=0} + C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(e^{-(k_{2\text{fast}} + k_{2\text{slow}}) \times (t - t_c)} - e^{-(k_{2\text{fast}} + k_{2\text{slow}}) \times t} \right) \quad t < t_{\text{end}k_{2\text{fast}}} \quad (9a)$$

$$C_{\text{org}'} = C_{\text{org}'=0} + C_{\text{exposure}} \times \left(\frac{k_1}{k_2} \right) \times \left(e^{-(k_{2\text{fast}} \times (t_{\text{end}k_{2\text{fast}}} - t_c) + k_{2\text{slow}} \times (t - t_c))} - e^{-(k_{2\text{fast}} + k_{2\text{slow}}) \times t} \right) \quad t \geq t_{\text{end}k_{2\text{fast}}} \quad (9b)$$

In addition to the above described models, other processes may need to be covered. It is possible to estimate uptake and elimination rate constants accounting for a steady

decline of exposure concentrations. This is done by adding k_{deg} (day^{-1}) to eqn (1) to yield eqn (10).⁷³

$$C_{\text{org}'} = C_{\text{org}'=0} + C_{\text{exposure}} \times \left(\frac{k_1}{k_2 - k_{\text{deg}}} \right) \times \left(e^{-(k_{\text{deg}} \times t)} - e^{-(k_2 \times t)} \right) \quad (10)$$

Such models may also be rewritten to account for a steady change in exposure concentration, as may be the case when only the free ions released from a metal-based NM would be taken up. The rate at which the metal ions are released may be included in the equations for uptake and elimination kinetics. For carbon fullerenes, such an approach was followed to model the uptake kinetics with inclusion of declining exposure concentrations due to settling of the fullerenes during the experiment.⁴⁸ This was done by including the linear regression slope of the natural logarithm of the fullerene concentration in the aqueous phase *versus* exposure time in the model formulations.

Another factor that may need to be accounted for is the biogenic formation of NMs inside the exposed organisms, a

process which has been suggested for Ag to occur in earthworms as part of the detoxification of metals in insoluble granules.¹⁴ In such a case, the model may take the form of a two-compartment model (eqn (4a) and (4b)) and k_i could be considered the rate at which NMs are formed from ionic metals taken up by the organism.

A final case to mention is when exposed organisms show a considerable change in biomass during exposure to the nanomaterials. A significant increase of biomass may affect the uptake kinetics as it may lead to so-called growth dilution, while in the case of considerable mass loss the compound may become more concentrated in the biological tissues (process 4 in Fig. 2). To account for these situations, a growth rate (k_g) has to be calculated, which then can be included in the equations for uptake and elimination by simply replacing k_2 in eqn (1) and (2) with $k_2 + k_g$.⁷³

In the following cases the applicability of PBPK or biodynamic models will be illustrated, based on existing datasets and references.



Case study i: Ag uptake from enchytraeids exposed to Ag₂S NMs with and without correction for mass loss.

In this case, the effect of biomass changes in the organisms on the prediction of rate constants will be illustrated. Adult age-synchronized *Enchytraeus crypticus* was exposed for 14 days to Lufa 2.2 soil spiked with 20 nm Ag₂S NMs at 2.5 mg Ag per kg dry soil (for details, see ESI†). At different time intervals, animals were sampled for the determination of Ag uptake kinetics. After 14 days, the remaining animals were transferred to clean soil to assess Ag elimination for 14 days. Three replicate samples were taken at each sampling time, and animals were allowed to void their guts after, freeze dried, weighed, acid digested and analysed for Ag. Fig. 3 (left) shows the Ag concentrations in the animals and the fit of the one-compartment model (eqn (1) and (2)) to the data. This resulted in $k_1 = 0.057 \text{ g}_{\text{soil}} \text{ g}_{\text{animal}}^{-1}$ per day and $k_2 = 0.370 \text{ day}^{-1}$. Upon analysis, it turned out that the animals gradually lost weight during the experiment. Fig. 3 (right) shows the mass over time of the animals, from which a negative k_{growth} value of -0.032 day^{-1} was derived. Correcting the uptake and elimination kinetics for weight change was done by replacing k_2 in eqn (1) and (2) with ' $k_2 + k_{\text{growth}}$ '. This did not affect the fit of the curve to the data and did not affect k_1 , but the k_2 value increased from 0.370 to 0.402 day^{-1} , so did the value of k_{growth} . This case clearly indicates the potential to over- or underestimate kinetic rate constants when not including all relevant processes that may drive the internal concentrations of NMs.

Case study ii: modelling approaches with different PBPK model definitions

For the different model formulations as described in eqn (1) to (9b), specific kinetic rate constant parameters are needed. To assess potential effects of different model definitions and kinetic parameters on the analysis and interpretation of experimental results, data on the uptake of different forms of Ag in earthworms¹⁴ were analysed with different model formulations, including scenarios with either separate uptake rates for different forms of NMs (e.g. ionic and particulate uptake of Ag) or two elimination rates (fast and slow elimination). In Baccaro *et al.* (2018), different expo-

sure experiments were conducted in which earthworms were exposed to AgNO₃, pristine Ag-NMs (which showed dissolution) and Ag₂S-NMs.¹⁴ The experimental data shown in Fig. 4, and used in this example, were taken from the pristine Ag-NM exposure experiment. When a single compartment was fitted with a single k_1 and k_2 (eqn (1) and (2)) the model described the data with a significant fit, according to Baccaro *et al.* (2018)¹⁴ (Fig. 4: 'Predict' $p < 0.01$; r^2 : 0.75; for k_1 and k_2 see Table 1). The fit (as r^2) improved when an additional excretion rate was included in the model (eqn (8), (9a) and (9b)), although the significance of the regression decreased due to the lower degrees of freedom (Fig. 4 'Predict separate k_2 '; $p < 0.05$; r^2 : 0.85; for k_1 , $k_{2\text{slow}}$ and $k_{2\text{fast}}$ see Table 1). The uptake rate constant was slightly greater when a second elimination rate constant was included, while the fast elimination rate constant was slightly greater than the original one and the slow elimination rate constant was smaller. Directly after the transfer of the worms to clean soil, the Ag-concentrations decreased rather fast (driven by both fast and slow elimination routes⁷⁷), however, after a longer time the elimination rate decreased. The modelled fast elimination only occurred up to 7 days after placing the worms in clean soil, after which only the slow elimination remained (End $_{k_{2\text{fast}}} = 7$ days, Table 1).

These models were fitted to the uptake data of Ag from the pristine particle experiment. The accumulation patterns in this experiment in which the earthworms were exposed to two forms of Ag (Ag-NMs and dissolved Ag⁺), however, may also be predicted by using parameters from the other two experiments specifically on Ag⁺ and Ag-NM exposure, in Baccaro *et al.* (2018),¹⁴ by using a model with two uptake rate constants derived from those two experiments (eqn (5a), (5b), (6a) and (6b)). The uptake rate constant for the ionic uptake ($k_{1\text{ion}}$) could be derived from the experiment with Ag-NO₃,¹⁴ while $k_{1\text{NM}}$ was based on the experiment with Ag₂S-NMs in that study (for parameters see Table 1). When applying $k_{1\text{ion}}$, $k_{1\text{NM}}$, an estimate of the dissolution rate of the pristine Ag-NMs had to be made, the source of ionic Ag in the soil, using eqn (11).

$$C_{\text{NM}_t} = C_{\text{NM}_{t=0}} \times \exp(-k_{\text{dis}} \times t) \quad (11)$$

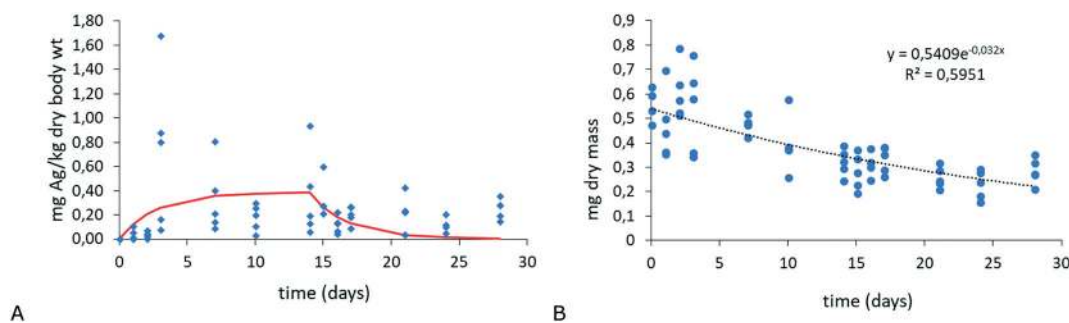


Fig. 3 A: Ag uptake and elimination in *E. crypticus* exposed to Ag₂S-NMs (20 nm, 2.5 mg Ag per kg dry soil) (x-axis: days; y-axis: mg kg⁻¹). B: Mass over time of animals during the uptake and elimination kinetics test (x-axis: days; y-axis: mg dry mass).



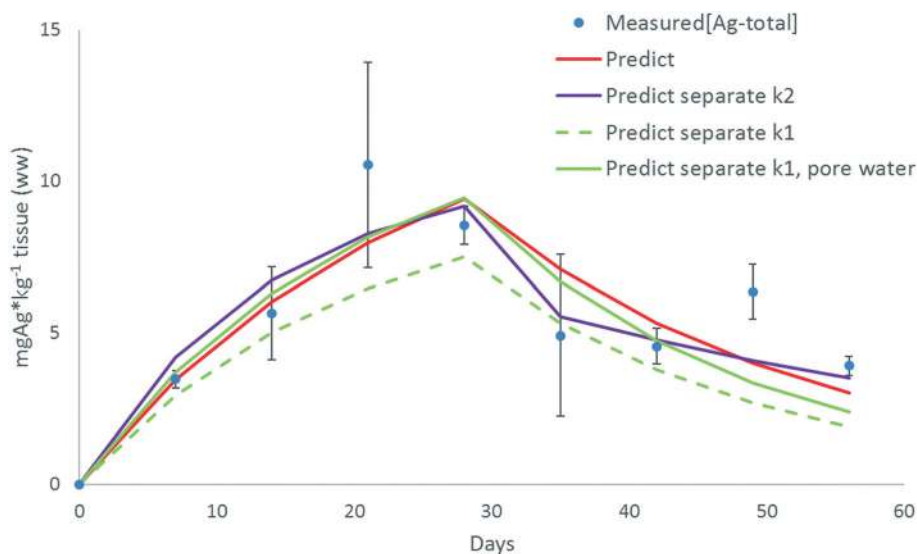


Fig. 4 Modelling of $[Ag_{total}]$ in earthworms exposed to pristine Ag-NMs (20 nm , 10 mg kg^{-1}); measured $_{[Ag-total]}$: empirical data Baccaro *et al.* (2018)¹⁴ (average and stdev); for equations and parameters for the different scenarios see Table 1. Day 0–28: earthworms in spiked soil, days 28–56: earthworms transferred to clean soil.

Table 1 Parameters used in and statistical output from the models describing the accumulation patterns of Ag in earthworms exposed to Ag-NMs, as shown in Fig. 4

Model	k_1	k_{1ion}	k_{1NM}	k_2	k_{2fast}	k_{2slow}	k_{dis}	End $_{k_{2fast}}$	Equations	Significance (p -value)	R^2	Parameters
Scenario, Fig. 4	kg kg^{-1} per day	kg kg^{-1} per day	kg kg^{-1} per day	Day^{-1}	Day^{-1}	Day^{-1}	Day^{-1}	Days	(Uptake phase, elimination phase)			
Predict	0.063	—	—	0.041	—	—	—	—	(1) and (2)	<0.001	0.75	k_1, k_2 fitted to data
Predict separate k_1	—	0.055	0.008 (ref. 14)	0.054	—	—	0.82	—	(6) and (2)	<0.001	0.74	$k_{1ion}^{14}, k_{1NM}^{14}, k_{dis}$ (ref. 17)
Predict separate k_1 , pore water	—	16.8 (L kg^{-1} per day)	0.008	0.044	—	—	—	—	(6) and (2)	<0.001	0.74	$k_{1ion}^{14}, k_{1NM}^{14}$, recalculated pore water concentrations ¹⁴
Predict separate k_2	0.085	—	—	—	0.055	0.018	—	7	(8), (9a) and (9b)	<0.001	0.83	$k_1, k_{2fast}, k_{2slow}, \text{End}_{k_1}$ fitted to data

k_{dis} was set at 0.82 day^{-1} .⁷⁸ The modelling with these parameters resulted in somewhat lower concentrations in the earthworms (Fig. 4, 'Predict separate k_1 ', $p = 0.15$; r^2 : 0.74; Table 1). The relatively high r^2 value of 0.74 indicates that the modelled uptake and elimination patterns did follow the patterns of the empirical data, which would indicate that the ionic uptake was mainly driving the overall uptake in both original experiments on Ag-salts and pristine Ag-NMs in Baccaro *et al.* (2018).¹⁴ Soil pore water concentrations could be indicative of the bioavailable fraction of the ionic Ag in the experiments, which were comparable between the original experiments ($40.5\text{ }\mu\text{g L}^{-1}$ for Ag-NM exposure *versus* $37.9\text{ }\mu\text{g L}^{-1}$ for the Ag- NO_3 exposure). When using the pore water concentrations as proxy for exposure levels for deriving k_{1ion} ($37.9\text{ }\mu\text{g L}^{-1}$), it results in a high k_{1ion} value of 16.8 (Table 1). When applying this k_{1ion} to predict Ag_{total} in the earthworms based

on pore water concentrations in the pristine Ag-NM experiment ($40.5\text{ }\mu\text{g L}^{-1}$), the resulting modelling fitted the data significantly (Fig. 4, 'Predict separate k_1 , pore water' $p < 0.05$; r^2 : 0.74). The fitted curve is close to the single k_1 modelling exercise.

The modelling approaches in this case, using the different uptake rate constants, indicate the possibility of modelling the uptake of different forms of metal-based NMs and released ionic forms by organisms. In dynamic settings, rate constants derived in single-form experiments (*i.e.* ionic exposure or exposure to non-soluble NMs) may be used to predict accumulation patterns in experiments in which different forms may be included (in the case of soluble NMs). However, for such approaches, the fate of the NMs in the soil needs to be included in the modelling as well as their availability. This will be illustrated in greater depth in the following case.



Case study iii: modelling uptake of Ag by earthworms, including dissolution of Ag-NMs and adsorption/desorption onto the soil

Uptake experiments with NMs are mostly performed based on total concentrations, not taking into account the form of the material that is present. In the case of carbon based NMs the form is likely either fullerenes, carbon nanotubes or graphene, unless the NM is degraded by exogenous enzymes from the organisms. For metal-based NMs, both particulate and ionic metal forms may be taken up by the organisms, as was illustrated in Case study ii. Hence, when using NM concentrations to predict the uptake, the dissolution of the particles and the adsorption/desorption of ions onto soil particles needs to be taken into account. This can be done with the conceptual model, as depicted in Fig. 5. Based on this model, the uptake of Ag from different sources (ionic, dissolving NMs; stable NMs) by earthworms was modelled in three experimental scenarios using data from Baccaro *et al.* (2018):¹⁴ i) exposure of earthworms to AgNO₃ ([Ag]_{tot}: 9.3 mg kg⁻¹, assuming all dissolved at the start); ii) exposure of earthworms to pristine Ag-NMs ([Ag]_{tot}: 9.0 mg kg⁻¹); iii) exposure of earthworms to (non-dissolving) Ag₂S-NMs ([Ag]_{tot}: 3.7 mg kg⁻¹), all measured as total [Ag] in the samples. Based on this experimental data, the hypothesis that the Ag-uptake of earthworms is mainly *via* the ionic form can be explored.

Model description: In the model, Ag-NM concentrations decrease over time, due to dissolution, according to eqn (11). k_{dis} differs among the types of NMs. For pristine Ag-NMs this was 0.82 day⁻¹,⁷⁸ while for sulphidised Ag-NMs (Ag₂S-NMs), a much lower dissolution rate of 4.6×10^{-4} day⁻¹ was derived from ref. 79 based on data for particles with a S/Ag ratio of 0.0192 (see ESI[†]). The S/Ag ratio of the sulphidised Ag-NMs used in Baccaro *et al.* (2018)¹⁴ was around 0.5 at fully sulphidised sites. However, the NMs were not fully sulphidised with sites with much lower S/Ag ratios, as low as 0.015 (unpublished data), so close to 0.0192 from ref. 79. Dissolved Ag may adsorb onto soil particles, diminishing its bioavailability since it is assumed that the biouptake of Ag ions is only from free Ag⁺ ions in the pore waters. The adsorption kinetics are described by the adsorption and desorp-

tion rate constants (k_{ads} , k_{des} (day⁻¹)) which were derived based on eqn (12) and (13):

$$k_{ads} = k_d \times k_{des} \quad (12)$$

$$k_d = k_f \times \text{ratio}_{\text{watertosoil}} \quad (13)$$

where k_{ads} as the adsorption rate constant (day⁻¹), k_{des} is the desorption rate constant (day⁻¹), k_f is the Freundlich distribution constant (L kg⁻¹) and a $\text{ratio}_{\text{watertosoil}}$ is needed to correct from pore water to soil (kg L⁻¹, $\text{ratio}_{\text{watertosoil}} = 10$). k_{ads} , k_f and $\text{ratio}_{\text{watertosoil}}$ were taken from ref. 80 using the soil type that reflected the properties of the soil used by ref. 14 as much as possible (Olivier soil: derived k_{ads} : 0.0288 day⁻¹; derived k_{des} : 0.000218 day⁻¹).

Using the models on dissolution and adsorption/desorption, and the inputs from ref. 14, the soil concentrations of the different forms (particulate, dissolved Ag adsorbed to soil particles and non-adsorbed ionic Ag) were predicted (Fig. 6). Concentrations in the earthworms could be fitted to the modelled variable dissolved Ag-concentrations in the soil, quantifying k_1 and k_2 based on (modelled) dissolved Ag-concentrations and not on total soil concentrations as was done by Baccaro *et al.* (2018).¹⁴ The expectation was that the variation of the k_1 and k_2 would be relatively small among the different scenarios, because the most important form of Ag driving the uptake (dissolved ionic Ag) was used as a base for the modelling.

Fig. 6 shows the distribution of the different forms of Ag in the soil while Fig. 7 shows the total Ag concentrations in the earthworms (modelled and measured) for the three scenarios. In the ionic exposure scenario, the initial dissolved Ag-ion concentration was 9.3 mg kg⁻¹ which decreased over time due to adsorption to the soil. For the pristine Ag-NMs, a dissolution rate of 0.82 day⁻¹ resulted in over 97% dissolution within three days, so although the dissolved Ag ionic concentration in this scenario started at 0 mg kg⁻¹, it rapidly followed the same kinetics as the ionic exposure. For the Ag₂S-NM exposure, the concentrations of dissolved Ag ions were orders of magnitude lower those for the other two scenarios, due to the low rate of dissolution. When the worm concentrations were fitted to the variable desorbed Ag-

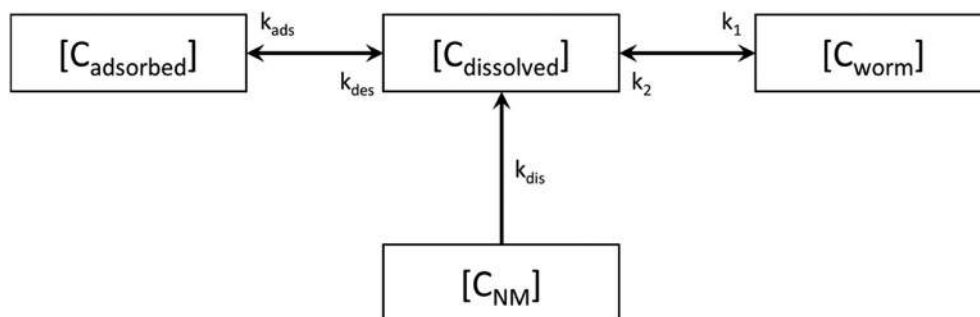


Fig. 5 Conceptual model used in the current case, depicting the modelled fate of Ag-NMs in soil, and the potential accumulation of ionic Ag in earthworms. For the dissolution process, no equilibrium is assumed.



concentrations (Fig. 7), significant k_1 and k_2 parameters could be derived for the different forms of Ag (Table 2).

Uptake and elimination rate constants for the dissolving pristine Ag-NMs are similar to the ones for the ionic exposure, indicating similar uptake kinetics. This was also concluded by Baccaro *et al.* (2018).¹⁴ The uptake rate constants for Ag-NM and AgNO₃ exposure only differed by approximately 10% within each study. These uptake rate constants are slightly higher in the current study when compared to Baccaro *et al.* (2018)¹⁴ (0.087 versus 0.061 and 0.079 versus 0.055 (kg kg⁻¹ per day) for Ag-NMs and AgNO₃, respectively). This is expected, since the uptake rate constants of the current study are based on available ionic Ag concentrations, while Baccaro *et al.* (2018)¹⁴ used (higher) total concentrations as inputs for the modelling. Ratios between the rate constants of the two studies are similar among the forms (1.42 and 1.43 respectively), indicating similar impacts of the inclusion of the availability of the Ag-ions for uptake for both forms. The overall modelled uptake of Ag from exposure to Ag₂S-NMs is low, yet the uptake rate (k_1) is larger in comparison with the other forms (2.3 kg kg⁻¹ per day), which is also higher than was found by Baccaro *et al.* (2018)¹⁴ (0.008 kg kg⁻¹ per day). The high rate constant of the current study is due to the extremely low concentrations of available Ag, indicating that sole uptake *via* available ionic Ag may not fully explain the uptake in the case of these non-soluble Ag₂S-NMs. This would imply that in the case of such insoluble NMs, other routes of uptake (*e.g.* particulate) may also play a significant role, although the absolute accumulation of particulate Ag is much lower than that of the ionic form (the measured concentrations in the Ag₂S exposed worms are significantly lower than in worms exposed to the other forms). The relatively large k_1 value for the uptake of Ag from Ag₂S-NMs in the current study may also be due to underestimation of the dissolution of the Ag₂S-NMs used, although this is less likely considering the extent of the differences. Excretion rates (k_2) are similar between the current study and Baccaro *et al.* (2018)¹⁴ for all forms (0.036 versus 0.04, 0.113 versus 0.064, and 0.038 versus 0.044 for Ag from Ag-NM, Ag₂S and AgNO₃, respectively) which is expected since k_2 is mainly dependent on the internal worm concentrations. The higher excretion rate for Ag from Ag₂S-NMs could suggest that a different form

of Ag is excreted faster than the form in the ionic and pristine Ag-NM exposed earthworms, although this could not be confirmed in Baccaro *et al.* (2018).¹⁴

The results of this case study indicate that, in concurrence with ionic metal exposures, bioavailability is important to consider in the case of the accumulation assessment of dissolving metal-based NMs. Furthermore, uptake of particulate NMs seems to be relevant in the case of non-dissolving NMs, although the absolute accumulation of non-dissolving NMs may be significantly lower than that of dissolving NMs.

Recommendations and guidance

The assessment of bioaccumulation potential is an important facet of the environmental risk assessment of NMs, and currently regulatory bodies include the use of invertebrates for bioaccumulation testing. For example, the OECD technical guidance (TG) 317 on earthworm bioaccumulation testing indicates that uptake curves can be drawn and bioaccumulation factors derived. However, such technical guidance documents may need amending for NMs. Based on the available literature, neither BLMs nor bioaccumulation factors based on measured data are to be recommended for modelling of (longer term) bioaccumulation of different forms of nanomaterials. Assumptions underlying these modelling approaches, including equilibrium theory that relates to uptake of solutes, are not met in the case of NMs. Dynamic PBPK-modelling approaches are more suitable for nanomaterials. Different uptake and elimination processes can be included in the modelling frameworks, including fast (active) uptake and elimination but also slow (passive) processes. Storage as inert fractions, biogenic transformation of NMs, growth dilution and other internal kinetic processes can also be incorporated into the modelling formulations, although the determination of specific rate constant parameters may be a challenge. Accumulation experiments to assess kinetic parameters should include uptake and elimination phases and the analyses should in principle only include materials that cross epithelia, unless the focus is on bio-magnification in food chains.

In order to allow read across of data and parameters between studies, it is essential to quantify concentrations of the

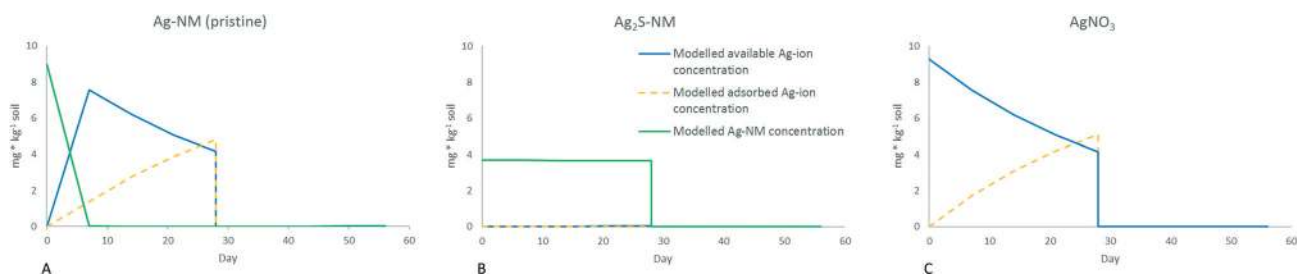


Fig. 6 Modelled concentrations of different forms of Ag in the soil. A: Exposure to Ag-NM pristine, B: exposure to Ag₂S-NM, C: exposure to AgNO₃. For all graphs: red line: available desorbed Ag-ions, orange dotted line: Ag-ions adsorbed to soil particles, not available, green line: modelled Ag-NM concentrations. All concentrations in mg kg⁻¹, initial concentrations used as inputs for modelling were taken from Baccaro *et al.* 2018.¹⁴ Day 0–28: earthworms in spiked soil, days 28–56: earthworms transferred to clean soil.



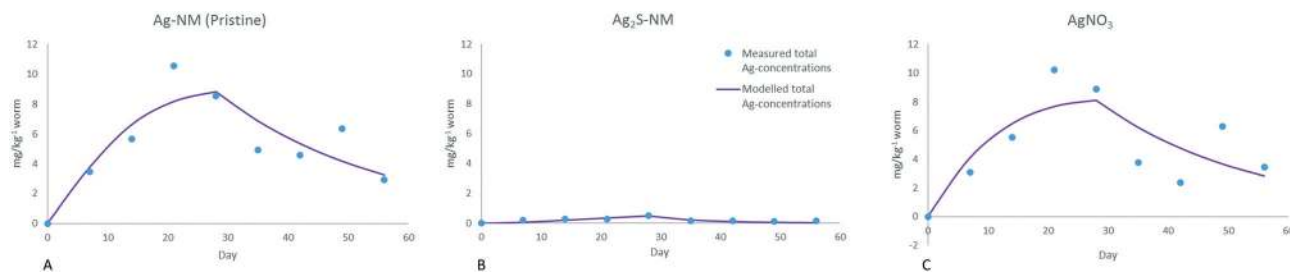


Fig. 7 Modelled total Ag-concentrations in earthworms (mg kg^{-1} ww), based on different exposure scenarios and soil concentrations as shown in Fig. 6. Day 0–28: earthworms in spiked soil, days 28–56: earthworms transferred to clean soil.

different forms of nanomaterials. Modelling of available metal ion concentrations in the pore water has been illustrated to be a useful proxy for bioavailability in experiments on soil organisms in the case of dissolving NMs. For particulate forms of NMs the main driver of uptake is phagocytosis, however, little is known on the drivers of NM bioavailability. Uptake of particulate forms is generally much lower than the ionic uptake but may still be relevant in modelling the total uptake of NM, especially for slowly, or non-dissolving NMs. The quantification of the availability of the different forms demands accurate protocols *e.g.* for pore-water extraction, with methodologies able to separate particles from ions to detail NM speciation. Filling this data gap is essential in order to be able to model the accumulation of different forms of specific NMs in an integrated way, coupling the environmental fate of NMs with uptake in biota. In addition, biogenic formation of particulate materials in tissues is a well-known protection mechanism by which organisms can store metals in a less toxic form. When addressing uptake of metallic NMs, it should always be confirmed that particles found in the organisms are similar to the ones they were exposed to. For this, the size, shape and elemental composition of NMs need to be established. Without this information, particulate uptake may be overestimated since biogenic particles are included in the derivation of the parameters.

More complex PBPK models demand more parameters that need to be quantified. In complex cases, exposure experiments should be designed with ample statistical power. It was shown in the examples that although the r^2 value of a modelling approach increased (*i.e.* scenario 'Predict separate k_2 ' versus 'Predict'), its significance decreased due to decreased degrees of freedom (Table 2). Furthermore, only processes should be included for which kinetic rate constants can be quantified. For instance, the biogenic formation of

particulate metal in organisms may depend on the internal metal concentration, hence, this may only be induced at higher concentrations. In this case, the kinetic rate constant of the biogenic formation is dependent on the internal concentration, which may hamper the modelling of form specific accumulation of NMs. In such cases, it may be needed to restrict the modelling to total concentrations of the material.

Author contributions

All the authors have contributed to the discussions leading to the manuscript and the writing of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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References

- S. J. Klaine, A. A. Koelmans, N. Horne, S. Carley, R. D. Handy, L. Kapustka, B. Nowack and F. v. d. Kammer, Paradigms to assess the environmental impact of manufactured nanomaterials, *Environ. Toxicol. Chem.*, 2012, **31**, 3–14.
- K. Hund-Rinke, A. Baun, D. Cupi, T. F. Fernandes, R. Handy, J. H. Kinross, J. M. Navas, W. Peijnenburg, K. Schlich, B. J. Shaw and J. J. Scott-Fordsmand, Regulatory ecotoxicity testing of nanomaterials – proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles, *Nanotoxicology*, 2016, **10**, 1442–1447.
- R. D. Handy, N. W. van den Brink, M. Chappell, M. Mühling, R. Behra, M. Dušinská, P. Simpson, J. Ahtiainen, A. N. Jha, J. Seiter, A. Bednar, A. Kennedy, T. F. Fernandes and M. Riediker, Practical considerations for conducting ecotoxicity

Table 2 Uptake (k_1) and elimination (k_2) rate constants of single compartment modelling of Ag-uptake modelling in earthworms from available Ag-ions, based on three exposure scenarios. For description of scenarios, see text

Ag source	k_1 (kg kg^{-1} per day)	k_2 (day^{-1})
Ag-NM	0.0866	0.0355
Ag ₂ S-NM	2.309	0.113
AgNO ₃	0.0787	0.0383



- test methods with manufactured nanomaterials: what have we learnt so far?, *Ecotoxicology*, 2012, **21**, 933–972.
- 4 E. Lahive, K. Jurkschat, B. J. Shaw, R. D. Handy, D. J. Spurgeon and C. Svendsen, Toxicity of cerium oxide nanoparticles to the earthworm *Eisenia fetida*: subtle effects, *Environ. Chem.*, 2014, **11**, 268–278.
 - 5 Z. M. Świątek, C. A. M. van Gestel and A. J. Bednarska, Toxicokinetics of zinc-oxide nanoparticles and zinc ions in the earthworm *Eisenia andrei*, *Ecotoxicol. Environ. Saf.*, 2017, **143**, 151–158.
 - 6 C. W. Isaacson, L. Sigg, A. A. Ammann, J. Stadnicka-Michalak and K. Schirmer, Interactions of TiO₂ nanoparticles and the freshwater nematode *Plectus aquatilis*: particle properties, kinetic parameters and bioconcentration factors, *Environ. Sci.: Nano*, 2017, **4**, 712–719.
 - 7 J. R. Velicogna, E. E. Ritchie, R. P. Scroggins and J. I. Princz, A comparison of the effects of silver nanoparticles and silver nitrate on a suite of soil dwelling organisms in two field soils, *Nanotoxicology*, 2016, **10**, 1144–1151.
 - 8 J. Garcia-Alonso, F. R. Khan, S. K. Misra, M. Turmaine, B. D. Smith, P. S. Rainbow, S. N. Luoma and E. Valsami-Jones, Cellular Internalization of Silver Nanoparticles in Gut Epithelia of the Estuarine Polychaete *Nereis diversicolor*, *Environ. Sci. Technol.*, 2011, **45**, 4630–4636.
 - 9 P. S. Tourinho, C. A. M. van Gestel, A. J. Morgan, P. Kille, C. Svendsen, K. Jurkschat, J. F. W. Mosselmans, A. M. V. M. Soares and S. Loureiro, Toxicokinetics of Ag in the terrestrial isopod *Porcellionides pruinosus* exposed to Ag NPs and AgNO₃ via soil and food, *Ecotoxicology*, 2016, **25**, 267–278.
 - 10 M. J. C. van der Ploeg, R. D. Handy, P. L. Waalewijn-Kool, J. H. J. van den Berg, Z. E. Herrera Rivera, J. Bovenschen, B. Molleman, J. M. Bavco, P. Tromp, R. J. B. Peters, G. F. Koopmans, I. M. C. M. Rietjens and N. W. van den Brink, Effects of silver nanoparticles (NM-300K) on *Lumbricus rubellus* earthworms and particle characterisation in relevant test matrices, including soil, *Environ. Toxicol. Chem.*, 2014, **33**, 743–752.
 - 11 P. L. Waalewijn-Kool, K. Klein, R. M. Fornies and C. A. M. van Gestel, Bioaccumulation and toxicity of silver nanoparticles and silver nitrate to the soil arthropod *Folsomia candida*, *Ecotoxicology*, 2014, **23**, 1629–1637.
 - 12 L. M. Rossbach, B. J. Shaw, D. Piegza, W. F. Vevers, A. J. Atfield and R. D. Handy, Sub-lethal effects of waterborne exposure to copper nanoparticles compared to copper sulphate on the shore crab (*Carcinus maenas*), *Aquat. Toxicol.*, 2017, **191**, 245–255.
 - 13 S. Makama, R. Peters, A. Undas and N. W. van den Brink, A novel method for the quantification, characterisation and speciation of silver nanoparticles in earthworms exposed in soil, *Environ. Chem.*, 2015, **12**, 643–651.
 - 14 M. Baccaro, A. K. Undas, J. de Vriendt, J. H. J. van den Berg, R. J. B. Peters and N. W. van den Brink, Ageing, dissolution and biogenic formation of nanoparticles: how do these factors affect the uptake kinetics of silver nanoparticles in earthworms?, *Environ. Sci.: Nano*, 2018, **5**, 1107–1116.
 - 15 S. Böhme, M. Baccaro, M. Schmidt, A. Potthoff, H.-J. Stark, T. Reemtsma and D. Kuhnelt, Metal uptake and distribution in the zebrafish (*Danio rerio*) embryo: differences between nanoparticles and metal ions, *Environ. Sci.: Nano*, 2017, **4**, 1005–1015.
 - 16 J. M. Unrine, S. E. Hunyadi, O. V. Tsyusko, W. Rao, W. A. Shoultz-Wilson and P. M. Bertsch, Evidence for Bioavailability of Au Nanoparticles from Soil and Biodistribution within Earthworms (*Eisenia fetida*), *Environ. Sci. Technol.*, 2010, **44**, 8308–8313.
 - 17 M. Baalousha, G. Cornelis, T. A. J. Kuhlbusch, I. Lynch, C. Nickel, W. Peijnenburg and N. W. van den Brink, Modeling nanomaterial fate and uptake in the environment: current knowledge and future trends, *Environ. Sci.: Nano*, 2016, **3**, 323–345.
 - 18 C. Schultz, K. Powell, A. Crossley, K. Jurkschat, P. Kille, A. J. Morgan, D. Read, W. Tyne, E. Lahive, C. Svendsen and D. J. Spurgeon, Analytical approaches to support current understanding of exposure, uptake and distributions of engineered nanoparticles by aquatic and terrestrial organisms, *Ecotoxicology*, 2015, **24**, 239–261.
 - 19 A. T. Wray and S. J. Klaine, Modeling the influence of physicochemical properties on gold nanoparticle uptake and elimination by *Daphnia magna*, *Environ. Toxicol. Chem.*, 2015, **34**, 860–872.
 - 20 F. R. Khan, K. B. Paul, A. D. Dybowska, E. Valsami-Jones, J. R. Lead, V. Stone and T. F. Fernandes, Accumulation Dynamics and Acute Toxicity of Silver Nanoparticles to *Daphnia magna* and *Lumbricus variegatus*: Implications for Metal Modeling Approaches, *Environ. Sci. Technol.*, 2015, **49**, 4389–4397.
 - 21 M. Diez-Ortiz, E. Lahive, P. Kille, K. Powell, A. J. Morgan, K. Jurkschat, C. A. M. Van Gestel, J. F. W. Mosselmans, C. Svendsen and D. J. Spurgeon, Uptake routes and toxicokinetics of silver nanoparticles and silver ions in the earthworm *Lumbricus rubellus*, *Environ. Toxicol. Chem.*, 2015, **34**, 2263–2270.
 - 22 M.-N. Croteau, S. K. Misra, S. N. Luoma and E. Valsami-Jones, Silver Bioaccumulation Dynamics in a Freshwater Invertebrate after Aqueous and Dietary Exposures to Nanosized and Ionic Ag, *Environ. Sci. Technol.*, 2011, **45**, 6600–6607.
 - 23 R. D. Handy, G. Cornelis, T. Fernandes, O. Tsyusko, A. Decho, T. Sabo-Attwood, C. Metcalfe, J. A. Steevens, S. J. Klaine, A. A. Koelmans and N. Horne, Ecotoxicity test methods for engineered nanomaterials: Practical experiences and recommendations from the bench, *Environ. Toxicol. Chem.*, 2012, **31**, 15–31.
 - 24 A. Praetorius, N. Tufenkji, K.-U. Goss, M. Scheringer, F. von der Kammer and M. Elimelech, The road to nowhere: equilibrium partition coefficients for nanoparticles, *Environ. Sci.: Nano*, 2014, **1**, 317–323.
 - 25 E. E. Ruppert, R. S. Fox and R. D. Barnes, *Invertebrate Zoology: A Functional Evolutionary Approach*, Thomson-Brooks/Cole, Belmont, CA, 7th edn, 2004.
 - 26 P. Cardoso, T. L. Erwin, P. A. V. Borges and T. R. New, The seven impediments in invertebrate conservation and how to overcome them, *Biol. Conserv.*, 2011, **144**, 2647–2655.



- 27 R. G. Morgado, S. Loureiro, M. N. González-Alcaraz, A. C. Duarte, A. Cachada and T. Rocha-Santos, in *Soil Pollution*, Academic Press, 2018, pp. 59–87, DOI: 10.1016/B978-0-12-849873-6.00003-0.
- 28 K. W. Cummins and M. J. Klug, Feeding ecology of stream invertebrates, *Annu. Rev. Ecol. Syst.*, 1979, **10**, 147–172.
- 29 K. W. Cummins, R. W. Merritt and P. C. N. Andrade, The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil, *Stud. Neotrop. Fauna Environ.*, 2005, **40**, 69–89.
- 30 R. C. Brusca, W. Moore and S. M. Shuster, *Invertebrates*, Sinauer Associates, Sunderland, Massachusetts U.S.A., 3rd edn, 2016.
- 31 A. Laycock, M. Diez-Ortiz, F. Larner, A. Dybowska, D. Spurgeon, E. Valsami-Jones, M. Rehkämper and C. Svendsen, Earthworm Uptake Routes and Rates of Ionic Zn and ZnO Nanoparticles at Realistic Concentrations, Traced Using Stable Isotope Labeling, *Environ. Sci. Technol.*, 2016, **50**, 412–419.
- 32 J. R. Velicogna, D. M. Schwertfeger, A. H. Jesmer, R. P. Scroggins and J. I. Princz, The bioaccumulation of silver in *Eisenia andrei* exposed to silver nanoparticles and silver nitrate in soil, *NanoImpact*, 2017, **6**, 11–18.
- 33 R. D. Handy and F. B. Eddy, in *Physicochemical Kinetics and Transport at Biointerfaces*, ed. J. Buffle, H. P. Leeuwen and W. Koster, John Wiley, Chisester, 2004, DOI: 10.1002/0470094044.ch7.
- 34 F. R. Khan, S. K. Misra, N. R. Bury, B. D. Smith, P. S. Rainbow, S. N. Luoma and E. Valsami-Jones, Inhibition of potential uptake pathways for silver nanoparticles in the estuarine snail *Peringia ulvae*, *Nanotoxicology*, 2015, **9**, 493–501.
- 35 G. Cornelis, K. Hund-Rinke, T. Kuhlbusch, N. van den Brink and C. Nickel, Fate and Bioavailability of Engineered Nanoparticles in Soils: A Review, *Crit. Rev. Environ. Sci. Technol.*, 2014, **44**, 2720–2764.
- 36 M. J. van der Ploeg, J. H. van den Berg, S. Bhattacharjee, L. H. de Haan, D. S. Ershov, R. G. Fokkink, H. Zuilhof, I. M. Rietjens and N. W. van den Brink, In vitro nanoparticle toxicity to rat alveolar cells and coelomocytes from the earthworm *Lumbricus rubellus*, *Nanotoxicology*, 2014, **8**, 28–37.
- 37 I. Lynch, A. Salvati and K. A. Dawson, PROTEIN-NANOPARTICLE INTERACTIONS What does the cell see?, *Nat. Nanotechnol.*, 2009, **4**, 546–547.
- 38 D. Drobne and A. Fajgelj, Use of Tc-99m-Perchnetate to follow liquid water uptake by *Procellio Scaber*, *J. Exp. Biol.*, 1993, **178**, 275–279.
- 39 H. M. Fox, Anal and oral intake of water by crustacea, *J. Exp. Biol.*, 1952, **29**, 583–599.
- 40 M. R. Warbur, *Evolutionary Biology of Land Isopods*, Springer-Verlag, Berlin, Germany, 1993.
- 41 M. G. Vijver, J. P. M. Vink, C. J. H. Miermans and C. A. M. van Gestel, Oral sealing using glue: A new method to distinguish between intestinal and dermal uptake of metals in earthworms, *Soil Biol. Biochem.*, 2003, **35**, 125–132.
- 42 S. P. Hopkin, *Biology of the Springtails (Insecta: Collembola)*, OUP Oxford, Oxford, UK, 1997.
- 43 G. Eisenbeis, Physiological absorption of liquid water by collembola - absorption by the ventral tube at different salinities, *J. Insect Physiol.*, 1982, **28**, 11–20.
- 44 T. Mesaric, C. Gambardella, T. Milivojevic, M. Faimali, D. Drobne, C. Falugi, D. Makovec, A. Jemec and K. Sepcic, High surface adsorption properties of carbon-based nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses in *Artemia salina* larvae, *Aquat. Toxicol.*, 2015, **163**, 121–129.
- 45 J. Baumann, J. Koser, D. Arndt and J. Filser, The coating makes the difference: Acute effects of iron oxide nanoparticles on *Daphnia magna*, *Sci. Total Environ.*, 2014, **484**, 176–184.
- 46 S. Novak, A. Jemec Kokalj, M. Hočevár, M. Godec and D. Drobne, The significance of nanomaterial post-exposure responses in *Daphnia magna* standard acute immobilisation assay: Example with testing TiO₂ nanoparticles, *Ecotoxicol. Environ. Saf.*, 2018, **152**, 61–66.
- 47 S. R. Tangaa, H. Selck, M. Winther-Nielsen and F. R. Khan, Trophic transfer of metal-based nanoparticles in aquatic environments: a review and recommendations for future research focus, *Environ. Sci.: Nano*, 2016, **3**, 966–981.
- 48 K. Tervonen, G. Waissi, E. J. Petersen, J. Akkanen and J. V. K. Kukkonen, Analysis of fullerene-C60 and kinetic measurements for its accumulation and depuration in *Daphnia magna*, *Environ. Toxicol. Chem.*, 2010, **29**, 1072–1078.
- 49 W.-C. Hou, P. Westerhoff and J. D. Posner, Biological accumulation of engineered nanomaterials: a review of current knowledge, *Environ. Sci.: Processes Impacts*, 2013, **15**, 103–122.
- 50 T. L. Rocha, T. Gomes, J. P. Pinheiro, V. S. Sousa, L. M. Nunes, M. R. Teixeira and M. J. Bebianno, Toxicokinetics and tissue distribution of cadmium-based Quantum Dots in the marine mussel *Mytilus galloprovincialis*, *Environ. Pollut.*, 2015, **204**, 207–214.
- 51 C. A. Garcia-Negrete, J. Blasco, M. Volland, T. C. Rojas, M. Hampel, A. Lapresta-Fernandez, M. C. J. de Haro, M. Soto and A. Fernandez, Behaviour of Au-citrate nanoparticles in seawater and accumulation in bivalves at environmentally relevant concentrations, *Environ. Pollut.*, 2013, **174**, 134–141.
- 52 T. Gomes, C. G. Pereira, C. Cardoso, J. P. Pinheiro, I. Cancio and M. J. Bebianno, Accumulation and toxicity of copper oxide nanoparticles in the digestive gland of *Mytilus galloprovincialis*, *Aquat. Toxicol.*, 2012, **118**, 72–79.
- 53 Y. H. Shu, G. R. Zhang and J. W. Wang, Response of the common cutworm *Spodoptera litura* to zinc stress: Zn accumulation, metallothionein and cell ultrastructure of the midgut, *Sci. Total Environ.*, 2012, **438**, 210–217.
- 54 S. Novak, D. Drobne, M. Golobič, J. Zupanc, T. Romih, A. Gianoncelli, M. Kiskinova, B. Kaulich, P. Pelicon, P. Vavpetič, L. Jeromel, N. Ogrinc and D. Makovec, Cellular Internalization of Dissolved Cobalt Ions from Ingested CoFe₂O₄ Nanoparticles: In Vivo Experimental Evidence, *Environ. Sci. Technol.*, 2013, **47**, 5400–5408.



- 55 I. Giska, C. A. M. van Gestel, B. Skip and R. Laskowski, Toxicokinetics of metals in the earthworm *Lumbricus rubellus* exposed to natural polluted soils - relevance of laboratory tests to the field situation, *Environ. Pollut.*, 2014, **190**, 123–132.
- 56 M. Diez-Ortiz, I. Giska, M. Groot, E. M. Borgman and C. A. M. Van Gestel, Influence of soil properties on molybdenum uptake and elimination kinetics in the earthworm *Eisenia andrei*, *Chemosphere*, 2010, **80**, 1036–1043.
- 57 L. Dai, *Toxicity and bioaccumulation of silver and copper oxide nanoparticles in two deposit feeders, a polychaete Capitella teleta and a mollusk, Macoma balthica, compared to other metallic forms*, Roskilde University, Roskilde, Denmark, 2013.
- 58 R. Dallinger and P. S. Rainbow, *Ecotoxicology of metals in invertebrates*, Taylor and Francis, Boca Roca, United States, 1993.
- 59 P. S. Rainbow, Trace metal concentrations in aquatic invertebrates: why and so what?, *Environ. Pollut.*, 2002, **120**, 497–507.
- 60 S. H. Liang, S. C. Chen, C. Y. Chen, C. M. Kao, J. I. Yang, B. S. Shieh, J. H. Chen and C. C. Chen, Cadmium-induced earthworm metallothionein-2 is associated with metal accumulation and counteracts oxidative stress, *Pedobiologia*, 2011, **54**, 333–340.
- 61 P. S. Rainbow, Trace metal bioaccumulation: Models, metabolic availability and toxicity, *Environ. Int.*, 2007, **33**, 576–582.
- 62 F. R. Khan, S. K. Misra, J. Garcia-Alonso, B. D. Smith, S. Strekopytov, P. S. Rainbow, S. N. Luoma and E. Valsami-Jones, Bioaccumulation Dynamics and Modeling in an Estuarine Invertebrate Following Aqueous Exposure to Nanosized and Dissolved Silver, *Environ. Sci. Technol.*, 2012, **46**, 7621–7628.
- 63 N. Adam, F. Leroux, D. Knapen, S. Bals and R. Blust, The uptake and elimination of ZnO and CuO nanoparticles in *Daphnia magna* under chronic exposure scenarios, *Water Res.*, 2015, **68**, 249–261.
- 64 F. R. Khan, G. M. Kennaway, M. N. Croteau, A. Dybowska, B. D. Smith, A. J. A. Nogueira, P. S. Rainbow, S. N. Luoma and E. Valsami-Jones, In vivo retention of ingested Au NPs by *Daphnia magna*: No evidence for trans-epithelial alimentary uptake, *Chemosphere*, 2014, **100**, 97–104.
- 65 F. Ribeiro, C. A. M. Van Gestel, M. D. Pavlaki, S. Azevedo, A. M. V. M. Soares and S. Loureiro, Bioaccumulation of silver in *Daphnia magna*: Waterborne and dietary exposure to nanoparticles and dissolved silver, *Sci. Total Environ.*, 2017, **574**, 1633–1639.
- 66 K. R. Timmermans and P. A. Walker, The fate of trace-metals during the metamorphosis of chironomids (Diptera, Chironomidae), *Environ. Pollut.*, 1989, **62**, 73–85.
- 67 M. O. Montes, S. K. Hanna, H. S. Lenihan and A. A. Keller, Uptake, accumulation, and biotransformation of metal oxide nanoparticles by a marine suspension-feeder, *J. Hazard. Mater.*, 2012, **225**, 139–145.
- 68 S. Thakali, H. E. Allen, D. M. Di Toro, A. A. Ponizovsky, C. P. Rooney, F.-J. Zhao, S. P. McGrath, P. Criel, H. Van Eeckhout, C. R. Janssen, K. Oorts and E. Smolders, Terrestrial Biotic Ligand Model. 2. Application to Ni and Cu Toxicities to Plants, Invertebrates, and Microbes in Soil, *Environ. Sci. Technol.*, 2006, **40**, 7094–7100.
- 69 M. M. Ardestani and C. A. M. van Gestel, Using a toxicokinetics approach to explain the effect of soil pH on cadmium bioavailability to *Folsomia candida*, *Environ. Pollut.*, 2013, **180**, 122–130.
- 70 M.-N. Croteau, S. K. Misra, S. N. Luoma and E. Valsami-Jones, Bioaccumulation and Toxicity of CuO Nanoparticles by a Freshwater Invertebrate after Waterborne and Dietborne Exposures, *Environ. Sci. Technol.*, 2014, **48**, 10929–10937.
- 71 J. C. McGeer, K. V. Brix, J. M. Skeaff, D. K. DeForest, S. I. Brigham, W. J. Adams and A. Green, Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment, *Environ. Toxicol. Chem.*, 2003, **22**, 1017–1037.
- 72 S. N. Luoma and P. S. Rainbow, Why Is Metal Bioaccumulation So Variable? Biodynamics as a Unifying Concept, *Environ. Sci. Technol.*, 2005, **39**, 1921–1931.
- 73 M. M. Ardestani, N. M. van Straalen and C. A. M. van Gestel, Uptake and elimination kinetics of metals in soil invertebrates: A review, *Environ. Pollut.*, 2014, **193**, 277–295.
- 74 M. G. Vijver, C. A. M. van Gestel, N. M. van Straalen, R. P. Lanno and W. J. G. M. Peijnenburg, Biological significance of metals partitioned to subcellular fractions within earthworms (*Aporrectodea caliginosa*), *Environ. Toxicol. Chem.*, 2006, **25**, 807–814.
- 75 M. G. Vijver, J. P. M. Vink, T. Jager, N. M. Van Straalen, H. T. Wolterbeek and C. A. M. Van Gestel, Kinetics of Zn and Cd accumulation in the isopod *Porcellio scaber* exposed to contaminated soil and/or food, *Soil Biol. Biochem.*, 2006, **38**, 1554–1563.
- 76 T. Ramskov, A. Thit, M.-N. Croteau and H. Selck, Biodynamics of copper oxide nanoparticles and copper ions in an oligochaete – Part I: Relative importance of water and sediment as exposure routes, *Aquat. Toxicol.*, 2015, **164**, 81–91.
- 77 M. N. Croteau, S. N. Luoma, B. R. Topping and C. B. Lopez, Stable metal isotopes reveal copper accumulation and less dynamics in the freshwater bivalve *corbicula*, *Environ. Sci. Technol.*, 2004, **38**, 5002–5009.
- 78 A. Taghavy, A. Mittelman, Y. Wang, K. D. Pennell and L. M. Abriola, Mathematical Modeling of the Transport and Dissolution of Citrate-Stabilized Silver Nanoparticles in Porous Media, *Environ. Sci. Technol.*, 2013, **47**, 8499–8507.
- 79 C. Levard, B. C. Reinsch, F. M. Michel, C. Oumahi, G. V. Lowry and G. E. Brown, Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on Dissolution Rate, *Environ. Sci. Technol.*, 2011, **45**, 5260–5266.
- 80 L. Zhan, *MSc thesis*, Louisiana State University and Agricultural and Mechanical College, 2013.
- 81 D. M. Di Toro, H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin and R. C. Santore, Biotic ligand model of the acute toxicity of metals. 1. Technical basis, *Environ. Toxicol. Chem.*, 2001, **20**, 2383–2396.

