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Nanopesticides: Guiding Principles for Regulatory Evaluation of Environmental Risks

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ABSTRACT: Nanopesticides or nano plant protection products represent an emerging technological development that, in relation to pesticide use, could offer a range of benefits including increased efficacy, durability, and a reduction in the amounts of active ingredients that need to be used. A number of formulation types have been suggested including emulsions (e.g., nanoemulsions), nanocapsules (e.g., with polymers), and products containing pristine engineered nanoparticles, such as metals, metal oxides, and nanoclays. The increasing interest in the use of nanopesticides raises questions as to how to assess the environmental risk of these materials for regulatory purposes. Here, the current approaches for environmental risk assessment of pesticides are reviewed and the question of whether these approaches are fit for purpose for use on nanopesticides is addressed. Potential adaptations to existing environmental risk assessment tests and procedures for use with nanopesticides are discussed, addressing aspects such as analysis and characterization, environmental fate and exposure assessment, uptake by biota, ecotoxicity, and risk assessment of nanopesticides in aquatic and terrestrial ecosystems. Throughout, the main focus is on assessing whether the presence of the nanoformulation introduces potential differences relative to the conventional active ingredients. The proposed changes in the test methodology, research priorities, and recommendations would facilitate the development of regulatory approaches and a regulatory framework for nanopesticides.

KEYWORDS: *nanopesticides, environmental risk, ecotoxicity, environmental fate*

INTRODUCTION

Engineered nanoparticles (ENPs) are being, or have the potential to be, used in many industrial sectors including defense, energy generation and storage, agriculture, and environmental remediation.[1] One sector where the use of ENPs is receiving increasing interest is the pesticide sector with the development of a range of plant protection products that are termed "nanopesticides".[2−5] Nanopesticides "involve either very small particles of a pesticide active ingredient (ai) or other small engineered structures with useful pesticidal properties".[6] For the purpose of this paper, we regard a nanopesticide as a plant protection product in which nanotechnology (e.g., use of materials that have a physical form with at least one size dimension in the range of 1−100 nm) is employed to enhance the efficacy or reduce the environmental footprint of a pesticide ai. It is noteworthy, however, that so-called "nano" formulations of patented pesticides often contain size fractions beyond the 100 nm conventional "nanorange".[7]

Nanopesticides represent an emerging technological development that, in relation to pesticide use, could offer a range of

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Table 1. Potential Applications of Nanotechnology in the Pesticides Sector

function	how this can be achieved	current examples
enhanced apparent solubility	nano- and microemulsions	emulsion-based registered pesticides, Banner MAXX of Syngenta[64]
faster decomposition in soil and/or plant	nanocatalyst-conjugated ai in microcapsules	SDS-modified TiO_2/Ag conjugated with ai such as dimethomorph;[65] imidacloprid and avermectin[66]
controlled release	nanocapsules, nanospheres	polymeric stabilized bifenthrin;[67] nanocomposite 2,4-D;[68] porous hollow Si-encaged validamycin[69]
targeted delivery	nanocapsules	nanoenapcsulated glyphosate or sulfonylurea herbicide[2]
protection against premature degradation	nanocapsules with catalyst ai conjugate	TiO_2-M262 polymer metaflumizone;[70] porous hollow Si-encaged validamycin[69]
enhanced uptake/efficacy	nano- and microemulsions, nanospheres	nanopermethrin;[71] nanosphere insecticides[72]
enhanced toxicity to target organism (lower dose)	nanodispersions; nanosuspensions	nanodispersed triclosan[73]
nanoparticle as ai	nanometals and nanoclays	registered Nano-Ag biocide;[74] Nano-Si[75,76]

benefits including increased efficacy, durability, and a reduction in the amounts of active ingredients that need to be used.[2] A range of formulation types have been suggested including emulsions (e.g., nanoemulsions), nanocapsules (e.g., with polymers), and inorganic ENPs, such as metals, metal oxides, and nanoclays.[3−5] These products, which are at different stages in the product development cycle, can be used to improve the efficacy of existing pesticide active ingredients or to enhance their environmental safety profiles, or both (Table 1). In some cases, the ENP itself (or a solubilized form of the ENP) may "drive" the biological effect (e.g., nanosilver when used as a pesticide where the active component is the ionic silver that is released from the ENP), whereas in other cases nanotechnology is used to protect an ai or enhance its delivery to the site of action. In the future, nanotechnology may also provide benefits for precision farming through "smart field systems"; for example, wireless sensors could be linked via satellite to a laptop computer to detect and locate crop infestation with pathogens and to trigger application of pesticide as needed.[5] These systems have the potential to overcome the need to apply a pesticide to the entire crop, thereby protecting the environment through the use of reduced quantities and targeted application of the pesticide ai.

The increasing interest in the use of nanopesticides raises questions over how the environmental risk of these materials should be assessed for regulatory purposes. There is an increasing body of evidence (including from other applications of ENPs) that the factors and processes affecting the environmental behavior and effects of ENPs may differ from "conventional" substances that do not contain ENPs and, consequently, that methods used in current risk assessment approaches may need refining or replacing to deal with these differences. For example, the REACH framework for assessment of chemicals is considering amendments to its Annexes to account for some of the unique factors associated with ENPs. With respect to pesticides, the Scientific Advisory Panel of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of the United States[8] discussed the adequacy of current procedures of hazard and exposure evaluation and concluded that (i) the potential risks of pesticides containing ENPs to humans and the environment may be different from those of conventional pesticides; (ii) the existing environmental fate and effects models are generally inadequate for predicting the behavior of nanopesticides; and (iii) additional metrics and parameters are needed for improved understanding of the environmental fate and effects processes associated with nanopesticides. European Union (EU) Regulation 1107/2009 for marketing authorization of plant protection products in Europe also requires the novel characteristics of a nanopesticide to be considered in the assessment process as it calls for due consideration of the interactions between the ai, safener (chemical substances used in combination with pesticides to make them "safer" and more targeted), synergists, and coformulants during the evaluation of a plant protection product.

In this paper, the current approaches for environmental risk assessment (ERA) of conventional pesticides are reviewed and their fitness for assessment of nanopesticides is analyzed (Table 2). In light of the insights generated, potential adaptations to existing ERA tests, as well as suitable alternative procedures for use with nanopesticides, are discussed. For the purpose of this review we consider three broad categories of nanopesticides: (i) where the nanoformulation is simply used to increase the apparent solubility/dispersion of the pesticide or to protect it from degradation in the formulation, but upon spraying is no longer associated with the ai; (ii) where the ENP is utilized to alter either the toxicokinetics or toxicodynamics of the pesticide, and as such is associated with the ai at least until it reaches the site of action; and (iii) where either the ENP itself or the nanopesticide complex is itself the active ingredient. It is the last category that presents the most complex scenario from a regulatory perspective. In all cases there exists also the potential for mixtures of "free" and nanoparticle-associated ai to coexist in the environment, the ratio of which may not be constant but change over time or as a function of the formulation and environmental conditions, thereby confounding measurements of efficacy and toxicity. Therefore, a key criterion for risk assessment purposes is the "durability" of the nano−ai complex following application of the nanopesticide.

CURRENT APPROACHES TO ENVIRONMENTAL RISK ASSESSMENT

Environmental risk assessments of pesticides are required in many regions of the world before a product can be placed on the market.[8,9] To limit the time, costs, and logistics needed for these risk assessments, a tiered approach focusing on key drivers of impact (e.g., Figure 1) is typically used. A tier is defined as a complete effect and exposure assessment resulting in an appropriate assessment end point. Each tier will involve the estimation of a predicted environmental concentration (PEC), which is the estimated concentration of an active substance in key environmental compartments including surface water, groundwater, and soil. The PECs are then compared to an effect end point such as a predicted no effect concentration (PNEC), the median effective concentration (EC_{50}), or no observed effect

Table 2. Different Environmental Fate Parameters Used for Conventional Pesticides and Their Relevance for Nanopesticides

characteristic	parameter/test currently used	is the characteristic relevant for NPs?	comments
distribution between soil, water, and air	K_{oc}/K_d	not appropriate	colloidal characteristics (aggregation, settlement, interaction with surfaces, matrix effects) drive the distribution, so other parameters may be needed; behavior will be concentration dependent, so studies should reflect likely environmental exposures; environmental parameters will affect particle behavior, so studies should cover environmentally relevant concentration ranges
	K_H	not appropriate	
	VP	not appropriate	
stability in soil, water, and sediment	DT_{50} soil	yes	half-lives of ENPs in different environmental matrices are relevant; the challenge is how to measure the ENPs; characteristics of the test matrices could be important in determining persistence, so a range of matrices should be tested
	DT_{50} water	yes	
	DT_{50} sediment	yes	
bioaccumulation	K_{ow} BCF	not appropriate	other parameters may emerge as understanding develops; rate constants for uptake and depuration may be a more suitable descriptor of potential accumulation; uptake is likely to be concentration dependent, so studies should reflect likely environmental exposures
		not appropriate	
environmental concentrations	PEC_{soil}	yes	mass concentration may not be the best metric for ENP risk, so it may be necessary to express exposure concentrations in other units such as particle number concentration or surface area concentration; exposure models may need refining to account for the processes important in transport of particles
	PEW_{sw}	yes	
	PEC_{sed}	yes	
	PEC_{gw}	yes	
	PEC_{air}	yes	
ecotoxicity	EC50, EC10	yes	maintenance of constant exposure conditions may be particularly challenging for nanopesticides; analytical characterization to support the tests may need to be considered; other units for effects concentration (e.g., particle size concentration) may be needed

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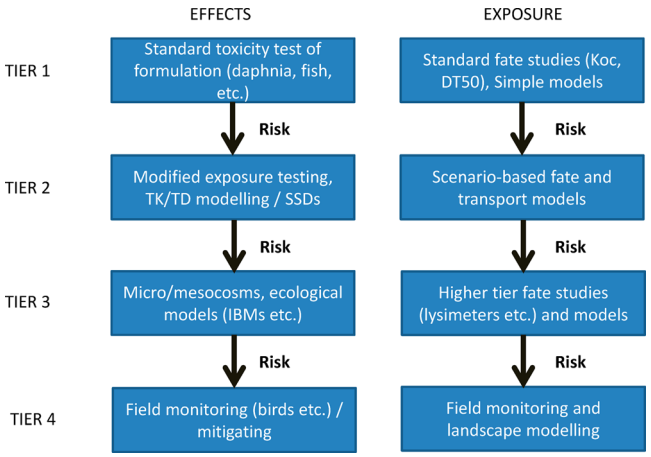

Figure 1. Tiered approach to environmental risk assessment of a pesticide.

concentration (NOEC) to derive a risk characterization ratio, which is compared with a regulatory trigger value. Uncertainty factors are incorporated into the process to account for the many uncertainties associated with extrapolating from regulatory fate and effect studies to impacts in the real environment.[10]

Under current regulations, PECs in soil, surface water, and groundwater need to be calculated for ERA purposes (e.g., FOCUS).[11] These concentrations are dependent on the use patterns of the product and the persistence and phase partitioning (e.g., sorption) of the ai in/between environmental compartments (e.g., soil, sediment, pore water). The movement of pesticide through the soil profile and ultimately to drainage systems or groundwater is described by models based on the convection−dispersion processes, whereas macropore transport is considered in some circumstances, for example, in the pesticide leaching model MACRO.[12] Pesticide runoff to surface streams is governed by off-site transport of pesticides in the solution phase, although erosion of the surface soil may also contribute to surface water contamination. Additional exposure pathways include off-target drifts during application, movement through subsurface drainage channels, and volatilization to, and deposition from, the atmosphere. Once in a water body, processes such as sorption, transformations, and flow dynamics influence the fate and risk of pesticides. These processes are characterized using the key parameters summarized in the following paragraphs.

Phase partitioning of the ai is often quantified with the sorption coefficients K_d or K_{oc}, (Table 2) and to characterize the nonlinear sorption behavior, the Freundlich isotherm is often measured. These measurements are usually carried out using the batch equilibration approach, where the soil and water phases are mixed to reach phase equilibrium.[13] Half-life ($t_{1/2}$) or half-dissipation time (DT_{50}) is used to indicate a pesticide's persistence in soil and can be measured in a laboratory incubation experiment or a field dissipation study.[14] The decrease of pesticide concentration in soil measured over time is used to fit kinetics models to derive $t_{1/2}$ or DT_{50}. Similarly, persistence is measured in water and sediment systems, and vapor pressure and Henry's law constant are required to estimate the potential for volatilization from soil or water.

The bioconcentration factor (BCF) is required to assess potential risks to high trophic levels and to classify compounds in terms of their persistence, bioaccumulation potential, or toxicity (PBT). Hydrophobicity (i.e., high K_{ow}) may trigger bioaccumulation tests that are conducted in accordance with relevant guidelines.[15] A BCF value for an ai can be derived in one of two ways. If bioaccumulation into nontarget organisms, such as fish, is viewed as a phase partitioning process, BCF can be calculated as the ratio of the uptake rate constant (K_{in}) to the depuration rate constant K_{out} measured in an exposure test that does not need to reach equilibrium. Alternatively, BCF can be calculated as the ratio of body residue to the concentration in water at the steady state.

The first tier of the assessment process typically uses simple exposure models with defined scenarios. For aquatic organisms, tests include studies with pelagic microalgae (green algae, blue-green algae, diatoms), aquatic plants (a monocotyledon and a dicotyledon), invertebrates (the crustaceans *Daphnia magna*, *Americamysis bahia*, the insect *Chironomus riparius*, oligochaeta *Lumbriculus variegatus*), and fish. For the terrestrial compartment, tests with earthworms (*Eisenia* sp., *Enchytraeus* sp.) and springtails (*Folsomia candida*) and studies into the effects on microbial community functions are most commonly conducted.

In the second tier, more complex exposure modeling may be used and more complex laboratory effect studies are likely to be employed such as modified exposure studies and/or toxicokinetic−toxicodynamic experiments and modeling or the development of species sensitivity distributions using additional nonstandard test species, preferably from taxa not included in the initial set of studies.

The third tier is used to assess processes such as biomagnification, recovery, and indirect effects using semirealistic exposure regimes and biological communities through the use of semifield experiments (i.e., microcosms, mesocosms) and ecological models. The last tier can include field monitoring of concentrations and effects of pesticide, although it is often difficult to get a clear view of the effects of a single pesticide given the presence of multiple stressors in agro-ecosystems. A diagrammatic representation of the tiered risk assessment approach currently used for pesticides is presented in Figure 1.

WHY MIGHT NANOPESTICIDES NEED A DIFFERENT APPROACH?

Over the past few years, understanding of the factors affecting the risks posed by ENPs to the environment has developed significantly. It is now recognized that many of the approaches and assumptions used to assess the risks of conventional chemicals may not be appropriate for ENPs and that some modifications may be required to risk assessment guidelines.[16,17] The OECD has a large work program that is considering how test guidelines may need to be adapted to assess the hazard of ENPs. In the following section we draw on some of this work, as well as the wider literature and the authors' expertise, to highlight how nanopesticides may differ from conventional pesticides in terms of their fate and effects and the implications of these differences for the ERA process.

For a conventional pesticide, it is usually assumed that ecotoxicity is related to ai mass concentration, and risk is therefore characterized using exposure and effects data expressed in terms of mass per volume or mass per mass of ai. However, for nanopesticides, other parameters such as particle number concentration and particle size distribution (PSD), as well as the ratio of "free" and nanoparticle-bound ai, may be important in determining the pesticide bioavailability and toxicity.[18−20] Analytical methodologies used to characterize the levels of available pesticides over time in regulatory fate and effect studies may therefore need to be supplemented with additional

methodologies that provide an understanding of the particle number concentration and particle size distribution over time. Approaches could include scattering methods (e.g., DLS), particle tracking methods (e.g., NTA), centrifugal methods (e.g., DCS), or fractionation methods (e.g., FFF), the latter method allowing for determination of ai size when coupled with appropriate chemical selective detectors. However, as these methods are not routine in pesticide analysis laboratories, this represents a significant change in approach. It is also important to note that nanopesticides will often undergo changes in their degree of dispersion or agglomeration over time, depending on the concentration of the nanopesticide and environmental factors such as pH, ionic strength, dissolved molecules of the test media, and as such are not equilibrium systems. Thus, characterization of a nanopesticide at different stages in its environmental life cycle and throughout fate and effect studies may be very important.

In standardized ecotoxicity studies, it is recommended that the test concentration should be kept within ±20% of the nominal test concentrations.[21] This is an important issue when considering products containing ENPs and so is also an issue for nanopesticides. For products containing ENPs, there is the question whether it is the mass concentration or the particle number concentration (and mean size) that should be maintained. It is clear that agglomerate/aggregate size and type will change throughout exposures. Furthermore, particle number varies markedly for ENPs of different sizes at a constant mass: for example, a particle mass of 100 ng corresponds to only 2.4×10^4 particles (spheres of unit density) of 2 μm in diameter, but to 2.4×10^{10} particles of 20 nm, or to 2.4×10^{13} particles of 2 nm.[22] Although the maintenance of a particle concentration to within ±20% of a starting concentration for a test is likely to be extremely challenging, it could be highly informative in understanding the risks of a nanopesticide to the natural environment.

The behavior of ENPs is similar to that of colloids, and therefore phase partitioning between environmental matrices cannot be assumed. For example, sorption and uptake into organisms are unlikely to be partitioning processes but rather involve active and receptor-mediated processes for uptake and aggregation and deposition processes for association with suspended matter, soil, or sediment. These processes will be dynamic,[23] whereas the phase partitioning driven process that occurs for conventional pesticides can to a large extent be modeled by equilibrium processes. Consequently, the use of soil sorption coefficients such as K_d or K_{oc} may not be relevant for modeling the movement of a nanopesticide within the environment (Table 2). Other factors such as the aggregation rate, sedimentation rate, and interaction of an ENP with surface receptors may need to be considered. The release rate of the ai from the ENP complex will also need to be characterized, as well as the ratio of free versus ENP-bound ai. Furthermore, transport of nanopesticides in air could be very different; for a conventional pesticide volatilization will be the process whereby the pesticide molecules transfer from soil and plant surfaces to the air, whereas for a nanopesticide the release of particles is likely to be important.

For aquatic and soil-dwelling organisms, dermal and respiratory surfaces represent a significant (often dominant) route of pesticide exposure. Partitioning principles govern the rate of entry across biological membranes such that the extent of absorption is driven by the chemical properties of the molecule (e.g., hydrophobicity) and the physiological structure (e.g., surface area, extent of sclerotization) of the biological surface. Upon entering the body, fugacity-driven distribution of the pesticide across different organs and tissues occurs, with fate in tissues determined by the organism's potential to biotransform molecules as a step toward elimination and by the potential to interact with the relevant cellular receptors. The primary (e.g., cytochrome P450), secondary (e.g., glutathione-s-transferase), and tertiary (e.g., ABC transporter) systems governing this metabolism and excretion are among the best studied biological pathways, whereas key uptake receptors are known as a function of the known mode(s) of action.

The diffusion and equilibrium partitioning that often dominate the cellular uptake of pesticides may not always be relevant for nanopesticides where the ai is bound to the ENP or is itself the ENP. As intact particles, there are a number of general mechanisms, such as passive and facilitated diffusion for direct transfer across biological membranes without enclosure[26] and active transport and endocytosis through membrane carrier proteins and channels,[27] that can result in the internalization of ENPs.[23,25]However, it is the mechanisms of endocytosis pathways that have received most attention through the use of pharmacological inhibitors[28] and gene activity disruption[29] to tease out the mechanisms and fluorescent labeled ENPs and antibody colocalization[30] studies to understand uptake kinetics and subcellular localization. Such studies have revealed that the size of ENPs taken up depends on the type of endocytosis and the corresponding vesicle sizes associated with their transport. Macropinosomes are generally 1−5 μm in diameter; calveolin-coated vesicles in the 50−80 nm range[31] and clathrin-mediated endocytosis structures are typically 120 nm in size. Most ENP have been found to localize in lysosomes following uptake,[32,33] which are sites of destruction for foreign or no longer useful materials, and there is thus the potential for release of locally high concentrations of pesticide from the lysosomes via the so-called "Trojan-horse" mechanism. The uptake pathway of a nanopesticide into organisms could therefore be very different from that of a conventional pesticide.

For conventional pesticides, the octanol/water partition coefficient (K_{ow}) is an important characteristic that indicates the affinity for lipid-rich tissues of nontarget organisms. Determining the K_{ow} values of ENPs is generally difficult because ENPs do not partition into either phase (Table 2). Rather, ENPs accumulate at the octanol/water interface because of their high surface energy.[34,35] Whereas the phase partitioning of organic molecules is driven partly by thermodynamics associated with water molecules versus the energy associated with partitioning to a surface, colloidal interactions are dependent on the net energy of attraction/repulsion with a surface.[36]

For pesticides it is usually assumed that dissipation, uptake, and distribution behavior is independent of the concentration. For nanopesticides it can be expected that phase partitioning, the relationship of mass concentration to particle concentration, uptake into biota, and distribution within organisms are all highly concentration dependent. It is likely that phase partitioning across membranes depends on the agglomeration status, size, and surface charge of ENPs; these parameters in turn depend on the concentration. For example, Handy et al.[37] in their idealized scenario of uptake of chemicals and ENPs by gills of freshwater fish, highlighted the key differences between lipophilic organic chemicals and ENPs. Prior to transfer across the gill epithelium, the substances must diffuse into the so-called unstirred layer (USL) made of water/mucous secretions. They observed that while small lipophilic organic molecules can easily diffuse into the USL and then through or between the

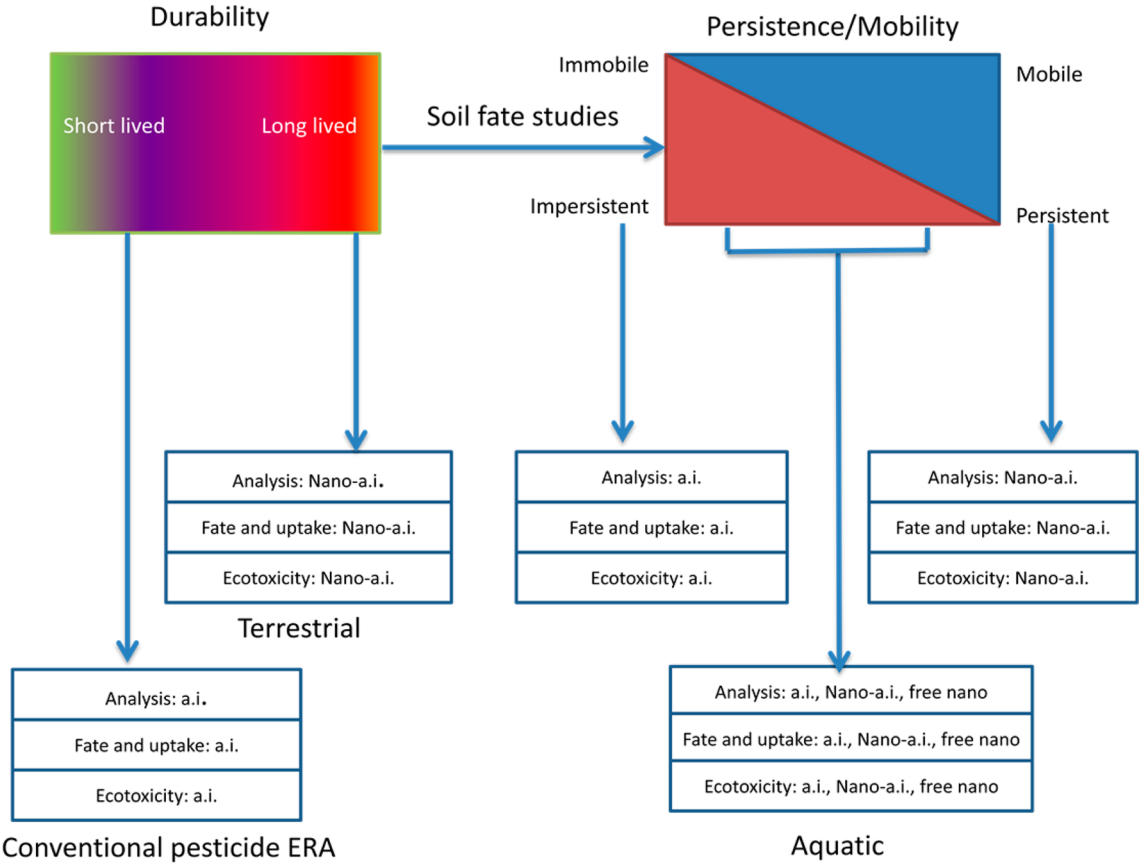

Figure 2. Conceptual strategy for assessing the environmental risks of a nanopesticide.

cells, ENPs are expected to diffuse at a slower rate that is influenced by humic substances and mucoproteins. In addition, ENPs may get entangled with mucoproteins, leading to prevention/retardation of their uptake. The size of ENPs precludes their uptake via ion or other transporters on the cell membranes. Furthermore, the Ca^{2+}- and Mg^{2+}-rich environment in the tight junctions between cells may trigger the aggregation of ENPs, thereby reducing their diffusion. Such processes at biological surfaces have the potential to alter the absorption and distribution of nanopesticides in comparison to traditional forms, with resultant effects on tissue distributions and biomagnification potential.

Quantifying internal exposure to ENPs may be required to understand the relationship between ENP properties and toxicity. For example, the rate of release of the ai will likely differ in different matrices. A consequence may be different exposure dynamics at target sites versus in the environment (e.g., fast release of ai in cellular compartments and slow release of ai in soil pore water due to different pH regimens and cellular enzymatic action). Quantitative property−toxicity relationships would help in the decision of how toxicity testing for ENPs should be standardized, but are not yet sufficiently developed or validated.

HOW COULD THE POTENTIAL RISK POSED BY A NANOPESTICIDE TO THE ENVIRONMENT BE ASSESSED?

It is clear from the above discussion that nanopesticides are likely to behave differently from conventional pesticides and that some of the traditional metrics used in ERA may be inappropriate for nanopesticides. Therefore, to adequately assess the environmental risk of a nanopesticide, alternative test methods and metrics may be required. Although in the future it may be possible to develop highly complex test methods and modeling approaches that accurately estimate the exposure and effects of a nanopesticide in a particular situation, we do not believe that the current knowledge allows us to do this at this time. It is also important to recognize that current approaches for conventional pesticide risk assessment employ many assumptions to address uncertainties and are by no means perfect themselves. To move forward in a pragmatic and implementable manner, we therefore propose a rational framework that accounts for the key differences between nanopesticides and conventional pesticides and where additional information may be required for their regulation. This aspect is further expanded in the sections below and illustrated schematically in Figure 2.

The proposed approach uses information on the durability of a nanopesticide product and the fate of the product in soil to make decisions on what component or mixture of components (e.g., conventional ai, nano−ai complex, or the free ENP acting as ai) should be tested and analyzed in aquatic fate studies and in aquatic and terrestrial ecotoxicity studies (Figure 2). The scientific rationale for this approach is that nanoformulation of a pesticide could be utilized to alter the toxicokinetics (i.e., the (rate of) uptake, biotransformation, distribution, and elimination) or the toxicodynamics (i.e., the mechanism or mode of toxic action of the ai), and by knowing which of these (or both) may be affected, rational decisions regarding modifications to existing tests or introduction of new tests could be made.

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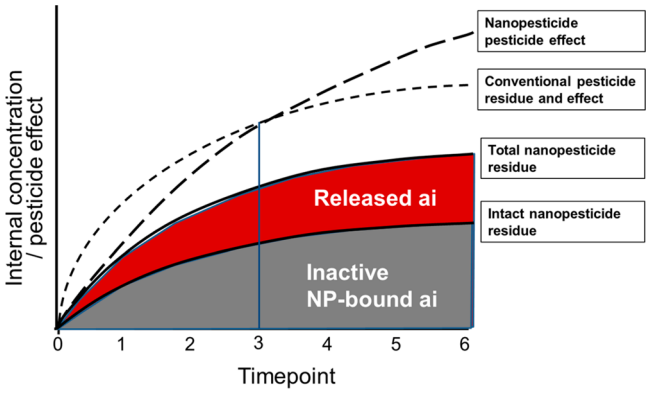

Figure 3. Schematic illustration of potential time-dependent (*x*-axis) effects on pesticide internal concentration and toxicity (*y*-axis) of nanoformulation pesticide preparations as compared to conventional forms. On the basis of the assumption that the ai is only active when released from the nanoformulation (which occurs over time), internal concentrations of released ai derived from the nanoformulation will increase over time as the pesticide is released from the nanocarrier. As a result, when exposure times are short in a test bioassay (e.g., up to timepoint 3), then the relatively small amount of ai that has been released may result in an observed toxicity that is lower than for the conventional ai formulation. In contrast, for longer term exposures (timepoints 4−6), the greater time allowed for ai release may result in higher internalized concentrations that can be associated with potential receptors to cause toxic effects that can be greater than for the conventional formulation.

Figure 3 provides an illustration of one scenario whereby the rate of release of ai from nanoformulation affects the duration of assessment required, assuming that the ai is only active in the free form. Other scenarios are also possible, such as the ai being active in the bound form also or even more active in the bound form, which would result in further alterations to the toxicokinetics diagram, but again an understanding of these effects would facilitate decision-making regarding appropriate testing durations and approaches. There is also potential for so-called Trojan-horse effects, whereby the uptake is of the nano−ai complex and the release of the ai occurs inside the plant cells; depending on the local environment, the release rate may be more dramatic, leading to extreme concentrations of ai that not occur via uptake of the free ai or to accumulation of NPs containing nonreleased ai. Such scenarios are not shown in Figure 3. Additionally, there is the potential that the ai may be more active in the bound state (nano−ai complex), in which case the nanoformulation would lead to higher local concentrations even at shorter time points.

If the nano component of a nanopesticide simply protects the ai from degradation in the formulation, then the fate and behavior of the ai will remain the same as in a conventional pesticide formulation and the nanocomponent will likely degrade during the application. Emulsions are a case in point. If the nanoformulation itself is the ai, or if the pesticide is more active when bound to the nanoparticle (e.g., as a result of increased local concentration, avidity effects, or optimal presentation of active site), then information about the conventional formulation will make only a minor contribution to understanding the nanoformulation. Thus, an understanding of where and how the nanoformulation is likely to affect the action of the ai will guide additional studies into characterization (e.g., particle number, ratio of free and nanoparticle-bound ai as a function of time/suspension conditions), fate and behavior, and efficacy, which may be required to gain regulatory approval. In the following sections we describe the different components of the scheme and the test approaches that could be employed. We start by considering the important elements of ENP characterization and quantification that are prerequisite to sound fate and effect assessment of nanopesticides.

CONSIDERATIONS FOR THE CHARACTERIZATION AND QUANTIFICATION OF NANOPESTICIDES

Similar considerations regarding the applicability of risk assessment approaches utilized for conventional chemicals to nanopesticides arise in terms of characterization, due to the challenges in analysis and characterization of nanobased materials in complex matrices.[38] Analysis of even conventional pesticides can be challenging at times.[39,40] Coupling this with the fact that techniques suitable for the detection, quantification, and characterization of nanopesticides (and indeed ENPs in general) in water, soil, and biota have not yet been validated or even developed in many cases leads to the conclusion that we are currently unable to adequately characterize these materials to support their risk assessment. Challenges are encountered due to the wide variety of nanopesticides and their presence as particle-bound forms making additional analysis (such as PSD, particle mass or number concentration, or surface charge) necessary. Expected environmental concentrations of nanopesticides will be low, and thus method sensitivity also becomes a challenge in the performance of fate and effect studies at environmentally relevant concentrations.

Techniques potentially suitable for the analysis of ENPs in environmental or food matrices have been extensively discussed in many reviews (e.g., Tiede et al.[41] and Hassellöv et al.[42]). To our knowledge no studies to date have examined the applicability of these methods of analysis to nanopesticides. Despite this, adequate analysis and characterization of nanopesticides are crucial for meaningful durability, fate, and ecotoxicity studies. This is indeed challenging, for not only the ai (conventional or ENP based) needs to be considered but the nano component (e.g., porous silica, metal oxide) also has to be characterized. Therefore, new methodologies will be required for nanopesticides, because extraction methods may alter the form of the nanopesticide, precluding any meaningful information on particle size distribution. Given the relatively high hydrophobicity of most pesticides,[43] strong nonpolar solvents under enhanced temperature and pressure conditions are often used to extract the residues.[39] However, such extraction procedures may affect the stability of the nano−ai complex.

On the basis of the assumption that various physical forms of the ai will display differences in mobility and toxicity, we propose a characterization scheme that employs the fractionation of the nanopesticides into three components: particulate (homo- and heteroaggregates incorporating ai), nanobound ai, and free ai. Size fractionation procedures (e.g., filtration, centrifugation) leading to operationally defined size classes are common in environmental analyses where size is relevant.[38,44] In particular, size fractionation, coupled to conventional pesticide analysis, could be used to determine the portion of the ai that remains associated with the nanocarrier and the agglomeration state of a nanocarrier, which will also influence ai fate and behavior. Although we propose that in principle this approach would in many cases provide an adequate level of characterization for risk assessment, current centrifugation and filtration methodologies require validation, and it is likely that further method development would be required to allow quantitative application.

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In some cases further characterization of the sample, or its size fractions, might be required. Physicochemical properties such as ENP size or shape could be characterized using the techniques outlined below and discussed in the literature. However, many conventional nanocharacterization methods (e.g., DLS, TEM) will lack the specificity needed to distinguish the nanopesticide from background particles as well as the sensitivity (limit of detection in terms of particle numbers) required for realistic environmental exposure concentrations.

Binary size separations (e.g., ultrafiltration, centrifugation), combined with appropriate physicochemical analysis of the fractions, can give information on the state of the ai as well as characteristics of the nanocarrier. Currently, two high-resolution size fractionation methods appear promising for application to nanopesticides. These are hydrodynamic chromatography (HDC),[45] which is somewhat analogous to the more commonly used size exclusion chromatography, and field flow fractionation (FFF). These approaches might find utility in cases when artifacts from filtration or centrifugation might be problematic or when a higher level of nanocharacterization (e.g., PSD of ai or nanocarrier) is desired. For some nonmetal based nanocomponents (e.g., polymers), detection, size characterization, and mass and/or particle number concentration analysis in environmental matrices could be particularly challenging.

For a nanopesticide consisting solely of a metal or metal oxide based ENP (i.e., where the ENP itself/dissolution of the ENP is the pesticide), conventional pesticide analysis is not required; however, chemical analysis for the element of interest (e.g., Ag, Si) using ICPMS will likely be possible. Element-specific size analysis is possible with specialized ICPMS techniques including FFF-ICPMS, HDC-ICPMS, and single particle ICPMS (spICP-MS) or even a combination of HDC-spICP-MS.[46−49] However, the use of complementary techniques such as TEM, NTA, and DLS is recommended to validate the measurements.

Recommended Approach for Characterization. Here we suggest a tiered analytical approach analogous to the tiered approach used in fate and ecotoxicology assessments. The degree to which one applies the methodology depends on the durability, mobility, and persistence of the respective nanopesticide. The tiered approach is detailed in Figure 4. At the simplest level (tier 1) the analytical approach is identical to what is currently performed for a pesticide ERA, namely, exhaustive extraction followed by analysis to quantify ai present in the samples. This is the most likely level needed for analysis of emulsions and short-lived, nonpersistent nanopesticides. However, if differences between nanoformulation and non-nanoformulation are seen in terms of durability, transport, and uptake, then additional "nano-analysis" of ai and carrier is required. This approach would likely be applied to polymer and "hard" ENPs (porous silica, metals, metal oxides). Although the approach involves methods that fractionate the nanocarrier, given the likely much higher toxicity of the ai over the nanocarrier, the chemical analysis of the fractions, or the online analysis in the case of HDC and FFF, is focused on quantification of the ai in each associated size fraction and on determination of whether the ai is free or nanobound. The exception is where the ai is the nanopesticide, for example, nano-Ag, in which case the additional physical characterization obtained by these methods (e.g., PSD of Ag) may prove valuable in the risk assessment.

EXPOSURE ASSESSMENT

Durability of the Product. The durability of the product will determine the extent to which the environment will be exposed to the nanopesticide. For some product types, for example, nanoemulsions or nanodispersions, that are used to enhance the apparent solubility, it is likely that following application the "nano" properties of the pesticide will be lost. In these cases we would recommend that the product be treated as a conventional pesticide in the risk assessment process. Information should be available from the product development studies that assist in assessing the likely durability of the nanopesticide following application. If the product is long-lived, we recommend that the terrestrial component of the risk assessment is carried out with the nano−ai complex. If available, persistence studies undertaken during product development would allow quantification of the release rates of the ai from the ai−nano complex as well as the ai and ENP degradation rates. Such measurements can be performed using the separation techniques described above.

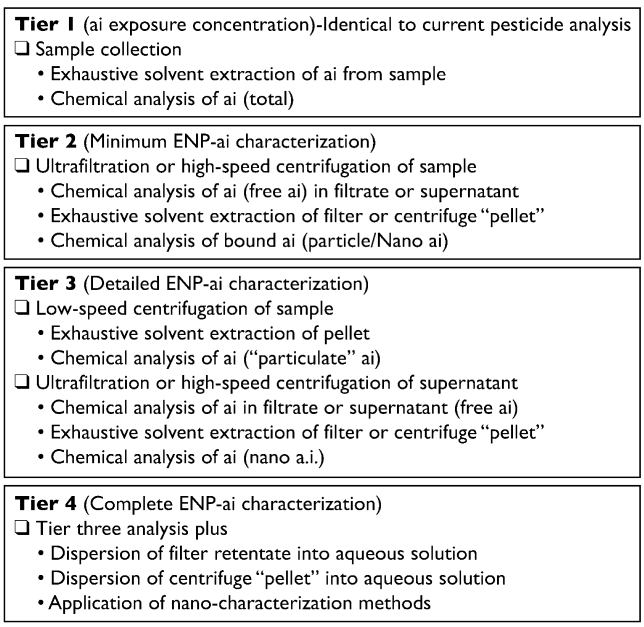

Figure 4. Tiered approach for analysis and characterization of nanopesticides.

Persistence. Using soil fate studies (e.g., column experiments, see Exposure Assessment below) nanopesticides can be characterized as persistent versus nonpersistent and mobile versus immobile. The outcome triggers how to continue the aquatic component of the risk assessment. If the nanopesticide (i.e., its nanoform) is nonpersistent, then the aquatic risk assessment may be conducted using only the ai. By contrast, if the nanopesticide is persistent, then the aquatic risk assessment has to be conducted using the nano−ai complex as well. If the persistence of the nano−ai complex is unclear or between those two clear-cut cases and threshold or trigger values need to be derived, then the aquatic risk assessment also needs to be carried out twice, that is, with the nano−ai complex and with the ai alone to cover possible worst case scenarios.

Persistence ($t_{1/2}$ or DT_{50}) of an ai may be influenced by its release from the carrier material. The release rate of the ai from the carrier and the degradation rate of the ai need to be quantified as both are key parameters in the transport models. We recommend incubation experiments with the ai as well as with the formulated product (i.e., the ENP−ai complex), where

the total concentration of the ai is measured. The difference between the experiment with the ai alone and with the ENP−ai complex would allow parametrization of the fate model by allowing estimates of release rate and degradation rate to be derived. However, it should be noted that this approach requires assumptions about the bioavailability of the ai when in the nano−ai complex. Two limit cases are possible: the ai in the nano−ai complex is equally accessible (bioavailable) for degradation as the ai without the nanocomplex, or the ai in the nano−ai complex is not degradable at all. An independent estimation of both the release rate and the degradation in the nano−ai complex is not possible from incubation experiments alone. However, an approach such as limited enzymolysis/proteolysis applied to the nano−ai complex and compared to the conventional ai would provide insights regarding the impact of the nanoformulation on the degradation/metabolism of the ai. This approach has been successfully applied to assess the impact of ENP on protein confirmation and degradation but has yet to be confirmed for environmental degradation. This approach could be a promising direction for initial testing.[50,51]

Mobility. The batch adsorption approach is not suitable for ENPs that are determined to undergo nonequilibrium processes. We suggest the use of column breakthrough experiments to derive nanopesticide mobility parameters as transport model inputs to estimate leaching or runoff/erosion movement. The experiments should not use excessive porewater velocity, as this may diminish the environmental relevance as velocity influences ENP fate.[52] These experiments should be undertaken with both the ai and the nanoformulation. In both cases, the concentration of ai in the leachate is measured and the fraction associated with the ENP−ai complex could be quantified using separation techniques, possibly in combination with advanced separation equipment such as FFF or HDC (as discussed in the characterization section above) to ensure robust quantification without interference from naturally occurring particles in the leachate. The fraction associated with soil colloids can be estimated from the difference in supernatant concentrations in the treatments (ai alone vs ENP−complex). Modeling techniques should be employed to derive the retardation (as opposed to sorption) parameters, which are based on the difference between the treatments using the release and degradation rates from durability studies, as described above.

Soils and Sediment−Water Test Systems for Sorption and Degradation Studies. The characteristics of soils for regulatory degradation and sorption studies are described in OECD guidelines.[13] Commonly used test soils are, in principle, suitable for studies with nanopesticides. However, additional criteria may be needed to ensure that soils with contrasting influences on ENP behavior are included in the studies. Important factors include pH, pore size distribution, ionic strength, dissolved organic carbon concentration, and clay content.[24] Characterization of persistence and sorption in sediment−water systems would follow the same principles as for soil, testing both the ai and the ENP−ai complex.

Fate and Exposure Modeling. The fate model would generally quantify free ai as well as the fraction of ai bound within the ENP−ai complex in different media. Existing fate models for pesticides could not be used without substantial revision and adaption to accommodate nanopesticide specific processes that are different from conventional pesticides. These differences include release of ai from nanopesticide complex, phase partitioning, agglomeration and changes in the ENP size distribution, possible interdependencies of particle size distribution, phase partitioning and degradation, as well as concentration dependence of all the above parameters.[36] The development of appropriate fate models for nanopesticides represents a significant challenge that will require substantial quantitative data from laboratory and field studies on nanopesticide behavior under various environmental conditions for model testing.

Uptake into Biota. Similarly to other ENPs, bioaccumulation cannot be viewed as a straight phase partitioning process, and K_{ow} is unlikely to be a good indicator for bioaccumulation potential of nanopesticides.[35] Therefore, BCF may not be estimated as the ratio of K_{in} and K_{out} in a short exposure tests and has been found to be not a relevant parameter to describe the bioaccumulation of ENPs.[53] It is generally considered that current standard protocols for bioaccumulation (e.g., fugacity-driven biomagnification) do not apply to ENPs (see below for more information; this has been reviewed previously[16,17]). As uptake processes for nanopesticides differ from those of conventional pesticides, the choice of test organisms may also have to be reconsidered. For example, filter feeders in aquatic environments (e.g., daphnids, bivalves) may be potentially vulnerable to elevated exposure due to their ability to process a large volume of media. For terrestrial systems, traditional test species such as earthworms that have a known potential for metal and organic chemical biomagnification are likely to be most relevant as they may accumulate larger amounts of nanopesticides. In the absence of a good predictor, such as K_{ow} for conventional pesticides, one has to rely on the testing of individual products. A test that reaches steady state may still yield the necessary information for assessing bioaccumulation potential, where bioaccumulation is calculated as the ratio of body residue over aqueous phase concentration at steady state. The experimental uptake test methods that explore uptake and depuration over time may be appropriate, but they need to (a) be designed using relevant dosing levels, (b) monitor steady state ENP concentrations in the media, (c) be representative of species likely to be exposed, and (d) be interpreted differently so as to reflect the uptake/depuration rates rather than the conventional BCFs. Whereas the need for global descriptors and predictive modeling frameworks for bioaccumulation assessment of ENPs is clear, considerable challenges remain in developing new or adapting existing protocols and approaches.[53] Time series based bioaccumulation studies to determine toxicokinetic parameters for nanopesticides are needed so the accumulation patterns of conventional pesticides and nanopesticides can be compared. These studies need to consider the form of the accumulated material (free versus nanobound ai) because this may have implications for the progression of toxicity over time (Figure 3).

EFFECTS ASSESSMENT

Are Current Standard Regulatory Approaches Applicable to Nanopesticides? As described above, there are standard regulatory approaches and tests that will need to be followed when the risks of conventional pesticides are assessed. A key question, therefore, is "are these standard ecotoxicity testing approaches appropriate for use on nanopesticides and are any modifications needed to these approaches?". The regulatory protection goals for a nanopesticide will be no different from those of a traditional pesticide, so the typical testing paradigm where acute and chronic effects data are generated for a range of trophic levels in different environmental compartments will be equally applicable to a nanopesticide. Questions

do, however, arise over which species should be tested and over whether specific test protocols need adaptation to cope with nanopesticides. The suitability of current standard test protocols for the assessment of hazard of products containing ENPs has been subject to much research and discussion. A technical workshop on the suitability of standard test methods for nanotoxicology[17] concluded recently that the standardized methods available for aquatic, sediment, and soil samples and mammalian and avian species provide a useful basis from which to develop appropriate effects studies for use with ENPs but that some modifications may be required. Some of these are discussed below.

Which Species Should Be Tested? Although the broad approach to testing may be relevant for effect assessment based on the range of currently utilized test organisms, the fate and form of a nanopesticide in an environment such as a surface water body could mean that certain organism types will have a higher degree of exposure than others. The requirement to include particle feeding organisms (e.g., filter or suspension feeders) in the test battery, as well as "conveyor-belt" feeders and benthic organisms, has already been proposed for general hazard assessment of ENPs, and these proposals should also apply to nanopesticides.

What Technical Modifications to Standard Test Protocols Could Be Needed? A wide range of technical modifications to standard test protocols have been proposed for ENPs,[16,17] which are likely to be equally required for nanopesticides. For example, appropriate references and controls (e.g., metal salts for metallic ENPs, larger scale (non-nanoscale) "bulk" materials, solvent controls) may be needed for comparative assessments; new dosing methods may be required to ensure homogeneous distributions of the material in the test systems; there may be a need for increased stirring/mixing of the water, or water changes, to maintain exposure concentrations in aquatic tests throughout a study; the duration of studies may need to be refined to reflect differences in the uptake kinetics of nanopesticides compared to conventional pesticides; and new characterization methods to track the dynamic behavior and fate of an ENP during an ecotoxicity study are likely to be needed and characterization may need to be done more frequently during a study than for a traditional pesticide. Some of the analytical approaches described under Recommended Approach for Characterization may be appropriate here. Two additional challenges associated with the application of standard test protocols for nanoformulations are (1) identification of the most appropriate dose metric and (2) accounting for nanoformulation agglomeration or aggregation, which also affects the available "nano" dose. As discussed earlier, mass is the metric utilized for ai and, indeed, for ENP to date, although there is considerable debate in the literature as to whether particle number, particle surface area, or reactive particle surface area would be more appropriate dose metrics for ENP[54] for dose–hazard and dose–exposure correlations. Aside from this, there is the unresolved question of how to compare the dose of conventional ai (free) versus nanoparticle-encapsulated/nanoparticle bound ai, which, as indicated in earlier sections, may be active in the bound form only or upon "release" only. Similar considerations apply to certain nanopharmaceuticals, and perhaps the approaches taken by medical regulators might be instructive in this respect. Considerations could include mass ai/ENP or mass ai/mass ENP while still reflecting the issue of durability of the nano–ai complex, as per Figure 2. Finally, the agglomeration potential of the nano–ai complex under actual exposure conditions would need to be factored into the assessment to correlate dose with response.

Role of Higher Tier Laboratory Ecotoxicity Testing Approaches. One of the main challenges in the risk assessment of nanopesticides will be how to combine the results from the standard effects studies and the exposure predictions. The use of higher tier laboratory ecotoxicity studies using, for example, natural waters rather than standard media or sediment–water test systems may overcome these issues. These approaches have been proposed for use in pesticide environmental risk assessment for some time now.[55] If designed correctly, the advantage of the approaches is that the test integrates the fate and effects processes that are likely to occur in the natural environment. Such approaches could be useful for assessing risk of nanopesticides until more mechanistic approaches are developed.

How Could Nanopesticides Interact with Other Environmental Contaminants? Whereas pesticides are assessed on a product by product basis, just like any other contaminant, in the real environment, they will co-occur with other substances, so mixture interactions are possible. Although risk assessment of environmental mixtures is not usually required for product risk assessment, it is possible that a nanopesticide will interact synergistically with other contaminants through the Trojan-horse effect.[56] This effect occurs when an ENP interacts with another substance and carries the substance into an organism or an organism tissue type. The effect can result in increased internal exposure to contaminants, which might not usually be accumulated. As our knowledge of these interactions increases, it may be necessary to incorporate these types of interactions into regulatory risk assessment schemes.

THE WAY FORWARD

Nanopesticides represent an attractive technological advancement from the standpoint of increased efficacy and protection of the environment and human health. The discussion in the preceding sections demonstrates that the factors and processes affecting the environmental behavior and effects of nanopesticides may differ from "conventional" pesticides, and therefore new or refined risk assessment approaches are needed. Unlike conventional pesticides, the uptake, bioavailability, and toxicity of nanopesticides are dependent on the particle number concentration and particle size distribution, as well as ratio of "free" and ENP-bound ai. Therefore, adaptation of existing methodologies and additional methodologies for analysis, characterization, environmental fate, and effect assessment are needed. However, considerable challenges exist, including maintenance of constant (±20%) particle concentration during the ecotoxicological tests, adequate characterization of the ENPs, identification and measurement of appropriate phase partitioning parameters (as conventional parameters may not apply), standardization of fate and toxicity testing protocols, and development of models (e.g., using quantitative structure–activity and property–toxicity relationships). Because current knowledge does not allow the accurate estimation of the exposure and effects of a nanopesticide in a particular situation, a pragmatic approach such as the one proposed here (Figure 2), coupled with a theoretical understanding of how nanoformulation might affect the toxicokinetics and/or toxicodynamics of the pesticide (Figure 3), is needed to move forward.

To implement this approach, a crucial decision-making step relates to the durability of a nanoformulated pesticide product and the fate of the product in soil. This allows identification of

whether the new product could be treated as a conventional pesticide or differently as a nanopesticide that demonstrates more activity as the ENP−ai complex. This step also guides the additional requirements in terms of ENP characterization and residue analysis as well as the fate and effect assessment. As discussed above, in several circumstances existing approaches can be used with some modifications and adaptations. Overall, the existing tiered risk assessment approach, such as that used in the EU, remains a useful framework for the risk assessment of nanopesticides, although with adaptions as presented above to account for potential alterations in the fate and behavior resulting from the nanoformulation. As the science of ENPs advances and new tools and techniques become available, the quality of data underpinning risk assessment will improve and further inform the regulatory requirements. In this regard, the collaborative efforts of the OECD Working Party on Manufactured Nanomaterials, the U.S. EPA, the APVMA, and other agencies may provide future guidance.

FUTURE NEEDS AND RECOMMENDATIONS

- Recent reviews on nanopesticides and ENPs used in crop protection and agricultural production[3−5] have discussed research priorities[57] and needs regarding nanospecific regulations.[58,59] Building on these, and including considerations of exactly where and how the nanoformulation might affect the environmental fate, behavior, and effects of a pesticide ai, we make the following recommendations relating to the development of a regulatory framework for nanopesticides. These are by no means exhaustive but merely indicative of different elements of an ERA (e.g., characterization, environmental fate and exposure, effect) required for an appropriate regulatory response for nanopesticides.
- A clear definition of what is/what is not a nanopesticide is needed for regulatory purposes. Definitions developed recently for nanomaterials in general may help, but these typically consider the size dimensions ranging between 1 and 100 nm and are only partially applicable to nanopesticides. Emerging pesticide nanoformulations are not only increasingly complex and biologically active but may also exhibit a potential change in the physicochemical properties and/or biological effects at a size range that is larger than the nanoscale (>100 nm). The need for inclusion of additional parameters[60] and larger size limits, for example, up to 1000 nm, has also been proposed for some nanomaterials.[61,62]
- Tools and techniques to characterize properties (such as primary particle shape, size range, surface properties) of complex formulations of nanopesticides are lacking. Without adequate analytical tools, regulatory requirements for robust data for risk assessment cannot be generated. Current ecotoxicological assessments have limited relevance due to a poor ability to characterize ENPs in complex environmental compartments at present, although this area is developing quickly. Despite greater attention to inorganic ENPs (compared to organic), detection and quantification of nanometal and metal oxides in complex matrices (e.g., formulations, environmental media) at environmentally realistic concentrations remain challenging. Nanopesticides are more complex products by design (Table 1) and therefore pose greater challenges to analysts. However, experience gained in the field of nanomedicine may be helpful for characterizing nanopesticides and should be integrated into the nanopesticide regulatory framework.
- Little advancement in exposure and effect modeling has so far occurred on ENPs and especially nanopesticides. Current models fail to take into consideration material sizes, shapes, functionalization, and surface properties and alterations in both ENPs and environmental matrices during exposure assessment.[63] Exposure models that differentiate the free and nanocomplex and the ENP size distribution and other toxicologically/fate relevant properties over time need to be developed as a matter of priority. Until we better understand the relationship between ENP properties and toxicity, we can only resort to toxicity testing case by case under conditions similar to those in the environment. As this may quickly become infeasible, sound ERA requires first the establishment of property−toxicity relationships based on comprehensive empirical data.
- Given the added uncertainty in risk assessment (such as that associated with detection and quantification, characterization, and exposure assessment) compared to "conventional" pesticides, the definition as well as the regulatory framework should consciously be made more adaptive and responsive to advancements in science and knowledge on nanopesticides. A pragmatic approach is to focus on understanding the persistence of the nano−ai complex and whether the nano−ai complex alters the activity of the ai relative to the conventional formulation as described here for reformulated pesticides. However, where the ENP itself is the ai, additional challenges remain.

Evidence-based decision-making is an expectation of contemporary regulators and demands that decisions are supported by science, and precaution is used where evidence is incomplete. To this end, regulators are warranted in erring on the side of caution until such time as the properties and behavior of nanopesticides are better understood. This may curtail the realization of full potential economic and environmental benefits of this rapidly expanding technology. It is also important that the product developer is aware that the nanoformulation of an already approved pesticide will require a separate ERA.

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Notes

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ABBREVIATIONS USED

ai	active ingredient
APVMA	Australian Pesticides and Veterinary Medicines Authority

BCF	bioconcentration factor
DCS	differential centrifugal sedimentation
DLS	dynamic light scattering
DT_{50}	dissipation half-life
EC	European Commission
EC50	half-maximal effective concentration
EFSA	European Food Safety Authority
ENP	engineered nanoparticle
ERA	Environmental Risk Assessment
EU	European Union
FDA	U.S. Food and Drug Administration
FIFRA-SAP	Federal Insecticide, Fungicide, and Rodenticide Act−Scientific Advisory Panel (USA)
FFF	field flow fractionation
FOCUS	forum for the co-ordination of pesticide fate models and their use
HDC	hydrodynamic chromatography
IBMs	individual-based models
ICPMS	inductive coupled plasma mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
K_d	soil/water partitioning coefficient; sorption coefficient
K_{oc}	soil organic carbon:water partitioning coefficient
K_{ow}	octanol/water partition coefficient
NOEC	no observed effect concentration
NTA	nanoparticle tracking analysis
MACRO	model designed to incorporate preferential flow
OECD	Organisation for Economic Co-operation and Development
PBT	persistent, bioaccumulative and toxic
PEC	predicted environmental concentration
PSD	particle size distribution
SEM	scanning electron microscopy
spICPMS	single-particle inductive coupled plasma mass spectrometry
SSD	species sensitivity distribution
$t_{1/2}$	decay half-life
TK/TD	toxicokinetics/toxicodynamics
TEM	transmission electron microscopy
USL	unstirred layer

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