Comprehensive framework for human health risk assessment of nanopesticides

Melanie Kah [1]✉, Linda J. Johnston [2], Rai S. Kookana [3,4], Wendy Bruce[5], Andrea Haase [6], Vera Ritz[7], Jordan Dinglasan[8], Shareen Doak[9], Hemda Garelick[10] and Vladimir Gubala[11]

Nanopesticides are not only in an advanced state of research and development but have started to appear on the market. Industry and regulatory agencies need a consolidated and comprehensive framework and guidance for human health risk assessments. In this perspective we develop such a comprehensive framework by exploring two case studies from relevant product types: an active ingredient delivered with a nanocarrier system, and a nanoparticle as an active ingredient. For a nanocarrier system, three entities are tracked during the assessment: the nanocarrier–active ingredient complex, the empty nanocarrier remaining after the complete release of the active ingredient, and the released active ingredient. For the nanoparticle of pure active ingre-dient, only two entities are relevant: the nanoparticle and the released ions. We suggest important adaptations of the existing pesticide framework to determine the relevant nanopesticide entities and their concentrations for toxicity testing. Depending on the nature of the nanopesticides, additional data requirements, such as those pertaining to durability in biological media and potential for crossing biological barriers, have also been identified. Overall, our framework suggests a tiered approach for human health risk assessment, which is applicable for a range of nanopesticide products to support regulators and industry in making informed decisions on nanopesticide submissions. Brief summaries of suitable methods including references to existing standards (if available) have been included together with an analysis of current knowledge gaps. Our study is an important step towards a harmonized approach accepted by regulatory agencies for assessing nanopesticides.

To feed nearly 10 billion people by 2050, food production would need to increase by at least 50% from the 2012 levels[1]. Clearly, innovations in the agricultural sector will continue to be required, which include the development of effective plant protection products (for example, pesticides), to achieve this target. The agrochemical industry is constantly seeking novel active ingredients (AIs) as well as new approaches to formulate and deliver pesticides. Nanoscience and nanotechnology can harness the extraordinary properties of materials at the nanoscale (<100 nm) to make an important contribution in such innovations[2,3].

Nanopesticides are currently an area of intense interest in nanotechnology and agriculture and food communities, as reflected by several reviews on this topic during the last five years[2–6]. Nanotechnology offers new opportunities to facilitate development of novel AIs and reuse existing chemistries through nanoformulations (for example, using a nanocarrier (NC) delivery system) that enable new pesticide functionalities, such as slow release of AI, increased stability, enhanced penetration (through cell membranes) and a greater efficacy of the AI in controlling the target organisms[4,5], often with a view to reducing application rates through greater efficacy and/or targeted delivery. For example, nanoparticles (NPs) of metal and/or metal oxides of Ag, Cu and Zn, as AIs, have been found to be effective antimicrobial and antifungal agents[3]. In addition, the slow-release (nanoencapsulated) and nanocomposite formulations of metal oxides have been found to be more potent in disease control than conventional formulations[3]. Double-stranded RNA loaded on designer, non-toxic, degradable, layered double hydroxide clay nanosheets not only offered greater stability to the AI (RNA) against plant viruses, but also resulted in reduced wash-off in rain and enhanced systematicity (uptake and transfer inside the sprayed plant)[7]. Nanopesticides (for example, with NCs or novel AIs) are in an advanced state of research, development and testing and are likely to be presented for regulatory evaluation.

Indeed, some nanopesticides are already commercially available[2]. For instance, Vive Crop Protection uses polymer-based delivery systems to design nanoformulations for enhancing the stability of the AI in salt solutions to allow their application with fertilizers through irrigation (chemigation) and also as a mixture of various nanopesticides, if needed.

There is currently no internationally accepted definition of nanopesticides, and thus regulatory agencies may adopt different size ranges and different limits for the fraction of nanosized particles[8–10]. For our purpose, we use an operational definition of a nanopesticide as a plant protection product in which a nanomaterial is used to enhance the functionality, increase the utility and/or alter the risk profile of a conventional AI or is presented as a new AI. This perspective does not cover materials that are called 'biocides' in the EU, and which include substances used in livestock breeding, food packaging and household kitchen or canteen settings. Some current nanopesticide formulations have sizes larger than the 1–100 nm nanoscale size range, similar to the situation with nanomedicines[11,12]. On the other hand, some products (for example,

[1]School of Environment, The University of Auckland, Auckland, New Zealand. [2]Metrology Research Centre, National Research Council Canada, Ottawa, Ontario, Canada. [3]CSIRO, Glen Osmond, South Australia, Australia. [4]University of Adelaide, Glen Osmond, South Australia, Australia. [5]Health Evaluation Directorate, Health Canada's Pest Management Regulatory Agency, Ottawa, Ontario, Canada. [6]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany. [7]Department of Pesticides Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany. [8]Vive Crop Protection, Mississauga, Ontario, Canada. [9]Institute of Life Science, Swansea University Medical School, Swansea, UK. [10]Department of Natural Science, Faculty of Technology, Middlesex University, London, UK. [11]Medway School of Pharmacy, University of Kent, Chatham Maritime, UK. ✉e-mail: melanie.kah@auckland.ac.nz

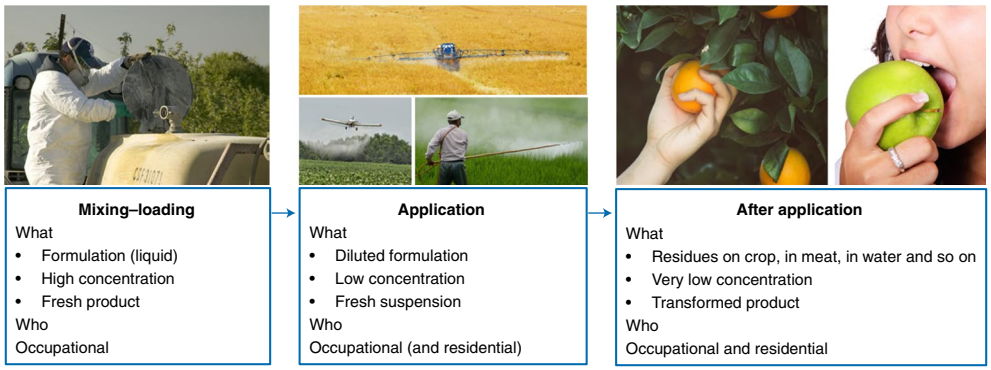

Fig. 1 | The three stage of human exposure to pesticides. Exposure during mixing–loading, application and after application must be considered in risk assessment. Image (left): Inga Spence/Science Source.

microemulsions) may contain fractions in the 1–100 nm range and have been on the market for decades without previously being classified as 'nano'[13,14].

At the time of introducing pesticides into modern agriculture (in the middle of the last century), it was acknowledged that the use of pesticides potentially presents a risk to human and ecosystem health[15], as they are bioactive agents by design (targeting insects, fungi, weeds and so on) and are applied intentionally in the environment. Ever since, new pesticide products have had to undergo rigorous safety testing to prevent unacceptable risks as part of a premarket risk assessment, and this also applies to nanopesticides. Although nanopesticides can potentially decrease this risk by reducing the level of exposure (for example, by reducing the required applied dose), they can also increase the potential for health risks by increasing the bioavailability and/or the bioactivity of the AI, changing its mechanism of action or introducing coformulants/carriers that may also be bioactive.

Conventional pesticides are evaluated by regulatory agencies primarily on the basis of the AI and its representative formulations. In contrast to the AI, other components of the formulations (coformulants or excipients) have generally been considered 'inerts'; however, safety data may be required by some regulatory agencies (for example in the European Union, Canada), in particular for safeners (chemicals added to a plant protection product to eliminate or reduce phytotoxic effects on certain plants) and synergists (chemicals with no or weak pesticidal activity, which enhance activity of the active substance(s) in a plant protection product). Nevertheless, for nanopesticides, it can be expected that the coformulants/excipients may contribute to a larger extent to the effectiveness of the pesticide. Therefore, they must be evaluated in all cases for potential risks to humans and ecosystem health. In fact, the European Food Safety Authority (EFSA) guidance for risk assessment of nanomaterials in food and feed[16] stipulates that for nanopesticides all coformulants/excipients (for example, surfactants, solvents, carriers, wetting agents) that contribute to the formulation must be considered. Moreover, the safety of all the components of the nanopesticide entity (that is, AI + coformulants) must be evaluated, regardless of whether the AI or coformulants separately have previously been evaluated as safe. A framework for ecological risk assessment for nanopesticides has already been developed and published[17], along with some guidance on its application to specific case studies, especially in problem formulation[18]. This study, therefore, focuses on human health risk assessment only.

The human safety testing of nanopesticides thus requires special attention and additional considerations as compared with the safety evaluation of typical, conventional chemicals. This is primarily because the physicochemical characteristics of nanomaterials (for example, size, shape, surface area and surface chemistry) have a strong bearing on their interactions with biological tissues and hence can influence their pharmacology, toxicokinetics and subsequently potential toxicity[19]. Furthermore, these characteristics may undergo changes in the biological environment, thereby altering the stability and durability of the surface and core of nanomaterials and consequently their toxicological response[19]. Difficulties in evaluation of nanomaterial toxicity that include pharmaco- and toxicokinetics of nanomaterials are well recognized[20].

The OECD Working Party on Manufactured Nanomaterials concluded that the existing human health risk assessment paradigm used for chemicals (with a few exceptions) can be adapted to determine the potential for human health risks of nanomaterials[21]. This group also suggested that modified or alternative testing strategies were necessary in some cases for risk analysis to inform human health, ecosystem health and exposure data needs for manufactured nanomaterials[21]. In this context, our aim was to identify key considerations that are crucial for adaptation of existing human health risk assessment paradigms to develop a comprehensive framework suitable for human health risk assessment of nanopesticides and applicable to industry, academic and regulatory agencies. The specific goals were (1) to identify key additional considerations associated with nanopesticides for human health risk assessment, (2) to develop a comprehensive framework for testing and assessment of nanopesticides for human health risk assessment, including suggestions for suitable methods and standards (if existing), and (3) to highlight knowledge gaps (including lack of methodology) that require urgent attention.

In this Perspective we have taken a pragmatic approach by building on the existing conventional risk assessment paradigm for pesticides, as well as considering frameworks and guidance that are currently available for NPs in other areas, such as cosmetics[22] and the food and feed chain[16]. Our goal is not to present an exhaustive list of scientific knowledge gaps in the field. Instead, we apply a top-down strategy to support decision making and meet regulatory needs, as recommended by Grieger et al.[23]. Here, we provide a comprehensive framework for human health risk assessment that will support regulators, researchers and industry in making informed decisions on nanopesticide submissions.

Human health risk assessment for conventional pesticides

Human health risk assessment of pesticide AIs and formulated products is a very well established process that forms an integral part of pesticide regulatory frameworks in many countries. Risk assessment typically consists of three key activities: exposure assessment, hazard identification and characterization, and risk characterization[24]. Human exposure to pesticides may occur from occupational and non-occupational (residential) uses of pesticides as well as via the diet and drinking water, which may contain residual traces of

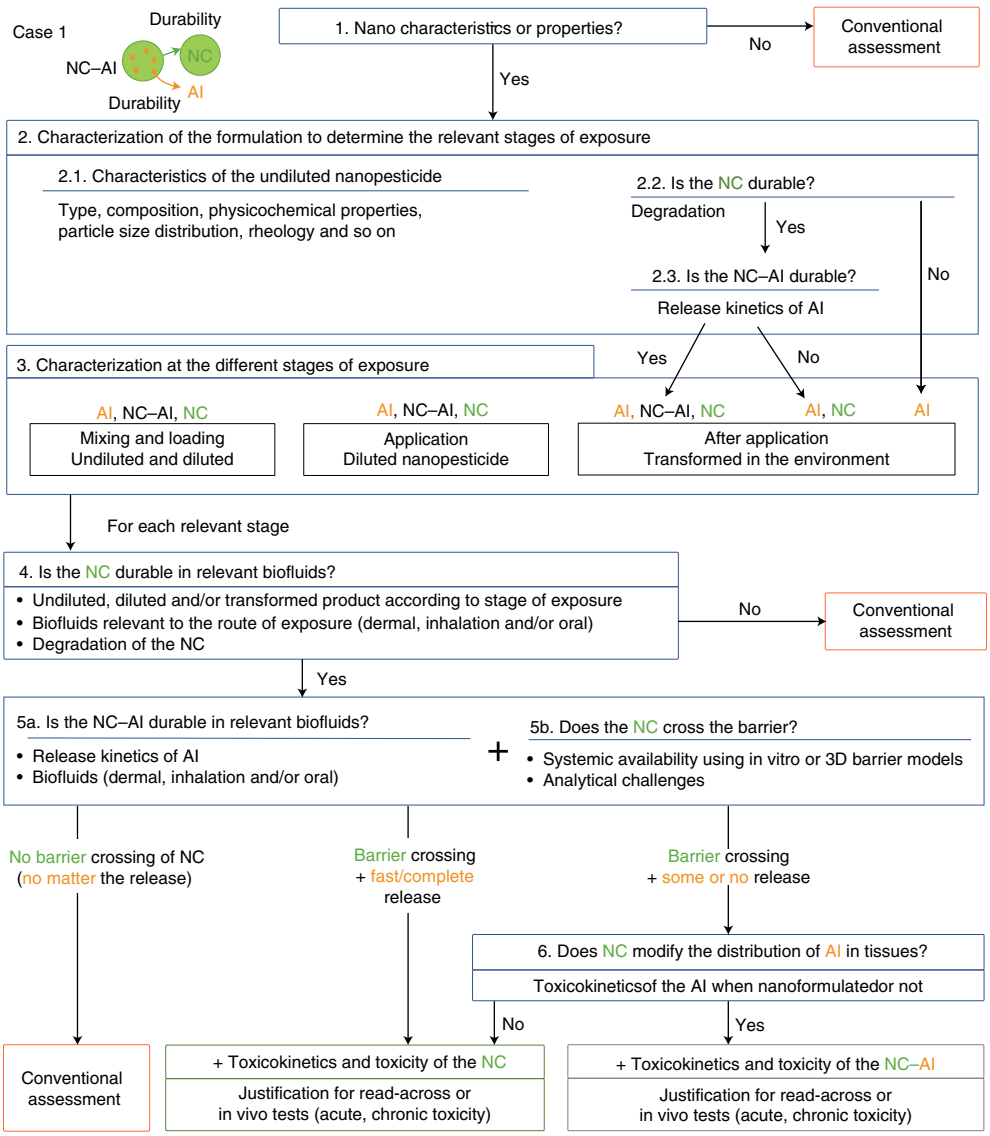

Fig. 2 | Decision tree considering case 1: a polymer NC associated with a pesticide AI. Steps 1–3 aim to determine the characteristics and species of nanopesticides relevant to the three stages of human exposure (who is exposed to what and when?). For each relevant stage, steps 4–6 help to identify the requirements for toxicokinetic and toxicity data to support human health risk assessment.

pesticides. Figure 1 summarizes the three key stages of potential human exposure to pesticides: (1) mixing–loading, (2) application and (3) after application. Each stage involves different forms of the pesticide (for example, concentrated, diluted, transformed in the field), and exposed population (professional, residential). Occupational exposure can be mitigated through the use of adequate personal protection equipment, whereas mitigation strategies to reduce non-occupational exposure (including bystanders, residents and consumers) are limited. Dermal, inhalation and ingestion exposure are all relevant to each stage, but depending on the type of pesticide and its intended use one of the routes may become of major concern and require more detailed investigations. Identifying scenarios that may lead to exposure to a specific pesticide is an important stage of the problem formulation.

Hazard identification and characterization are performed by using results from toxicology studies, typically in vivo but increasingly via alternative approaches including in vitro and in silico. Safety factors are applied to account for uncertainty and variability (for example, normal versus impaired individuals). The requirements for the AI include toxicokinetic studies (absorption, distribution, metabolism and excretion, both intravenous and oral), acute systemic toxicity (oral, dermal, inhalation), skin and eye irritation, skin sensitization, short-term and long-term toxicity, genotoxicity (in vitro and in vivo), carcinogenicity and reproductive and developmental toxicity as well as other endpoints (for example, neurotoxicity and immunotoxicity)[16]. Similar data requirements exist in the European Union and Canada for some coformulants (that is, safeners and synergists) but not for others (for example, inerts, excipients). For formulations (AI + coformulants), the toxicity data requirements are currently limited to acute systemic toxicity (oral, dermal, inhalation), skin and eye irritation, skin sensitization and sometimes dermal absorption[25,26]. The required toxicology studies to support pesticide registrations are broadly similar in the European Union[25,27,28], Canada[29] and the United States[26]. Finally, the exposure and hazard assessments are used together to characterize the overall risk associated with the use of a pesticide product. If the risk is considered acceptable, the results from the assessment are also used to define a set of detailed instructions appearing on the label (for example, dose, mode of application, protective equipment), which aim to minimize exposure and ensure safe use.

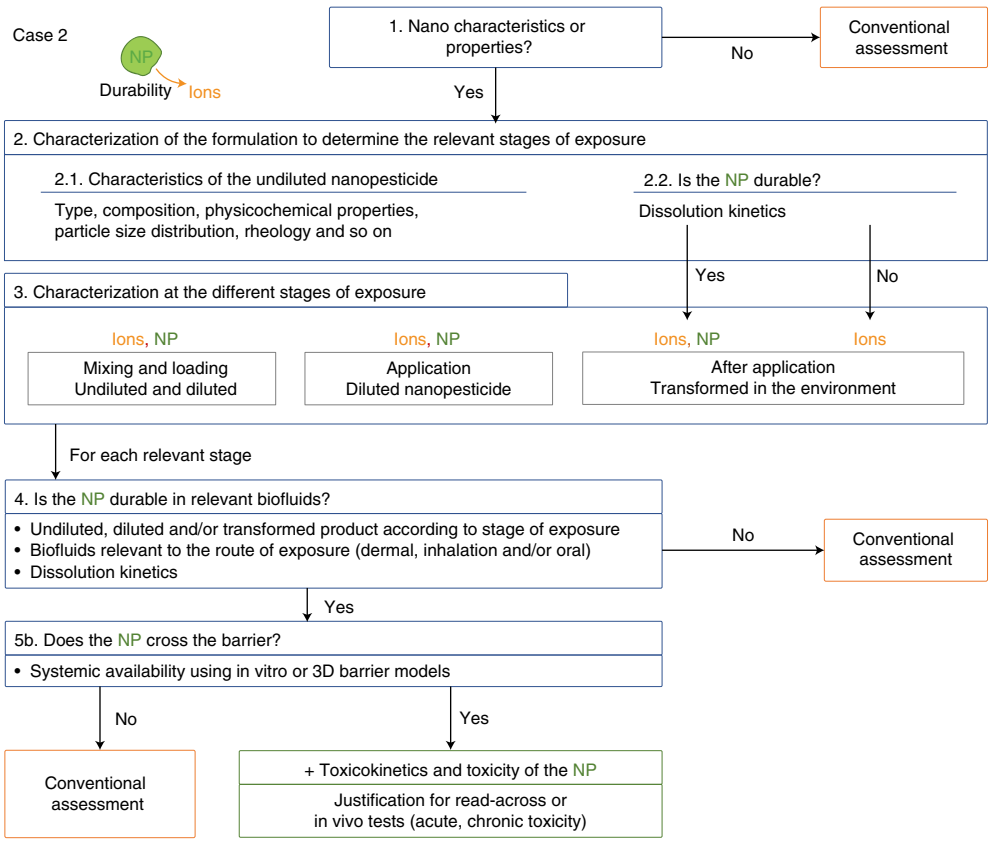

Fig. 3 | Decision tree considering case 2: metal or metal oxide NPs releasing ions over time. Steps 1–3 aim to determine the characteristics and species of nanopesticides relevant to the three stages of human exposure (who is exposed to what and when?). For each relevant stage, steps 4–6 help to identify the requirements for toxicokinetic and toxicity data to support human health risk assessment.

Rationale for adapting the existing framework to nanopesticides

The majority of nanopesticides proposed until now consist of reformulations of existing AIs that are already authorized for use and that have gone through the human health risk assessment described above. Other nanopesticides aim to deliver novel AIs (for example, RNA interference, natural substances) or explore new functionalities of inorganic elements (for example, Cu, Zn) when they are in NP form[2]. In all cases, the nano character of the nanopesticides can substantially affect their fate, biointeractions and effects on human health. Such nanospecific aspects can be easily missed when applying the conventional risk assessment framework. The properties, fate and effects of nanopesticides cannot be assumed to be similar to those of a conventional pesticide, even when the individual ingredients of the formulation are already considered safe on their own[16]. It is thus essential that additional and suitable tests are conducted to ensure the robust risk assessment of nanopesticides.

Considering the range of nanopesticides currently at various stages of development, two relevant case studies covering a range of properties were considered to develop and test the proposed framework for human health risk assessment (illustrations in the top left corners of Figs. 2 and 3): the two case studies represent two of the more commonly discussed types of nanopesticide[2].

Case 1 is an NC system for the slow release of a pesticide AI, for example, a polymer NC[30] releasing an AI (for example, an insecticide or herbicide) over time after application to soil or foliage. Case 1 (NC–AI complex) includes examples where the AI is either encapsulated within or entrapped by the polymer NC. The characterization methods developed up to now for metal and metal oxide NPs may not be applicable for nanoscale polymers. In addition, the degradability of the NC itself has to be determined. There are three entities to track during the assessment: (1) the NC–AI complex, which is likely to dominate in the early stage of exposure (mixing–loading and application), (2) the empty NC and/or its degradation products remaining after the complete release of the AI and (3) the released AI. In our case study, the latter may be considered to behave similarly to the conventional pesticide AI, keeping in mind that exposure patterns in space and time may be different relative to an AI applied as a conventional formulation, as highlighted in previous work on ecological risk assessment[17,18].

Case 2 is an NP made of a pure, nanoscale AI (for example, metal or metal oxide NPs) stabilized with, for example, salts, surfactants or polymers. The application of similar products has been proposed to suppress pathogen infections[2,31]. For this case study, there are two entities to track: the metal or metal oxide NP and the released ions. In some cases the effects of stabilizers may also require assessment. In the environment, the dissolution kinetics of some materials (for example, copper oxide) is generally considered to be slow compared with zinc oxide or silver NPs. However, dissolution may be substantially faster in biological media[32,33].

Nanoformulation of a pesticide may decrease or increase the risk to human health. Often, the quantity of AI applied in the field can be reduced for nanoformulations (case 1) or nanoscale AI (case 2), thereby reducing the level of (external) exposure to the AI. However, at the same time, the dose reaching a specific target organ (that is, internal dose) might increase, for example, by altering the penetration of the AI through biological barriers (also relevant for cases 1 and 2). Thus, both effects must be taken into account carefully. Note that the term ‘dose’ requires careful consideration for nanotoxicology studies, since mass, particle number and surface area metrics

are used. It is also important to distinguish the characteristics of the nanopesticide at different stages of exposure, as a range of transformation processes resulting from exposure to environmental conditions can substantially affect toxicological responses.

In line with previous conclusions on the ecological risk assessment of nanopesticides[17,18], the existing general human health risk assessment paradigm can be applied to nanopesticides. However, additional data on novel properties may be required and testing methods may need some adaptations[16]. For instance, one additional requirement when dealing with nanopesticides is related to the simultaneous occurrence of three processes with different kinetics that determine the species of nanopesticides and their concentrations, all of which are relevant for toxicity testing: (1) the degradation of the AI, (2) the degradation of the NC (case 1) or NP (case 2) and (3) the degradation or dissociation of the NC–AI complex (case 1). These three processes and their kinetics determine the different entities to which humans may be exposed (that is, the AI, the NC/NP and/or the NC–AI complex) at different stages of pesticide use (Fig. 1). Transformation processes of the AI, NC, NP and NC–AI complex (for example, dissolution, hydrolysis, formation of corona and so on) should also be considered when characterizing exposure and testing toxicity, especially in a postapplication scenario.

When dealing with AI or NC/NP systems that have already been assessed and are thus data rich, performing comparative exposure assessments may allow some degree of bridging and read-across, especially for acute toxicity tests[16,34,35]. In the framework below, we identify a sequence of steps that aim to guide risk assessors in the identification of necessary additional testing. When possible, existing data should be used to avoid unnecessary animal use for toxicity testing.

Overview of the framework

The decision trees presented in Figs. 2 (case 1) and 3 (case 2) were constructed by systematically considering the additional factors and processes that may need to be taken into account when assessing the risk of nanopesticides to human health. The stepwise approach is briefly presented below, while more details on the methods proposed and challenges are presented in the following sections. As the vast majority of pesticide formulations are liquid, only nanopesticides supplied as a liquid formulation are considered.

The first step of the decision tree is a determination on whether the novel product requires additional assessment relative to a conventional pesticide. The procedure and criteria are expected to differ according to the jurisdiction. In some cases a declaration by the applicant is sufficient to determine whether additional investigations are needed. However, in other cases a decision will be based on whether the material meets the definition of a nanomaterial in the relevant legislation. Nanomaterial definitions typically specify a size range but may also include other factors. For example, EFSA[16] requires physical chemical characterization to determine whether the material meets the nanomaterial definition in the European Union regulation and therefore requires a series of additional in vitro and in vivo tests. However, additional testing may also be required in cases where a material does not meet the definition (for example, having size above the order of 100 nm) but does display properties characteristic of the nanoscale. If the initial response to question 1 indicates that additional assessments are warranted, then the various questions in Figs. 2 and 3 should be considered. Following characterization of the undiluted nanopesticide (step 2.1), the durability of the NC/NP should be determined (step 2.2). If degradation/dissolution is very rapid on the timescale of mixing–loading or application, postapplication assessment will only be required for the AI/ion and possibly NC degradation products. For case 1, the association between the NC and AI should also be characterized to determine whether the NC–AI complex is durable during the mixing–loading, application and postapplication stages (step 2.3). The results from the durability tests will identify the species (AI/ion, NC–AI, NC/NP) that must be considered and characterized for each of the three exposure stages (step 3). As an example, an NC–AI complex that releases AI rapidly (that is, complete release during the time required for application) will not require further assessment at the postapplication stage. However, for many materials it is likely that a mixture of intact and dissolved or transformed material will have to be considered.

Figures 2 and 3 also outline the necessary steps to consider if human exposure to the NC, NP or NC–AI complex is likely to occur. Steps 4–6 should be considered for each relevant entity and stage of exposure identified in Figs. 2 and 3 (that is, AI/ion, NC–AI and/or NC/NP as undiluted, diluted and/or transformed in the environment). First, the durability of the NC/NP (step 4) is evaluated in a relevant medium for the application route (dermal, inhalation and/or oral). If the NC/NP degrades rapidly, for case 1 a conventional assessment of the AI is sufficient and for case 2 we can rely on data existing for the ions. If the NC/NP does not degrade rapidly, then two additional considerations must be taken into account. The first only applies to case 1 and is the durability of the NC–AI complex in biological media representing the relevant exposure pathways (dermal, inhalation and/or oral, step 5a). The second (step 5b) applies to both cases 1 and 2, and is whether the NC/NP can cross a biological barrier (for example, dermal). Note that here it is assumed that the NC and NC–AI complex will have similar barrier-crossing capabilities. This is likely to hold true provided that the AI does not substantially modify the size, shape or surface charge of the NC, which are the key properties that influence the crossing of particles through dermal[36], intestinal[37] and pulmonary barriers[38]. If no barrier crossing occurs, then a conventional assessment of the AI should be sufficient as we then assume that only the AI/ion alone will be able to penetrate the barrier. If the barrier crossing occurs and the rate of release/dissolution is slow, then the impact of the NC on the toxicokinetics of the AI needs to be investigated for case 1 (step 6, Fig. 2). If the toxicokinetics of the AI are not affected by the NC, then the conventional assessment for the AI must be complemented by a nanospecific assessment of the NC (green box in Fig. 2). In cases where the toxicokinetics of the AI are modified by the NC (for example, increased amount of AI in tissues), the toxicity of the NC–AI complex also needs to be investigated (black box in Fig. 2). Both acute and chronic toxicity testing may be required, unless justification for read-across to existing toxicological data is available.

It should be noted that case 2 (metal or metal oxide NP) is slightly simpler than case 1 (polymer NC). Dissolution tests in biofluids can provide information to estimate the durability of the NP (step 4) and to inform on release kinetics of the ion (step 5a). If the NP dissolves rapidly or it does not cross biological barriers, a conventional assessment focusing on the ion toxicity should be sufficient. Barrier crossing combined with slow dissolution requires toxicological testing of the NP in relevant acellular or cellular models (green box in Fig. 3).

Key questions and associated methods and limitations

The sections below detail the key steps of the framework presented in Figs. 2 and 3, and summarize the methods and approaches required.

Step 2.1. Characterization of the undiluted nanopesticide formulation. Currently, physicochemical characterization is required for the AI but is typically not expected for coformulants that are not safeners or synergists[16]. For nanopesticides, the undiluted nanopesticide formulation should be characterized in all cases (step 2.1) and a rationale for developing the novel product is also typically requested at this stage. In addition to describing the type of formulation (for example, encapsulation, nanodispersion, emulsion),

the ingredients and their proportions, the physicochemical properties of the formulation should be thoroughly characterized. The required properties depend on the type of nanopesticide and its intended use. Characterization will typically include assessment of properties such as chemical composition, crystal structure (where relevant), primary particle size, shape and aspect ratio, which are typically independent of the medium. A range of properties such as surface charge, surface chemistry, dissolution and agglomeration/aggregation level must also be assessed. Those depend on the properties of the medium in which they are placed and on time and environmental conditions such as temperature and sunlight. A clearer correlation with exposure and hazard assessment is often observed for medium-dependent properties[39].

Standards developed by ISO and the ongoing development of new (or adaptation of existing) test guidelines by OECD[40] should be applicable for characterization in some cases. However, it should be noted that many of these methods may require modification when dealing with soft polymer NCs (case 1). Several recent reviews[20,41–43] summarize the key properties for risk assessment for nanomaterials and the recommended analytical methods, along with a critical evaluation of their range of applicability, advantages and limitations. A different combination of techniques may be required to characterize different nanopesticides[14]. Some methods have specific concentration requirements that may require dilution of the formulation before measurement, and this should be done with a diluent relevant to the mixing–loading stage, which may have a different composition from the initial formulation (for example, added salt or dispersants).

Step 2.2. Durability of the NC/NP in environmental media. The durability of the NC (case 1) or NP (case 2) should be determined using degradation or dissolution assays. It should be noted that the NC/NP is unlikely to be unstable in the initial formulation. However, there may be cases where the NC/NP is designed to facilitate mixing–loading and application but to then degrade rapidly after application. Assessing durability in the diluted formulation can minimize testing at the postapplication stage and in biological media.

For case 1, NCs may be made of naturally occurring polymers such as polysaccharides and proteins that are easily degraded in the environment or biological tissues[5,30,44]. The use of these materials is likely to be of minimal concern for adverse side effects. Other NCs are made of synthetic polymers that are used for encapsulation, and these NCs may persist for an extended time period after application. The degradation of many of these polymers will have been studied in some environmental media and possibly also in biological fluids, although not necessarily in their nanoform[45]. Options for bridging and read-across from existing data should be considered when possible. For polymer NCs for which the persistence is unknown, studies of carbon dioxide release from isotopically labelled polymer can be used to estimate persistence, as, for example, in studies of biodegradation of polyacrylates in soil[46]. Many available studies have not focused specifically on polymer NCs, and some adaptation of existing methods used for larger polymer particles and films may be required.

For case 2, measurement of dissolution kinetics will be required. Methods for assessing dissolution rates for metal and metal oxide NPs are well established and generally rely on measuring ionic concentration in the aqueous phase using inductively coupled plasma–atomic emission spectroscopy (ICP-AES) or mass spectrometry (ICP-MS)[19,47,48]. The use of a continuous flow-through system is preferable to a static system with a restricted volume of fluid that may lead to saturation of the sample with one of the solutes, preventing further dissolution. Considerable literature data are available for metal and metal oxide NPs. It is important to note that dissolution rates are sensitive to the concentration of the material, the presence of additives (for example, salt) and pH, potentially requiring measurement under conditions relevant to the formulation, as well as environmental and biological samples. Dissolution measurements will be more challenging under postapplication conditions where the ion concentration may be low, the composition of the environmental sample (soil, water and so on) is not well known and contamination by naturally occurring ions may be an issue. In such cases the use of simple soil models may prove adequate (for example, ref. [49]); otherwise, indirect lines of evidence may be necessary. It is important to note that exposure to environmental conditions is more likely to cause degradation and transformations for pesticides than for other nanoenabled products.

Step 2.3. Durability of the NC–AI complex (case 1 only). For case 1, determination of AI release kinetics will indicate whether the NC–AI complex is durable during the mixing–loading, application and postapplication stages. Release kinetics from a nanoformulation before and after dilution can be assessed by adapting methods currently employed to measure release from polymer NCs used for drug delivery. Most approaches rely on separation of the free and complexed drug by methods such as ultrafiltration, centrifugation, dialysis or continuous flow, followed by quantification of residual free drug in the filtrate or supernatant using standard analytical techniques such as high-performance liquid chromatography or gas chromatography–mass spectrometry[50–52]. A separation method that does not disturb the integrity of the NC or perturb the equilibrium between free and bound AI should be selected. Careful method validation and well designed controls are essential to account for the effect of dilution on the release of the AI and the potential adsorption of the AI on the filtration/centrifugation device. Analytical ultracentrifugation is a promising alternative to separation methods; it does not require a previous separation step and can detect and quantify the unbound material by changes in UV–visible absorbance or refractive index[52].

The methods described above can also be adapted to examine NC–AI durability in some simple soil models, which are needed in step 3. However, measuring release kinetics of AI under realistic field conditions (after application) is very challenging, and indirect methods should also be considered to demonstrate whether the association is durable or not (see, for example, refs. [53,54]). Examination of release of AI in biological media should be feasible with the same tests, taking into account the possibility of complications due to any of the components (see step 5a). As noted above, it is important to consider that rapid release kinetics upon dilution or application means that the NC–AI complex as a whole will not require assessment in the postapplication scenario. Examples of this would be cases where the NC is used to facilitate mixing–loading or application but not to further modify the AI behaviour.

Step 3. Comprehensive characterization at the three stages of human exposure. The characterization methods described for steps 2.1–2.3 can generally be applied to determine the key characteristics of the nanopesticides at the first stage of human exposure (that is, undiluted and diluted nanopesticide). Additional aspects relevant at the mixing–loading and application stages include the formation of degradation products and aerosols, which require assessment of exposure through inhalation. For the postapplication stage, the nanopesticide transformed in the environment (residues on crop surface, soil, groundwater, possible transfer to meat, fish and so on) must also be characterized, and investigations into the formation of dislodgeable residues on crops or dust may be required.

It should be noted that, if the durability (that is, kinetics of degradation/release/dissolution) is sufficiently short during the mixing–loading and application stages, postapplication investigations are only needed for the AI or ion, leading to a conventional assessment and read-across if relevant data already exist. For nanopesticides

with slow transformation kinetics, investigations into the environmental fate of the NC–AI complex and the NC itself may be necessary to determine the relevant exposure levels and characteristics. The detection, characterization and quantification of nanopesticides after application in the field is currently limited by the lack of adequate analytical techniques, especially for case 1. Although the total residues of AI can be determined with conventional methods, determining whether the AI is still associated with the NC or is in a nanoform, and whether transformation processes have occurred, is very challenging[55].

Steps 4 and 5a. Characterization of nanopesticides in relevant biofluids. The biodurability of the NC/NP (step 4) and NC–AI (step 5a, for case 1 only) refers to the dissolution, enzymatic degradation or chemical disintegration of the nanopesticide[47]. It can be assessed with acellular in vitro assays that utilize simulated biological fluids to identify cases for which it will be necessary to obtain toxicokinetic and toxicity data for the nanopesticide. These measurements will identify cases for which the durability of the NC/NP and NC–AI is sufficiently short that one can rely on pre-existing data for the AI or ion. Many of the analytical challenges mentioned earlier for environmental media (steps 2.2, 2.3 and 3) are also relevant for biofluids[56].

A range of model biological fluids have been used for dermal, inhalation and oral exposure routes[19]. For inhalation, two lung compartments should be considered: the fluid in the extracellular airway lining to which the particles are initially exposed and the phagolysosomal fluid found in alveolar macrophages, which rapidly scavenge inhaled particles. Models for both are based on the original Gamble solution (a neutral electrolyte solution with added glycine) and an acidified analogue that better mimics the phagolysosomal environment. Oral exposure can be assessed using saliva, gastric fluid (acidic) and intestinal (neutral) fluid models that typically contain a mixture of salts and several enzymes), although it should be noted that the composition of each of these fluids varies considerably as a function of time and diet. Dermal exposure models include simulated sweat formulations and simulated sebum formulations. A compilation of simulated biological fluids that have been used for durability studies is available in an ISO technical report[19]; this report summarizes several examples from the literature where model biofluids have been used to assess nanomaterial dissolution. A more detailed summary of available studies on nanomaterial dissolution in model biofluids is provided in an OECD report[47]. It is important to keep in mind that, although these simulated models have been shown to provide reasonable mimics for human biofluids, they have defined compositions that do not match the dynamic in vivo conditions and typically do not include the range of in vivo enzymes and proteins that may modify nanomaterial behaviour.

Step 5b. Barrier crossing by the NC/NP (systemic availability). If the NC/NP does not degrade/dissolve (for instance, >12% persistent[16]), it is important to establish whether it can cross biological barriers at the site of exposure and enter the human body. Human biological barriers, particularly those that are directly exposed to the surrounding environment (skin, lungs and gastrointestinal tract) have evolved to protect us from infection, bacteria and parasites. However, entities on the nanoscale have the potential to penetrate deeper into the human body, and persistent nanomaterial may remain in biological compartments for extended periods of time[57]. Thus, NCs/NPs that are both biopersistent and able to enter the systemic circulation require more rigorous hazard evaluation to understand their long-term impact on human health. Evaluation of bioavailability is a priority for assessing potential toxicity, independent of the exposure route and endpoint[58].

Measurement of the absorption or penetration of an exogenous agent across the skin is evaluated according to the OECD Test Guideline 428: Skin Absorption: In Vitro Method[59]. This approach is used for the evaluation of pesticides, biocides and other industrial chemicals applied as formulations to human or animal skin preparations. The guideline is very broad, and additional guidance on the application of this method and reduction of variability in data sets has been provided by EFSA[60]. However, this document specifically states that "the issue of nanoformulations in plant protection products is not addressed", and thus evaluation of nanopesticides should be performed on a case-by-case basis.

Three-dimensional (3D) and advanced cell culture systems for a variety of human tissues are commercially available or can be grown in standard tissue culture laboratory facilities. These in vitro tools exhibit more natural cell–cell contacts, improved metabolic activity and transcriptomic profiles that more closely represent the in vivo situation[61]. The importance of 3D reconstructed skin models for risk assessment is prominent for cosmetics hazard assessment, where testing in vivo is prohibited[61]. Skin-equivalent models typically consist of a fully differentiated and stratified epidermal barrier that closely resembles normal human skin with a dry surface stratum corneum. Thus, exogenous test materials are applied to the skin model in a similar manner to human dermal exposure. The skin irritation and corrosion endpoints are now evaluated in vitro using 3D reconstructed skin-based protocols (for example, OECD Test Guidelines 439[62] and 431[63]). International validation efforts to facilitate genotoxicity testing in 3D reconstructed skin models for chemicals have also been expanded to nanomaterials[61,64]. Interestingly Wills and colleagues[64] demonstrated the utility of the 3D reconstructed skin model for evaluation of dermal barrier penetration of silica NPs using transmission electron microscopy (TEM).

Other biological barriers of importance are those presented by the pulmonary system and gastrointestinal tract, following inhalation and/or ingestion of the nanopesticide, respectively. For the lung, advanced coculture systems that include multiple pulmonary cell types are grown at the air–liquid interface, supporting exposure to aerosols[65,66]. More complex 3D culture systems and 3D in vitro respiratory tissue models are also commercially available[67,68]. These models have been applied for characterizing nanomaterial toxicity and present advantages over standard two-dimensional cell culture, as they allow for the detection of damage mechanisms such as those associated with chronic inflammation that usually only arise in vivo[69]. Similarly, triple-culture models of the gastrointestinal tract have been developed, which actively produce mucins[70,71]. Barrier penetration can be assessed in these pulmonary and gastrointestinal tract in vitro coculture systems, by exploring the passage of materials across the model system when applied to the top surface using imaging (for example, TEM) or chemical analysis techniques (for example, ICP-MS)[66,69,72,73].

While the above methods for assessing barrier crossing are suitable for studies of many NPs (case 2), NCs made of polymers (case 1) are very challenging to detect and track in biological tissues using conventional imaging techniques (TEM, scanning electron microscopy), mainly due to the lack of contrast as both carrier and tissues are carbon-rich materials. The Wills study[64] that used advanced TEM imaging modes to study uptake of silica NPs in 3D dermal models illustrates the challenges, even for NPs that are detectable by TEM without staining. Radiolabelling approaches (for example, ^{14}C) can also be used to detect NC in biological matrices, but they must be used with care and are expensive and time consuming. Producing labelled NC is often impossible at an industrial scale, as this implies an excessive use of radioactive label. Smaller-scale production processes can lead to NCs that have different properties than the one for which regulatory authorization is required. Fluorophore labelling and fluorescence imaging are attractive alternatives, but it is important to ensure that the added fluorophore remains attached to the NC during the barrier-crossing study. Furthermore, labelling the NC may alter its fundamental physicochemical characteristics,

thereby modulating its barrier penetration capacity. In some cases, step 5b may thus be best addressed using indirect lines of evidence to indicate whether barrier crossing occurs.

It is important to understand if NC/NPs have the ability to cross biological barriers at the potential sites of exposure using in vitro approaches, such as those described above. If step 5b demonstrates that there is no barrier crossing of the NC (case 1) or NP (case 2), then a conventional assessment of the AI or ion(s) should be sufficient, regardless of the release/dissolution kinetics. If the NC/NP can cross biological barriers, then the conventional assessment must be complemented by characterization of the toxicokinetics and toxicity of the NC or NP.

Step 6. Modification of the AI tissue distribution by the NC (case 1 only). At this stage of the framework, we have demonstrated that the NC can cross relevant biological barriers, while it is still associated with some AI (no or incomplete release as determined in step 5a). Toxicokinetic tests should be conducted to investigate whether the nanoformulation modifies the distribution of the AI (case 1) in different organs and the possibility that it may increase the concentration of AI in certain tissues. Change in the toxicokinetic behaviour was previously recognized by the EFSA as an important trigger for nanospecific assessment for nanopesticides with size range above 100 nm (ref. [16]). The current OECD Test Guideline 417 on in vivo toxicokinetics is not applicable to nanomaterials[74]. The underlying processes determining the toxicokinetics of NPs require a dedicated test design[75] and several projects are currently developing a new guideline adapted to nanomaterials[40,58].

When considering the AI in case 1, however, using Test Guideline 417[74] can already be very informative at this stage. For instance, comparing the toxicokinetics of the nanoformulated AI and a conventional formulation (or the unformulated AI) will indicate whether the nanoformulation modifies the distribution of the AI in animals. If not, toxicity tests carried out on the NC and AI separately may be sufficient. If it does, toxicokinetic and toxicity tests (acute and chronic) for the NC–AI complex are also needed, unless read-across from existing data is deemed acceptable.

Toxicity testing. According to the results obtained from steps 1–6, three levels of toxicity assessment may be required for nanopesticides.

1. The simplest case corresponds to the conventional toxicity assessment currently applied to pesticides and focusing on the AI or ion (orange box in Figs. 2 and 3). The current assessment is warranted for products that do not fall within the regulatory definition of nanopesticides, and nanopesticides whose NCs/NPs do not cross biological barriers or NPs (case 2 only) that are not durable (that is, rapid dissolution).
2. Additional toxicokinetic and toxicity testing of the empty NCs or NPs using adapted protocols are required when barrier crossing occurs.
3. In cases similar to case 1 where the nanoformulation modifies the toxicokinetics of the AI, toxicological studies of the NC–AI complexes are also required.

For levels 2 and 3, toxicokinetic and toxicity testing need to consider nanospecific aspects (for example, dispersion, agglomeration/aggregation), and this may require adaptation of the protocols that have been developed and validated for solubilizable substances. Some tests (for example, toxicokinetics) are currently being adapted through OECD programmes, and a new toxicokinetics guideline is being developed. For others, guidance with respect to possible modifications of existing tests is presented in ref. [16], and they often require case-by-case considerations based on expert judgement. For in vitro studies, data on stability are essential to ensure that exposure levels are maintained during the test to avoid false negative results due to, for example, sedimentation or agglomeration.

Knowledge gaps and concluding remarks. As discussed under various sections above, there is a range of limitations in the current state of knowledge for human health risk assessment of nanopesticides. Some of these limitations are briefly described below to provide an impetus to future work in this area. Several organizations (for example, OECD, EFSA) active in this space have ongoing work to address these gaps, to some extent. For example, new OECD test guidelines for nano-relevant properties are currently being developed[40].

- The framework has been largely developed on the basis of two large classes of nanopesticide products (that is, polymer NC–AI complexes and inorganic metal or metal oxide NPs). In due course, different types of nanopesticide (for example, dendrimer technology, solid formulations) may emerge, warranting additional considerations.
- Many nanopesticides are based on complexation of an AI with an NC (for example, a polymer). Such nanopesticides will require characterization of the amount of AI incorporated into the carrier and the kinetics for its release. Methods for the reliable measurement of release kinetics are currently limited. The methods employed for nanomedicines (polymer–drug complexes) may allow adaptation for nanopesticides.
- Tests and procedures employed in risk assessment of conventional pesticides, while being useful, may be insufficient for nanopesticides. New tests are likely to be needed, depending on the nature of nanoproducts and as the experience with such products grows. Current adaptations of toxicokinetic and toxicity tests for nanomaterials are largely based on inorganic materials (case 2) and thus may not be suitable for nanopesticides represented by case 1.
- Many formulations currently contain relatively large amounts of inerts, including nanoforms of silica (typically used as a rheology modifier). These and other non-nano inerts can potentially (unintentionally) alter the bioavailability of the pesticide.
- While it is recognized that the biological environment can alter the physicochemical characteristics of nanomaterials and consequently their toxicological response, such potential changes are currently not well understood for a range of nanopesticides.
- Validated approaches to detect and track nanomaterials in biological matrices are currently lacking, and the detection of polymer NC complexes will present even more challenges than that of inorganic NPs. Potential use of indirect approaches to collect evidence should be explored. For example, a comparative assessment of a nanopesticide with non-nano or unformulated product may help discern the effect of excipients (inerts) on the fate of the chemical. An early dialogue between applicants and regulators may help design scientifically sound and relevant experiments for such purposes.

We have noted that regulatory agencies use different definitions for nanopesticides, and some also take into account parameters in addition to size for identification of nanopesticides. Independent of the criteria applied to decide whether a nano assessment is needed, the framework that we have developed can be applied to all cases where nanotechnology has been harnessed to offer novel formulation properties that lead to desirable outcomes (for example, targeted delivery, greater efficacy, lower environmental footprint—to name a few).

New guidelines and guidance documents will certainly have to be established to deal with nanopesticides. However, existing knowledge on assessing conventional products plays a valuable role in their development. In some cases, conventional AIs are being

reformulated (for example, hydrophobic pyrethroids in a hydrophilic carrier to facilitate their targeted delivery), and knowledge about the fate and behaviour of the AIs may thus already exist. In such cases, some of the data and information required to employ the above framework may already be available. Additionally, using bridging arguments or read-across from conventional to nanoformulations can help to speed up the regulatory process.

One of the key benefits intended from this framework is a better understanding of the portfolio of data and information required for sound assessment of human health risk that regulators will expect industry to provide. A clear communication between the two parties is therefore expected to be mutually beneficial. We hope this framework will facilitate an early dialogue between the regulators and industry to develop strategies to adequately address the regulatory requirements for emerging nanopesticides. In addition to focusing on the technical potential of novel nanopesticides, developers should consider the contextual applicability of the products at the earlier stages of development; this includes how the products can be assessed for regulatory authorization[76]. The application of more holistic evaluation tools such as life-cycle assessment[77] or One Health approaches[78] can also help maximize the benefits of nanopesticides relative to conventional products.

Received: 20 January 2021; Accepted: 23 July 2021;
Published online: 13 September 2021

References

1. FAO *The Future of Food and Agriculture: Trends and Challenges* (2017); https://reliefweb.int/report/world/future-food-and-agriculture-trends-and-challenges
2. Kah, M., Tufenkji, N. & White, J. C. Nano-enabled strategies to enhance crop nutrition and protection. *Nat. Nanotechnol.* **14**, 532–540 (2019).
This review summarizes current challenges in crop nutrition and protection, and the possible solutions offered by nanotechnology.
3. Adisa, I. O. et al. Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. *Environ. Sci. Nano* **6**, 2002–2030 (2019).
4. Kah, M., Kookana, R. S., Gogos, A. & Bucheli, T. D. A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat. Nanotechnol.* **13**, 677–684 (2018).
A critical evaluation of nanofertilizers and nanopesticides against their conventional analogues indicates that lack of information on the efficacy and environmental impact of nanoagrochemicals under field conditions is a critical knowledge gap.
5. Camara, M. C. et al. Development of stimuli-responsive nano-based pesticides: emerging opportunities for agriculture. *J. Nanobiotechnology* **17**, 100 (2019).
6. Singh, H. et al. Recent advances in the applications of nano-agrochemicals for sustainable agricultural development. *Environ. Sci. Process. Impacts* **23**, 213–239 (2021).
7. Mitter, N. et al. Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nat. Plants* **3**, 16207 (2017).
8. Health Canada *Policy Statement on Health Canada's Working Definition for Nanomaterial* (2011); https://www.canada.ca/en/health-canada/services/science-research/reports-publications/nanomaterial/policy-statement-health-canada-working-definition.html
9. Miernicki, M., Hofmann, T., Eisenberger, I., Kammer, Fvonder & Praetorius, A. Legal and practical challenges in classifying nanomaterials according to regulatory definitions. *Nat. Nanotechnol.* **14**, 208–216 (2019).
The current limitations of the European Union definitions for 'nanomaterial' are outlined along with recommendations for a more coherent approach to classifying nanomaterials for regulatory purposes.
10. US EPA *Control of Nanoscale Materials under the Toxic Substances Control Act* (2015); https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/control-nanoscale-materials-under
11. Boverhof, D. R. et al. Comparative assessment of nanomaterial definitions and safety evaluation considerations. *Regul. Toxicol. Pharmacol.* **73**, 137–150 (2015).
12. Etheridge, M. L. et al. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine* **9**, 1–14 (2013).
13. Kah, M. Nanopesticides and nanofertilizers: emerging contaminants or opportunities for risk mitigation? *Front. Chem.* **3**, 64 (2015).
14. Bocca, B. et al. Nanopesticides: physico-chemical characterization by a combination of advanced analytical techniques. *Food Chem. Toxicol.* **146**, 111816 (2020).
15. Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. & Polasky, S. Agricultural sustainability and intensive production practices. *Nature* **418**, 671–677 (2002).
16. Hardy, A. et al. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. *EFSA J.* **16**, e05327 (2018).
This European Food Safety Authority guidance document provides detailed information on the physical chemical characterization and toxicological testing required for risk assessment of the impact of nanoscience and nanotechnology applications in the food and feed chain on animal and human health.
17. Kookana, R. S. et al. Nanopesticides: guiding principles for regulatory evaluation of environmental risks. *J. Agric. Food Chem.* **62**, 4227–4240 (2014).
This paper presents the framework for ecological risk assessment of nanopesticides.
18. Walker, G. W. et al. Ecological risk assessment of nano-enabled pesticides: a perspective on problem formulation. *J. Agric. Food Chem.* **66**, 6480–6486 (2018).
This perspective article summarizes the relevant considerations for problem formulation in the ecological risk assessment of nanoenabled pesticides.
19. ISO *ISO/TR 19057:2017* (2017); https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/06/38/63836.html
20. Gubala, V. et al. Engineered nanomaterials and human health: part 1. Preparation, functionalization and characterization (IUPAC Technical Report). *Pure Appl. Chem.* **90**, 1283–1324 (2018).
21. OECD *Important Issues on Risk Assessment of Manufactured Nanomaterials (Series on the Safety of Manufactured Nanomaterials No. 33)* (2012); http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)8&doclanguage=en
22. Scientific Committee on Consumer Safety (SCCS) *Guidance on the Safety Assessment of Nanomaterials in Cosmetics* SCCS/1611/19 (Publications Office, 2019).
23. Grieger, K. et al. Best practices from nano-risk analysis relevant for other emerging technologies. *Nat. Nanotechnol.* **14**, 998–1001 (2019).
24. International Programme on Chemical Safety (IPCS) *Principles for the Assessment of Risks to Human Health from Exposure to Chemicals* (1999); http://www.inchem.org/documents/ehc/ehc/ehc210.htm
25. EC *Commission Regulation (EU) No 284/2013 of 1 March 2013 Setting Out the Data Requirements for Plant Protection Products, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market* (2013); https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32013R0284
26. US EPA *Data Requirements for Pesticide Registration* (2013); https://www.epa.gov/pesticide-registration/data-requirements-pesticide-registration
27. EC *Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 Concerning the Placing of Plant Protection Products on the Market and Repealing Council Directives 79/117/EEC and 91/414/EEC* (2009).
28. EC *Commission Regulation (EU) No 283/2013 of 1 March 2013 Setting out the Data Requirements for Active Substances, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market (Text with EEA Relevance)* (2013).
29. Health Canada. Regulatory Directive (DIR2005-01) Guidelines for Developing a Toxicological Database for Chemical Pest Control Products. https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/regulatory-directive/2005/developing-toxicological-database-chemical-pest-control-products-dir2005-01.html (2005).
30. Shakiba, S. et al. Emerging investigator series: polymeric nanocarriers for agricultural applications: synthesis, characterization, and environmental and biological interactions. *Environ. Sci. Nano* **7**, 37–67 (2020).
31. Ma, C. et al. Advanced material modulation of nutritional and phytohormone status alleviates damage from soybean sudden death syndrome. *Nat. Nanotechnol.* **15**, 1033–1042 (2020).
32. Avramescu, M.-L., Chénier, M., Palaniyandi, S. & Rasmussen, P. E. Dissolution behavior of metal oxide nanomaterials in cell culture medium versus distilled water. *J. Nanoparticle Res.* **22**, 222 (2020).
33. Koltermann-Jülly, J. et al. Abiotic dissolution rates of 24 (nano)forms of 6 substances compared to macrophage-assisted dissolution and in vivo pulmonary clearance: grouping by biodissolution and transformation. *NanoImpact* **12**, 29–41 (2018).
34. Health Canada *Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides* (2015); https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/guidance-waiving-bridging-mammalian-acute-toxicity-tests-pesticides.html

35. US EPA *Bridging or Waiving Data Requirements* (2020); https://www.epa.gov/pesticide-registration/bridging-or-waiving-data-requirements
36. Gimeno-Benito, I., Giusti, A., Dekkers, S., Haase, A. & Janer, G. A review to support the derivation of a worst-case dermal penetration value for nanoparticles. *Regul. Toxicol. Pharmacol.* **119**, 104836 (2021).
37. Beloqui, A., des Rieux, A. & Préat, V. Mechanisms of transport of polymeric and lipidic nanoparticles across the intestinal barrier. *Adv. Drug Deliv. Rev.* **106**, 242–255 (2016).
38. Paranjpe, M. & Müller-Goymann, C. C. Nanoparticle-mediated pulmonary drug delivery: a review. *Int. J. Mol. Sci.* **15**, 5852–5873 (2014).
39. Steinhäuser, K. G. & Sayre, P. G. Reliability of methods and data for regulatory assessment of nanomaterial risks. *NanoImpact* **7**, 66–74 (2017).
40. Rasmussen, K., Rauscher, H., Kearns, P., González, M. & Riego Sintes, J. Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data. *Regul. Toxicol. Pharmacol.* **104**, 74–83 (2019).
41. Gao, X. & Lowry, G. V. Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. *NanoImpact* **9**, 14–30 (2018).
Progress towards standardization and validation of methods to characterize the intrinsic and extrinsic properties of nanomaterials for risk assessment purposes is reviewed.
42. Johnston, L. J., Gonzalez-Rojano, N., Wilkinson, K. J. & Xing, B. Key challenges for evaluation of the safety of engineered nanomaterials. *NanoImpact* **18**, 100219 (2020).
43. Rasmussen, K. et al. Physico-chemical properties of manufactured nanomaterials—characterisation and relevant methods. An outlook based on the OECD Testing Programme. *Regul. Toxicol. Pharmacol.* **92**, 8–28 (2018).
44. Sampathkumar, K., Tan, K. X. & Loo, S. C. J. Developing nano-delivery systems for agriculture and food applications with nature-derived polymers. *iScience* **23**, 101055 (2020).
45. Liang, D. et al. Degradation of polyacrylate in the outdoor agricultural soil measured by FTIR-PAS and LIBS. *Polymers* **10**, 1296 (2018).
46. Zumstein, M. T. et al. Biodegradation of synthetic polymers in soils: tracking carbon into CO_2 and microbial biomass. *Sci. Adv.* **4**, eaas9024 (2018).
47. OECD *Assessment of Biodurability of Nanomaterials and their Surface Ligands (Series on the Safety of Manufactured Nanomaterials No. 86* (2018); http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2018)11&doclanguage=en
This OECD report summarizes current in vitro and in vivo methods to measure biodurability of nanomaterials as well as the effects of surface coatings and ligands on dissolution and degradation processes.
48. OECD *Guidance Document for the Testing of Dissolution and Dispersion Stability of Nanomaterials and the Use of Data for Further Environmental Testing and Assessment Strategies* (2020); http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2020)9&doclanguage=en
49. OECD *Test No. 106: Adsorption–Desorption Using a Batch Equilibrium Method* (Organisation for Economic Co-Operation and Development, 2000).
50. D'Souza, S. A review of in vitro drug release test methods for nano-sized dosage forms. *Adv. Pharm.* **2014**, e304757 (2014).
51. European Nanomedicine Characterisation Laboratory (EUNCL) *Verification of Expected Lipid Composition in Nanomedical Controlled Release Systems by Liquid Chromatography–Tandem Mass Spectrometry* EUNCL-PCC-032 (2017).
52. Gioria, S. et al. Are existing standard methods suitable for the evaluation of nanomedicines: some case studies. *Nanomedicine* **13**, 539–554 (2018).
53. Kah, M., Weniger, A.-K. & Hofmann, T. Impacts of (nano)formulations on the fate of an insecticide in soil and consequences for environmental exposure assessment. *Environ. Sci. Technol.* **50**, 10960–10967 (2016).
54. Kah, M., Walch, H. & Hofmann, T. Environmental fate of nanopesticides: durability, sorption and photodegradation of nanoformulated clothianidin. *Environ. Sci. Nano* **5**, 882–889 (2018).
55. Zhang, P. et al. Nanomaterial transformation in the soil–plant system: implications for food safety and application in agriculture. *Small* **16**, 2000705 (2020).
56. Marques, M. R. C., Loebenberg, R. & Almukainzi, M. Simulated biological fluids with possible application in dissolution testing. *Dissolution Technol.* **18**, 15–28 (2011).
57. Oberdörster, G. et al. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol.* **2**, 8 (2005).
58. OECD *Developments in Delegations on the Safety of Manufactured Nanomaterials* (2019); https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2019)11&doclanguage=en
59. OECD *Test No. 428: Skin Absorption: In Vitro Method* (2004); https://www.oecd-ilibrary.org/environment/test-no-428-skin-absorption-in-vitro-method_9789264071087-en
60. EFSA. Guidance on dermal absorption. *EFSA J.* **10**, 2665 (2012).
61. Singh, N., Wills, J. W. & Doak, S. H. in *Nanotoxicology* 248–275 (Royal Society of Chemistry, 2017).
The advantages of 3D cell culture models for in vitro nanotoxicity testing are reviewed, along with an overview of available 3D models to mimic the physiological environment of a variety of tissues and organs.
62. OECD *Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method* (2020); https://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method_9789264242845-en
63. OECD *Test No. 431: In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method* (2014); https://www.oecd-ilibrary.org/environment/test-no-431-in-vitro-skin-corrosion-reconstructed-human-epidermis-rhe-test-method_9789264264618-en
64. Wills, J. W. et al. Genetic toxicity assessment of engineered nanoparticles using a 3D in vitro skin model (EpiDerm™). *Part. Fibre Toxicol.* **13**, 50 (2016).
65. Barosova, H., Drasler, B., Petri-Fink, A. & Rothen-Rutishauser, B. Multicellular human alveolar model composed of epithelial cells and primary immune cells for hazard assessment. *J. Vis. Exp.* **159**, e61090 (2020).
66. Chortarea, S. et al. Repeated exposure to carbon nanotube-based aerosols does not affect the functional properties of a 3D human epithelial airway model. *Nanotoxicology* **9**, 983–993 (2015).
67. Barosova, H. et al. Use of EpiAlveolar lung model to predict fibrotic potential of multiwalled carbon nanotubes. *ACS Nano* **14**, 3941–3956 (2020).
68. Willoughby, J. A. Predicting respiratory toxicity using a human 3D airway (EpiAirway™) model combined with multiple parametric analysis. *Appl. Vitr. Toxicol.* **1**, 55–65 (2014).
69. Evans, S. J. et al. In vitro detection of in vitro secondary mechanisms of genotoxicity induced by engineered nanomaterials. *Part. Fibre Toxicol.* **16**, 8 (2019).
70. Kämpfer, A. A. M. et al. Development of an in vitro co-culture model to mimic the human intestine in healthy and diseased state. *Toxicol. Vitr.* **45**, 31–43 (2017).
71. Ude, V. C., Brown, D. M., Stone, V. & Johnston, H. J. Using 3D gastrointestinal tract in vitro models with microfold cells and mucus secreting ability to assess the hazard of copper oxide nanomaterials. *J. Nanobiotechnol.* **17**, 70 (2019).
72. Clift, M. J. D. et al. A novel technique to determine the cell type specific response within an in vitro co-culture model via multi-colour flow cytometry. *Sci. Rep.* **7**, 434 (2017).
73. Modrzynska, J. et al. In vivo-induced size transformation of cerium oxide nanoparticles in both lung and liver does not affect long-term hepatic accumulation following pulmonary exposure. *PLoS ONE* **13**, e0202477 (2018).
74. *Test No. 417: Toxicokinetics, OECD Guidelines for the Testing of Chemicals* Section 4 (OECD, 2010).
75. *Toxicokinetics of Manufactured Nanomaterials: Report from the OECD Expert Meeting* (OECD, 2016); http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)24&doclanguage=en
76. Kah, M. & Kookana, R. Emerging investigator series: nanotechnology to develop novel agrochemicals: critical issues to consider in the global agricultural context. *Environ. Sci.: Nano* **7**, 1867–1873 (2020).
77. Lowry, G. V., Avellan, A. & Gilbertson, L. M. Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nat. Nanotechnol.* **14**, 517–522 (2019).
78. Lombi, E., Donner, E., Dusinska, M. & Wickson, F. A One Health approach to managing the applications and implications of nanotechnologies in agriculture. *Nat. Nanotechnol.* **14**, 523–531 (2019).

Acknowledgements

We are grateful to several experts who made contributions to the early stages of this project, including D. Anderson, P. Demokritou, G. Mahler, L. Parsons, A. Byro, D. McShan, P. Reeves, A. Tobia, U. Nasini, S. Qu, G. Walker, H. Xiao and B. Xing. The development of this framework was facilitated by financial support from IUPAC via a project on Human Health Risk Consideration of Nano-enabled Pesticides for Industry and Regulators (project number 2017-035-2-600).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to Melanie Kah.

Peer review information *Nature Nanotechnology* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.