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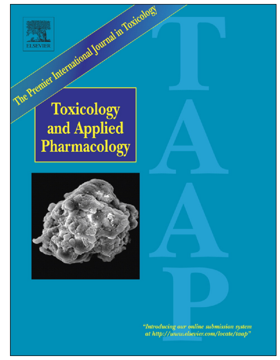


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PII: S0041-008X(23)00199-0

DOI: https://doi.org/10.1016/j.taap.2023.116560

Reference: YTAAP 116560

To appear in: *Toxicology and Applied Pharmacology*

Received date: 28 February 2023

Revised date: 6 May 2023

Accepted date: 19 May 2023

Please cite this article as: A. Ale, V.S. Andrade, M.F. Gutierrez, et al., Nanotechnology-based pesticides: Environmental fate and ecotoxicity, *Toxicology and Applied Pharmacology* (2023), https://doi.org/10.1016/j.taap.2023.116560

**Nanotechnology-based pesticides: environmental fate and ecotoxicity**

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ABSTRACT

The imminent increase in global food demand inevitably leads to an increase in agricultural practices, with an emphasis on pesticide applications. Nanotechnology-based pesticides, or nanopesticides, have gained importance as they are more efficient and, in some cases, less toxic than their conventional counterparts. However, concerns about these novel products have arisen as evidence about their (eco)safety is controversial. This review aims to: (1) introduce the currently applied nanotechnology-based pesticides and their mechanisms of toxic action; (2) describe their fate when released into the environment, with an emphasis on aquatic environments; (3) summarize available research on ecotoxicological studies in freshwater non-target organisms through a bibliometric analysis; and (4) identify gaps in knowledge from an ecotoxicological perspective. Our results show that the environmental fate of nanopesticides is poorly studied and depends on both intrinsic and external factors. There is also a need for comparative research into their ecotoxicity between conventional pesticide formulations and their nano-based counterparts. Among the few available studies, most considered fish species as test organisms, compared to algae and invertebrates. Overall, these new materials generate toxic effects on non-target organisms and threaten the integrity of the environment. Therefore, deepening the understanding of their ecotoxicity is crucial.

**Keywords:** algae, bibliometric analysis, ecotoxicity, fish, invertebrates, nanopesticides.

## Introduction

In the last decades, nanotechnology and its multiple applications have become a key feature among the current fields of studies. Agriculture has not been the exception as high-tech practices based on nanotechnology and the development of novel nanoproducts have gained importance in a context where there is a need for both developing efficient strategies for pest control (by preventing economic and production losses) and coping with its resistance to conventional formulas (Zhang and Goss, 2022). Indeed, nanotechnology has revolutionized the field of agriculture through far-reaching applications. It has been extensively reviewed that nano-enabled products, such as nanofertilizers and nanopesticides, present desirable properties like great stress tolerance and reduced transportation costs, apart from being "ecofriendly" (Arora et al., 2022). However, a major challenge when considering pesticides is reducing their environmental footprint in a context where their use inevitably increases in response to growing global food demand and pest resistance. In fact, it has been recorded that the use and production of pesticides have tripled in the last fifty years (Wang et al., 2022). In this context, degraded soils and climate change are also flattening yield curves for many crops, while it is estimated that a 50-80% increase in production will be needed by 2050 (Lowry et al., 2019). This goal becomes even more difficult to achieve when considering the lack of efficiency in food usage, with one-third of harvested food being thrown away (Tscharntke et al., 2012).

Unfortunately, it has been estimated that less than 2% of conventional pesticides actually reach the target crop and less than 1% reach the ultimate pest. As a result, nanotechnology-based innovations have gained importance and have been adopted worldwide (Arora et al., 2022). Among the advantages of nanotechnology-based pesticides are a reduction in the amounts of active ingredients, an enhancement of their solubility, and a decrease in the use of surfactants and adjuvants commonly applied in commercial formulations (Cáceres et al., 2019). Furthermore, nanoformulations provide controlled release of agrochemicals, offering long-lasting and more consistent effects while potentially reducing application and consequent leaching and runoff (Zhang and Goss, 2022). This is particularly important since pesticide waste, manufacturing costs, and environmental emissions must all be minimized while extending the period during which they are active in crops (Sun et al., 2016). As such, nanotechnology-based pesticides have been proposed as a more efficient and sustainable way to both control crop diseases and increase production.

Metal-based nanopesticides are one of the most common types, often employing Au, Ag, Cu, magnetic, and silica nanoparticles (NP) due to the ease of introducing functional groups onto their surfaces, leading to high-affinity interactions between NP and pesticide molecules. Furthermore, the use of NP in the synthesis of pesticides results in uniform particles of different sizes. The smaller the nano-pesticides are, the better they are distributed on crop leaves, and thus the higher their efficacy becomes (Cáceres et al., 2019; Peixoto et al., 2021; Sun et al., 2019).

Nanotechnology-based pesticides were recently reviewed by Abdollahdokht et al. (2022) such as nano-emulsions, nano-encapsulates, nano-gels, and electrospun nano-fibers. For example, nano-emulsions are biphasic dispersions systems formed by mixing surfactants, and they are kinetically stable and highly desirable to dissolve poorly water-soluble pesticides into small oil droplets so improve their bioavailability and efficacy. Nano-encapsulates consist of encapsulating active ingredients and releasing them in a controlled manner while preventing the compound from premature degradation. Nano-gels, in particular, have been widely used as nanoscopic drug carriers for delivering bioactive mediators in a time-controlled or site-specific manner, such as pheromones and essential oils. It is worth mentioning that nanocarriers not only improve the dispersibility and stability of pesticides but also facilitate the delivery of effective ingredients to target organisms, improving their bioavailability. Lastly, electrospun nano-fibers are more recently being developed and highly employed for plant protection purposes (Abdollahdokht et al., 2022).

However, concerns have been raised about the "dark side" of nanomaterials and their true (eco)safety, as their effects on health are not yet known and even less attention has been given to their runoff into water bodies and potential toxic effects on non-target organisms. Although it has been suggested that nanoformulations are a sustainable way to control crop disease and increase production, there is little research on the ecotoxicity of nanotechnology-based pesticides, which often lacks conclusive results (Arora et al., 2022). Therefore, this review aims to provide an updated understanding of these novel high-tech pesticides from an ecotoxicological perspective. Therefore, our specific goals are to: (1) introduce the currently applied nanotechnology-based pesticides and their mechanisms of toxic action; (2) describe their fate when released into the environment, with an emphasis on aquatic environments; (3) summarize available research on ecotoxicological studies in freshwater non-target organisms through a bibliometric analysis; and (4) identify gaps in knowledge from an ecotoxicological perspective.

**Toxicity mechanisms of nanotechnology-based pesticides**

Nanopesticides can reach surface water bodies and affect their quality, generating deleterious impacts on ecosystems. Particularly, nanoparticles (NP), which are widely used in nanopesticide formulations, can cause toxicological effects not only due to their nanoscale-intrinsic properties but also because of their widespread distribution and bioaccumulation in water and soil environments. As a result, their negative effects on biota have been extensively reported (Corsi et al., 2022; Kuhlbusch et al., 2018; Zielińska et al., 2020). The situation becomes more complex when considering that NP can behave as a "Trojan horse" mechanism, contributing to the toxic effects of nanopesticides. Moreover, different parameters must be estimated, and the uncertainties are sometimes very important. For example, lipophilic, hydrophilic, and hydrophobic silica NP (SiNP) were proposed as larvicidal, pupicidal, and insect growth inhibitor (Barik et al., 2012). The insecticidal activity of SiNP is mainly attributed to the desiccation provoked by their adsorption to the insect cuticular lipid and then breaking the structures (Cáceres et al., 2019). The size, surface area, charge, and materials employed also affect their biocidal functions. Indeed, it was reported that SiNP toxicity against *Spodoptera frugiperda Sf9* cells increased with the reduction in particle size. The lethal dose 50 ($LD_{50}$) decreased from 4.709 to 0.133 mg $mL^{-1}$ for 1430 and 14 nm-sized NP, respectively. In addition, the graft of the NP with amine groups significantly augmented the $LD_{50}$, further confirming the lower toxic effect of the modified SiNP (Santo-Orihuela et al., 2016).

Nanostructured alumina was also identified as an outstanding nanopesticide material. The mechanism involves the adsorption of the nanostructured alumina, favored by its high specific surface area, to the body and cuticle of the insect. Once again, the protective wax layer is disrupted, leading to water loss and the inevitable dehydration and death of the insects (Stadler et al., 2017, 2010).

Alternative nanopesticides including those obtained with Ag, Cu, and Zn were also proved to be effective. In those cases, the most plausible mechanism of actions is due to the release of metal ions and their effect over the biological structures. For example, Ag ions can bind to cysteine-containing proteins; interact with DNA bases, provoke structural changes in cell membrane; and inhibit cell division and reproduction (Mikhailova, 2020). However, it is worth adding that metal ions in environments could interact with organic matter and further toxic effects on biota may be mitigated, apart from stabilize nanoparticles (Ale et al., 2021a; Wang et al., 2023). In addition, another well-known mechanism of toxicity involves the production of reactive oxygen species (ROS) on the surface of the NP which can severely damage the organisms. Indeed, the increase of ROS disrupts cell homeostasis, that damages cells at several levels and ultimately lead to apoptosis and death (Adisa et al., 2019).

Pesticidal nanoemulsions have received a great deal of attention mainly due to the possibility of improving the uptake of pesticides. Indeed, the toxicity of pesticide nanoemulsions was reported to be higher than the toxicity of conventional formulas. For example, the $LC_{50}$ of permethrin to *Culex quinquefasciatus* mosquito were 0.117 and 0.715 mg $L^{-1}$ for the nanoemulsified and conventional pesticides, respectively (Anjali et al., 2010). Interestingly, droplet size also conditioned the toxic effects in terms of $LC_{50}$. It was reported that for nanoemulsions of neem oil with sizes between 31 nm and 251 nm, the $LC_{50}$ diminished with droplet size (Anjali et al., 2012). In addition, toxicity of nanoemulsions were evaluated against non-target organisms, confirming the absence of restraint in root length and germination percentage to the seeds of *Cucumis sativus*, *Lycopersicum esculentum*, and *Zea mays*. Thus, they may represent a safe alternative to bulk permethrin in agricultural practices (Suresh Kumar et al., 2013).

Polymers were also employed in the formulation of pesticides and, in this case, the lower toxicity against non-target organism was mainly attributed to the slow release of the active ingredients which resulted in lower environmental exposure and residues. Different reports further confirmed that these polymeric formulations do not affect non-target organism, soil microbiota, or plants (Pasquoto-Stigliani et al., 2017; Pradhan et al., 2013; Regina Assalin et al., 2022). Solid lipid nanoparticles are currently being proposed for the formulation of pesticides (Nemati et al., 2022). Notably, Jacques et al. (2017) evaluated these particles loaded and unloaded with pesticide and observed that the detrimental effects on *Caenorhabditis elegans* nematode were caused by intrinsic

NP properties. Similarly, these NP were proved to cause morphological alterations in non-target organism such as bees (Oliveira et al., 2019). Alternatively, assays with non-target organisms revealed that solid lipid NP did not affect plant growth (de Oliveira et al., 2015). Despite the progress in the field of nanotechnology, with emphasis on agricultural formulations, the comprehension of their plausible toxicity mechanisms is still insufficient to address the pros and cons of nanotechnology-based pesticides.

**Environmental fate of nanotechnology-based pesticides**

Nowadays, the use of nanopesticides is widely extended all over the world, especially in relation to the application as agrochemical for croplands and control of plagues involved in human and veterinary health (Zaheer et al., 2022). However, their rapid development has led to a new type of environmental impact due to the creation of another kind of pollution. The knowledge about the environmental fate is scarce, which leads to incomplete assessment of the risk and benefits related to the current use of agrochemical. One example of the risk is that it is frequently assumed for traditional pesticides that formulants and active ingredients separate rapidly upon application in the field, therefore, such validity should be verified for nano-enabled formulations that are designed to modify the environmental fate of active ingredients. Moreover, there is uncertainty whether nanopesticides can be adequately evaluated within the current pesticide regulatory framework (Kah et al., 2016, 2013).

Based on the primary objectives of nanoformulations (increased solubility, slow/targeted release, and protection against degradation of active ingredients), it is possible to reduce their application rates compared to conventional ones. This could be an advantage as may lead to a lower environmental impact (Yadav et al., 2022). On the other hand, the properties described above may generate different contamination problems in soils and aquatic bodies due to improved transportation, persistence, and toxicity.

With the aim to move forwards to the understanding about the fate of nanopesticides in the environment and support the development of robust exposure assessment procedures; Kah et al. (2018) investigated the extent to which three nanoformulations can affect the photodegradation adsorption of the insecticide clothianidin (neonicotinoid) and evaluated several approaches to estimate durability; a key parameter for the exposure assessment of nanopesticides. In this sense, the authors showed that the nanoformulations increased the photodegradation half-life in water by a maximum of 21% relative to the conventional formulation. Sorption to soil was also investigated, and results showed that the sorption was increased by up to 51% and 10% for the unformulated clothianidin and the commercial formulation, respectively. According to this study, the results indicated that nanoformulations may have a greater impact on the fate of pesticide active ingredients than commercial formulations. Otherwise, they suggested that the durability of nanoformulations after their application in the environment is an essential parameter that needs to be characterized for the development as well as for the evaluation of nano-enabled agrochemicals. In addition, if the durability of nanoformulation is of short-term, it is commonly assumed that compounds of nanopesticides will dissociate and behave independently upon application in the field. On the contrary, if the nanopesticides are environmentally persistent, the possible scenario of evaluation is more complex and the exhaustive analysis of nanocarriers properties will be necessary.

Furthermore, an essential factor to consider is the complex interaction between nanopesticides and microorganisms, which play an important role in the environmental fate of these nanomaterials. Microbes present in soil contribute to organic matter decomposition, nutrient recycling, and growth enhancement (Calder et al., 2012; Santaella and Plancot, 2020; Yadav et al., 2022). In particular, the soluble compounds of a pesticide have been considered key for transportation and biodegradation. Therefore, it is important to study the possible environmental fate of nanoformulations that aim to increase solubility.

Finally, another important fact to consider is that, as mentioned before, nanopesticides containing nanoparticles (NP) that could deliver them and their release could undergo a process of aggregation. This homoaggregation (between NP) or heteroaggregation (NP reacting with natural mineral and organic colloids) could produce significant changes to their fate and potential toxicity in the environment. This phenomenon has already been described, especially for aquatic environments, where nanomaterials accumulate in bottom sediments, facilitated in natural systems by heteroaggregation (Doucet et al., 2006; Filella, 2006; Lead and Wilkinson, 2006). Moreover, it has been demonstrated that aquatic and terrestrial environments contain natural NP, including colloidal clays, iron and manganese hydrous oxides, and dissolved organic matter, as well as fibrillar colloids that are exudates from algae and other microorganisms (polysaccharides and proteins), which contribute to the phenomenon of aggregation.

In conclusion, the behavior and fate of nanopesticides when released into the environment represent a major issue of vital importance that needs to be resolved (Kumar et al., 2019). The complexity of this issue lies in the lack of studies with robust results, as well as in the intrinsic properties of nanopesticides, the synthesis methods and ingredients used, and the environmental scenarios in which these substances will ultimately be released. A summary of the most commonly studied nanopesticides, their main applications, and fate is shown in Table 1. Additionally, the development of new methods that provide reliable results and measurements about the environmental fate of nanopesticides represents an important and significant challenge. These results could form the basis for establishing regulatory rules aimed at minimizing environmental risk to natural water, sediment, soil systems, and biota.

Table 1. Main types of currently applied nanopesticides, applications, and environmental fate.

| Nanopesticide | Applications | Environmental fate | References |
|---|---|---|---|
| Silica-based | Insecticide by provoking desiccation by adsorption to the cuticular lipid. | They are rather stable. No photo/chemical degradation nor speciation occur in aquatic media, while only the particle size may change because of aggregation or agglomeration. Dissolution may occur depending on pH and organic acid salts. Low toxicity was suggested on non-target organisms. Their persistence in organisms' tissues is low. They may adsorb to cellular surfaces and may alter membrane structure and integrity. | Ale et al. (2021b), Santo-Orihuela et al. (2016) |
| Metallic nanoparticle-based (e.g., silver, copper). | Biocide, mainly with antibacterial purposes, by cellular membranes damage, ion release and the "Trojan horse" mechanism. | Persistent in environments, nanoparticle dissolution (and ion release) could happen. It can also occur agglomeration and/or sedimentation. The particles could be also stabilized with organic substances (humic acids, bacteria, fungi organisms). Algae presence enhances the release of ions because of the interaction with their exudates. | Andrade et al. (2023), Corsi et al. (2022), Zhang and Goss (2022) |

| | | | |
|---|---|---|---|
| Nanocarriers based on natural polymers, polysaccharides or lipids | They are applied as insecticide (e.g., acetamiprid-based), herbicides (e.g., halloysite nanotubes, clothianidin-based), or fungicide (e.g., lignin and ethylene glycol-based). | It was assumed their low persistence when reach environments, despite they have been poorly studied and they fate will depend on the remaining ingredients. However, the photodegradation half-life in water and sorption to soil could be increased in comparison with conventional formulas. | Ding et al. (2023), Liang et al. (2022), Wang et al. (2022), Yadav et al. (2022) Zeng et al. (2019), Kah et al. (2018) |
| Alumina nanostructure-based | Insecticide, by adsorption to the body and cuticle of the insect, therefore, the protective wax layer is disrupted, leading to water loss and dehydration. | Not studies regarding the environmental fate were found. In case of organisms' exposure, their toxicity was suggested as low (in terms of cyto- and genotoxicity). | Rani et al. (2023), Stadler et al. (2017, 2010) |
| Solid lipid-based | Dependent on the pesticide loading (e.g., with atrazine herbicide, or oil-loaded antiparasitic). | Poorly studied, highly dependent on the pesticide loading. | Nemati et al. (2022), de Albuquerque et al. (2021), Oliveira et al., (2019), de Oliveira et al., (2015) |
| Nanoemulsions | Generally loaded with conventional pesticides (e.g., | Toxic effects on target and non-target organisms were higher in comparison with the conventional counterparts | Anjali et al. (2012, 2010) |

| | | | |
|---|---|---|---|
| | neem oil and permethrin insecticides). | as their absorption is greater. | |

**Eco(un)safety of nanotechnology-based pesticides**

According to some research, it has been suggested that the ecosafety of nanotechnology-based pesticides is guaranteed due to their optimization in terms of lower application amounts and the prolonged and sustained release of their active ingredients (de Albuquerque et al., 2021; Amjad et al., 2018; Clemente et al., 2014). Based on these assumptions, it is hypothesized that the environmental consequences are fewer compared to conventional formulations (Lowry et al., 2019; Zhang and Goss, 2022). However, the global warning lies in the limited information about the environmental fate and unknown impacts of nano-based pesticides, which hampers our ability to precisely estimate the benefits and risks of these novel formulations (Peixoto et al., 2021). In this sense, there are a few but still inconclusive cases of studies suggesting that the intrinsic properties of nanomaterials, such as particle size or crystalline phases, directly influence the ultimate toxicity on non-target species (de Albuquerque et al., 2021; Nogueira et al., 2020; Zhang et al., 2020). Unfortunately, there are few reports comparing the exposure of the same test species to both environmentally relevant concentrations of a nano-pesticide and its conventional counterpart (further details in the sections below). Therefore, there is still a long way to go before we can finally elucidate the ultimate ecosafety of nanotechnology applications in the agricultural field. Due to their higher persistence and lower mobility in soil and water, nanopesticides may travel via protozoans and non-target organisms such as insects and fish, and ultimately reach humans (Gottschalk and Nowack, 2011). As a result, the (eco)safety of these novel products have been called into question. Since nanometric materials have been shown to be able to enter organisms through various routes (e.g., inhalation, ingestion, absorption) (Ale et al., 2023), further studies considering environmentally relevant concentrations of nanopesticides, as well as the time and mode of exposure through simulations under field and experimental conditions, are critical.

**Bibliometric analysis**

We conducted an exhaustive literature search to summarize the available research into ecotoxicological studies. Published documents (articles and reviews) available in the PubMed, Science Direct, Scopus, and Google Scholar databases, were used for analysis. Additional articles were further identified by checking reference lists of relevant papers. The following keywords (and their combinations) were used: nanopesticide, nanoinsecticide, nanoherbicide, nanofungicide, toxicity, aquatic organisms, freshwater, fish, invertebrate, microcrustacean, and algae.

The results obtained show, in accordance with other bibliometric reviews on nanoparticles (e.g., Ale et al., 2021b; Cazenave et al., 2019; Gutierrez et al., 2021; Kahru and Dubourguier, 2010), that the cumulative number of studies on nanopesticides has increased substantially in recent years. These studies have primarily focused on reporting methodological aspects of their production and synthesis, as well as their effectiveness and potential as promising environmentally safe pesticides. However, little progress has been made in understanding their potential ecotoxicity in non-target organisms (Figure 1).

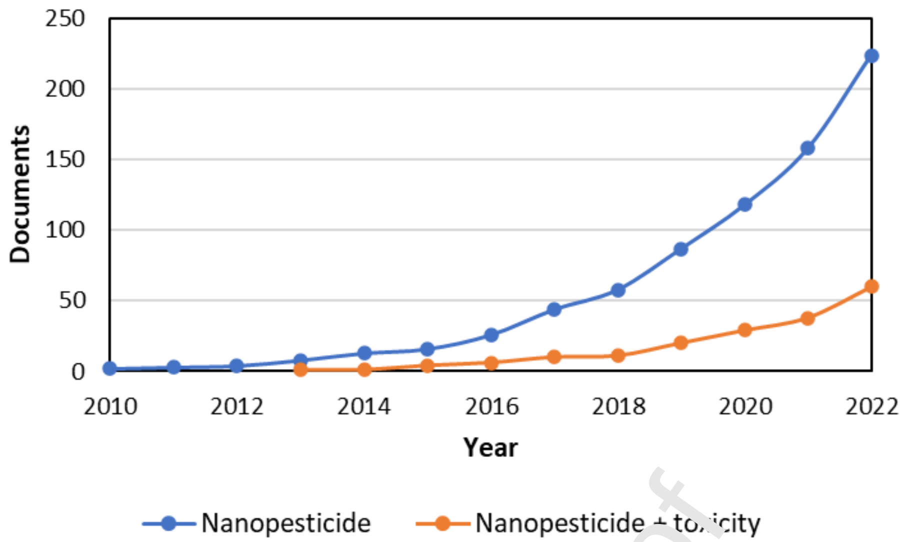


Figure 1. Cumulative number of documents (review and research articles) per year published on “Nanopesticide” and “Nanopesticide + toxicity” (data available in Scopus database until December 2022).

Based on the results obtained from the aforementioned analysis, we selected research papers that assessed the effects of nano-based pesticides in freshwater biota, obtaining ecotoxicological data from 21 articles published between 2010 and 2022. Figure 2 shows a word cloud built from words that appear on titles and keywords of the selected papers. This representation provides an insight into the main issues addressed in toxicological studies, giving greater size to words that appear more frequently. Thus, “toxicity” is the most used word in the publications, followed by “nanopesticide” and “nanoparticles”, while the terms “effect” and “copper” (which is mostly connected to copper oxide nanopesticide) are in fourth place. The standardized species (“zebrafish” and “*Daphnia magna*”) had greater prominence among the tested aquatic organisms.

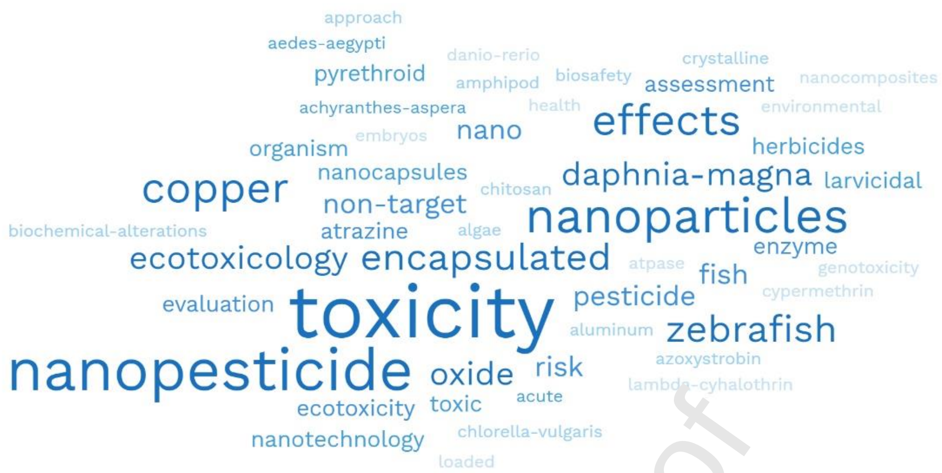


Figure 2. Word cloud showing the frequency of words appearing in the titles and keywords of the 21 selected articles on the toxicological effects of nanopesticides.

The ecotoxicological studies were classified into the main taxonomic groups (algae, invertebrates, and fish). The ecotoxicity of nanopesticides has been mostly investigated in fish followed by invertebrates and algae (Figure 3). Across the different groups, the main ecotoxicological effects were analyzed (further details in sections below).

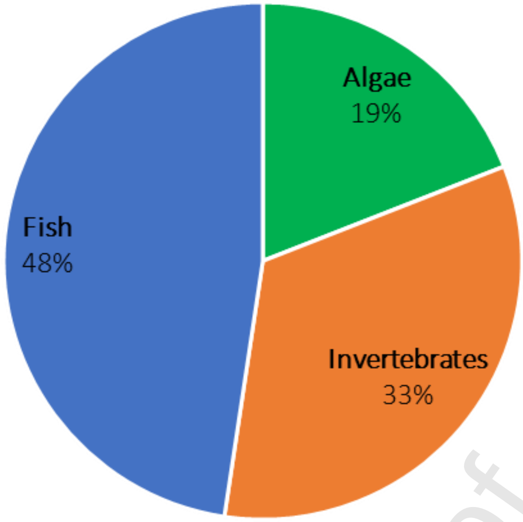


Figure 3. Schematic representation of the distribution of the main taxonomic groups investigated and included in our analysis (articles cited in the text).

**Ecotoxicity of nanotechnology-based pesticides**

Based on the references obtained from the bibliometric analysis, the up-to-date ecotoxicology of nano-based pesticides was summarized in Figure 4 and followed detailed.

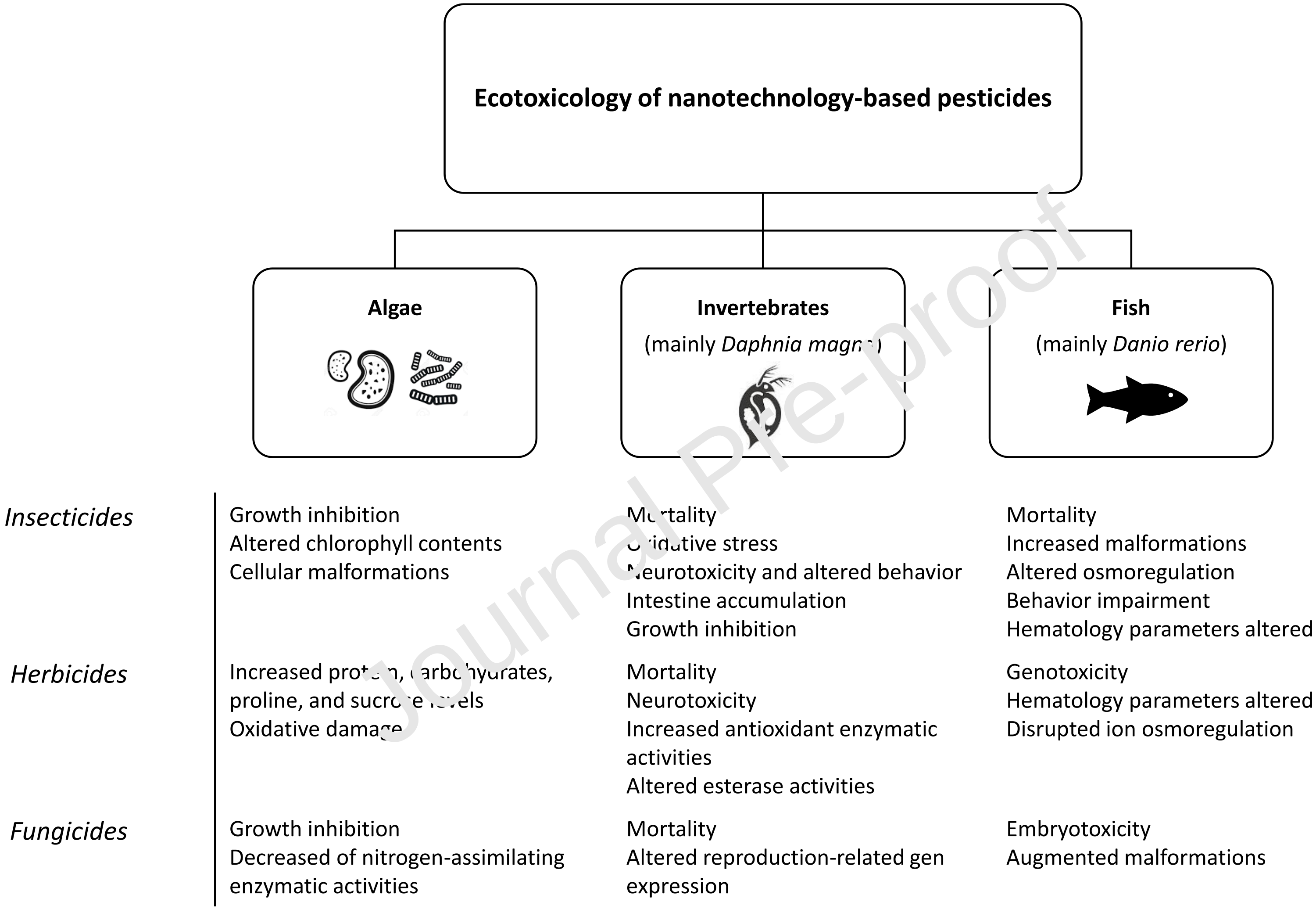


Figure 4. Summary of the main ecotoxicological effects of nanotechnology-based pesticides on non-target organisms.

*Algae*

Insecticides

Nanoinsecticide effects on alga are still unknown. The only study focused on this issue reported that permethrin nano-emulsion caused no effects on *Closterium* sp. in terms of mortality, cell viability, chlorophyll content, and cellular morphology under the concentrations applied for mosquitos' control ($\leq 1$ mg $L^{-1}$) (Mishra et al., 2019).

Herbicides

Encapsulation of herbicides atrazine and ametryn resulted in a lower toxicity than their respective non-encapsulated active ingredients to the algae *Pseudokirchneriella subcapitata* (Clemente et al., 2014). Interpretations suggested that cell walls of algae could provide a barrier to the entry of nanocapsules, thus, being less toxic than the chemical compounds alone. However, contradictory results were found in another study where the nano-form of the herbicide pendimethalin was slightly more toxic than a commercial traditional formulation for the freshwater alga *Chlorella vulgaris* based on the half maximal effective concentration ($EC_{50}$) toxicity test (19 and 20 μg $L^{-1}$ for the nano and non-nano form, respectively) (Noaman et al., 2020). Additional toxicological tests in this survey showed that both, the traditional and nano-form of this herbicide decreased chlorophyll a and b, and increased carotenoid, protein, carbohydrates, proline, and sucrose algal content. Also, both herbicides induced oxidative stress as lipid peroxidation (in terms of malondialdehyde levels, MDA) and increased activities of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) antioxidant enzymes. The study also reported changes in cell organelles such as compacted organelles, heavy distributed starch grains and fatty bodies, and destroyed chloroplasts.

Fungicides

Among nano-fungicides effects on algae, the only report available carried out by Kumar et al. (2016) showed that the recommended dose of nano-hexaconazole did not have significant inhibitory effect on the growth of nitrogen fixing blue green algae *Anabaena*, *Nostoc*, *Aulosira*, and *Tolypothrix* spp. in terms of biomass and chlorophyll content. While the nano-fungicide caused inhibition in activity of nitrogen assimilating enzymes, the traditional non-nano formulation led to larger decrease in these enzymatic activities.

*Invertebrates*

Insecticides

Most of the studies analyzing freshwater invertebrate mortality by nano-pesticides focused on insecticides effects on *Daphnia magna*, although these studies are still incipient (Figure 5). Encapsulated pyrethroid (lambda-cyhalothrin) caused higher mortality to *D. magna* at the smaller hydrometric diameter or at a the most stable condition, varying these characteristics according to environmental pH and ionic strength (Son et al., 2015). However, a biogenic nano-insecticide based on silver nanoparticles (AgNP) with extracts of *Achyranthes aspera* leaves as reducing and capping agents, showed to be effective to control the dengue vector larvae (*Ae. Aegypti*) but did not inflict any lethal effects on *D. magna* and *Moina macrocopa* at concentrations that affected the vector larvae (Sharma et al., 2020).

A contradictory effect was found for another natural nano-insecticide used for malaria vectors consisting of cadmium sulfide (CdS) nanoparticles (NP) with extract of *Valoniopsis pachynema* algae as capping agent (Sujitha et al., 2017). This nano-insecticide exerted several toxicological effects on the non-target species mud crab

*Scylla serrate* by affecting its survival and generating oxidative stress (increased glutathione S- transferase -GST- enzymatic activity) and neurotoxicity (decreased acetylcholinesterase -AChE- activity).

In a more recent study, aluminum oxide ($Al_2O_3$) NP, usually applied as nano-insecticide, showed to be ingested by *D. magna* and accumulated in the intestine. These NP also showed to generate oxidative stress (alteration of antioxidant enzymatic activities and ROS -reactive oxygen species- generation), mortality, growth inhibition, and alterations in reproduction and swimming behavior (Nogueira et al., 2020). However, the toxicological effects of this nano-pesticide were dependent on their different crystalline phases, as the η-$Al_2O_3$ NP were found to be more toxic than α-$Al_2O_3$ NP. It is important to highlight that all the tested concentrations were well above the predicted environmental concentrations.

Herbicides

Nanocapsules containing atrazine or ametryn were more toxic to *D. similis* than their respective non-encapsulated active ingredients in acute toxicity tests (Clemente et al., 2014). Similar results were found for *Chironomus sancticaroli* larvae, for which solid lipid nanoparticles loaded with atrazine caused greater mortality and biochemical alterations than atrazine active ingredient, which can be related to the fact that the nanoparticle control (without atrazine) also induced biochemical alterations and mortality (de Albuquerque et al., 2021).

Fungicides

Exposure of *D. magna* to copper hydroxide ($Cu(OH)_2$) nanopesticide (Kocide 3000®), used as fungicide and bactericide, induced mortality ($LC_{50}$: 2.4 mg $L^{-1}$, Figure 5), modified the expression of detoxification related genes, and decreased the expression of genes related to reproduction (Aksakal and Arslan, 2020). In outdoor mesocosms, Carley et al. (2020) found that the same commercial copper-hydroxide nanopesticide (Kocide 3000®) caused shifts in the composition, richness, and abundance of aquatic bacteria, fungi, and eukaryote. However, no similar effects were found for terrestrial communities.

*Fish*

Insecticides

In recent years, a series of pyrethroid nano-formulations of traditional active ingredients (i.e., nanoemulsions, nanospheres, and nanocapsules), have been to some extent investigated (Figure 5). Huang et al. (2022) showed that lambda-cyhalothrin-loaded nanocapsules (average diameter: 209.4 nm) caused higher mortality rate and malformations of zebrafish (*Danio rerio*) larvae and embryos when compared to the micro-sized counterpart (2.4-12.4 μm) after 96 hours of exposure. Moreover, the authors highlighted that the release and sedimentation behavior of both compounds were particle size-dependent, being the micro scale release slower but sunk more quickly in the water media. In addition, the nanocapsules were more accumulated in gill of fish because of their smaller size and stronger permeability. In a former study with zebrafish embryos, Meredith et al. (2016) found similar results in a 24-h trial. After isolating and concentrating the nano-sized capsules (~250 nm) of lambda-cyhalothrin, they found no difference in the acute toxicity compared to larger particles of the same composition (~2.2 μm); however, the technical active ingredient exposure resulted in significantly less fish experiencing tremors compared to any of the encapsulated product exposures. This suggests that the capsule size does not influence the toxic response of the entrapped lambda-cyhalothrin, but the presence or absence of the capsules does. Different results were found with the pyrethroid bifenthrin in a study which investigated the relative toxicity of both allosperse-encapsulated bifenthrin and the base pesticide in juvenile rainbow trout (*Oncorhynchus mykiss*). Overall, nano-bifenthrin demonstrated a reduced lethality ($LC_{50\text{-}96h}$: 12.1 μg $L^{-1}$, Figure 5) and causes reduced physiological stress (in terms of gill ATPase activity) in comparison to its conventional counterpart ($LC_{50\text{-}96h}$: 6.2 μg $L^{-1}$) (Blewett et al., 2019).

A comparative toxicity assessment was performed to evaluate the sublethal effects (geno- and hepatotoxicity) of cypermethrin nanoparticles and conventional cypermethrin on murrel, *Channa punctatus*, after 15 days of exposure (Amjad et al., 2018). A higher percentage of blood micronuclei and increased serum transaminases and alkaline phosphatase activities in fish exposed to cypermethrin indicated that the nano-formulation is less toxic than the conventional form. Similar results were registered by Mishra et al. (2019), who exposed juvenile zebrafish to a nano-emulsion of permethrin (mean droplet diameter: 12.4 ± 1.1 nm) at a mosquitocidal concentration which was found to be non-toxic.

Studies including the effects of other classes of nano-insecticides than pyrethroid on fish are more limited. Vallim et al. (2022) investigated encapsulated dimethoate (organophosphate) in alginate chitosan nanoparticles and evaluated its toxicological effects on embryos and larvae of zebrafish in terms of lethality, morphology, and behavior. Nanoparticle toxicity was evaluated in comparison with dimethoate technical grade, empty nanoparticles and dimethoate, and a commercial formulation. Major toxic effects on embryo and larval development were observed in commercial dimethoate exposure followed by the technical pesticide, suggesting that nanoencapsulation may be safer for fish. In a recent study, both silver and graphene oxide nanomaterial were selected to produce nanocomposites from the natural pesticide chlorophyllin and to evaluate their toxic effect on *Clarias gariepinus* (Abbas et al., 2022). All of them altered the hematological, immunological, and biochemical functions of *C. gariepinus*, compromising fish health.

Herbicides

Health hazards and biological effects of nano-based herbicides on fish have been scarcely investigated. Only a single study was found after the bibliographic search, where the effects of atrazine and nano-encapsulated atrazine were compared. Freshwater teleost *Prochilodus lineatus* were exposed to atrazine, nano-atrazine, or the nanocapsules for 24 and 96 hours. The evaluated endpoints (genotoxic, biochemical, and physiological biomarkers) showed that nano-atrazine was less toxic compared to atrazine, concluding that the nanoencapsulation of atrazine protected the fish from the herbicide effects (Andrade et al., 2019).

Fungicides

Current knowledge on the ecotoxicity of nano-based fungicides in fish is also extremely limited. Up to date, two studies have focused on these compounds. Firstly, Zhang et al. (2020) investigated the lethal and sublethal effects of nano-enabled azoxystrobin in zebrafish (*Danio rerio*) embryos and larvae during their early developmental stages. It was demonstrated that nano-azoxystrobin had higher lethality ($LC_{50\text{-}96h}$: 1031 µg $L^{-1}$, Figure 5) when compared to the conventional form of the fungicide ($LC_{50\text{-}96h}$: 334 µg $L^{-1}$). However, sub-lethal effects at different biological levels (including molecular, biochemical, morphological, and organismal levels) showed little to no ecotoxicological differences between the two exposures. Afterward, Aksakal and Sisman (2020) evaluated the embryotoxicity of a copper-based nano pesticide (Kocide 3000®) in the early life stages of zebrafish. Although this compound is employed to prevent the development of various fungal and bacterial plant diseases, results demonstrated the toxic effects of this nano-pesticide on non-target aquatic organisms. In particular, the copper-based nano pesticide induced developmental toxicity with malformations in zebrafish embryos and larvae and disturbed innate immune system functions, as the expression of several immune system-related genes were altered.

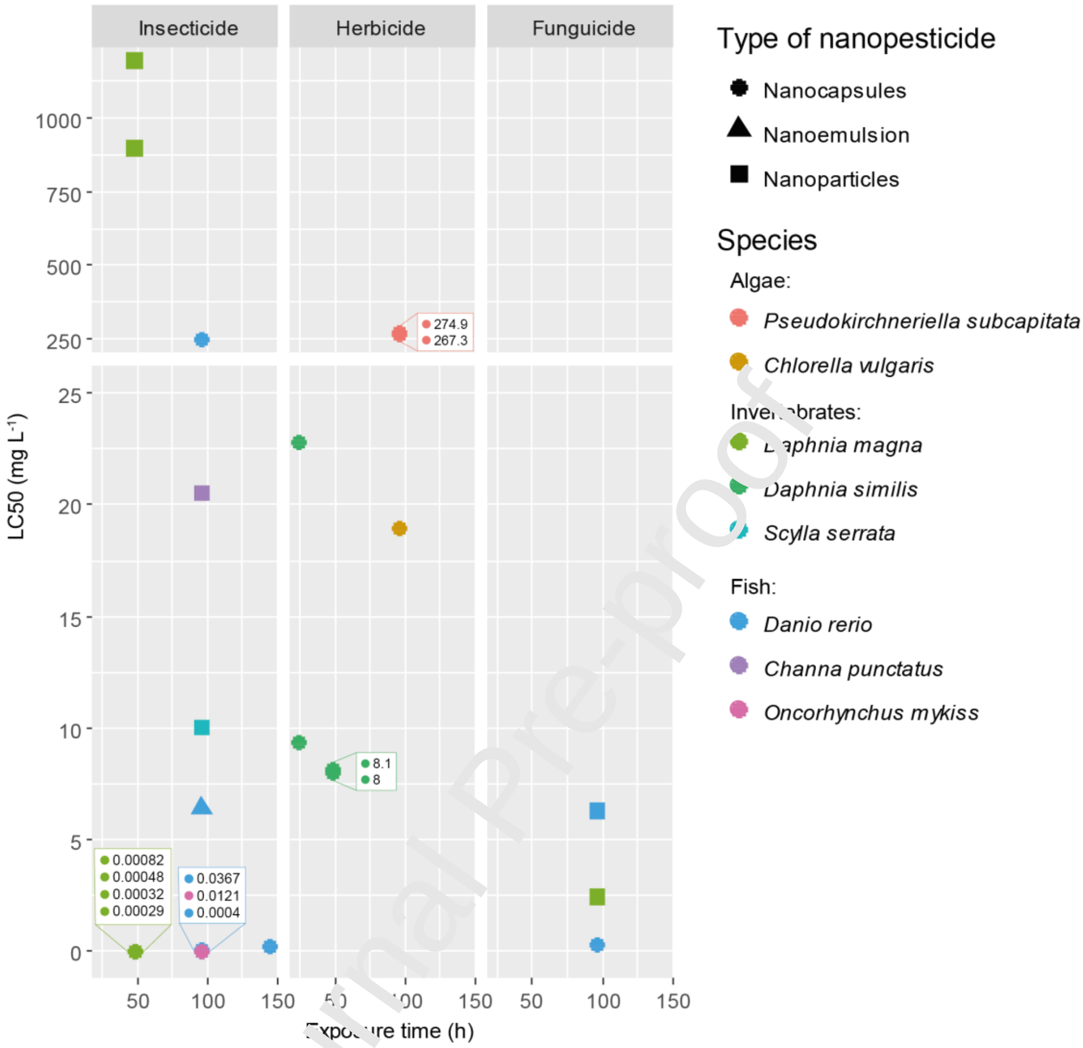


Figure 5. Lethal concentrations 50% ($LC_{50}$, mg $L^{-1}$) for algae, invertebrates, and fish, of nanocapsules and nanoparticles applied as insecticides, herbicides, and fungicides.

**Gaps of knowledge and further needs for research**

From the analysis carried out through this review, several gaps were identified in the knowledge of the ecosafety of nanotechnology-based pesticides. Their environmental fate has been poorly assessed, and further research is needed to better understand the behavior when these high-tech nanoproducts reach natural environments. The importance of this challenge lies in the complexity of the nanopesticides, which have different intrinsic characteristics such as their ingredients (e.g., metallic, alumina, silica-based), which determine their persistence; the synthesis method, and the external factors of the environment they reach (e.g., ionic strength, presence of organic matter and/or algae). Moreover, the available studies on the ecotoxicity of these novel materials are still very limited, and in some cases, the results obtained are not conclusive. Despite the fact that some authors have suggested that nano-forms are more ecofriendly than conventional formulations, we suggest that real-world exposure scenarios could be underestimated under such assumptions. In this sense, another major challenge lies in the development of ecotoxicological tests with sensitive test species and comparisons of exposures between nanopesticides and their conventional counterparts (and active ingredients), including any of the organisms described in this study (algae, invertebrates, fish). We believe that there is a lack of robust and conclusive results at all biological levels and that studies with different test species would complement each other. In addition, we encourage the use of test species other than those used in standard tests (e.g., *Daphnia magna* microcrustacean and *Danio rerio* fish), as holarctic or holotropic regions do not represent realistic scenarios and functional groups, so environmental tolerance ranges may be underestimated (Ale et al., 2021b; Cazenave et al., 2019; Gutierrez et al., 2021). Finally, to fully understand the potential environmental risks of nano-based agrochemicals, it is imperative to analyze their effects at higher levels of biological organization, such as populations, communities, and ecosystems.

**Funding**

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica: PICT 2018-01271 (PI: Jimena Cazenave) and PICT-2020-01206 (PI: Analía Ale).

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**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**CRediT author statement**

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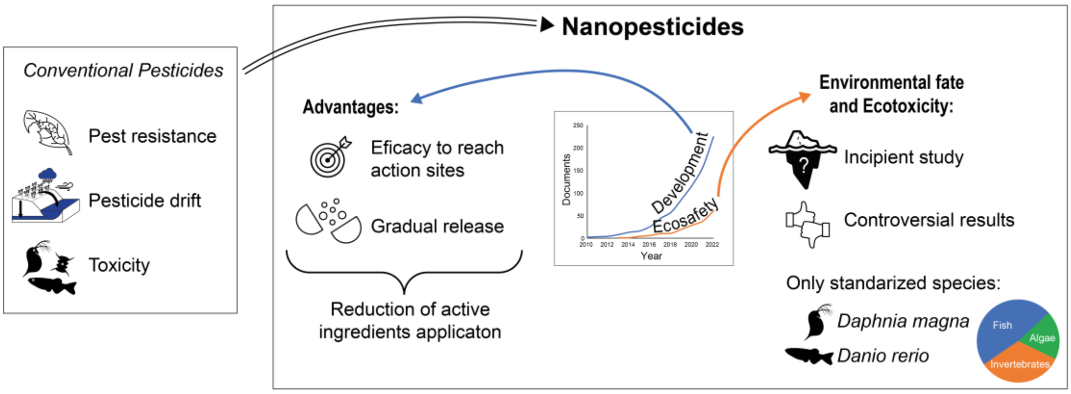
**Declaration of interests**

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract



## Highlights

- Ecosafety of nanopesticides is not guaranteed.
- Nanopesticides are more efficient that their conventional counterparts.
- Fish are the most test species up-to-date studied.
- Further ecotoxicological comparative studies (nano-based *vs* conventional) are needed.
- The environmental fate of nanopesticides is poorly understood.