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Joint toxicity prediction of nanoparticles and ionic counterparts: Simulating toxicity under a fate scenario

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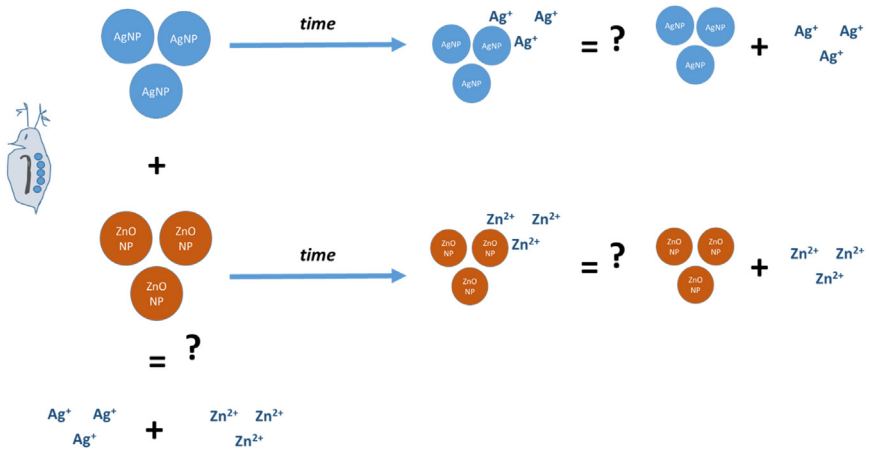
HIGHLIGHTS

- A mixture toxicity approach was used with ZnO and Ag nanoparticles (NPs).
- A dissolution scenario and toxicity patterns of NP mixtures was assessed.
- NPs mixture patterns did not reflect their ionic counterparts mixture responses.
- NP mixture prediction should not rely on available information for regular chemicals.
- Ag+ was responsible for a synergistic pattern in an Ag NP and $AgNO_3$ mixture.

GRAPHICAL ABSTRACT



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ABSTRACT

The joint effects of NPs with other chemicals is not fully yet understood along with the joint effects of the particulate and dissolved forms/fractions. The predictability of joint effects is of great importance for environmental risk assessment. Therefore this study aimed at inferring on the predictability of NPs binary mixture toxicity based on their ionic counterparts' mixtures, and evaluating if the joint toxicity of two forms of the same element (NP and ion) acts as dilution of each other. Effects of individual and mixtures of ZnO and Ag NPs and their respective salts ($AgNO_3$ and $ZnCl_2$) were studied in immobilization and feeding tests using *Daphnia magna*. NPs mixture toxicity patterns did not mirror their ionic counterparts' mixture toxicity responses and therefore their prediction should not rely on the available knowledge for regular chemicals. Regarding mixtures from the same element with different forms (NP and ions), both Zn and Ag mixtures showed a deviation from additivity, relying on the interaction between NP and ions. A synergistic effect was depicted when the concentrations of Ag ions increased, while antagonism was observed with AgNP increase in suspension. This is an expected pattern in long term studies due to dissolution, relating fate and toxicity.

1. Introduction

Nanomaterials (NMs) are currently described as materials containing particles that, for 50% or more of the particles in the number

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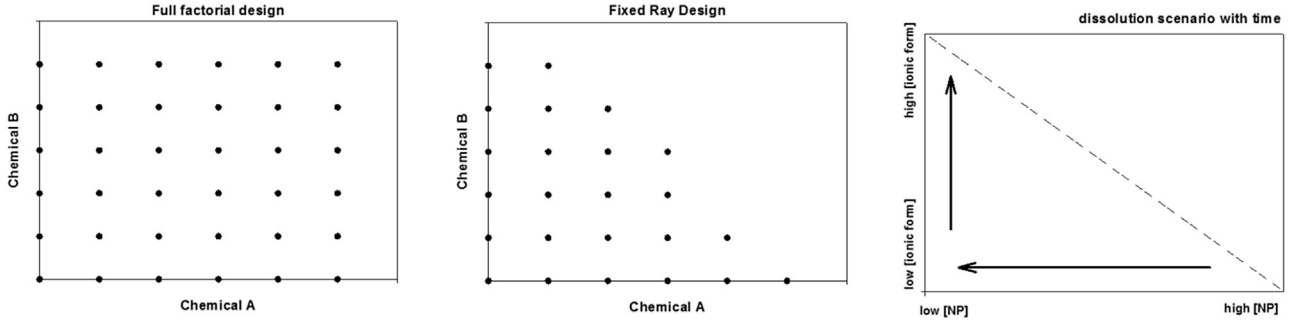

Fig. 1. Schematic examples for the experimental designs of binary mixtures used for immobilization tests (left graph, full factorial design), feeding inhibition tests (middle graph, fixed ray design) and the dissolution scenario, with time (right graph), showing that with time the concentration of nanoparticles ([NP]) decrease, increasing the concentration of the ionic form.

size distribution, have at least one of their dimensions between 1 and 100 nm (nanoparticles—NPs) or have a specific surface area by volume greater than 60 m^2/cm^3, according to the European Commission Recommendation of 18 October, 2011 on the definition of nanomaterial (2011/696/EU). These characteristics along others also of major importance (i.e. dissolution rate, surface charge, shape) increased their application in (new) products over the last decades [1,2] with the promise that they will continue to grow in the future, leading therefore to a continuous release into the aquatic environment [3].

Once in the aquatic compartment, NPs may undergo different physicochemical processes that will ultimately change their initial characteristics and consequently affect their bioavailability to aquatic organisms [4]. As a result they can become more or less toxic, due to the formation of agglomerates and/or aggregates when in contact with other chemical forms [5], or due to dissolution [6–8] where ions can be released from their surface and become more bioavailable to aquatic organisms. Therefore, for the science where it is *all about* size [9], it is important to know the potential ecotoxicological hazards and exposures, to predict or evaluate risks once NPs reach the environment.

Several studies have already investigated the adverse effects of many types of NPs to innumerous species of aquatic organisms [10–12]. However, most of the ecotoxicological experiments performed in laboratory mainly focus on single exposures of NPs which do not truly reflect their toxic effects to aquatic organisms [13]. In "realistic scenarios" organisms are rarely exposed exclusively to single chemicals but rather to a combination of other stressors (e.g., other chemicals, other small structures, abiotic factors, organic matter and others) and thereby exert joint effects to the aquatic organisms [13–15]. In addition, even when exposed to one NP, organisms will have to deal with different metal forms, being NP speciation an important issue to consider. Therefore, it is important to study the combined effects of NPs and their coexisting forms to achieve a comprehensive and realistic understanding of the adverse effects of NPs.

Different models have been developed to predict the toxicity of chemical mixtures. The most common and used models are concentration addition (CA) [16] and independent action (IA) [17]. Both theoretical models describe additive joint toxicity of chemicals based on the similarity or dissimilarity concept of their mode of action (MoA), respectively [18,19]. However, when the MoA of the tested chemicals are unknown or not fully understood, both the CA and IA models are usually advised and the choice is based on the best data fits [19,20]. Recently, an EFSA report on mixture toxicity highlighted the CA model as the most conservative conceptual model, and also that ecotoxicological approaches should rely on an overall mechanistic pathway (e.g. Adverse Outcome Pathways) when dealing with environmental mixtures, instead of focusing on specific modes of action [21]. Despite this, deviations from the conceptual models can occur, describing stronger (synergism) or weaker (antagonism) effects than expected, or those that depend on the levels of both chemicals (different deviations at low and high doses of the chemicals) or on the ratio of both chemicals, relying on the composition of the mixture [19,22].

ZnO and Ag NPs are two types of NPs that have gained great attention over the last years due to their useful properties. ZnO is a metal oxide nanoparticle that has been used in a large scale in personal care products (e.g. sunscreens), food additives, biosensors, electronic materials as well as in coatings and paints [10,23,24]. As for Ag NPs, they are metal based NPs mostly known for their antimicrobial properties and are applied in medical devices, clothes and personal care products [2,25]. For both types of NPs, there is already an extensive literature reporting adverse effects to several aquatic organisms, such as algae [7,26], invertebrates [12,27] and fish [28,29]. Despite the fact that it is often reported that the ionic release from NPs is the major source of toxicity, till now only few studies considered the potential joint effects of particulate and ionic forms [30,31], and also few have looked at NPs mixture toxicity, and if this can be predict relying on the mixture effects of their (ionic) counterparts.

Therefore, the two main aims of the present study were: (i) to infer on the predictability of NPs binary mixture toxicity based on their ionic counterparts' mixtures assessment, and (ii) to evaluate if the joint toxicity of two forms of the same element (NP and ion) act as dilution of each other. The toxicity of four binary mixtures build up from two NPs (ZnO and Ag) and their respective ionic counterparts ($ZnCl_2$ and $AgNO_3$) was evaluated using as test-organism the cladoceran *Daphnia magna*. For that an experimental design was based on previously reported results on single exposures for both forms of Ag and Zn [25,32]. Short term acute (48 h) and sublethal (24 h) tests were carried out and both models IA and CA and potential deviations, considering changes in concentrations and forms present, were tested to predict toxicity patterns. The short term approach allowed a more accurate estimation of the joint toxicity of NPs and ions, as time is an important factor regarding dissolution (Fig. 1, right graph).

2. Material and methods

2.1. Test organism

All tests were performed using the freshwater cladoceran *Daphnia* magna Straus, clone Beak, as test organism. *D. magna* cultures were kept in glass beakers of 1 L with 800 mL of ASTM hard water [33] and 20 daphnids per beaker, at a constant room temperature of 20 ± 1 °C and a 16:8 h light:dark photoperiod. Cultures were renewed three times a week and fed daily with *Raphidocelis*