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Converging hazard assessment of gold nanoparticles to aquatic organisms

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HIGHLIGHTS

- Toxicity of gold to freshwater algae, daphnia and zebrafish was examined.
- Naked and functionalized Au nanoparticles resulted harmless to these organisms.
- Gold chloride resulted toxic after acute exposure, with LC50 between 1 and 2 mg L^{-1}.
- Surface charge and size of Au nanoparticle are key factors to explain its toxicity.

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ABSTRACT

The gold nanoparticles (Au-NPs) are being increasingly used because of their huge diversity of applications, and consequently, elevated levels in the environment are expected. However, due to their physico-chemical properties and functionalization a high variety of Au-NPs can be found, and complete toxicological information for each type of Au-NPs still lacks, and even, the toxicological information for the same species is sometimes contradictory. Therefore, hazard assessment should be done case by case. Hence, the objective of this study was to obtain ecotoxicological information of the same Au-NPs in aquatic organisms and to find a rationale for Au-NPs toxicity. For such a purpose, bare and hyaluronic acid capped Au-NPs (12.5 nm) along with Au-NPs bulk material were tested on freshwater algae, *Daphnia* and zebrafish. Results showed that while gold nanoparticles were found to be harmless to the tested organisms, the soluble gold showed to be toxic to algae and *Daphnia*, with an LC_{50} between 1 and 2 mg L^{-1}. Comparing our results with those gathered in the literature, it appears that a common hazard assessment of Au-NPs on the studied organisms can be elucidated.

1. Introduction

There is an on-going debate about whether the current approach for chemicals is appropriate or not to perform NPs risk assessment. The group of NPS comprises nanoparticles of different nature, and each with infinite possibilities of having different size, shape and surface functionalization, and hence, hazard assessment has become an arduous task. For Stone et al. (2010), the substance-based classification system for NPs is the most pragmatic way for testing strategy, but considering the multiple combinations of NPs, it seems that hazard assessment should be done case by case. Considering that a specific study for every new NP is not a practical proposition, instead, the European network NanoImpactNet (http://www.nanoimpactnet.eu/) advocates a more rational scientific approach, where the properties of NPs should be critically considered with respect to test method execution, while trying to find common properties for different NPs in order to share a common hazard assessment (Handy et al., 2012).

Specific concerns have also been raised about whether the ecotoxicological tests presently used are adequate or not to assess the toxic properties of NPs (Scown et al., 2010; Sovova and Koci, 2012; Euronanoforum, 2009). However, it is not surprising, since the available information on NPs toxicology have usually arisen from standardized ecotoxicological tests, designed primarily for bulk chemicals, and on the other hand, the toxicological information is somehow contradictory for some types of nanoparticles.

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We can find an example in gold nanoparticles (Au-NPs) and their potential risks to the aquatic environment. As a result of a variety of uses, environmental release of Au-NPs is anticipated and therefore any impact on environment and human health deserves great attention. Literature has reported that Au-NPs -with dissimilar size and coatings- have been tested in freshwater algae (Renault et al., 2008), *Daphnias* (Lovern et al., 2008; Li et al., 2010), zebrafish embryos (Harper et al., 2008; Bar-Ilan et al., 2009; Browning et al., 2009) and adult zebrafish (Geffroy et al., 2012); however, authors concluded differently regarding Au-NP toxicity. In addition, while some authors state that the Au-NPs resulted to be toxic to certain organisms (Harper et al., 2008; Browning et al., 2009), other authors conclude that the Au-NPs were not toxic to the same organisms (Bar-Ilan et al., 2009; Asharani et al., 2011). Finally, we have not found experiments investigating the degradation of Au-NPs, and therefore nothing is known about chronic hazards or why not, eventual changes in toxicity.

Therefore, a critical review of the ecotoxicology of Au-NPs in order to find a rationale for Au-NPs toxicity is needed. In this sense, the first step for a critical hazard assessment of one type of Au-NPs is to test the same and well characterized Au-NP on freshwater algae, *Daphnia magna* and fish under acute standardized test.

The objective of this study was to investigate the ecotoxicity of fully characterized Au-NPs on the freshwater algae, *Daphnia* and zebrafish in order to provide the basis for an ecotoxicological hazard assessment of the Au-NPs tested. We also aimed to discuss whether common toxicological properties emerge for different Au-NPs. Finally, we tested the soluble gold used for the Au-NPs synthesis under study, which might be present in the environment as a result of the Au-NPs degradation.

2. Materials and methods

2.1. Materials

The nanoparticles studied were suspensions of non-functionalised gold nanoparticles (Au-NPs) or hyaluronic acid functionalised gold nanoparticles (HA-Au-NPs) in citrate buffer, provided by Endor Nanotechnologies S.L. (http://www.pcb.ub.es/homepcb/live/es/p2808.asp). Citrate-stabilised Au-NPs were prepared following the standard methodology described by Turkevich et al. (1953). Functionalised Au-NPs were prepared according to Lee et al. (2006); the Au-NP suspension was mixed with modified OligoHA (5 kDa), and the mixture stirred at room temperature for 1 h.

The molar concentration of Au nanoparticles was 96.62 nM, calculated on the basis of the number of nanoparticles. In addition, Au concentration in the solution was determined by ICP-Ms. giving 1 mg mL^{-1}.

The test solutions/suspensions were prepared the same day of the experiment by adding the NP suspensions into the specific medium to attain final nominal concentrations of 9.6 nM Au-NPs (≈100 mg L^{-1} Au), 1.9 nM Au-NPs (≈20 mg L^{-1} Au) and 0.96 nM Au-NPs (≈10 mg L^{-1} Au).

Gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$; batch 40398PJ, purity > 99.9%) was obtained from Sigma–Aldrich. Potassium dichromate (batch 82403G, 99.94% purity) was obtained from Merck. Gold chloride or potassium dichromate were dissolved firstly in deionized water (stock solution) and further dissolved in each test media to achieve test concentrations.

2.2. Characterization of the NPs in test media

The size, shape as well as the stability of the Au-NPs in the test media was evaluated at 0, 24, 48 and 96 h. The stability of the Au-NPs included in the test solutions/suspensions was assessed by UV–VIS (UV-2501 PC, UV–VIS recording Spectrophotometer Shimadzu®). The Au-NPs size/shape was evaluated initially by TEM (JEOL 1010 with 80 kV), and then characterized by Dynamic Light Scattering (Zetasizer serie nano, Malvern®).

2.3. Toxicological testing

Au-NPs were tested in unicellular algae (*Scenedesmus subspicatus*), *D. magna* and fish (*Brachydanio rerio*), following standard OECD test guidelines (OECD 201, 202 and 203, respectively). In our laboratory, these bioassays are carried out under UN-EN ISO 17025 quality standards, for which they are accredited by the National Accreditation Entity (ENAC; http://www.enac.es/).

Due to the nature of the tested material (turbid/coloured substance), two modifications to the algal test procedure were incorporated following Cleuvers and Weyers (2003) recommendations. First, the volume of the test beakers was reduced from 100 mL to 20 mL, in order to reduce the light path through the coloured solution. Second, the cell concentration was determined by fluorescence instead of cell counting, as this latter technique was unable to discriminate between algae and agglomerated Au-NPs. Fluorescence measurements of the algal solutions were recorded every 24 h with a reader Tecan Infinite M200, and correlated to the cell concentration (ISO 8692:1989; OCDE 201; Mayer et al., 1997) with the following parameters: λ excitation of 437 nm and λ emission of 686 nm.

Fish were sacrificed at the end of the exposure period (96 h), fixed in 10% buffered formalin and processed in paraffin for the histopathology study at the Fish Disease Diagnostic Service (Veterinary School, Universitat Autonoma de Barcelona). Fixed fish were decalcified for 48 h in an 8% formic acid solution, dehydrated in an increasing ethanol series, cleared with Histo-clear (R) and embedded in paraffin. Blocks of 4 μm were sectioned and stained with haematoxylin-eosin for evaluation.

Au-NPs were tested at 9.6 nM Au-NPs (≈100 mg L^{-1} Au) in the *Daphnia* test, at 9.6 nM Au-NPs and 1.9 nM Au-NPs (≈20 mg L^{-1} Au) in the fish test, and at 0.96 nM Au-NPs (≈10 mg L^{-1} Au) in the algae test. The concentration selected in the algal test was based on results of a pre-screening experiment, which showed that higher NPs concentrations increased turbidity to levels (>400 NTU) that influenced negatively the algal growth.

Gold (III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$) was tested in the range of 0.5–5 mg L^{-1}, 0.5–5 mg L^{-1} or at 2 mg L^{-1} (0.6–6.3, 0.6–6.3 and 2.5 μM Au) in algae, *Daphnia* and fish, respectively.

In compliance with accredited methods, and in order to check the quality of the experimental development, positive control groups, containing potassium dichromate, were also tested. For such a purpose, concentrations of 0.3 mg L^{-1}, a range between 0.98 and 1.18 mg L^{-1} and 150 mg L^{-1} were tested in algae, *Daphnia* and fish, respectively.

2.4. Incorporation of NPs into the test organisms

Incorporation of Au-NPs into algae was investigated by means of transmission electron microscopy. For such a purpose, one replicate of the test was taken every 24 h and processed by the Electron Microscopy Unit of the Carlos III Health Institute. Briefly, after obtaining the cell pellet by centrifugation, the samples were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, post-fixed in 1% osmium tetroxide, and, after dehydration in serial ethanol solutions (with an intermediate "en bloc" staining step with uranyl acetate in 70% ethanol), they were embedded in epoxydic resin and polymerized at 45 °C and 60 °C. Ultrathin section were obtained in an Ultracut U6 ultramicrotome, contrasted with uranyl acetate and lead citrate, and studied in a CM12 Philips transmission electron microscope at 120 kV.