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Gold-nanoparticles ingestion disrupts reproduction and development in the German cockroach

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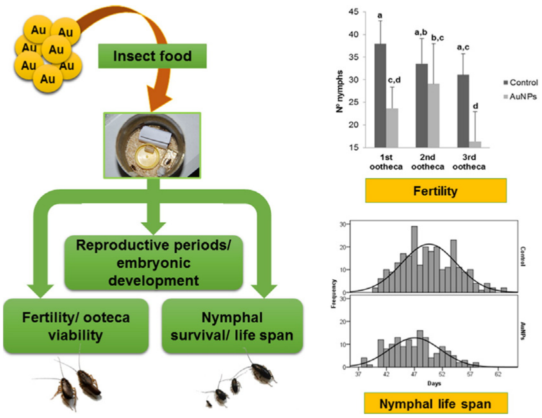
HIGHLIGHTS

- Gold from Au-nanoparticles in food was accumulated by adult *Blattella germanica*.
- Ingestion of Au-nanoparticles modified ootheca viability and fecundity.
- Nymphal life span of *B. germanica* was reduced by gold-nanoparticle ingestion.
- *B. germanica* is proposed as a model in anthroposphere nanotoxicological studies.

GRAPHICAL ABSTRACT



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ABSTRACT

The present work shows the effects of gold nanoparticles (AuNPs) orally administered on reproduction and development of the insect *Blattella germanica*. Newly emerged females were provided with food containing AuNPs (87.44 μg/g) of a size between 15 and 30 nm (mean 21.8 nm), and were allowed to mate with males. Food ingestion, mortality, reproductive parameters (time to ootheca formation and eclosion, ootheca viability and fertility) as well as postembryonic developmental parameters of the first ootheca (nymphal survival and life span) were recorded throughout the experiment. Gold from AuNPs was accumulated by adults of *B. germanica* with a bioaccumulation factor of 0.1. Ingestion of AuNPs did not disturb the time for ootheca formation nor ootheca eclosion. However, ootheca viability was decreased almost by 25% in AuNPs treated females in comparison to controls. At the same time the number of hatched nymphs was decreased by 32.8% ($p < 0.001$) in AuNP group respect to control one. The postembryonic developmental parameters were also affected by AuNPs treatment, with a 35.8% of decrease ($p < 0.01$) in number of nymphs that moulted to second and third instars and a reduction of their life span. Ingestion of AuNPs causes sublethal effects in *B. germanica* that compromises life-traits involved in population dynamics. *B. germanica* is proposed as a model species in nanotoxicological studies for urban environments.

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1. Introduction

In the last decade the use of nanoparticles (NPs)[3] and nanomaterials has increased exponentially, and nowadays these materials are used in industrial, electronics, pharmaceutics, biomedicine, cosmetics and many other fields (Golbamaki et al., 2015; Kaminskas et al., 2012; Li et al., 2015; Oberdörster et al., 2005; Shah et al., 2013). The physicochemical properties derived from the increase in NPs surface area enhance their biological activity, mobility and bioavailability and make NPs ideal for its use as drug carriers (Margulis-Goshen and Magdassi, 2013). But the same properties that make NPs so interesting may also make them harmful because their toxicity could also increase (Golbamaki et al., 2015; Nel et al., 2006).

Therefore, the potential interactions and risk of nanomaterials to the environment and human health should be assessed before be used (Golbamaki et al., 2015; Lapresta-Fernández et al., 2012). Although the data on the toxicological implications of many already produced nanomaterials are rising (Juganson et al., 2015) the information of their toxicity and their underlying mechanisms are still insufficient (Golbamaki et al., 2015). Acute exposure to nanomaterials may have only transient effects that do not apparently affect the health of an organism, but a long term exposure may affect its life history through impacts on its development, viability and reproduction (Posgai et al., 2011). Nevertheless, studies of the effects of chronic exposure at low-dose of nanomaterials are scarce.

The synthesis of gold nanoparticles (AuNPs)[4] has increased in the last years due to their chemical properties and biocompatibility (Daniel and Astruc, 2004; Das et al., 2009; Wang and Wang, 2014) that have made possible their application in many areas (biochemical sensing and detection, bioimaging, biolabels) (Mayavan et al., 2011; Zhao et al., 2013). Also they are widely used in consumer products (cosmetics, pregnancy tests, food, clothing care, supplements in food and beverages, maintenance of automobiles, etc.) (Sung et al., 2011; Vance et al., 2015). In fact, global AuNPs market size was estimated around 1,078.5 million dollars in 2013 with a perspective to reach 4,999.2 million dollars by 2020 (Grandviewresearch, last accessed 17/12/2015). Because the list of consumer products that contain nanomaterials is far to be completed, few data are known about the quantities and types of nanomaterials that are currently present in domestic, clinical and industrial solid wastes.

Gold has been considered biologically inert, but recently allergic reactions in mammals after dermal contact or ingestion (Eisler, 2004; Forte et al., 2008; Sung et al., 2011) have been detected. Furthermore, toxicological results after different ways of AuNPs exposure in invertebrates (Judy et al., 2012; Lapresta-Fernández et al., 2012; Pompa et al., 2011; Rocha et al., 2011) demonstrate that gold is not harmless.

Current literature points fundamentally on ecotoxicological data of the AuNPs effects in aquatic environments; on the other hand, published results about terrestrial ecosystems are limited (Agtuca, 2014; Lapresta-Fernández et al., 2012). However, several studies show evidence that AuNPs are transferred through dietary exposure from plants to their primary consumer with the result of biomagnification and bioaccumulation of gold material in the tissues of the consumer (Judy et al., 2011, 2012).

Cockroaches are tropical and subtropical insects, but they are present in nearly all of the climatic regions of the world, many of them have adapted to living indoors becoming widespread pests. As far as food habits are concerned, cockroaches are opportunistic scavengers with limited foraging ranges and their diet is dependent upon the vagaries of their environment (Raubenheimer and Jones, 2006). In this sense, the cockroach *Blatella germanica* has been considered as an extreme generalist omnivore (Jensen et al., 2015). Cockroaches are an important source of food for a number of organisms, including arthropods, birds, and mammals. As such, they are an important part of the food chain playing an extremely important role in nutrient cycling. All these characteristics together with the fact that cockroaches have been recently proposed as useful bioindicators of indoor pollution (Wang et al., 2015) make *B. germanica* a good candidate for nanoparticles toxicity studies.

The growing global concern about the use of vertebrate animals in experimentation, together with severe regulations worldwide about this matter, makes it difficult to address in depth toxicological studies of nanomaterials. Therefore, it has been proposed to use insects as biological models for assessing the toxicity of nanoparticles (Posgai et al., 2011; Zhou et al., 2012). In a similar way, insects (cockroaches) have been proposed as good candidates for neurobiology, endocrine and neurotoxicological studies (Huber et al., 1990; Peterson et al., 2008; Scharrer, 1987). Also insects have been proposed as biological models to assess nanoparticle's toxicity (Zhou et al., 2012).

Little is known about the effects of AuNPs in insects and the available data indicate that in a cockroach they could affect the insect's behaviour (Rocha et al., 2011) and in a fly they produce significant in vivo toxicity, with reduction of their life span and fertility, DNA fragmentation and overexpression of the stress proteins (Pompa et al., 2011).

The aim of this work was to investigate the life history responses of the insect *Blattella germanica* chronically exposed to AuNPs in a laboratory experiment. Sublethal effects of this kind of NP through a complete life cycle were observed, and the ability of the AuNPs exposed insects to properly develop and reproduce were shown to be affected.

2. Material and methods

2.1. Insects

Blatella germanica (Linnaeus 1767) were reared under laboratory conditions (25 ± 2 °C and 65–70% relative humidity). Water was supplied in glass vials plugged with cotton, and insects were fed with mouse chow as described elsewhere (Garcerá-Zamorano et al., 1981).

2.2. Nanoparticles

$HAuCl_4 \cdot 3H_2O$ (99%) and trisodium citrate (99%) were purchased from Sigma-Aldrich. Milli-Q water was used in all experiments.

The synthesis of AuNPs was based in the previous article of Puntes team (Bastús et al., 2011). A solution of 2.2 mM sodium citrate in Milli-Q water (50 mL) was heated in a three-necked round-bottomed flask to boiling point. A condenser was utilized to avoid the evaporation of the solvent. After boiling started, 0.33 mL of $HAuCl_4$ (25 mM) was added to the citrate solution. The colour of the solution changed from yellow to the typical gold colloidal colour in a few minutes, indicating the presence of AuNPs. In order to increase the particle size of the gold seeds produced in the early stage, the reaction was cooled until the temperature of the solution reached 90 °C and later on 0.33 mL of $HAuCl_4$ solution (25 mM) and 0.33 mL of citrate solution (60 mM) were added. These steps were repeated 6 times more every 2 min.

AuNPs were characterized by UV–Vis and Transmission Electron Microscopy (TEM). UV–Vis absorption spectra were recorded using an Agilent 8453 spectrometer in the range from 190 to 900 nm, 0.1 nm resolution. HRTEM studies of the samples were carried out on a TECNAI F20 microscope operating at 200 kV. The samples were prepared by dropping the colloidal solution on a carbon-coated copper grid.

2.3. Food preparation

AuNPs were administered in the food to adults and nymphs belonging to the experimental group. Four milliliter of AuNPs solution (65.58 mg/L) in sodium citrate (45 mM) were mixed with 3 g of mouse chow powder. The resulting dough was placed in moulds (12 × 5 × 4 mm) and allowed to dry into an oven at 37 °C for 24 h.

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[3] NPs: Nanoparticles.

[4] AuNPs: Gold nanoparticles.

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