http://informahealthcare.com/nan
ISSN: 1743-5390 (print), 1743-5404 (electronic)

Nanotoxicology

Nanotoxicology, Early Online: 1–10
 DOI: 10.3109/17435390.2014.889238

informa
healthcare

ORIGINAL ARTICLE

Impact of gold nanoparticles on zebrafish exposed to a spiked sediment

Amina Dedeh[1], Aurélie Ciutat[1], Mona Treguer-Delapierre[2], and Jean-Paul Bourdineaud[1]

[1]CNRS, UMR EPOC 5805, University of Bordeaux, Arcachon, France and [2]Institute of Chemistry of Condensed Matter of Bordeaux, University of Bordeaux, CNRS, UPR 9048, Pessac, France

Abstract

Increasing use of metallic nanomaterials is likely to result in release of these particles into aquatic environments; nevertheless it is unclear whether these materials present a hazard to aquatic organisms. The impact of contaminated sediment containing 14-nm gold nanoparticles (AuNPs) was investigated in the zebrafish *Danio rerio* exposed for 20 days to two concentrations, 16 and 55 μg/g dry weight. AuNPs were released from the sediment to the water column, and during this period the mean concentrations of AuNP in the filtered water fraction were 0.25 ± 0.05 and 0.8 ± 0.1 μg/L, respectively. A similar experiment with ionic gold contamination was simultaneously performed to obtain a positive control. AuNP exposure triggered various effects in fish tissues including modifications of genome composition, shown using a random amplified polymorphic DNA-PCR genotoxicity test. Expression of genes involved in oxidative stress, mitochondrial metabolism, detoxification and DNA repair were also modulated in response to AuNP contamination. Gold altered neurotransmission, since brain acetylcholine esterase activity increased for both tested doses of AuNP but not for ionic gold. Gold accumulation in fish tissues demonstrated the lower bioavailability of AuNP compared to ionic Au, and underlined the higher toxic potential of the nanoparticle form.

Keywords

Bioaccumulation, gene expression, genotoxicity, gold nanoparticles, neurotransmission, zebrafish

History

Received 8 November 2013
Revised 20 December 2013
Accepted 22 January 2014
Published online 20 February 2014

Introduction

Nanotechnology is a highly promising and exciting interdisciplinary molecular technology that spans many areas of science and technological application because of the surface properties and very small size of nanoparticles (Moore, 2006). Technology has created an impressive increase in the production of nanomaterial (NM) in many sectors of the society (Klaine et al., 2008). These industrial products and wastes tend to end up in waterways (e.g. drainage ditches, rivers, lakes, estuaries and coastal waters) (Daughton, 2004; Moore, 2006). Consequently, as nanotechnology industries start to come on line with larger scale production, it is inevitable that more and more nanoscale products will enter the aquatic environment (Daughton, 2004; Howard, 2004; Moore, 2002; Moore & Noble, 2004; Royal Society and Royal Academy of Engineering, 2004). The global market for gold nanoparticles (AuNPs) in biomedical, pharmaceutical and cosmetic applications was worth $204.6 million in 2006 (www.industryweek.com).

AuNPs are already in use for numerous applications, ranging from biosensors to catalysts, in electronics, new paints, cosmetics and cancer treatments, etc. Their unique properties, chemical stability, their capacity to exhibit a multiplicity of shapes, particle sizes and surface chemistry will ensure that they will be key nanoscale components in many technologies (Daniel & Astruc, 2003; Cobley et al., 2011; Gonzalez et al., 2011; Henry et al., 2011). In fact, AuNPs exhibit unique optical and electrical properties which are of great interest for drug delivery, cellular imaging diagnostics and therapeutic agents (Giljohann et al., 2010; Murphy et al., 2008). Generally, AuNPs are stabilized with organic compounds allowing them to cross cell membranes readily and to escape phagocytic clearance by the reticulo-endothelial system. They are also functionalized with anionic compounds to produce an electrostatic repulsion which avoids conglomerate formation and keeps the desired properties. As with any pharmaceutical or other health technology, it is imperative to understand the full scope of AuNP biocompatibility and to ensure that hazard potential is minimized (Giljohann et al., 2010; Murphy et al., 2008; Kim et al., 2013).

Nanoparticles fate and behavior are dominated by aggregation to particles (>1 μm) sufficiently large so that their transport is dominated by sedimentation (Gustafsson & Gschwemd, 1997; Buffle & Leppard, 1995). The aggregation is defined as the direct attraction between particles. Sedimentation process is important in the self-purification of water bodies and results in pollutant loss from surface waters and accumulation in the sediments and is analog to the likely behavior of manufactured NPs, with aggregation and subsequent sedimentation, an important process in their ultimate fate (Klaine et al., 2008). The estimated amount of nanoparticles released into the aquatic environment is around 65 tons per year (Blaser et al., 2008). A significant portion of NPs in waste waters is expected to partition to sewage sludge (Kiser et al., 2009).

Zebrafish (*Danio rerio*) has been recommended as an inexpensive, quick and easy model to assess the NM toxicity (Fako & Furgeson, 2009). This fish is a small cyprinid with many advantages for toxicological research (Bourdineaud et al., 2013), such as transparency of the embryo, a completely sequenced

Correspondence: Jean-Paul Bourdineaud, CNRS, UMR EPOC 5805, University of Bordeaux, Arcachon Marine Station, Place du Dr Peyneau, 33120 Arcachon, France. Tel: +33 556223930. Fax: +33 556835104. E-mail: jp.bourdineaud@epoc.u-bordeaux1.fr

genome, numerous eggs and no major rearing difficulties. Moreover, zebrafish possess a high degree of homology to the human genome. Effects of AuNPs and other nanoparticles on zebrafish were investigated demonstrating that *D. rerio* is a good animal model for assessing NM impacts from different exposure routes, at various life stages and concentration pressures (Table 1). Most studies investigating AuNP impacts on zebrafish used waterborne exposure and did not represent realistic environmental concentrations. In fact, contamination values tested reached levels up to 250 mg/L in the water column, and the majority of AuNPs used were functionalized with organic compounds (Asharani et al., 2008, 2010; Bar-Ilan et al., 2009; Kim et al., 2013; Harper et al., 2011; Truong et al., 2012a,b, 2013). Dietary exposure of zebrafish to AuNPs demonstrated a toxic impact at much lower concentrations (Geffroy et al., 2012).

The sediment constitutes a very important storage reservoir, it can have a very strong complexing capacity and contaminants come mainly from the deposit of particles in suspension in the water column (Ciutat & Boudou, 2003). Indeed, terrestrial ecosystems are expected to be an ultimate sink for a large portion of NPs (Gottschalk et al., 2009). It has been observed that marine and earthworms can accumulate NPs from contaminated sediments; support that sediment presents a source of NM contamination (Unrine et al., 2010; García-Alonso et al., 2011). In mesocosms modeling an estuarine food web, it was shown that a single AuNP dose could be transferred from the water column to the sediment (Ferry et al., 2009) (the fraction of AuNP in sediment reached 24.5%). However, the reverse situation has never been assessed, that in which metal NPs could be made bioavailable to the water column from the sediment. It is thus of great interest to determine if intact NPs can be taken up from the sediment by organisms.

Therefore, the purpose of this study was to assess bioavailability and toxic effects of AuNPs (14 nm) from spiked sediment (16 and 55 μg/g dry weight) on zebrafish exposed for 20 days. Contaminations concentrations did not reflect the natural levels

Table 1. A literature survey of metal nanoparticles doses recently used in zebrafish.

Life stage	Exposure route and metal nanoparticles characteristics	Contamination pressures	Reference
Zebrafish embryos	Waterborne Ag NPs (5–20 nm) Capping with BSA	5, 10, 25, 50 and 100 mg/L	Asharani et al. (2008)
Zebrafish embryos	Waterborne Au NPs (15–35 nm) Ag NPs (5–35 nm) Pt NPs (3–10 nm) Capping with PVA	10, 25, 50, 75 and 100 mg/L	Asharani et al. (2010)
Zebrafish embryos	Waterborne Au NPs (1.5 nm) Surface functionalization with TMAT	0.08, 0.4, 2, 10 and 50 mg/L	Kim et al. (2013)
Zebrafish embryos	Waterborne Au NPs (0.8, 1.5 and 15 nm) Surface functionalization with TMAT, MES, MEE and MEEE	0.016, 0.08, 0.4, 2, 10, 50 and 250 mg/L	Harper at al. (2011)
Zebrafish embryos	Waterborne Au NPs (1.5 nm) Surface functionalization with TMAT, MES and MEEE	50 mg/L	Truong et al. (2012a)
Zebrafish embryos	Waterborne Au NPs (1.2 nm) Surface functionalization with MPA	0.08, 0.4, 2, 10 and 50 mg/L	Truong et al. (2012b)
Zebrafish embryos	Waterborne Au NPs (1.5 nm) Surface functionalization with TMAT, MES and MEEE	0.016, 0.08, 0.4, 2, 10, 50 and 250 mg/L	Truong et al. (2013)
Zebrafish embryos	Waterborne Au NPs (3,10, 50 and 100 nm) Surface functionalization with TPPMS	50, 5, 0.5 and 0.05 mg/L	Bar-Ilan et al. (2009)
Adult zebrafish	Waterborne Cu NPs (80 nm)	0.25 and 1.5 mg/L	Griffitt et al. (2007)
Adult zebrafish	Waterborne AgNPs (5–20 nm)	30 and 120 mg/L	Choi et al. (2010)
Adult zebrafish	Waterborne TiO_2 (34 nm), ZnO (68 nm) and CeO_2 (10 nm) NPs	0.5 and 5 mg/L	Johnston et al. (2010)
Adult zebrafish	Dietary AuNPs (12–50 nm) Capping with citrate	0.04 and 0.1 μg/day/g fish body weight	Geffroy et al. (2012)
Adult zebrafish	Dietary CdS NPs (8–50 nm)	0.04 and 0.1 μg/day/g body weight	Ladhar et al. (2013)
Adult zebrafish	Sedimentary AuNPs (14 nm) Capping with citrate	16 and 55 μg/g dried sediment weight; 0.25 and 0.8 μg/L released in water column	This study

Abbreviations: PVA, polyvinyl alcohol; TMAT, *N*,*N*,*N*-trimethylammoniumethanethiol; MES, 2-mercaptoethanesulfonic acid; MEEE, 2,2-mercaptoethoxyethoxyethanol; TPPMS, monosulfonated triphenylphosphane; MPA, mercaptopropionic acid.

and were selected in reference to a previous study which investigated the effects on earthworms of a sediment spiked with AuNPs and that used 5, 20 and 50 mgAuNPs/kg dry weight (Unrine et al., 2010). The originality of this work lies in the fact that for the first time an NP-spiked sediment was used as a contamination source for a vertebrate model living in the overlying water column. Only few authors have used aquatic sediment as an NP contamination source and always with invertebrate animal models such as annelids (García-Alonso et al., 2011). The bioaccumulation of gold transferred from sediment to water column, neurotransmission perturbation, gene expression assessment in various tissues and DNA damage in whole body of zebrafish were investigated using microcosms made up of a mixed biotope consisting of water column and contaminated sediment. For comparative purposes, and to provide a control for ionic form, zebrafish were also exposed simultaneously to a sediment containing the same levels of gold salt ($KAuCl_4$).

Materials and methods

Preparation of AuNPs

AuNPs were prepared according to the refined Turkevich method, which is based on the reduction of gold ions in aqueous solution with trisodium citrate (Kimling et al., 2006). Potassium gold(IIII)chloride (100.13 mg, $KAuCl_4$, 99.9%, Aldrich, St. Louis, MO) was dissolved in 100 mL ultrapure water and added under vigorous stirring to 400 mL boiling distilled water. When boiling resumed, 50 mL of 0.88% sodium citrate (441 mg) was added under vigorous stirring and this suspension was boiled for an additional 20 min. The final colloidal suspension contained gold NPs and reached a gold concentration of 90 ± 4 mg of gold/L which represented 2.99×10^6 particles/L. Nanoparticle structural and size characterizations were investigated using conventional and high-resolution transmission electron microscopy (TEM and HRTEM). Preliminary TEM and HRTEM observations were performed with a JEOL 2200 FS equipped with a field emission gun, operating at 200 kV. High resolution transmission electron microscopy micrographs were acquired with a Gatan Ultrascan CCD 2k x 2k. Samples were prepared by drop casting a 2.5-µL aliquot of the NP suspension onto a 300 mesh carbon-coated copper grid, which was allowed to evaporate under ambient conditions. In order to be representative and statistically meaningful, many images from several regions of various samples were recorded and the most characteristic results are presented in Figure 1(a–c). The size distribution was obtained by analysis of TEM images of 200 nanoparticles located at different regions of the grid. A log-normal distribution typical of this synthetic method is observed, with an average diameter of 14 nm and a standard deviation of 2 nm. Nanoparticles had a spherical shape. The zeta potential of AuNPs in suspension was determined using dynamic light scattering and phase analysis light scattering, respectively (Zetasizer 3000HS, Malvern Instruments, Worcestershire, UK) and reached −50 mV in a suspension of pH 8.

Experimental design and sediment sampling

The experiment used glass aquaria of 12 cm × 12 cm × 24 cm containing 5–6 cm of sediment and filled with 3 L (16 cm) of fresh water representing a water column height of 16 cm laid above a 6 cm thick layer of sediment. Average water parameters throughout the experiment were as follows: temperature of 21.06 ± 0.46 °C; conductivity of 465 ± 94 µS/cm and pH of 8.14 ± 0.1 (mean ± SD). The experimental units were permanently aerated by air bubbling in the superficial layer of the water column in order to produce an oxygen-saturated environment and exposed to a 12:12 h light:dark regime. Five replicates were set up for each condition (=5 independent aquaria per condition). A similar experiment with gold salt ($KAuCl_4$) was performed simultaneously to obtain a control for ionic form.

Time zero for the experiment was just after adding zebrafish to the experimental units. The experiment lasted 20 days with seven sampling times for water analysis: 0, 3, 7, 10, 13, 17 and 20 days. The complete experimental design was based on 25 experimental units set up simultaneously.

The sediment used had been collected in from the Garonne river upstream from Bordeaux at a site called Cadaujac (44°45′23″N, 0°31′44″W, France), and its main characteristics are reported in Table S1. It was homogenized, sieved at 2.5 mm diameter and stored at 4 °C in the dark for 72 h. Five samples were collected in order to determine the ''fresh weight/dry weight'' ratio (fw/dw = 1.58 ± 0.08, mean ± SD, after 72 h of desiccation at 60 °C) and the background gold concentration in sediment (0.050 ± 0.007 µg/g dw). The sediment was contaminated by adding one of the forms of gold solution followed by a 15 min mechanical homogenization to with a mixer (Peugeot, Pc20543, Paris, France) then stored at 4 °C for 72 h. We added, respectively, 111 and 555 mL of a suspension containing 90 mg/L AuNP per kg of sediment for C_1 and C_2 conditions. For the ionic gold conditions, we added, respectively, 19 and 95 mL of a solution containing 521 mg/L of $KAuCl_4$ per kg of sediment for C_1 and C_2 conditions. After sediment contamination, five sediment samples were collected randomly from each contamination condition at different places and depths and checked for Au distribution. The resulting quantifications gave the following values for the experimental conditions: a sediment contaminated with 15.6 ± 1.72 µg/g dry weight of AuNPs [C_1], and a sediment contaminated with 55.2 ± 3.74 µg/g dry weight of AuNPs [C_2]. For gold ionic spiking the following values were quantified: a sediment contaminated with 14.8 ± 2.53 µg/g dry weight of $KAuCl_4$ [C_1] and a sediment contaminated with 54.5 ± 4.33 µg/g dry weight of $KAuCl_4$ [C_2]. Eight hundred grams of control or contaminated sediment (fresh weight) was introduced into each experimental unit. Dechlorinated tap water (3 L) was then carefully added after 48 h, avoiding disturbance at the sediment surface. The experimental units were allowed to settle and equilibrate for 8 days before fish were placed.

Zebrafish culture and sampling

Wild-type adult zebrafish (*D. rerio*) were purchased from a commercial company (Exomarc, Lormont, France), and acclimatized for 7 days at 20 °C in a large tank filled with dechlorinated water (body weight: 0.79 ± 0.03 g, wet wt; standard length: 3.33 ± 0.07 cm, $n = 6$). ive fish were randomly placed in experimental units and fed 25 mg/fish every 3 days (Dr. Bassleer Biofish Food). Six fish were sampled to determine the background Au concentration. After 20 days of exposure, fish were collected, killed within seconds by immersion in melting ice in agreement with the ethical guidelines displayed and used by the NIH intramural research program, dissected and frozen at −80 °C for bioaccumulation, neurotoxicity assessment, genotoxicity and gene expression analyses.

Turbidity measurements

Turbidity in the water column was measured at the seven sampling times in each experimental unit. Ten milliliters of water was collected from the central part of the experimental units. Water samples were then processed for measurement with a turbidimeter (Turb 430 IR/T; WTW Company, Weilheim, Germany). Results were expressed in Formazine Turbidity Units

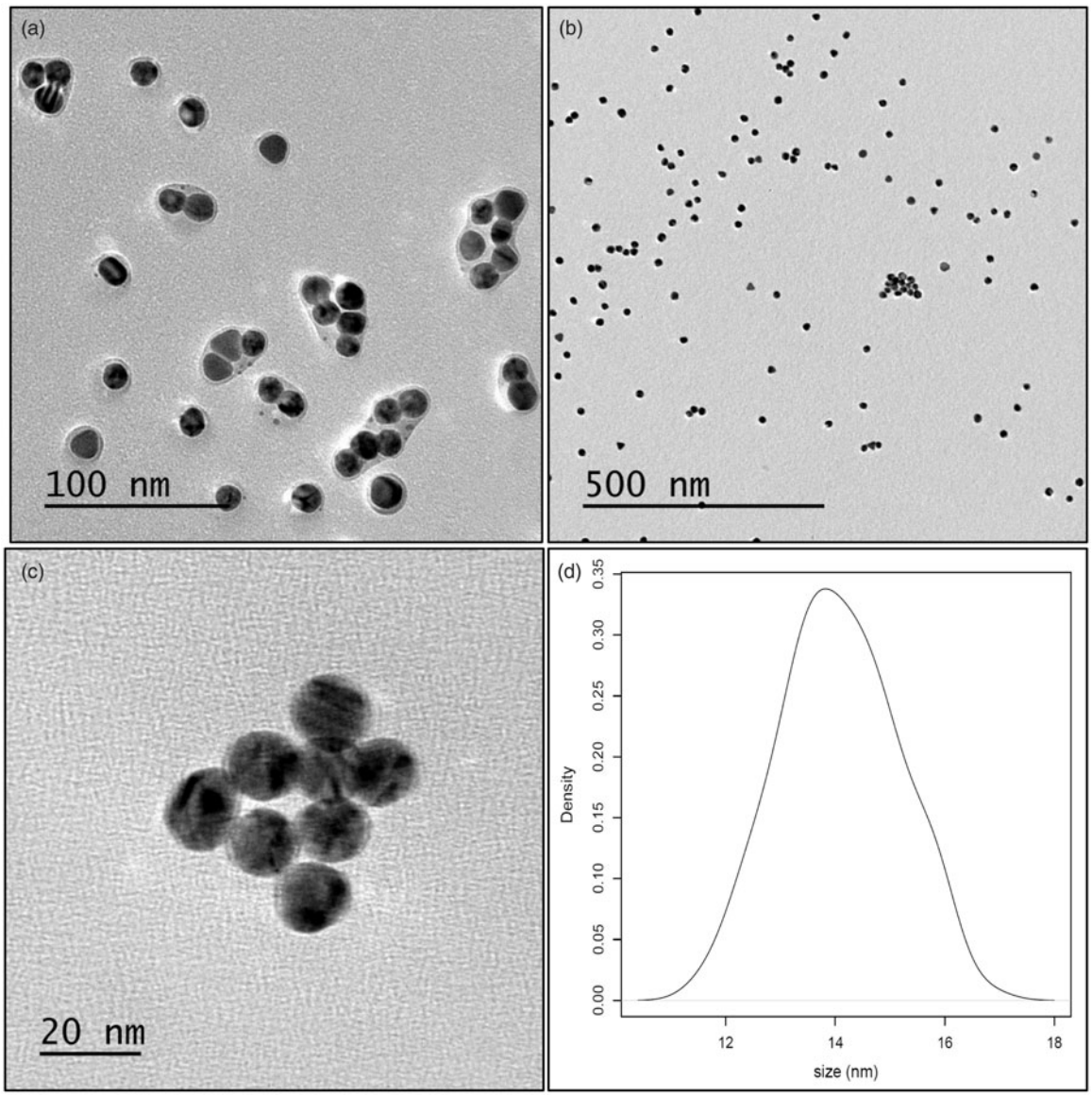

Figure 1. Panels a, b, and c show representative TEM micrographs of 14 nm gold NPs at three different magnifications. Panel d: Distribution of nanoparticle size.

(FTU), which are equivalent to Nephelometric Turbidity Units (NTU) (Ciutat et al., 2005). No significant differences in turbidity were observed for all treatments. Turbidity values were between 400 and 800 FTU during the 20 days of exposure (Figure S1).

Gold quantification

Gold quantifications in water and zebrafish tissues were carried out by electrothermic atomic absorption spectrophotometry with Zeeman correction using a graphite furnace (M6 Solaar AA spectrometer, Thermoptec, Mulgrave, Australia) with a detection limit of 25 ng Au/L. Gills (tissue weight between 10 and 20 mg) were digested in 1 mL of *aqua regia* (0.25volume of 65% HNO_3 – Merck, Darmstadt, Germany – and 0.75 volume of 37% HCl – Riedel de Haën, Sigma-Aldrich, Germany) at 100 °C for 3 h. Digestive tract and muscle samples (tissue patches weighing more than 20 mg) were digested in 3 mL of *aqua regia* at 100 °C for 3 h. Liquid from digested gill, digestive tract and muscle underwent a 6-fold dilution with ultrapure water (MiliQ, Bedford, MA). Fish brains (weighing less than 10 mg) were digested in 0.2 mL of *aqua regia* at 100 °C for 3 h then diluted 5-fold with ultrapure water. Detection limits were 5 ng/g for brains weighing 5 mg, 8 ng/g for gill samples weighing 15 mg, and 15 ng/g for muscle samples weighing 30 mg.

Gold determination was performed on water samples from the seven sampling times. Twenty milliliters was collected from each experimental unit, 10 mL was directly acidified with *aqua regia* (600 µL) for gold determination in unfiltered samples; the other 10 mL was filtered at 0.2 µm (cellulose acetate membrane SFCA) and then acidified with *aqua regia* (600 µL) for gold quantification in the filtered water fraction. The analytical methods were simultaneously validated for each sample series by analyzing standard solutions of gold (Prolabo, Nantes, France). Values were consistently within the certified ranges.

Acetylcholine esterase activity measurement

Brain and muscle acetylcholine esterase (AchE) activity, a neurotoxicity biomarker, was assessed according to the most common method, first described by Ellman et al. (1961), using microplate spectrometry. This assay makes use of the thiocholine-mediated cleavage of the chromogenic disulfide 5,5-dithiobis 2-nitrobenzoic acid. Before enzymatic assays, total protein

content of brain and muscle was measured according to the method described by Bradford (1976).

Quantification of genotoxic damages by RAPD-PCR and analysis of the melting temperature curves of the PCR products

Genotoxic effects of AuNP and ionic gold were assessed using a random amplified polymorphic DNA (RAPD)-based methodology. This method was successfully used on zebrafish exposed to cadmium, and gold or cadmium sulfide NPs (Cambier et al., 2010; Geffroy et al., 2012; Ladhar et al., 2013; Orieux et al., 2011). Genomic DNA isolation was performed by mincing frozen fish tissues o with a scalpel and using the DNeasy Blood & Tissue Kit (QIAGEN, Limburg, Netherlands) according to the manufacturer's instructions. Primers used for RAPD-PCRs were the decamer oligonucleotides OPB7 (5′-GGTGACGCAG-3′) and OPB11 (5′-GTAGACCCGT-3′). Reference β-actin probes were reverse 5′-AAGTGCGACGTGGACA-3′ and forward 5′-GTTT AGGTTGGTCGTTCGTTTG-3′ purchased from Sigma-Proligo (St. Louis, MO). Real time RAPD-PCRs were done with the Lightcycler apparatus (Roche, Basel, Switzerland) as described (Lerebours et al., 2013). Melting temperature curves analyses were done using the LightCycler Software 3.5 (Roche) as described (Lerebours et al., 2013).

Gene expression analysis in zebrafish

After 20 days, zebrafish were dissected and sampled tissues (brain, gills, digestive tracts and muscle) kept frozen in RNA-later (QIAGEN) at −80 °C until used. The expression of eight genes was analyzed; five samples were used for each condition. Samples were crushed and total RNA was extracted using the Absolutely RNA RT-PCR Miniprep kit (Stratagene, Santa Clara, CA), according to the manufacturer's instructions. In order to eliminate the maximum of lipids and proteins, we added a step of phenol–chloroform–isoamylic alcohol (25:24:1) extraction. At the end of this process, 30 µL of total RNA was collected. First-strand cDNA was synthesized from 14 µL of total RNA using the Affinity ScriptMulti Temperature cDNA Synthesis kit (Stratagene). The cDNA mixture was kept at −20 °C until it was used in real-time PCR reactions, which were performed in a thermocycler (Stratagene) following the manufacturer's recommendations. All primer pairs were designed with the Lightcycler probe designer setup (Table S2). Relative quantification of each gene expression level was normalized according to the β-actin gene expression. The choice of β-actin gene as a reference was relevant because its expression did not vary with contamination. In brain, for example the mean Ct remained constant for the different conditions (control: 21.7 ± 1.5; C_1AuNPs: 21.7 ± 2.4; C_2AuNPs: 23.2 ± 1.72; C_1Au: 23.2 ± 1.72 and C_2Au: 24.1 ± 2.6).

Statistical analysis

Significant differences in number of hybridizing sites and frequency PCR products when compared to control were determined with the *U*-test of Mann–Whitney or a *t*-test depending on data normality. Other significant differences in gold levels, acetylcholinesterase activity and relative gene expressions in fish tissues under different conditions were determined with the ANOVA on ranks using Tukey and Dunn's tests ($p < 0.05$). The statistical software used for all tests was Sigma stat 3.5 (San Jose, CA).

Results

During the experiment no mortality attributable to gold exposure was observed.

Gold release in the water column

Data for total and dissolved gold in the water column are shown in Figure S2(a) and (b). The mean gold concentration in unfiltered water samples reached 3.0 ± 0.8 and 11 ± 2 µg/L for C_1 and C_2 AuNP exposures, respectively, and 4 ± 1 and 33 ± 6 µg/L for C_1 and C_2 ionic exposures, respectively (means ± SE). In filtered water samples, in which gold is not associated with sedimentary particles, the filtered fraction of metal in the water column is bioavailable, therefore constituting the real contamination pressure. The mean gold concentration in filtered water samples reached 0.25 ± 0.05 and 0.8 ± 0.1 µg/L for C_1 and C_2 AuNPs exposures, respectively, and 0.33 ± 0.01 and 0.6 ± 0.04 µg/L for C_1 and C_2 ionic exposures, respectively (means ± SE). Thus, gold concentrations were similar for both forms of gold contamination. The fractions of bioavailable AuNPs over total gold in the water column were 8.3% and 7.3% for C_1 and C_2 exposures, respectively. The fractions of bioavailable AuNP over initial total gold in sediment were 6.0×10^{-5} and 5.4×10^{-5} for C_1 and C_2 exposures, respectively.

Gold (Au) accumulation in zebrafish tissues

In control fish, gold was above the detection limit only in the digestive tract. After AuNP exposure, Au remained unquantifiable in brain and muscle. In digestive tract, 11- and 70-fold increases in gold concentration compared to control were observed for C_1 and C_2 AuNPs exposures, respectively. In gill, bioaccumulation factors (calculated as the ratio of the concentration of gold in tissue over the concentration of filterable gold in the water column) reached 56 and 36 for C_1 and C_2 AuNP exposures, respectively. After exposure to ionic Au, Au remained unquantifiable in brain. In gill, bioaccumulation factors relative to control reached 43 and 210 for C_1 and C_2 ionic Au exposures, respectively. In muscle, bioaccumulation factors relative to the most bioavailable AuNP in the water column reached 200 and 266 for C_1 and C_2 ionic Au exposures, respectively. Thus, digestive tract accumulated the most of both forms of Au. For the same concentrations of filtered Au fraction in the water column, digestive tract accumulated twice as much ionic Au as AuNPs (Table 2).

AchE activity in brain and muscles

Although AuNP concentrations were below the detection threshold in brain, exposure to AuNP triggered a significant increase in AchE activity, reaching 24% and 25% above control for C_1 and C_2 AuNP exposures, respectively. In contrast, ionic Au exposures did not influence brain AchE activity. In muscle, despite the fact that Au concentration was below the detection threshold, exposure to the lower (but not higher) AuNP concentration resulted in a 48% increase in AchE activity. The higher ionic Au concentration triggered a 64% increase in muscle AchE activity (Table 3).

Genotoxicity analysis

After 20 days of exposure, the OPB7 probe showed differences in DNA composition between contaminated and control fish. Indeed, the number of probe hybridization sites displayed significant 3-, 2.5- and 2-fold increases after exposure to both AuNP concentrations and to C_1 ionic Au, respectively (Table 4). Using the OPB7 probe, the frequency of PCR product appearance from contaminated and control DNAs was different (Table S3). After exposure to both forms and concentrations of Au, PCR products of melting temperature (T_m) [85–86 °C] showed a significant decrease in frequency of appearance (from 0.8 for control down to 0.3 and 0.2 for contaminated fish), whereas a significant increase in frequency (from 0.1 for control up to 0.5 and 0.7 for

Table 2. Gold levels detected in zebrafish brain, gills, digestive tract and muscle (μg/g) fresh weight after 20 days of exposure[a].

	Brain	Gills	Digestive tracts	Muscle
Control	<dt	<dt	0.020 ± 0.001	<dt
Nano Au concentration in water (in sediment)				
0.25 μg/L (16 mg/kg)	<dt	0.0140 ± 0.0008*	0.22 ± 0.03*	<dt
0.8 μg/L (55 mg/kg)	<dt	0.029 ± 0.001*	1.4 ± 0.3*	<dt
Ionic Au concentration in water (in sediment)				
0.33 μg/L (16 mg/kg)	<dt	0.0120 ± 0.0008*	0.46 ± 0.05*[b]	0.060 ± 0.006*
0.6 μg/L (55 mg/kg)	<dt	0.21 ± 0.03*[b]	3.16 ± 0.52*[b]	0.16 ± 0.04*

<dt, below the detection threshold.
*Statistically significant values compared to control assessed by the ANOVA on ranks – Tukey and Dunn's test ($p < 0.05$).
[a]Metal concentration are means ± SEM, $n = 3$.
[b]Statistically significant values compared to AuNPs exposure assessed by Mann–Whitney test ($p < 0.05$).

Table 3. Acetylcholine esterase activity in brain and muscle extracts from zebrafish exposed to control and contaminated sediment[a].

	Brain	Muscle
Control	440 ± 17	720 ± 74
Nano Au concentration in water (in sediment)		
0.25 μg/L (16 mg/kg)	546 ± 8*	1068 ± 37*
0.8 μg/L (55 mg/kg)	551 ± 22*	920 ± 70
Ionic Au concenration in water (in sediment)		
0.33 μg/L (16 mg/kg)	421 ± 38	748 ± 109
0.6 μg/L (55 mg/kg)	467 ± 22	1183 ± 43*

*Statistically significant values compared to the control compared assessed by the ANOVA on ranks – Tukey and Dunn's test ($p < 0.05$).
[a]Data are presented as mean ± SEM ($n = 3$), and represent the specific initial velocity of acetylcholinesterase in nmol of hydrolyzed substrate per mg of protein per minute.

Table 4. Number of hybridization sites per genome unit of RAPD probes, after RAPD-PCR performed on individual genomic DNA from control and contaminated zebrafish.

	OPB7	OPB11
Control	0.52 ± 0.08	$(110 \pm 31.3) \times 10^{-5}$
Nano Au concentration in water (in sediment)		
0.25 μg/L (16 mg/kg)	1.5 ± 0.2*	$(383 \pm 106) \times 10^{-5}$
0.8 μg/L (55 mg/kg)	1.26 ± 0.16*	$(373 \pm 85) \times 10^{-5}$
Ionic Au concentration in water (in sediment)		
0.33 μg/L (16 mg/kg)	1.09 ± 0.19*	$(232 \pm 60) \times 10^{-5}$
0.6 μg/L (55 mg/kg)	0.73 ± 0.12	$(103 \pm 18.7) \times 10^{-5}$

Results are mean ± SEM ($n = 10$).
*Statistically significant values compared to control as assessed by the Mann–Whitney U-test $p < 0.05$.

contaminated fish) was observed for PCR products of T_m [86–87 °C].

Gene expression variations

Relative gene expression in brain, gill, muscle and digestive tract from exposed fish were compared to those from control fish (Table S4). From these values we determined differential gene expression, calculated as the ratio of relative expression in contaminated fish tissue over that of control (Table 5). Although Au accumulation within brain was below the detection threshold, a possible acclimative genetic response to both gold forms occurred at C_2 exposure. In fact, responsive genes were up-regulated several fold. Those genes were involved in oxidative stress (*sod1* and *sod2*), mitochondrial respiration (*cox1*), metal detoxification (*mt2*), DNA repair (*gaad*) and neurotransmission (*ache*) in brain exposed to both Au forms at C_2. Significant differences in gene regulation were observed between the two gold forms, some genes being three times more expressed in response to NPs than to ionic forms (*cox1*, *sod2*, *hsp70*, *ache*). In gill, both concentrations of AuNP triggered the repression of *sod2*, *cox1*, *rad51* and *gaad* genes. Contrary to NP exposures, the ionic form triggered the up-regulation of *mt2*, *gaad*, and *ache* genes at higher concentration suggesting a potential defense mechanism. In digestive tract, the two forms of gold resulted in different gene expression patterns. An up-regulation of DNA repair (*gaad* and *rad51*) and *ache* genes was observed for the lower NP exposure whereas *gaad* and *ache* genes returned to the basal level and *rad51* was 3-times less expressed at higher NP exposure. For the ionic gold exposures, contrary to the NP form, it was the higher exposure that resulted in the up-regulation of *mt2*, *gaad*, *rad51* and *ache* genes. In muscle, despite the fact that gold concentration was below the detection threshold, NP exposures triggered the down-regulation of several genes including *cox1*, *sod1*, *sod2*, *hsp70*, *ache* and *gaad.* Only the *mt2* gene was up-regulated 2-fold for both concentrations of NP. The lower ionic gold exposure, for which the gold accumulation level was quantifiable and equal to 60 pg/g, also caused the down-regulation of *cox1*, *ache*, *gaad* and *rad51* but the 20-fold up-regulation of the *sod2* gene.

Discussion

Metal and NP release from the sediment

Water turbidity displayed high values (400–800 FTU), which demonstrates the important resuspension of sediment particles due to fish swimming near the sediment water interface. Such a phenomenon had already been observed in the case of fathead minnows, who disturbed the sediment and increased benzo(a)pyrene concentration in the water compartment (McCarthy et al., 2003). This generated an increase of filtered gold in the water compartment; reaching values equal to 0.25 and 0.8 μg Au/L in filtered water for C_1 and C_2 nanoparticle gold concentrations in sediment. It indicates that for 50 ppm of nanoparticle gold in sediment, less than 1 μg/L of gold is released in the water column. Since the free and filterable water column NPs can be considered as bioavailable for fish, this concentration constitutes a very low contamination pressure compared with other published studies where concentrations reached, in some cases several orders of magnitude above 1 μg/L (Asharani et al., 2008, 2010; Bar-Ilan et al., 2009; Harper et al., 2011; Kim et al., 2013; Truong et al., 2012a,b, 2013).

The lowest observed adverse effect level (LOAEL) that had been described up to now in the literature was 10 mg/L for a *N*,*N*,*N*-trimethylammoniumethanethiol-covered AuNP (1.3 nm) after 114 h of exposure in zebrafish embryos and was related to embryo mortality, and 1 mg/L after 90 h of exposure when eye

Table 5. Differential gene expressions in various tissues from contaminated fish after 20 days of gold exposure.

Organs	Genes	0.25 µg/L nano Au	0.3 µg/L ionic Au	0.8 µg/L nano Au	0.6 µg/L ionic Au
Brain					
Oxidative stress	*sod 1*	=	2*	4*	3*
	sod 2	=	12*	12*[b]	4*
Mitochondrial metabolism	*cox1*	=	1/15*	7*[b]	2*[a]
Detoxification	*mt2*	=	1/6*	8*	8*[a]
	hsp70	=	=	11*[b]	3*
DNA repair	*gaad*	=	1/40*	9*	6*[a]
	rad51	=	1/38*	=	=
Neurotransmission	*ache*	=	4*	11*[b]	4*
Gills					
Oxidative stress	*sod 1*	=	=	=	=
	sod 2	1/4*[b]	3*	1/3*	=
Mitochondrial metabolism	*cox1*	1/4*[b]	1/11*	1/5*	1/4*
Detoxification	*mt2*	=	=	=	7*
	hsp70	=	=	=	=
DNA repair	*gaad*	3*[b]	1/12*	2*[b]	5*[a]
	rad51	1/3*	=	1/3*	=
Neurotransmission	*ache*	1/3*	=	=	3*
Digestive tract					
Oxidative stress	*sod 1*	=	4*	=	=
	sod 2	=	=	1/3*	1/5*
Mitochondrial metabolism	*cox1*	=	1/12*	1/11*	1/7*
Detoxification	*mt2*	=	1/8*	=	2*[a]
	hsp70	=	=	=	=
DNA repair	*gaad*	6*	=	=	20*
	rad51	6*	=	2*[ab]	5*
Neurotransmission	*ache*	5*	=	=	7*
Muscle					
Oxidative stress	*sod 1*	=	=	1/3*	nd
	sod 2	=	20*	1/9*	nd
Mitochondrial metabolism	*cox1*	1/5*	1/3*	1/10*	nd
Detoxification	*mt2*	2*	=	2*	nd
	hsp70	1/5*	=	1/16*[a]	nd
DNA repair	*gaad*	=	<1/100*	1/6*	nd
	rad51	1/3*[b]	1/10*	=	nd
Neurotransmission	*ache*	1/4*	1/3*	1/20*[a]	nd

The differential expression of a gene in a tissue is the ratio of its relative gene expression in fish maintained in the indicated contaminated conditions over that of fish maintained in uncontaminated conditions (mean, $n = 5$). =, identical to control condition; NE, no expression recorded, the Ct value was above that of the control without DNA; nd, not done.

*Statistically significant differential expression compared with control assessed by the ANOVA on ranks – Tukey and Dunn's test ($p < 0.05$).

[a]Statistically significant differential expression compared with 0.3 µg/L exposure.

[b]Statistically significant differential expression compared with ionic exposure assessed by Mann–Whitney test ($p < 0.05$).

pigmentation gene expression was considered (Kim et al., 2013). In contrast, in this study a much lower LOAEL for citrate-covered AuNP was revealed, a concentration as low as 0.25 µg/L in the medium for 20 days of treatment. This was observed in adult zebrafish, which that are considered much less sensitive to toxicants than embryos.

AuNP-bioaccumulation

Gold accumulation in fish tissues was highest in the digestive tract, which can be explained by the transfer of gold from gills to liver or by the fact that zebrafish can be contaminated by the dietary route while pecking surface sediment to feed on deposited organic matter like biofilms. These results are in agreement with metal accumulation in zebrafish exposed to a polymetallic gradient (Lot River, France) for 7 days, the highest cadmium concentrations were observed in the digestive tract despite the waterborne exposure (Orieux et al., 2011). Metal values recorded in zebrafish were, respectively, 4.6 ± 1.8 and 1.9 ± 0.5 nmol of Cd/g of tissue in the digestive tract and the gills after 3 days of exposure to 15 µg Cd/L. Bioaccumulation of gold NPs has been demonstrated in different animals such as filter feeding mussel *Mytilus edulis* where high gold levels were detected in the digestive gland (61 µg/g) and low levels in the gills (0.5 µg/g) and mantle (0.02 µg/g) after exposure to 750 µg AuNPs/L for 24 h (Tedesco et al., 2008). When zebrafish were exposed to food containing 4.5 µg/g AuNP (12 nm) for 36 days, higher accumulations were recorded in brain and liver reaching, respectively, 4.6 and 3.0 µg/g (Geffroy et al., 2012). In zebrafish embryos exposed to 50 mg/L AuNP for 24 h, to 5 mg/L (AuNPs) for 120 h, and to 50 mg/L of 2-mercaptoethanesulfonic acid-covered AuNP (1.5 nm) for 48 h, gold retention reached 0.6 µg/g (Asharani et al., 2010), 1.6 ng/embryo (Bar-Ilan et al., 2009), and 320 ± 30 ng/embryo (Harper et al., 2011), respectively. From bioaccumulation results, two potential contamination pathways can be deduced; the direct pathway which represent the bioavailable gold in the water column and the dietary pathway which can be the adsorbed gold on sediment particles.

AchE activity modification

AchE activity measured in brain extracts after 20 days of exposure to sediment contaminated with AuNPs increased compared to control. At first glance, this might appear contradictory with numerous data showing that most pollutants inhibit AChE activity in zebrafish, e.g. lead and cadmium (de Lima et al., 2013) and

mercury (Richetti et al., 2011). Nevertheless, some publications mention an activation phenomenon. For instance, in zebrafish exposed to different isotopic compositions of uranium, an increase in AchE activity was observed in brain extracts (Barillet et al., 2007). Romani et al. (2003) looked at possible changes in AchE activity in brain and white muscle of the Mediterranean bony fish *Sparus auratus* exposed for 20 days to sublethal copper concentrations (100 or 500 ng/L). Copper exposure led to increased specific activity and improved catalytic efficiency of AChE in those tissues. This increase in catalytic efficiency was also observed with Cu^{2+} ions. In particular, increased ionic strength enhanced the hydrolysis of acetylcholine by AChE in the pacific electric ray, *Torpedo californica* (Berman & Leonard, 1990).

Genotoxicity analysis

Recently, experiments on adult and embryonic life stages of zebrafish demonstrated that Cu and Ag NPs could cause acute toxicity to zebrafish (Griffitt et al., 2007; Asharani et al., 2008). In this work, using quantitative RAPD, we looked at the creation or elimination of probe hybridization sites on genomic DNA of contaminated fish compared to control, along with modifications of PCR products' T_m profiles, which are effective parameters in detecting small changes in DNA sequences (Cambier et al., 2010). Results of RAPD-PCR emphasized the genotoxicity of AuNPs and showed modifications in the number of OPB7 hybridization sites in fish genomic DNA and in frequency of PCR products. At high NP gold concentration, number of hybridization sites increased compared to control and ionic gold exposure. Studies have shown that AuNP can alter genomic material by binding to DNA and causing conformational changes (Goodman et al., 2006). Citrate-capped Au NP caused DNA damage in human HepG2 cells (Fraga et al., 2013) and in *D. rerio* (Geffroy et al., 2012). Other metallic NPs triggered genotoxicity in fish DNA such as silver NPs, which induced chromosomal aberrations and aneuploidy in medaka cells (Wise et al., 2010), and titanium NPs in rainbow trout cells (Vevers & Jha, 2008). Genomic alteration effects are due to single mutations of genomic DNA caused directly (NPs binding to DNA) or indirectly (oxidative stress) from NPs (Geffroy et al., 2012) because of ROS production, which can lead to DNA strand breaks, cross-linking and adducts of the bases or sugars (Cabiscol et al., 2000).

Genetic analysis

Various authors investigated gene expression alterations due to nanoparticles in *D. rerio*. In zebrafish embryos silver NPs caused significant alterations in the expression of genes involved in oxidative phosphorylation and protein synthesis after 24 h of exposure (Van Aerle et al., 2013), and AuNPs disrupted the expression patterns of key transcription factors regulating apoptosis, eye development and pigmentation (Kim et al., 2013). In adult zebrafish dietary exposure to AuNP and cadmium sulfide nanoparticles led to modulation in expression of genes involved in DNA repair, detoxification processes, apoptosis, mitochondrial metabolism and oxidative stress (Geffroy et al., 2012; Ladhar et al., 2013).

The gene expression patterns observed in this work show that AuNP exerts an influence on gene response bigger than ionic gold. This is illustrated in brain from fish exposed to 0.8 µg Au/L, where genes involved in oxidative stress scavenging (*sod2*), mitochondrial respiration (*cox1*), general stress response (*hsp70*) and neurotransmission (*ache*) displayed a 3-fold increased expression in response to NPs compared to ionic gold. Increased AChE activity correlates well with the increased expression of the *ache* gene in zebrafish brain despite the low concentration of gold detected in this tissue (below the detection threshold). In muscle and brain, the up-regulation of the *mt* gene might correspond to a detoxification response since it was demonstrated that metallothionein can bind various chemical forms of gold (Laib et al., 1985). In gill, ionic and nanoparticle gold induced similar effects on gene expression except for the *gaad* gene involved in DNA repair which was up-regulated when zebrafish were exposed to NPs, in accordance with genotoxicity results and, suggesting the onset of a DNA repair process activated after NP-induced DNA alterations.

Conclusion

This study is the first trial to evaluate effects of AuNPs -contaminated sediment on a vertebrate organism. Moreover concentration pressures were very low when considering the bioavailable gold released in the water column. The study showed that gold NP exposure led to various effects in zebrafish including gene expression level modifications, DNA alterations and AchE activity variation despite the low bioavailability of gold in fish tissues compared with the bulk form. These results highlight the potential harm of nanoparticles to aquatic organisms and also possibly to human health owing to the close homologies of the *D. rerio* genome with the human genome, and the important similarities of fish and mammalian cell metabolism.

Declaration of interest

Authors declare that we are not linked to private companies or organizations, and that we have got no financial or personal relationships with institutions or people that could influence the main results and conclusions of our work.

References

Asharani PV, Lianwu Y, Gong Z, Valiyaveettil S. 2010. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. Nanotoxicology 5:43–54.

Asharani PV, Wu YL, Gong Z, Valiyaveettil S. 2008. Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19:255102.

Bar-Ilan O, Albrecht RM, Fako VE, Furgeson DY. 2009. Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. Small 16:1897–910.

Barillet S, Adam C, Palluel O, Devaux A. 2007. Bioaccumulation, oxidative stress and neurotoxicity in *Danio rerio* exposed to different isotopic compositions of uranium. Environ Tox Chem 26:497–505.

Berman HA, Leonard K. 1990. Ligand exclusion on acetylcholinesterase. Biochemistry 29:10640–9.

Blaser SA, Scheringer M, Macleod M, Hungerbuhler K. 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci Total Environ 390: 396–409.

Bourdineaud JP, Rossignol R, Brèthes D. 2013. Zebrafish: a model animal for analyzing the impact of environmental pollutants on muscle and brain mitochondrial bioenergetics. Int J Biochem Cell Biol 45:16–22.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Chem 72:248–54.

Buffle J, Leppard GG. 1995. Characterisation of aquatic colloids and macromolecules. 1. Structure and behavior of colloidal material. Environ Sci Technol 29:2169–75.

Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3:3–8.

Cambier S, Gonzalez P, Durrieu G, Bourdineaud JP. 2010. Cadmium-induced genotoxicity in zebrafish at environmentally relevant doses. Ecotoxicol Environ Safe 73:312–19.

Choi JE, Kim S, Ahn JH, Youn P, Kang JS, Park K, et al. 2010. Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. Aquat Toxicol 100:151–9.

Ciutat A, Anschutz P, Gerino M, Boudou A. 2005. Effects of bioturbation on cadmium transfer and distribution into freshwater sediments. Environ Toxicol Chem 24:1048–58.

Ciutat A, Boudou A. 2003. Bioturbation effects on cadmium and zinc transfers from a contaminated sediment and on metal bioavailability to benthic bivalves. Environ Toxicol Chem 22:1574–81.

Cobley CM, Chen J, Cho EC, Wang LV, Xia Y. 2011. Gold nanostructures: a class of multifunctional materials for biomedical applications. Chem Soc Rev 40:44–56.

Daniel MC, Astruc D. 2003. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev 104:293–346.

Daughton CG. 2004. Non-regulated water contaminants: emerging research. Environ Impact Asses Rev 24:711–32.

de Lima D, Roque GM, de Almeida EA. 2013. *In vitro* and *in vivo* inhibition of acetylcholinesterase and carboxylesterase by metals in zebrafish (*Danio rerio*). Mar Environ Res 91:45–51.

Ellman GL, Courtney K, Andres VJR, Feather-Stone RM. 1961. A new and rapid colorimetric determination of AChE activity. Biochem Pharmacol 7:88–95.

Fako VE, Furgeson DY. 2009. Zebrafish as a correlative and predictive model for assessing biomaterial nanotoxicity. Adv Drug Deliv Rev 61: 478–86.

Ferry JL, Graig P, Hexel C, Sisco P, Frey R, Pennington PL, et al. 2009. Transfer of gold nanoparticles from water column to the estuarine food web. Nat Nanotechnol 4:441–44.

Fraga S, Faria H, Soares ME, Duarte JA, Soares L, Pereira E, et al. 2013. Influence of the surface coating on the cytotoxicity, genotoxicity and uptake of gold nanoparticles in human HepG2 cells. J Appl Toxicol 33: 1111–19.

García-Alonso J, Khan FR, Misra SK, Turmaine M, Smith BD, Rainbow PS, et al. 2011. Cellular internalization of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*. Environ Sci Technol 45:4630–6.

Geffroy B, Ladhar C, Cambier S, Treguer-Delapierre M, Brèthes D, Bourdineaud JP. 2012. Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: the role of size, concentration and exposure time. Nanotoxicology 6:144–60.

Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. 2010. Gold nanoparticles for biology and medicine. Angew Chem Int Ed Engl 49:3280–94.

Gonzalez E, Arbiol J, Puntes VF. 2011. Carving at the nanoscale: sequential galvanic exchange and kirkendall growth at room temperature. Science 334:1377–80.

Goodman CM, Chari NS, Han, G, Hong R, Ghosh P, Rotello VM. 2006. DNA binding by functionalized gold nanoparticles: mechanism and structural requirements. Chem Biol Drug Des 67:297–304.

Gottschalk F, Sonderer T, Scholz RW, Nowack B. 2009. Modeled environmental concenrtations of engineered nanomaterials (TiO_2, ZnO, Ag, CNT, fullerenes) for different regions. Environ Sci Technol 43: 9216–22.

Griffitt RJ, Weil R, Hyndman KA, Denslow ND, Powers K, Taylor D, et al. 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). Environ Sci Technol 41: 8178–86.

Gustafsson O, Gschwemd G. 1997. Aquatic colloids: concepts, definitions and current challenges. Limnol Oceanogr 42:517–28.

Harper SL, Carriere JL, Miller JM, Hutchison JE, Maddux BL, Tanguay RL. 2011. Systematic evaluation of nanomaterial toxicity: utility of standardized materials and rapid assays. ACS Nano 5:4688–97.

Henry AI, Bingham JM, Ringe E, Marks LD, Schatz GC, Van Duyne RP. 2011. Correlated structure and optical property studies of plasmonic nanoparticles. J Phys Chem C 115:9291–305.

Howard CV. 2004. Small particles – big problems. Int Lab News 34:28–9.

Johnston BD, Scown TM, Moger J, Cumberland SA, Baalousha M, Linge K, et al. 2010. Bioavailability of nanoscale metal oxides TiO_2, CeO_2, and ZnO to fish. Environ Sci Technol 44:1144–51.

Kim KT, Zaikova T, Hutchison JE, Tanguay RL. 2013. Gold nanoparticles disrupt zebrafish eye development and pigmentation. Toxicol Sci 133:275–88.

Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, Plech A. 2006. Turkevich method for gold nanoparticle synthesis revisited. J Phys Chem B 110:15700–7.

Kiser MA, Westerhoff P, Benn T, Wang Y, Perrez-Rivera J, Hristovski K. 2009. Titanium nanomaterial removal and release from wastewater treatment plants. Environ Sci Technol 43:6757–83.

Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, et al. 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 27:1825–51.

Ladhar C, Geffroy B, Cambier S, Treguer-Delapierre M, Durand E, Brèthes D, et al. 2013. Impact of dietary cadmium sulfide nanoparticles on *Danio rerio* zebrafish at very low contamination pressure. Natoxicology 8:676–85.

Laib JE, Shaw 3rd CF, Petering DH, Eidsness MK, Elder RC, Garvey JS. 1985. Formation and characterization of aurothioneins: Au, Zn, Cd-thionein, Au, Cd-thionein, and (thiomalato-Au)chi-thionein. Biochemistry 24:1977–86.

Lerebours A, Cambier S, Hislop L, Adam-Guillermin C, Bourdineaud JP. 2013. Genotoxic effects of exposure to waterborne uranium, dietary methylmercury and hyperoxia in zebrafish assessed by the quantitative RAPD-PCR method. Mutat Res Genet Toxicol Environ Mutagen 755:55–60.

McCarthy JF, Burrus LW, Tolbert VR. 2003. Bioaccumulation of benzo(a)pyrene from sediment by fathead minnows: effects of organic content, resuspension and metabolism. Arch Environ Contam Toxicol 45:364–70.

Moore MN, Noble D. 2004. Editorial: computational modelling of cell and tissue processes and function. J Mol Histol 35:655–8.

Moore MN. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ Int 32:967–76.

Murphy CJ, Gole AM, Stone JW, Sisco PN, Alkilany AM, Goldsmith EC, et al. 2008. Gold nanoparticles in biology: beyond toxicity to cellular imaging Acc Chem Res 41:1721–30.

Orieux N, Cambier S, Gonzalez P, Morin B, Adam C, Garnier-Laplace J, Bourdineaud JP. 2011. Genotoxic damages in zebrafish submitted to a polymetallic gradient displayed by the Lot River (France). Ecotoxicol Environ Safety 74:974–83.

Richetti SK, Rosemberg DB, Ventura-Lima J, Monserrat JM, Bogo MR, Bonan CD. 2011. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. NeuroToxicology 32:116–22.

Romani R, Antognelli C, Baldracchini F, De Santis A, Isani G, Giovannini E, Rosi G. 2003. Increased acetylcholinesterase activities in specimens of *Sparus auratus* exposed to sublethal copper concentrations. Chem Biol Interact 145:321–9.

Royal Society and Royal Academy of Engineering. Nanoscience and nanotechnologies: opportunities and uncertainties. RS policy document 19/04. London: The Royal Society; 2004. p. 113.

Tedesco S, Doyle H, Redmond G, Sheehan D. 2008. Gold nanoparticles and oxidative stress in *Mytilus edulis*. Mar Environ Res 66:131–3.

Truong L, Saili KS, Miller JM, Hutchison JE, Tanguay RL. 2012a. Persistent adult zebrafish behavioral deficits results from acute embryonic exposure to gold nanoparticles. Comp Biochem Phys C 155:269–74.

Truong L, Tilton SC, Zaikova T, Richman E, Waters KM, Hutchison JE, et al. 2013. Surface functionalities of gold nanoparticles impact embryonic gene expression responses. Nanotoxicology 7:192–201.

Truong L, Zaikova T, Richman EK, Hutchison JE, Tanguay RL. 2012b. Media ionic strength impacts embryonic responses to engineered nanoparticle exposure. Nanotoxicology 6:691–9.

Unrine JM, Hunyadi SE, Tsyusko OV, Rao W, Shoults-Wilson WA, Bertsch PM. 2010. Evidence for bioavailability of Au nanoparticles from soil and biodistribution within earthworms (*Eisenia fetida*). Environ Sci Technol 44:8308–13.

Van Aerle R, Lange A, Moorhouse A, Paszkiewicz K, Ball K, Johnston BD, et al. 2013. Molecular mechanisms of toxicity of silver nanoparticles in zebrafish embryos. Environ Sci Technol 47:8005–14.

Vevers WF, Jha AN. 2008. Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro. Ecotoxicology 17: 410–20.

Wise Sr JP, Goodale BC, Wise SS, Craig GA, Pongan AF, Walter RB, et al. 2010. Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquat toxicol 97:34–41.

Supplementary material available online

Supplementary Tables 1–4
Supplementary Figures 1–2