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Effects of diamond nanoparticle exposure on the internal structure and reproduction of *Daphnia magna*

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ABSTRACT

Nanomaterials have significant technological advantages but their release into the environment also carry potential ecotoxicological risks. Carbon-based nanoparticles and particularly diamond nanoparticles have numerous industrial and medical applications. The aim of the present study was to evaluate the toxic effects of diamond nanoparticles with an average particle size of 20 nm on the survival, reproduction and tissue structure of the freshwater crustacean *Daphnia magna*. The chronic toxicity test results showed 100% mortality at concentrations higher than 12.5 mg l^{-1} and that reproduction inhibition occurred in concentrations higher than 1.3 mg l^{-1}. Light microscopy showed that diamond nanoparticles adhere to the exoskeleton surface and accumulate within the gastrointestinal tract, suggesting that food absorption by the gut cells may be blocked. The results support the use of chronic approaches in environmental protection as part of an integrated environmental monitoring and assessment strategy.

1. Introduction

Nanomaterials have significant technological potential but an assessment of the different uses, effects and disposal methods is essential to any evaluation of the risks that they pose to environmental systems and human health. Nanoparticles (NPs) are currently used in many different areas such as electronics, pharmacology, biotechnology, cosmetics, energy devices and waste treatment [1]. The main concerns are whether the risks of engineered NPs exceed the benefits to society. Nanoparticles can enter the environment in different ways, e.g. through accidental release in engineered NP production processes or washing of nanobased cosmetics.

One of the key issues is to evaluate NP toxicity; various studies point to potential NP ecotoxicity when compared to bulk materials, probably due to their new physicochemical characteristics. Theoretical considerations suggest that smaller particles are likely to be more toxic on the account of their larger specific surface area and greater bioavailability [2]. The main issues for environmental hazard assessment may be summarized as, firstly, the need for the validation of laboratory test systems that characterize the effects of nanomaterials and, secondly, the need for studies on the impacts of specific nanomaterials on ecosystems [3]. It is also important to investigate whether or not current ecotoxicity test endpoints are adequate in the case of NP toxicity assessment.

Ecotoxicity studies have been conducted on a limited number of NPs and, only on a small number of aquatic species, so a significant knowledge gap exists for all aspects of NP-related environmental toxicology. In a recent review Kahru and Dubourguier [4] tried to identify the most harmful NPs and the most sensitive organisms. Their study mentions that most data available in the literature concerns TiO_2, ZnO and C60, and the effects on crustacea and bacteria. According to Zhu et al. [5] clear guidelines have not yet been established to quantify these effects.

In addition, NP uptake into the aquatic biota is a major concern, yet little information is available on the interaction between aquatic organisms and manmade NPs released [6]. Invertebrates have a key position as consumers in aquatic ecosystems and certain invertebrate species have been used as ecotoxicity test organisms. *Daphnia magna*, a freshwater crustacean, is one of the most sensitive test organisms to a variety of contaminants and has been used as a standard test organism in the protocols established by the US Environmental Protection Agency (EPA), the Organization for Economic Co-operation and Development (OECD), and the International Organization for Standardization (ISO).

D. magna were found to ingest NPs from the test suspensions through feeding behaviours, which indicates that the potential ecotoxicity and environmental health effects of NPs cannot be

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neglected [5,7–9]. Hund-Rinke and Simon [10] reported the immobilization of *D. magna* after exposure to TiO_2 nanoparticles in a study on photocatalytic activity at concentrations up to 3 mg l^{-1}. Oberdörster et al. [11] showed that carbon-based nanomaterials such as stable fullerene (C60) water suspensions can delay moulting and reduce offspring production in *Daphnia* at 2.5 ppm. Testing nC60 in the range of 0–0.8 mg l^{-1}. Tao et al. [12] reported accumulation and sublethal reproductive responses in *Daphnia*. The toxicity of single-walled carbon nanotube-based nanomaterials in *Daphnia* was also reported recently, in a study testing concentrations up to 5 mg l^{-1} [13].

In tests on *D. magna*, Zhu et al. [5] found that TiO_2, Al_2O_3 and carbon based NPs were more toxic than their bulk counterparts. In studies on the acute toxicity of C60 fullerenes and TiO_2 nanoparticles in *D. magna*, Lovern and Klaper [14] and Zhu et al. [5] found different toxicity thresholds which may be related to differences in particle size, preparation methods or test design. Shaking the suspension during exposure may be of particular significance. In the case of fullerenes Oberdörster et al. [11] state that solvents or sonication may potentially enhance their toxicity. The stirring/shaking of NPs in water may be more environmentally relevant, considering natural water flows in aquatic environments and the filter-feeding behaviour of *D. magna* [5].

Carbon nanomaterials have both industrial (e.g. polishing, energy conversion, oil and electrolyte additives and dry lubricants) and medical (e.g. coatings, biosensors and drug delivery) applications and are being produced in increasing quantities due to their novel characteristics. In a review on nanodiamond (ND) applications, Holt [15] refers that there is a wide range of applications in areas such as chemistry, materials, life sciences, medicine and physics. More recently Schrand et al. [16] stated that the attractive properties of ND will be exploited in a similar manner to other carbon nanoparticles, quantum dots, and metallic nanoparticles, for the development of therapeutic agents for diagnostic probes, delivery vehicles, gene therapy, anti-viral and anti-bacterial treatments and tissue scaffolds, and the development of novel medical devices such as nanorobots. More specifically, biotechnology applications have shown the prospective use of ND for bioanalytical purposes such as protein purification or biolabelling using highly fluorescent synthetic nanodiamond particles. There is however a lack of information on the potential amounts and routes of environmental release of ND.

As there is still a lack of information on the effects of ND on the biota, we used *D. magna* as a model organism to conduct a study of ND toxicity. The aim of the present study is to determine the potential toxicity of ND water suspensions in *D. magna* through the assessment of survival, reproduction and tissue structure.

2. Materials and methods

2.1. Nanoparticles

Detonation diamond nanoparticles were obtained from NanoCarbon Research Institute, Nagano, Japan. ND particles were provided in a polydispersed water colloidal solution (10%, w/w), with 90% diamond/10% other carbon forms, and an average particle size of 20 nm, according to the manufacturer.

Transmission electron microscopy (TEM) observations of nanodiamond particles after dispersion in ethanol and drying in carbon film were performed with a Hitachi H8100 microscope equipped with EDS.

A nanodiamond suspension for experiments was prepared by adding approximately 1 ml of the raw colloidal ND suspension to 1 l of Millipore – 0.2 μm filtered water and submitting to sonication in an ultrasound bath (Laborette 17, Fritsch GmbH, Germany) at 50–60 kHz for 24 h.

The physicochemical characterization of the test suspension was carried out by determining the concentration by gravimetry and the pH by potentiometry, in addition to the absorbance at 450 nm (DR/2000, HACH Company, USA) and the Zeta potential (Zeta-Meter System 3.0, Zeta-Meter Inc., USA), as described in the Standard Methods for the Examination of Water and Wastewater [17].

2.2. Ecotoxicity tests

To investigate the physiological and organism-level effects of ND particles on the freshwater crustacean *D. magna* (clone IRCHA-5), conventional chronic ecotoxicity tests were conducted using reproduction as the endpoint.

The test organisms were juvenile females, 6–24 h old, from the third to the sixth broods obtained from cultures in parthenogenic reproduction. Adult females are maintained in M7 culture medium [18] at 22 °C under a 16 h light:8 h dark photoperiod and reared individually in 150 ml containers. The culture medium is changed after each brood release. On a daily basis females are fed log phase green algae, *Chlorella vulgaris*, grown axenically in vitamin enriched Woods Hole MBL (pH 7.2) culture medium [19].

Chronic reproduction inhibition tests were performed according to ISO 10706: 2000 [20]. Five concentrations and a control group were used. Five replicates of one female were used in each group. Test concentrations were prepared immediately prior to use by diluting the stock ND suspension with test medium. In this procedure, the stock suspension was continuously stirred with a magnetic stirrer to maintain the ND in suspension. Each randomly selected neonate was placed in a test beaker with 50 ml of test suspension. Tests were conducted in the same temperature, light and photoperiod conditions as the culture.

A preliminary test in the concentration range [3.1–50 mg l^{-1}] and a definitive test in the concentration range [0.31–5.0 mg l^{-1}] were carried out with a 21-day-exposure period. During this time the individuals were checked and fed *C. vulgaris* on a daily basis and the test media were changed every other day. The number of living offspring per living parent and the time to first brood were registered. At the end of the exposure period, *D. magna* tested adults were also observed using a stereoscopic microscope.

2.3. Histological analysis

A structural study of 21-day-old *D. magna* females from chronic tests was carried out. In the preliminary test only individuals from the control and those exposed to 3.1 and 6.3 mg l^{-1} were observed.

The *Daphnia* were removed from the beakers via pipette and fixed in Bouin-Hollande's fluid (saturated solution of picric acid in 10% (v/v) formaldehyde and 7% (v/v) acetic acid) over 48 h. The samples were processed using the standard histological techniques according to the procedures described in Martoja and Martoja [21]. Briefly, after the period of fixation the samples were washed in a 6% (v/v) formic acid solution to promote decalcification for 24 h. Samples were then dehydrated in a progressive series of ethanol and embedded in xylene (Lab-Scan, Belgium). Following this, they were embedded in paraffin wax and sliced into 6–7 μm thick sections. The paraffin was removed using xylene as a solvent, the sections treated in a graduated series of alcohols, stained with hematoxylin and eosin (H&E) and mounted with DPX resin (DBH, Poole, England) for microscope observation. The histological observations were carried out using a light microscope (Leica-ATC 2000, Wetzal, Germany). At least 10 sections from each *Daphnia* were examined to assess the presence and abundance of nanoparticles within the gut.

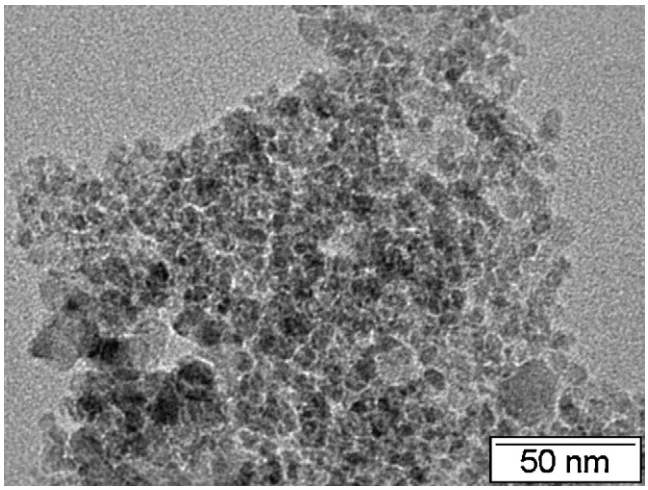

Fig. 1. TEM image of nanodiamond particles.

2.4. Data analysis

In the 21 days chronic tests on *D. magna*, fecundity was determined as the total number of neonates released per female. Significant differences between the results in each ND concentration and the control were assessed through the application of the non-parametric Mann–Whitney test [22]. Results are presented as: NOEC-21d ($mg\,l^{-1}$), the No Observed Effect Concentration, i.e. the highest concentration tested for which reproduction is not significantly different from the control, and LOEC-21d ($mg\,l^{-1}$), the Lowest Observed Effect Concentration, i.e. the lowest concentration tested for which reproduction is significantly different from the control.

Pairwise Pearson correlations between the mean number of juveniles per female and a qualitative parameter of ND abundance in the gut were determined using statistical analysis software (JMP® version 5.1) for six ND concentrations.

3. Results

3.1. Particle and suspension characterization

A relatively broad distribution of particle sizes is evidenced with TEM, the median particle size is probably smaller than 20 nm although occasionally larger particles are seen (Fig. 1).

Particles are constituted by 5 nm diamond crystals forming tightly bonded aggregates of slightly larger size, about 20 nm. This tight aggregation phenomenon has been discussed in the context of nanodiamond processing [23].

The prepared ND suspension was brownish, with no apparent aggregation. Settling was observed after 24 h. The physical and chemical characteristics of the suspension were determined: ND concentration = 500 $mg\,l^{-1}$, pH 4.3, absorbance (at 450 nm) = 0.175 and Zeta potential = +36.6 mV. The suspension can be considered electrically stabilized according to the Zeta potential value.

3.2. Chronic effects on reproduction

During the preliminary test, with concentrations in the range [3.1–50 $mg\,l^{-1}$], 100% mortality in *Daphnia* occurred for concentrations higher than 12.5 $mg\,l^{-1}$. Mortality was not higher than 20% during the definitive chronic test in the range [0.31–5.0 $mg\,l^{-1}$].

Six broods were obtained during the 21-day test period for the control females and females exposed to 0.31 $mg\,l^{-1}$ ND, the lowest test concentration. In the concentrations 0.31 and 0.63 $mg\,l^{-1}$ the total number of juveniles was not significantly different from the control (Fig. 2). A NOEC-21d = 0.63 $mg\,l^{-1}$ was obtained.

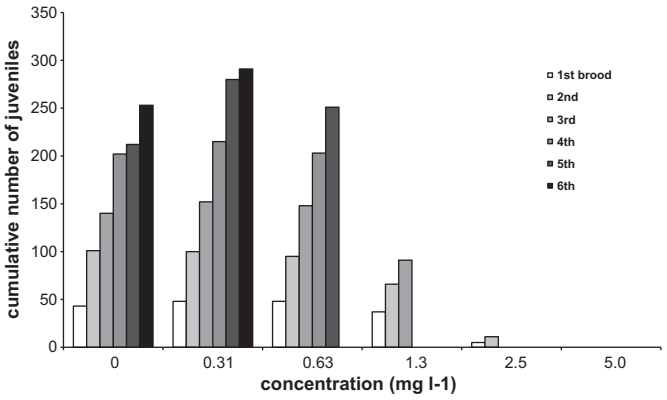

Fig. 2. Total cumulative number of *Daphnia magna* juveniles after each brood in the different concentrations of ND over the 21 days of the definitive chronic toxicity test.

Reproduction inhibition was observed for concentrations of 1.3 $mg\,l^{-1}$ and higher (Figs. 2 and 3), a LOEC-21d = 1.3 $mg\,l^{-1}$ was obtained.

In conclusion, the results of the 21-day chronic toxicity tests showed significant differences in reproduction between *Daphnia* exposed to ND suspensions at concentrations higher than 1.3 $mg\,l^{-1}$ compared to control *Daphnia*.

3.3. Chronic effects on tissue structure

Figs. 4 and 5 show representative images of the gut epithelium of *Daphnia* exposed to different ND concentrations in the preliminary and definitive chronic tests, respectively.

The controls in Fig. 4a and b show the typical structure of midgut wall cells, consisting of simple columnar cuboidal epithelium of one basic cell type. The epithelium lies on a thick basal lamina (Fig. 4b) which is surrounded by an external gut muscularis. According to Schultz and Kennedy [24] and Nogueira et al. [25] these columnar cells are approximately 20 μm high and 8 μm wide, but the digestive diverticula cells are more cuboidal, being approximately 10 μm in height. However, variations in size and shape along the entire length of the gut can be observed and the apical cell surface is modified as a regular border of microvilli throughout the length of the midgut. In the control *Daphnia* only small amounts of food residues are observed in the gut lumen (Fig. 4a and b). The epithe-

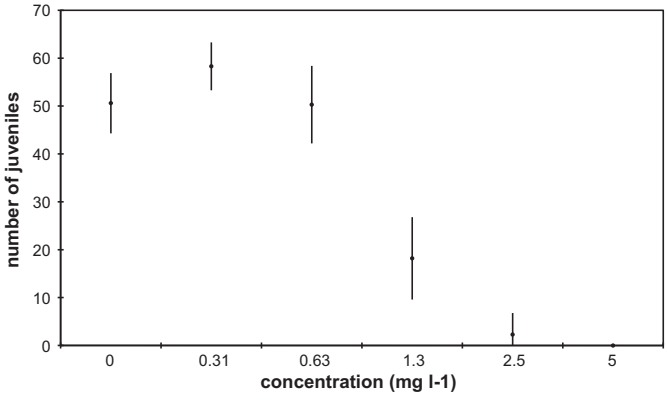

Fig. 3. Number of *Daphnia magna* juveniles (mean ± SD, $n = 5$) per female after 21 days exposure to the different concentrations of ND in the definitive chronic toxicity test.

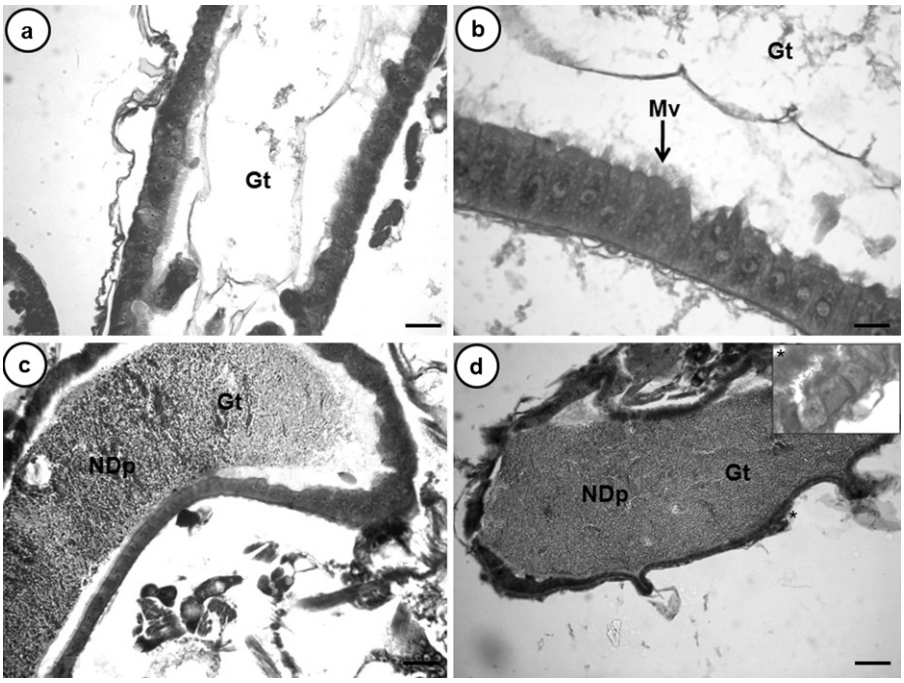

Fig. 4. Light microscopy images of the gut epithelium of *Daphnia magna* from the preliminary 21 days chronic toxicity test with nanodiamond (Gt: gut lumen; Mv: microvilli; NDp: aggregates of nanodiamond particles). Exposure concentrations: (a and b) [control]; (c) [3.1 mg l^{-1}]; (d) [6.3 mg l^{-1}]. Bars (a, c, and d) = 20 μm; (b) = 10 μm.

lium gut cells of the controls for the preliminary and definitive tests were similar.

The organisms exposed to 0.31 mg l^{-1} ND in the definitive test showed food residues inside the gut lumen but no ND was distinguishable with light microscopy (Fig. 5a). Figs. 4(c and d) and 5(b–f) show the distribution of particles in the gut of exposed organisms, with aggregated ND present in the gut lumen adjacent to the microvilli border but no ND particles observed inside gut cells. Figs. 4(c and d) and 5(c–f) are representative of larger aggregates of ND found in the gut lumen of exposed organisms. It is possible that the presence of food residues and organic matter within gut lumen facilitates ND aggregation. There is no evidence that ND crossed the gut epithelium in *D. magna*.

Histological assessment of females exposed to concentrations higher than 1.3 mg l^{-1} showed that the NPs adhere to the surface of the exoskeleton and accumulate within the gastrointestinal tract, mixed with food (Table 1), thus suggesting that food absorption by intestinal cells can be inhibited. Apparently the ND was not excreted during the exposure period or the elimination rate was not sufficient to clean the gut.

Table 1
Qualitative analysis, by light microscopy observation, of the ND abundance in *D. magna* gut after 21 days exposure in chronic toxicity tests.

Concentrations (mg l^{-1})	Abundance of ND aggregates	Description
Preliminary test		
0 (Control)	–	Normal morphology
3.1	++	ND present in lumen
6.3	+++	Cell degeneration
Definitive test		
0 (Control)	–	Normal morphology
0.31	–	Normal morphology
0.63	+	ND present in lumen
1.3	++	ND present in lumen
2.5	+++	ND present in lumen
5.0	+++	Cell degeneration

ND abundance: (–) absence; (+) residual; (++) abundant; (+++) very abundant.

Females grown under an exposure concentration of 0.31 mg l^{-1} ND showed normal tissue morphology and no ND particles in the gut. Females grown under an exposure concentration of 0.63 mg l^{-1} showed normal tissue morphology but residual ND particles were observed. In females exposed to the highest test concentration (6.3 mg l^{-1}), ND particles were abundant and the degeneration of intestinal cells could be observed (Fig. 4d). Some of the alterations detected in gut epithelial cells were the loss of their typical shape as shown in controls and the loss of cell adhesion to each other showing intercellular spaces (Figs. 4d* and 5f).

A statistically significant correlation ($r = -0.973$, $p = 0.001$, $n = 6$) was obtained between the mean number of juveniles per female and a qualitative parameter of ND abundance in the gut, presented in Table 1, showing a link between reproduction inhibition and ND ingestion.

4. Discussion

D. magna, an ecologically significant organism with an important role in the regulatory testing of chemicals, waters and wastes, can take in carbon based nanoparticles from test suspensions, namely single-walled carbon nanotubes [5,13], multi-walled carbon nanotubes [5] and fullerene (nC60) [5,8,12,26]. These results support our studies in suggesting that the exposure of aquatic organisms to NPs could pose a risk of bioaccumulation, especially for filter-feeding crustacea such as *D. magna*. *Daphnia* feed by creating a water column with their appendages that funnels the water towards their mouth, as well as circulating oxygen-rich water into the carapace to facilitate respiration [27].

Our results show the adhesion of ND to the *D. magna* exoskeleton, the presence of nanoparticles/aggregates in the digestive system and an accumulation in the gut of the *Daphnia* after 21 days exposure. ND adhesion to the body surface was observed, especially at high concentrations. Adhesion of the particles may be due to their hydrophobic properties. Hydrophobic substances have been reported to adhere easily to negatively charged biological material [28]. Therefore, the uptake and accumulation of NPs is hypothesized as resulting in the mechanical disruption of the feed-

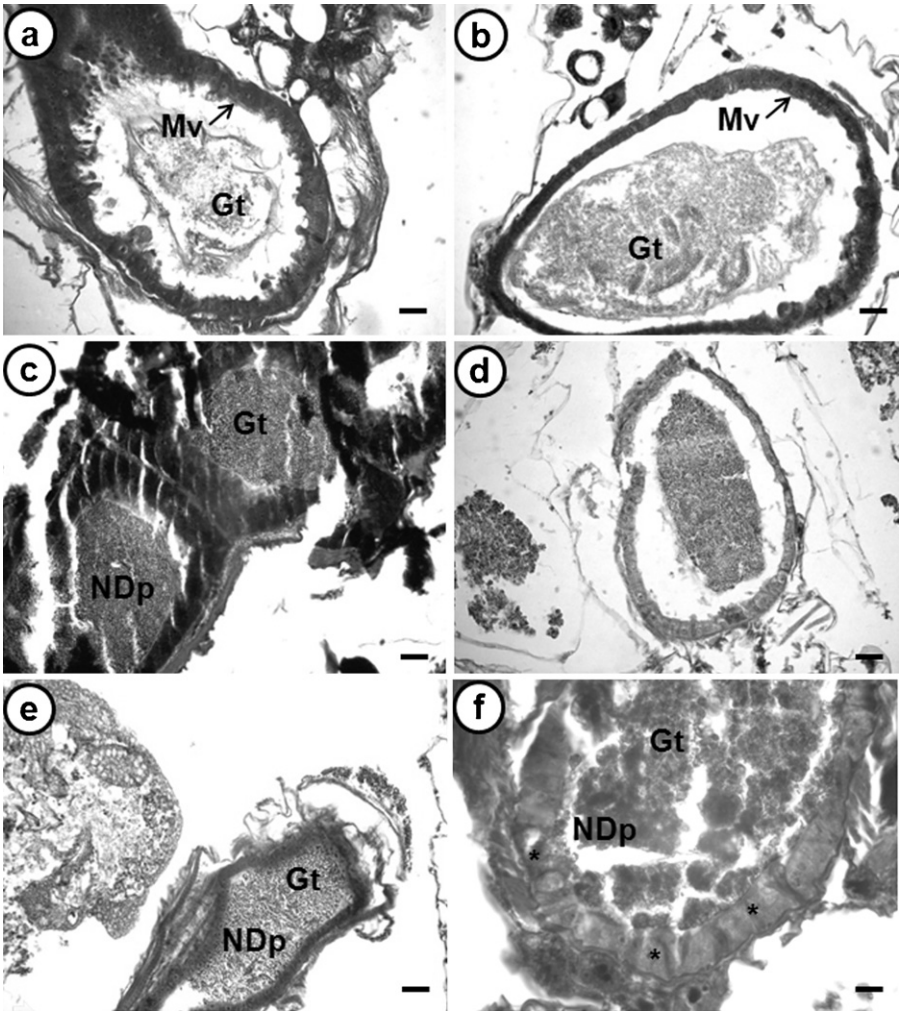

Fig. 5. Light microscopy images of the gut epithelium of *Daphnia magna* from the definitive 21 days chronic toxicity test with nanodiamond (Gt: gut lumen; Mv: microvilli; NDp: aggregates of nanodiamond particles; (*) cell degeneration). Exposure concentrations: (a) [0.31 mg l^{-1}]; (b) [0.63 mg l^{-1}]; (c) [1.3 mg l^{-1}]; (d) [2.5 mg l^{-1}]; (e and f) [5.0 mg l^{-1}]. Bars (a–e) = 20 μm; (f) = 10 μm.

ing appendages and penetration of the gut wall [5]. These could lead to the eventual immobilization and death observed in higher concentrations of ND and to reproduction inhibition and digestive tract damage in longer exposures to lower concentrations.

Ebert [29] and Hund-Rinke and Simon [10] considered that particles with a diameter of less than 50 μm are ingested by *D. magna* without any selective mechanism. However, larger particles are too difficult to process and daphnids prevent them from entering the filter chamber or reject them through movements of the postabdominal claw [30]. Also the flocculation tendency in larger particles or higher concentrations is likely to decrease ingestion, reducing the toxicity.

Microscopic observations suggest that the number of ND clumps increases in the gut lumen according to the different exposure treatments, showing higher ND concentrations in *Daphnia* exposed to the maximum concentrations tested. We could not detect ND inside the cells, however even at the higher magnification the light microscopy used in this study would not be able to provide accurate information about the uptake and distribution of ND in gut cells, due to the small size of the particles.

ND is not taken up by microvilli but remains in the cells' apical border, there is an accumulation of ND within the gut lumen, which is in agreement with the findings of Lovern et al. [8] for gold NPs, which support the hypothesis that undigested food residues and other gut content facilitate the clumping of NPs.

An epithelium, bearing well-developed microvilli, line the middle region of the *Daphnia* gut and this region is considered to be the main site of absorption [30]. In our study, the accumulation of ND in the gut lumen seems to cause significant changes in the apical cell region in that adsorptive epithelial gut cells lose their typical polarity. In addition, the cell integrity of some epithelial cells is altered and cell degeneration can be observed, particularly in organisms exposed to higher concentrations of ND (Fig. 5f).

Lovern et al. [8] also reported that nanoparticles are found in much higher concentrations in the gut than in the surrounding environment; however, no bioconcentration in the tissue of the *Daphnia* seemed to occur. These authors considered that the presence of gold nanoparticles in the gut potentially obstructed the absorption of nutrients or caused an excessive amount of energy to be used in the evacuation of these particles from the gut. The reduction in available energy would slow growth and possibly inhibit reproduction. Zhu et al. [31] reported that *Daphnia* displayed severe growth retardation, mortality and reproductive defects after chronic exposure for 21d to TiO_2.

Nanodiamond was previously reported to have low chemical reactivity and good biocompatibility with different cell lines, and not to be cytotoxic at concentrations up to 100 mg l^{-1} [32,33]. Accordingly, cell death is not expected to occur in the gut of the daphnids.

Our results also show that there are inhibitory effects of diamond nanoparticles on survival and/or reproduction in *D. magna*. After a 21 days exposure to ND water suspensions no significant mortality occurred in concentrations lower than 5.0 mg l^{-1}. We also observed reproduction inhibition, with a NOEC = 0.63 mg l^{-1} and a LOEC-21d = 1.3 mg l^{-1} ND with a decrease of 64% in the average number of juveniles when compared to the control. A statistically significant correlation was obtained between reproduction inhibition and ND ingestion. Tao et al. [12] also reported sublethal reproductive effects on *Daphnia* after exposure to nC60 at 0.2 mg l^{-1}.

Several studies have demonstrated that particle surface modifications, including functionalization, could mitigate the toxicity of nanomaterials [15,16,34–36]. Also Wiesner et al. [37] hypothesize that exposure to new types or concentrations of nanomaterials may have long-term, evolutionary influences on organisms on the basis of multigenerational exposures and multispecies interactions. To a large extent, we still lack an understanding of the interaction mechanisms at the molecular level between NP and biological systems [38].

Current regulations that focus on chemicals should be evaluated in terms of their applicability to nanomaterials and new regulations should be established with the focus not only on the chemical components but also on size, as chemical properties change at the nanoscale [5].

In the context of European regulation, there is a pressing need to establish whether or not REACH Regulation [39] applies to nanomaterials though *a priori*, REACH requirements can be applied directly to nanomaterials. Nanomaterials may require a different classification and labelling and current guidance does not consider their specific characteristics that may influence their behaviour and environmental impact.

As these particles could not be detected in cells by light microscopy, potential long-term accumulation cannot be assessed. Further investigation is underway, involving electronic microscopy. Even if there is no accumulation in tissues, predators would be exposed to the contents of the invertebrate's gut, and hence potentially receive a higher dose of ND than that present in the surrounding environment.

5. Conclusions

Our experimental results lead us to conclude that ND may have chronic effects on reproduction in freshwater zooplankton, even at low concentrations. We also conclude that *D. magna* can ingest ND from test suspensions, which accumulate in gut lumen as clumps of ND and food residues. Other grazing and filter-feeding aquatic organisms may respond in a similar manner. The potential for the subsequent transfer of NP to other trophic levels should therefore receive additional attention.

We also would like to point out the chronic approach as an added value in environmental protection as part of an integrated environmental strategy, and the relevance of histological evaluation in invertebrates, as a tool for detecting the presence of NPs and any associated cellular changes. This research also supports the idea that the potential ecotoxicity and environmental effects of NPs require further study and evaluation.

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References

[1] B. Nowack, T.D. Bucheli, Occurrence, behavior and effects of nanoparticles in the environment, Environ. Pollut. 150 (2007) 5–22.
[2] SCENIHR, The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies, Scientific Committee on Emerging and Newly Identified Health Risks, 2005, http://ec.europa.eu/comm/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf.
[3] SCENIHR, Risk assessment of products of nanotechnologies, Scientific Committee on Emerging and Newly Identified Health Risks, January 19, 2009, http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf.
[4] A. Kahru, H.-C. Dubourguier, From ecotoxicology to nanoecotoxicology, Toxicology 269 (2010) 105–119.
[5] X. Zhu, L. Zhu, Y. Chen, S. Tian, Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*, J. Nanopart. Res. 11 (2009) 67–75.
[6] M.N. Moore, Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environ. Int. 32 (2006) 967–976.
[7] T. Fernandes, Still life with nanoparticles, Environ. Sci. Technol. – Environmental News, July 15 (2006) 4328.
[8] S.B. Lovern, H.A. Owen, R. Klaper, Electron microscopy of gold nanoparticle intake in the gut of *Daphnia magna*, Nanotoxicology 2 (2008) 43–48.
[9] P. Rosenkranz, Q. Chaudhry, V. Stone, T. Fernandes, A comparison of nanoparticle and fine particle uptake by *Daphnia magna*, Environ. Toxicol. Chem. 28 (2009) 2142–2149.
[10] K. Hund-Rinke, M. Simon, Ecotoxic effect of photocatalytic active nanoparticles (TiO_2) on algae and daphnids, Environ. Sci. Pollut. Res. 13 (2006) 1–8.
[11] E. Oberdörster, S.Q. Zhu, T.M. Blickley, P. McClellan-Green, M.L. Haasch, Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C-60) on aquatic organisms, Carbon 44 (2006) 1112–1120.
[12] X. Tao, J.D. Fortner, B. Zhang, Y. He, Y. Chen, J.B. Hughes, Effects of aqueous stable fullerene nanocrystals (nC60) on *Daphnia magna*: evaluation of sub-lethal reproductive responses and accumulation, Chemosphere 77 (2009) 1482–1487.
[13] A.P. Roberts, A.S. Mount, B. Seda, J. Souther, R. Qiao, S. Lin, P. Chu Ke, A.M. Rao, S.J. Klaine, In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*, Environ. Sci. Technol. 41 (2007) 3025–3029.
[14] S.B. Lovern, R. Klaper, *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles, Environ. Toxicol. Chem. 25 (2006) 1132–1137.
[15] K.B. Holt, Diamond at the nanoscale: applications of diamond nanoparticles from cellular biomarkers to quantum computing, Philos. Trans. R. Soc. A 365 (2007) 2845–2861.
[16] A.M. Schrand, S.A. Ciftan Hens, O.A. Shenderova, Nanodiamond particles: properties and perspectives for bioapplications, Crit. Rev. Solid State Mater. Sci. 34 (2009) 18–74.
[17] L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, American Water Works Association, Water Environment Federation, Washington, USA, 1998.
[18] B.-P. Elendt, W.R. Bias, Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *D. magna*, Water Res. 9 (1990) 1157–1167.
[19] J.R. Stein, Handbook of Phycological Methods, Culture Methods and Growth Measurements, University Press, Cambridge, 1973.
[20] ISO, Water Quality – Determination of Long Term Toxicity of Substances to *Daphnia magna* Straus, ISO 10706, Paris, 2000.
[21] R. Martoja, M. Martoja, Initiation aux tecniques de lïhistologie animal, Masson & Cie, Paris, 1967.
[22] E.J. Duderwicz, S.N. Mishra, Modern Mathematical Statistics, Series in Probability and Mathematical Statistics, John Wiley, New York, 1988.
[23] A. Krüger, F. Kataoka, M. Ozawa, T. Fujino, Y. Suzuki, A.E. Aleksenskii, A.Ya. Vul', E. Ōsawa, Unusually tight aggregation in detonation nanodiamond: identification and disintegration, Carbon 43 (2005) 1722–1730.
[24] W. Schultz, J.R. Kennedy, The fine structure of the digestive system of *Daphnia pulex* (Crustacea: Cladocera), Tissue Cell 8 (1976) 479–490.
[25] I.C.G. Nogueira, A. Lobo-da-Cunha, V.M. Vasconcelos, Effects of *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* (cyanobacteria) ingestion on *Daphnia magna* midgut and associated diverticula epithelium, Aquat. Toxicol. 80 (2006) 194–203.
[26] S.B. Lovern, J.R. Strickler, R. Klaper, Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C_{60}, and $C_{60}HxC_{70}Hx$), Environ. Sci. Technol. 41 (2007) 4465–4470.
[27] K.G. Porter, J. Gerritsen, J.D. Orcutt Jr., The effect of food concentration on swimming paterns, feeding behaviour, ingestion, assimilation, and respiration by *Daphnia magna*, Limnol. Oceanogr. 27 (1982) 935–949.
[28] OECD, Draft Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, Environmental Health and Safety Publications, Series on Testing and Assessment, No. 23, 2000.
[29] D. Ebert, Ecology, epidemiology, and evolution of parasitism, in: Daphnia, National Library of Medicine (US), National Center for Biotechnology Information, Bethesda, MD, 2005, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books. Accessed 07 July 2009.

[30] R.H. Peters, R. de Bernardi (Eds.), *Daphnia*, Consiglio nazionale delle ricerche, Verbania Pallanza, Memorie dell'Istituto italiano di idrobiologia, vol. 45, 1987, pp. 1–502.
[31] X. Zhu, Y. Chang, Y. Chen, Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*, Chemosphere 78 (2010) 209–215.
[32] A.M. Schrand, H. Huang, C. Carlson, J.J. Schlager, E. Osawa, S.M. Hussain, L. Dai, Are diamond nanoparticles cytotoxic? J. Phys. Chem. B 111 (2007) 2–7.
[33] A.M. Schrand, L. Dai, J.J. Schlager, S.M. Hussain, E. Osawa, Differential biocompatibility of carbon nanotubes and nanodiamonds, Diamond Relat. Mater. 16 (2007) 2118–2123.
[34] C.M. Sayes, J.D. Fortner, W. Guo, D. Lyon, A.M. Boyd, K.D. Ausman, Y.J. Tao, B. Sitharaman, L.J. Wilson, J.B. Hughes, J.L. West, V.L. Colvin, The differential cytotoxicity of water-soluble fullerenes, Nano Lett. 4 (2004) 1881–1887.
[35] C.M. Sayes, F. Liang, J.L. Hudson, J. Mendez, W. Guo, J.M. Beach, V.C. Moore, C.D. Doyle, J.L. West, W.E. Billups, K.D. Ausman, V.L. Colvin, Functionalization density dependence of single-walled carbon nanotubes cytotoxicity *in vitro*, Toxicol. Lett. 161 (2006) 135–142.
[36] X. Zhu, L. Zhu, Y. Li, Z. Duan, W. Chen, P.J.J. Alvarez, Developmental toxicity in zebrafish embryos after exposure to manufactured nanomaterials: buckminsterfullerene aggregates (nC60) and fullerol, Environ. Toxicol. Chem. 26 (2007) 976–979.
[37] M.R. Wiesner, G.V. Lowry, K.L. Jones, M.F. Hochella, R.T. Digiulio, E. Casman, E.S. Bernhardt, Decreasing uncertainties in assessing environmental exposure, risk, and ecological implications of nanomaterials, Environ. Sci. Technol. 43 (2009) 6458–6462.
[38] A.D. Maynard, R.J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster, M.A. Philbert, J. Ryan, A. Seaton, V. Stone, S.S. Tinkle, L. Tran, N.J. Walker, D.B. Warheit, Safe handling of nanotechnology, Nature 444 (2006) 267–269.
[39] EC, Regulation No. 1907/2006 of the European Parliament and of the Council of 18th December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), OJ L 136, 29.05.2007, pp. 3–280.