Toxicity of engineered nanomaterials and their transformation products following wastewater treatment on A549 human lung epithelial cells

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A B S T R A C T

Here we characterize the toxicity of environmentally-relevant forms of engineered nanomaterials (ENMs), which can transform during wastewater treatment and persist in aqueous effluents and biosolids. In an aerosol exposure scenario, cytotoxicity and genotoxicity of effluents and biosolids from lab-scale sequencing batch reactors (SBRs) to A549 human lung epithelial cells were examined. The SBRs were dosed with nanoAg, nano zero-valent iron (NZVI), nanoTiO2 and nanoCeO2 at sequentially increasing concentrations from 0.1 to 20 mg/L. Toxicities were compared to outputs from SBRs dosed with ionic/bulk analogs, undosed SBRs, and pristine ENMs. Pristine nanoAg and NZVI showed significant cytotoxicity to A549 cells in a dose-dependent manner from 1 to 67 μg/mL, while nanoTiO2 and nanoCeO2 only exerted cytotoxicity at 67 μg/mL. Only nanoAg induced a genotoxic response, at 9, 33 and 53 μg/mL. However, no significant cytotoxic or genotoxic effects of the SBR effluents or biosolids containing nanomaterials were observed.

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1. Introduction

The concern that engineered nanomaterials (ENMs) may have adverse effects on human health is increasing as application of nanotechnology in consumer products expands [1,2]. Humans can be exposed to ENMs in the workplace and during use of nano-products, and also through contact with water, soil, or air to which ENMs may have been released [3]. Comprehensive risk assessment of ENMs requires characterization of the toxicity of ENMs under a wide range of exposure conditions, including environmental routes. Cytotoxicity and genotoxicity of common metal and carbon nanomaterials, such as nanoAg, nanoTiO2, and carbon nanotubes have been widely studied in human lung, dermal, and visceral cells [4–7]. However, in assessing the risks of ENMs released into the natural environment, available data on the toxicity of environmentally-relevant forms of ENMs is lacking [8,9]. Extrapolation of toxicity data based on testing of pristine ENMs may not be appropriate because ENMs are highly reactive by nature and can be chemically, physically and

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biologically transformed in the environment, potentially altering their toxicity [10,11].

Wastewater treatment plants (WWTPs) are a critical route of ENM receipt and release into the natural environment [12–14]. The complex wastewater matrix is likely to favor transformation of ENMs. A small portion of ENMs will remain in the wastewater effluent, while the majority will associate with the sludge [15,16] and eventually be disposed of by land-application, landfill, or incineration [12]. During the reuse of treated wastewater and land-application of waste sludge (biosolids), there is potential for humans to be exposed to transformed ENMs, especially through inhalation of aerosols generated [17,18]. However, to the knowledge of the authors, impacts of human exposure to transformed ENMs following wastewater treatment have not previously been reported.

This study examined cytotoxicity and genotoxicity of effluents and biosolids from lab-scale sequencing batch reactors (SBRs) receiving ENMs to A549 human lung epithelial cells. The SBRs were dosed with nanoAg, nano zero-valent iron (NZVI), nanoTiO2 and nanoCeO2 at sequentially increasing concentrations from 0.1 to 20 mg/L. Toxicities were compared to outputs from SBRs dosed with ionic/bulk analogs (Ag+, Fe2+, bulkTiO2 and bulkCeO2), outputs from undosed SBRs, and pristine ENMs.

2. Materials and methods

2.1. Preparation of samples

NanoAg (52 ± 12 nm, prepared using citrate reduction method), NZVI (46 ± 10 nm, NANOFER 255, Rajhrad, Czech Republic), nanoTiO2 (21 ± 12 nm, anatase nanopowder, Sigma–Aldrich, Saint Louis, MO), nanoCeO2 (33 ± 12 nm, Sigma–Aldrich) and their ionic/bulk analogs [Ag+ as AgNO3 (Fisher, Suwanee, GA), Fe2+ as FeSO4 (Fisher), bulkTiO2 (Sigma–Aldrich) and bulkCeO2 (Sigma–Aldrich)] were prepared/purchased and characterized as described in Ma et al. [35]. As previously reported (Ma et al. [35]), lab-scale nitrifying SBRs fed with synthetic wastewater were set up and operated at steady state under three conditions in duplicate: (1) SBRs dosed with nanoAg, NZVI, nanoTiO2, or nanoCeO2; (2) SBRs dosed with Ag+, Fe2+, bulkTiO2, or bulkCeO2; and (3) undosed SBRs. The dosing was initiated at 0.1 mg/L and sequentially increased to 1, 10 and 20 mg/L. The SBRs were actively nitrifying at the time of this study: further details on SBR performance are reported in Ma et al. [35]. Aqueous effluents and biosolids were sampled at the end of 20 mg/L dosing. In preparation for toxicity tests, samples from duplicate SBRs were combined and sterilized with concentrations of nanomaterials in SBR effluents and biosolids quantified by inductively coupled plasma mass spectrometry (ICP-MS), as described in the Supplementary Information.

2.2. Cell culture and treatment

A549 human lung alveolar epithelial cells were obtained from ATCC (#CCL-185, Manassas, VA). Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Thermo Scientific HyClone, Logan, UT) containing 10% heat inactivated fetal bovine serum (FBS, Atlanta Biologicals, Flowery Branch, GA) and 1% penicillin–streptomycin (Thermo Scientific HyClone, Logan, UT), and were maintained in a humidified incubator at 37 °C and 5% CO2. Cells were seeded at a density of 1 × 10^4 in 100 μL culture medium in each well of 96-well plates. At 80% confluency, they were treated for 24 h either with pristine nanomaterials, ionic/bulk analogs, SBR aqueous effluents, or SBR biosolids as indicated. The exposure concentrations of the pristine materials (Table S1) varied from 1 to 67 μg/mL for the cytotoxicity assay to estimate IC50 values. Genotoxicity assays were carried out at key concentration values based on the results of the cytotoxicity assays (Table S1). The exposure concentrations of nanomaterials and ionic/bulk materials in SBR effluents and biosolids are shown in Table 1. All samples were diluted with the culture medium to target concentrations.

2.3. Cytotoxicity assay

Cell viability was measured using the WST-1 assay (Roche, Indianapolis, IN) based on quantification of mitochondrial activity as an indicator of cytotoxicity. In viable cells, the tetrazolium salt WST-1 is converted to soluble formazan dye by mitochondrial succinate-tetrazolium reductase, which can be quantified by absorbance. After the A549 cells were treated with the samples for 24 h, WST-1 reagent was added to each well at 1/10 volume of the medium. The absorbance was quantified after incubating at 37 °C for 3 h using a Tecan Safire2 Microplate Reader (Tecan US Inc., Research Triangle Pa, NC) at 440 nm with a reference wavelength of 660 nm. All treated A549 cells (a) were tested in triplicate in three independent experiments with three controls: (b) untreated A549 cells in culture medium; (c) samples in culture medium without A549 cells; and (d) culture medium only. In a single experiment, cell viability was calculated as percentage of the average absorbance derived from triplicate runs of treated cells relative to untreated control cells, with absorbances of corresponding controls subtracted out to address possible matrix interferences: fraction cell viability = [(a – c)/(b – d)].

2.4. Genotoxicity assay

DNA damage in A549 cells was detected using immunofluorescent labeling of γH2AX foci as described elsewhere [19]. At sites of DNA double strand breaks, H2AX, a minor nucleosomal histone protein, is rapidly phosphorylated and forms γH2AX [20]. The experimental and imaging procedures are provided in the Supplementary Information. Three independent experiments were conducted with at least 200 cells imaged in a single test. Untreated cells and cells treated with 100 μM H2O2 for 10 min were included as negative and positive controls, respectively. Images were analyzed using ImageJ 1.47 (http://rsbweb.nih.gov/ij/) with a macro designed to subtract background and count the number of foci within the defined nucleus masks.
Table 1
Concentration of nanomaterials and ionic/bulk materials in SBR effluents and biosolids, and exposure concentration in cytotoxicity and genotoxicity assays.

| Nanomaterial | SBR Effluents (µg/L) | Exposure Concentration (µg/L)a | Biosolids (µg/µg total solids) | Exposure Concentration (µg/mL)b |
|-------------|----------------------|--------------------------------|-------------------------------|---------------------------------|
| NanoAg      | 28.43                | 9.48                           | 2332                          | 0.47                            |
| Ag⁺         | 43.67                | 14.56                          | 2212                          | 0.44                            |
| NZVI        | 7.10                 | 2.37                           | 38,327                        | 7.67                            |
| Fe⁺         | 4.70                 | 1.57                           | 48,061                        | 9.61                            |
| NanoTiO₂    | 0.95                 | 0.32                           | 49,201                        | 9.84                            |
| BulkTiO₂    | 1.02                 | 0.34                           | 67,649                        | 13.53                           |
| NanoCeO₂    | 0.25                 | 0.08                           | 22,360                        | 4.47                            |
| BulkCeO₂    | 0.86                 | 0.29                           | 26,454                        | 5.29                            |

a SBR effluents were diluted approximately 1:3 in the culture medium.
b SBR biosolids were exposed to A549 cells at 200 µg/µg total solids/mL.

Concentrations of nanoTiO₂ and bulkTiO₂ in SBR effluents were near the detection limit of Ti by ICP-MS and not significantly different from concentrations in the undosed SBR.

2.5. Statistical analysis

The data were presented as mean ± standard deviation of three independent experiments. Student’s t test or pairwise t test was conducted in R-2.8.1 (http://cran.r-project.org/bin/windows/base/old/2.8.1/) to determine statistical differences between samples at a significance level of 0.05 (p < 0.05).

3. Results and discussion

3.1. Toxicity of pristine nanomaterials

Based on the WST-1 assay, the viability of cells exposed to nanoAg, Ag⁺, NZVI and Fe⁺ for 24 h decreased significantly (p < 0.05) in a dose-dependent manner (Fig. S1). The IC₅₀ values for these materials were estimated to be 53 ± 2, 21 ± 0.1, 38 ± 2 and 55 ± 2 µg/mL, respectively. In particular, the IC₅₀ of Ag⁺ was significantly lower than that of nanoAg (p = 5 × 10⁻⁴), a result that is in agreement with other studies using A549 cell targets [21,4]. By contrast, NZVI was more toxic than its ionic analog, Fe⁺ (p = 6 × 10⁻⁴). In another study, the cytotoxicity of NZVI (synthesized through reduction of FeCl₃ by NaBH₄ and coated with Pd) to human bronchial epithelial cells 16HBE14o was not significantly different from Fe⁺ [22]. Differences between the studies could relate to differences in NZVI coatings (manufacturers in present study reports biodegradable organic and inorganic stabilizers) or the different cells used in the assays (A549 versus 16HBE14o). The viability of cells exposed to nanoTiO₂, nanoCeO₂ and their bulk analogs only decreased at a concentration of 67 µg/mL, by 10–20% (Fig. S1, p < 0.05). But in a few other studies, significant cytotoxicity of nanoTiO₂ and nanoCeO₂ to A549 cells was observed, even at concentrations up to 100 µg/mL [23,5,24].

Only cells that were exposed to 33 and 53 µg/mL nanoAg and 21 µg/mL Ag⁺ showed significantly higher numbers of γH2AX foci per cell (Fig. S2A) and greater percentages of cells containing γH2AX foci (Fig. S2B) (p < 0.05) relative to untreated control cells, suggesting genotoxicity resulting from DNA double strand breaks. The number of γH2AX foci per cell exposed to 9 µg/mL nanoAg was not significantly different from that of the control cells (Fig. S2A, p = 0.12), but the percentage of cells containing γH2AX foci was significantly higher than for the control cells (Fig. S2B, p = 0.03). Although IC₅₀ concentrations of NZVI and Fe⁺ (38 and 55 µg/mL, respectively), and nanoTiO₂, nanoCeO₂ and the bulk analogs at 67 µg/mL induced significant cytotoxicity to A549 cells, the number of γH2AX foci were not significantly different in cells treated with these materials (Fig. S2, p = 0.05). To the authors’ knowledge, genotoxicity of NZVI to human cells has not been investigated previously. Genotoxicities have been observed in A549 cells at concentrations of 2.5–15 µg/mL of nanoAg by ³²P post-labeling of DNA adducts [4], 10–50 µg/mL of nanoTiO₂ by cytokinesis block micronucleus assay [25], and 0.5–100 µg/mL of nanoCeO₂ by alkaline comet assay [23]. However, in this study, the genotoxicity of nanoAg could be demonstrated, but not nanoTiO₂ or nanoCeO₂ at 67 µg/mL. The distinct outcome of the nanoTiO₂ in the present study could be attributed to the different genotoxicity assays employed. With respect to nanoCeO₂, the prior study [23] utilized lab-synthesized nanoCeO₂ with a size range of 16–22 nm, whereas the present study utilized commercial nanoCeO₂ with an average particle size of 33 ± 12 nm.

3.2. Toxicity of SBR effluents and biosolids

Based on a previous study (Ma et al. [35]), >99% of nanomaterials and ionic/bulk analogs dosed into the SBRs partitioned into the sludge relative to the influent concentrations. Concentrations of nanomaterials in SBR aqueous effluents and biosolids, as well as exposed to A549 cells, are shown in Table 1. No significant decrease of cell viability or induction of γH2AX foci were observed in A549 cells exposed to SBR effluents (Fig. 1A and 2, p > 0.05). The exposure concentration of nanoAg in this study (9.48 µg/L) was much higher than predicted concentrations in WWTP effluents (<0.5 µg/L) [12–14], while the exposure concentrations of NZVI (2.37 µg/L), nanoTiO₂ (0.32 µg/L), and nanoCeO₂ (0.08 µg/L) were within or lower than the lower bound concentrations predicted (0.7–20 µg/L for NZVI [14], 1–70 µg/L for nanoTiO₂ [12–14], and 0.5 × 10⁻⁴ to 2 µg/L for nanoCeO₂ [13,14]. Results of this study indicated limited toxicity of nanoAg in wastewater effluents at higher than environmentally-relevant concentrations to A549 cells, while the effects of higher concentrations of NZVI, nanoTiO₂ and nanoCeO₂ remain to be determined. The concentrations of nanomaterials in biosolids in this study (>2000 µg/g dry mass, Table 1) were significantly
higher than concentrations predicted in biosolids from WWTPs (<1000 µg/g dry mass) [12–14]. Moreover, 200 µg total solids/mL represented a high exposure dose relative to other studies of effects of aerosolized biosolids to human lung cells [26]. The viability of cells exposed to biosolids at 200 µg total solids/mL decreased by 7–10% relative to untreated control cells (p < 0.05), except for biosolids containing NZVI (p = 0.08) (Fig. 1B). But, there was no significant difference between cells exposed to biosolids from undosed versus dosed SBRs (p > 0.05), indicating that the decrease in cell viability was not likely due to the nanomaterials or ionic/bulk materials, but probably instead to the high concentration of total solids. Cytotoxicity of biosolids was also examined, at 50 and 100 µg total solids/mL, and no significant effects were observed relative to control cells (p > 0.05, Fig. S3).

Genotoxicity of biosolids was examined at 200 µg total solids/mL. However, no significant differences were observed in terms of the number of γH2AX foci per cell or in the percentage of cells containing γH2AX foci in cells treated with biosolids relative to untreated control cells (Fig. 3, p > 0.05), suggesting little or no DNA damage to A549 cells at concentrations exceeding most likely aerosol exposure levels.

Uptake of pristine nanoAg, nanoTiO2, and nanoCeO2 by human cells [21,5,24,27], and NZVI by mammalian nerve cells [28] has been observed in previous studies, and the most commonly identified mechanism of toxicity was
the generation of reactive oxygen species, which induced oxidative stress [27,4,22,25]. Release of Ag⁺ was considered another potential cause of nanoAg toxicity [29]. Based on TEM–EDS mapping carried out in a previous study of the SBR biosolids (Ma et al. [35]), while a large portion of nanoAg remained dispersed, it mainly formed Ag–S complexes. Sulfidation has been reported to reduce toxicity of nanoAg to microbes, aquatic and terrestrial eukaryotic organisms due to low solubility of Ag–S complexes [30,31]. Similarly, transformation of nanoAg in this study may limit its reactivity and result in little toxicity of SBR biosolids to A549 cells. The majority of NZVI, nanoTiO₂ and nanoCeO₂ were aggregated, but not chemically modified. Epithelial cells are impervious to aggregated nanomaterials by diffusion or macropinocytosis [32]. Therefore, the absence of cytotoxicity and genotoxicity of SBR biosolids in this study could be attributed to the inability of aggregated NZVI, nanoTiO₂, and nanoCeO₂ to enter cells. However, the size of the nanomaterial aggregate can affect its physiological distribution and kinetics, cellular distribution (for example within the draining lymph node for an aerosolized particle), cellular uptake, and intracellular processing pathways [33]. Also, it is difficult to ascertain the fate of aerosolized nanomaterials from biosolids in the respiratory tract as particles can diffuse and convert during interstitial transport depending on their size [34]. Thus, future work may consider the toxicity under ex vivo and in vivo conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jtxrep.2014.08.017.

References

[1] T. Papp, D. Schiﬀmann, D. Weiss, V. Castranova, V. Vallyathan, Q. Rahman, Human health implications of nanomaterial exposure, Nanotoxicology 2 (1) (2008) 9–27.
[2] T. Xia, N. Li, A.E. Nel, Potential health impact of nanoparticles, Annu. Rev. Publ. Health 30 (2009) 137–150.
[3] V.L. Colvin, The potential environmental impact of engineered nanomaterials, Nat. Biotechnol. 21 (10) (2003) 1166–1170.
[4] R. Foldbjerg, D.A. Dang, H. Autrup, Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549, Arch. Toxicol. 85 (7) (2011) 743–750.
[5] A. Lankoff, W.J. Sandberg, A. Węgierska-Ciuk, H. Lisowska, M. Rofsnes, B. Sartowska, P.E. Schwarze, S. Mezczyńska-Wielgosz, M. Wojewodzka, K. Kruszewski, The effect of agglomeration state of silver and titanium dioxide nanoparticles on cellular response of HepG2, A549 and THP-1 cells, Toxicol. Lett. 208 (3) (2012) 197–213.
[6] N. Lewinski, V. Colvin, R. Drezek, Cytotoxicity of nanoparticles, Small 4 (1) (2008) 26–49.
[7] H.K. Lindberg, G.C.M. Falck, S. Suohon, M. Vippola, E. Vanhala, J. Capture, K. Savolainen, H. Norppa, Genotoxicity of nanomaterials: DNA damage and micronuclei induced by carbon nanotubes and graphite nanoparticles in human bronchial epithelial cells in vitro, Toxicol. Lett. 186 (3) (2009) 166–173.
[8] S.A. Blaser, M. Scheringer, M. MacLeod, K. Hungerbuhler, Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles, Sci. Total Environ. 390 (2–3) (2008) 396–409.
[9] S.J. Klaine, A.A. Koelmans, N. Horne, S. Carley, R.D. Handy, L. Kapustka, B. Nowack, F. von der Kammer, Paradigms to assess the environmental impact of manufactured nanomaterials, Environ. Toxicol. Chem. 31 (1) (2012) 3–14.

[10] B. Nowack, J.F. Ranville, S. Diamond, J.A. Gallego-Urrea, C. Metcalfe, J. Rose, N. Horne, A.A. Koelmans, S.J. Klaine, Potential scenarios for nanomaterial release and subsequent alteration in the environment, Environ. Toxicol. Chem. 31 (1) (2012) 50–59.

[11] M.R. Wiesner, G.V. Lowry, P. Alvarez, D. Dionysiou, P. Biswas, Assessing the risks of manufactured nanomaterials, Environ. Sci. Technol. 40 (14) (2006) 4336–4345.

[12] F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack, Modeled environmental concentrations of engineered nanomaterials (TiO₂, ZnO, Ag, CNT, Fullerene) for different regions, Environ. Sci. Technol. 43 (24) (2009) 9216–9222.

[13] F. Gottschalk, T.F. Sun, B. Nowack, Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies, Environ. Pollut. 181 (2013) 287–300.

[14] A.A. Keller, A. Lazareva, Predicted releases of engineered nanomaterials: from global to regional to local, Environ. Sci. Technol. Lett. 1 (2014) 65–70.

[15] M.A. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Perez-Rivera, K. Hristovski, Titanium nanomaterial removal and release from wastewater treatment plants, Environ. Sci. Technol. 43 (17) (2009) 6757–6763.

[16] L.K. Limbach, B. Beretier, E. Mueller, R. Krebs, R. Gaelli, W.J. Stark, Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency, Environ. Sci. Technol. 42 (15) (2008) 5828–5833.

[17] H.T. Bausum, S.A. Schaub, K.F. Kenyon, M.J. Small, Comparison of coliphage and bacterial aerosols at a wastewater spray irrigation site, Appl. Environ. Microbiol. 43 (1) (1982) 28–38.

[18] T. Paez-Rubio, A. Marariu, J. Sommer, H. Xin, J. Anderson, J. Peccia, Emission rates and characterization of aerosols produced during the spreading of dewatered class B biosolids, Environ. Sci. Technol. 41 (10) (2007) 3537–3544.

[19] Y.K. Yu, W. Zhu, H.L. Diao, C.X. Zhou, F.Q.F. Chen, J. Yang, A comparative study of using comet assay and gamma HAX Foci formation in the detection of N-nitrosodi-nitro-N-nitrosoquguanidine-induced DNA damage, Toxicol. In Vitro 20 (6) (2006) 950–965.

[20] E.P. Rogakou, D.R. Pilch, A.H. Orr, V.S. Ivanova, W.M. Bonner, DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139, J. Biol. Chem. 273 (10) (1998) 5858–5868.

[21] P. Cronholm, H.L. Karlsson, J. Hedberg, T.A. Lowe, L. Winnberg, K. Elhlin, L.O. Wallinder, L. Moller, Intracellular uptake and toxicity of ag and cuo nanoparticles: a comparison between nanoparticles and their corresponding metal ions, Small 9 (7) (2013) 970–982.

[22] C.R. Keenan, R. Goths-Goldstein, D. Lucas, D.L. Sedlak, Oxidative stress induced by zero-valent iron nanoparticles and Fe(II) in human bronchial epithelial cells, Environ. Sci. Technol. 43 (12) (2009) 4555–4560.

[23] L. De Marzi, A. Monaco, J. De Lapuente, D. Ramos, M. Borras, M. Di Gioacchino, S. Santucci, A. Poma, Cytotoxicity and genotoxicity of ceria nanoparticles on different cell lines in vitro, Int. J. Mol. Sci. 14 (2) (2013) 3065–3077.

[24] E. Moschini, M. Gualtieri, M. Colombo, U. Fascio, M. Camatini, P. Manteca, The modality of cell–particle interactions drives the toxicity of nanosized CuO and TiO₂ in human alveolar epithelial cells, Toxicol. Lett. 222 (2) (2013) 102–116.

[25] R.K. Srivastava, Q. Rahman, M.P. Kashyap, A.K. Singh, G. Jain, S. Jahan, M. Lohani, M. Lantow, A.B. Pant, Nano-titanium dioxide induces genotoxicity and apoptosis in human lung cancer cell line, A549, Human Exp. Toxicol. 32 (2) (2013) 153–166.

[26] E. Viau, F. Levi-Schaffer, J. Peccia, Respiratory toxicity and inflammatory response in human bronchial epithelial cells exposed to biosolids, animal manure, and agricultural soil particulate matter, Environ. Sci. Technol. 44 (8) (2010) 3142–3148.

[27] H.J. Eom, J. Choi, Oxidative stress of GeO₂ nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B, Toxicol. Lett. 187 (2) (2009) 77–83.

[28] T. Phenerat, T.C. Long, G.V. Lowry, B. Veronesi, Partial oxidation (aging) and surface modification decrease the toxicity of nanosized zerovalent iron, Environ. Sci. Technol. 43 (1) (2009) 195–200.

[29] P.V. AsaRani, G.L.K. Mun, M.P. Hande, S. Valljaveettil, Cytotoxicity and genotoxicity of silver nanoparticles in human cells, ACS Nano 3 (2) (2009) 275–290.

[30] C. Levard, E.M. Horze, B.P. Colman, A.L. Dale, L. Truong, X.Y. Yang, A.J. Bone, G.E. Brown, R.L. Tanguay, R.T. Di Giulio, E.S. Bernhardt, J.N. Meyer, M.R. Wiesner, G.V. Lowry, Sulfidation of silver nanoparticles: natural antidote to their toxicity, Environ. Sci. Technol. 47 (23) (2013) 13440–13448.

[31] B.C. Reinsch, C. Levard, Z. Li, R. Ma, A. Wise, K.B. Gregory, G.E. Brown, G.V. Lowry, Sulfidation of silver nanoparticles decreases Escherichia coli growth inhibition, Environ. Sci. Technol. 46 (13) (2012) 6992–7000.

[32] A. Albanese, W.C.W. Chan, Effect of gold nanoparticle aggregation on cell uptake and toxicity, ACS Nano 5 (7) (2011) 5478–5489.

[33] G. Oberdorster, J. Ferin, B.E. Lehnert, Correlation between particle-size, in-vivo particle persistence, and lung injury, Environ. Health Perspect. 102 (1994) 173–179.

[34] M. Semmler-Behnke, S. Takenaka, S. Fertsch, A. Wenk, J. Seitz, P. Mayer, G. Oberdorster, W.G. Kreyling, Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent re-entrainment onto airway epithelium, Environ. Health Perspect. 115 (5) (2007) 728–733.

[35] Y. Ma, J.W. Metch, E.P. Vejeras, I.J. Miller, E.C. Leon, L.C. Marr, P.J. Vikesland, A. Pruden, Microbial community response of nitirifying sequencing batch reactors to silver, zero-valent iron, titanium dioxide and cerium dioxide nanomaterials, Water Res. (2014), http://dx.doi.org/10.1016/j.watres.2014.09.008.