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# Cerium oxide nanoparticle aggregates affect stress response and function in *Caenorhabditis elegans*

Steven Rogers

Kevin M. Rice

Marshall University, rice9@marshall.edu

Nandini Manne

Marshall University, manne@marshall.edu

Tolou Shokuhfar

Kun He

*See next page for additional authors*

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**Authors**

Steven Rogers, Kevin M. Rice, Nandini Manne, Tolou Shokuhfar, Kun He, Vellaisamy Selvaraj, and Eric Blough

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Steven Rogers<sup>1</sup>, Kevin M Rice<sup>1</sup>, Nandini DPK Manne<sup>1</sup>, Tolou Shokuhfar<sup>2</sup>, Kun He<sup>2,3</sup>, Vellaisamy Selvaraj<sup>1</sup> and Eric R Blough<sup>1,4</sup>

## Abstract

**Objective:** The continual increase in production and disposal of nanomaterials raises concerns regarding the safety of nanoparticles on the environmental and human health. Recent studies suggest that cerium oxide (CeO<sub>2</sub>) nanoparticles may possess both harmful and beneficial effects on biological processes. The primary objective of this study is to evaluate how exposure to different concentrations (0.17–17.21 µg/mL) of aggregated CeO<sub>2</sub> nanoparticles affects indices of whole animal stress and survivability in *Caenorhabditis elegans*.

**Methods:** *Caenorhabditis elegans* were exposed to different concentrations of CeO<sub>2</sub> nanoparticles and evaluated.

**Results:** Our findings demonstrate that chronic exposure of CeO<sub>2</sub> nanoparticle aggregates is associated with increased levels of reactive oxygen species and heat shock stress response (HSP-4) in *Caenorhabditis elegans*, but not mortality. Conversely, CeO<sub>2</sub> aggregates promoted strain-dependent decreases in animal fertility, a decline in stress resistance as measured by thermotolerance, and shortened worm length.

**Conclusion:** The data obtained from this study reveal the sublethal toxic effects of CeO<sub>2</sub> nanoparticle aggregates in *Caenorhabditis elegans* and contribute to our understanding of how exposure to CeO<sub>2</sub> may affect the environment.

## Keywords

Cerium oxide nanoparticles, *Caenorhabditis elegans*, nanoparticles, toxicity, stress response

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## Introduction

The use of nanotechnology in industry is rapidly increasing, with a worldwide market size estimated to be in excess of \$1 trillion US by the year 2015.<sup>1</sup> Despite the swift progress and early acceptance of nanotechnology, the potential for adverse health effects in humans and the environment due to prolonged exposure at various concentration levels has not yet been established. Assessing the potential toxicity and the effects of nanoparticles on biological systems has become a relevant and quickly growing area of environmental toxicology research.<sup>2</sup>

Due to their smaller size and increased surface to volume ratio, nanomaterials oftentimes exhibit differences in their biological reactivity compared to that observed in “bulk” materials.<sup>3</sup> Previous work has suggested that material toxicity can vary in a size-dependent fashion with smaller features being associated with increased cellular dysfunction.<sup>3,4</sup> How exposure to nanoparticles may affect the environment and human health is still not fully understood.<sup>2</sup>

Cerium is a rare-earth element that in its oxide (CeO<sub>2</sub>) form is used as an industrial catalyst, in the automotive industry,<sup>5</sup> as an ultraviolet blocking material,<sup>6</sup> and an industrial polishing reagent.<sup>7</sup> Research on how CeO<sub>2</sub> may affect biological function when present as a nanoparticle is equivocal with some studies showing that these particles may be toxic

<sup>1</sup>Center for Diagnostic Nanosystems, Marshall University, Huntington, WV, USA

<sup>2</sup>Department of Mechanical Engineering and Engineering Mechanics, Michigan Technological University, Houghton, MI, USA

<sup>3</sup>School of Material Science and Engineering, Shandong University, Jinan, China

<sup>4</sup>Department of Pharmaceutical Sciences and Research, Marshall University, Huntington, WV, USA

### Corresponding author:

Eric R Blough, Department of Pharmaceutical Sciences and Research, Marshall University, 1700 3rd Ave, Huntington, WV 25755-1090, USA.  
Email: blough@marshall.edu



while others have shown little or no toxicity and even beneficial effects. In support of this latter possibility, CeO<sub>2</sub> nanoparticles have also been shown to exhibit antioxidant properties by acting as superoxide dismutase (SOD) and catalase mimetics.<sup>8</sup> CeO<sub>2</sub> nanoparticles demonstrate an autoregenerative capability to cycle between +3 and +4 valence states, which can allow for the scavenging of hydroxyl and superoxide radicals during each cycle.<sup>9,10</sup> However, other studies have demonstrated that exposure to CeO<sub>2</sub> nanoparticles can lead to increases in oxidative stress,<sup>11,12</sup> cellular inflammation, and DNA damage<sup>13–16</sup> and that CeO<sub>2</sub> nanoparticles are toxic to aquatic organisms.<sup>17,18</sup>

*Caenorhabditis elegans* is widely used in the laboratory for different types of investigations given its short lifespan, transparency, ease of cultivation, and high level of conservation with the vertebrate genome.<sup>19</sup> In the last decade or so, *C. elegans* has begun to be used as a model organism for the investigation of chemical toxicity given its sensitivity to oxidative stress.<sup>20</sup> How exposure to CeO<sub>2</sub> nanoparticle aggregates may affect biological function in *C. elegans* is not well understood. Recent data suggest that CeO<sub>2</sub> nanoparticle exposure in *C. elegans* is associated with decrease in longevity<sup>21</sup> and growth inhibition.<sup>22</sup> Although informative, it should be noted that only one size of CeO<sub>2</sub> nanoparticles was investigated in these publications. Given that nanoparticle size directly influences chemical and biological reactivity and that toxicological effects are concentration dependent, additional study is warranted. Similarly, while the measurement of growth inhibition and decreased longevity is important to understanding the toxicity of dispersed CeO<sub>2</sub>, how exposure to CeO<sub>2</sub> nanoparticle aggregates might affect *C. elegans* longevity, larval development, indices of stress, and fecundity is not known. This latter fact is particularly important given the potential roles that nematodes play in regulating ecosystem productivity. Therefore, the purpose of this study was to observe multiple endpoints for the toxicity of CeO<sub>2</sub> nanoparticles at both different sizes and concentrations in an aggregated state. We hypothesized that changes in CeO<sub>2</sub> aggregate, concentration, and size have the potential to alter *C. elegans* development, indices of stress response, external stress resistance, reproduction, and even viability. Our data suggest that exposure to higher levels of CeO<sub>2</sub> nanoparticle aggregates is associated with increased levels of organismal stress markers, decreases in fertility, and diminished worm growth. Taken together, these findings suggest that exposure to CeO<sub>2</sub> nanoparticle aggregates may be toxic to *C. elegans*.

## Materials and methods

### CeO<sub>2</sub> nanoparticle preparation and characterization

Previously characterized NanoActive CeO<sub>2</sub> (99.9% purity as determined by inductively coupled plasma mass spectrometry (ICP-MS); Lot #06-0118) was purchased from NanoScale

Corporation (Manhattan, KS, USA). Stock suspensions (3.5 mg/mL) were prepared in double-distilled water (ddH<sub>2</sub>O) by sonication for 2 min using a Vibra-Cell Sonicator (Sonics & Materials, Inc. Newton, CT) at room temperature and characterized.

### Transmission electron microscopy and energy dispersive X-ray spectroscopy

Particles were imaged in their native state using a JEOL JEM 3010 transmission electron microscope at 300 keV. For determining the atomic composition of the particles, energy dispersive X-ray spectroscopy (EDX) was performed using a detector fitted to a JEOL JSM-6320F Field Emission Scanning Electron Microscope that was equipped with Noran Voyager EDX software.

### Dynamic light scattering

The hydrodynamic size and size distribution of the CeO<sub>2</sub> nanoparticle aggregates were evaluated in ddH<sub>2</sub>O water using a Particle Size Analyzer (Model-LB-550; HORIBA, New Jersey, NJ) equipped with an He–Ne laser (633 nm) using back-scattered light. Experiments were performed in triplicate runs that were performed on three different days with freshly prepared samples.

### *C. elegans* strains and culturing conditions, chemicals, and materials

*C. elegans* strains were obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota. The CL2166 strain carries a *gst-4::GFP* reporter allowing fluorescent observation of glutathione S-transferase. The SJ4005 strain exhibits an *HSP-4::GFP* transgene that exhibits oxidative stress-inducible fluorescence of heat shock protein production (HSP-4) (the human equivalent to hsp70).<sup>23</sup> Age-synchronized populations of *C. elegans* were prepared using standard procedures.<sup>24</sup> Nematode strains were maintained at 20°C using *Escherichia coli* OP50-1 suspensions spread on nematode growth medium (NGM) plates 24 h prior to nematode transfer to ensure sufficient bacterial lawn growth.

### Determination of lifespan and fertility of *C. elegans* in presence or absence of CeO<sub>2</sub> nanoparticles

Age-synchronous eggs (*d*=0) were grown to L4 larval stage and then transferred to OP50-1-coated plates with or without CeO<sub>2</sub> nanoparticles (0.172 µg/mL (3.822 × 10<sup>-6</sup> µg/cm<sup>2</sup>), 1.72 µg/mL (3.822 × 10<sup>-5</sup> µg/cm<sup>2</sup>), and 17.21 µg/mL (3.822 × 10<sup>-4</sup> µg/cm<sup>2</sup>)). *C. elegans* were transferred to new plates during each day of the reproductive cycle. Just prior to the end of the reproductive

phase, nematodes were transferred to new plates every 3 days. Worms were observed daily and the number of live and dead counted. Nematodes were scored as dead when it no longer responded to being touched with a worm pick made from platinum wire. Nematodes that escaped the bacterial lawn or burrowed into agar were excluded from analysis. Lifespan experiments were performed with  $n=60-100$ .

Age-synchronous L4s were transferred to individual NGM plates with different doses of nanoparticles at the beginning of their reproductive cycle (~2.5 days) and then transferred to new plates every 24 h. Eggs were counted following each 24-h plate transfer. Reproduction experiments were performed in triplicate with  $n=30$ .

### Transgene GFP expression, growth, and development

After paralysis using 5  $\mu$ L of 5% hypochlorite solution, GFP reporter gene expression was observed using an Olympus BX51 fluorescence microscope (Olympus America, Melville, NY, USA). Images were captured under standardized conditions, and ImageJ software was used to quantify mean GFP intensity per unit area and animal length. Imaging experiments were performed in triplicate with  $n=30$ .

### Thermotolerance assay

Thermotolerance assays were performed as described by Lithgow et al.<sup>25</sup> Briefly, 3-day-old nematodes were exposed to 35°C. Surviving worms were counted after 8 h. Thermotolerance experiments were performed in triplicate with  $n=60$ .

### Statistical analysis

Results are presented as mean  $\pm$  standard error of mean (SEM). The log-rank test was performed using Prism 5.0 software (GraphPad Software, La Jolla, CA, USA) to determine differences in nematode survivability between groups. Comparisons between groups were performed using the Student's *t*-tests or one-way analysis of variance (ANOVA) with Newman-Keuls post hoc testing as appropriate. The level of significance accepted a priori was  $p<0.05$ .

## Results

### Characterization of CeO<sub>2</sub> nanoparticle aggregates

The mean hydrodynamic diameter of the CeO<sub>2</sub> nanoparticle aggregates as measured by dynamic light scattering (DLS) was  $184 \pm 75$  nm (Figure 1(a)). Transmission electron microscopy (TEM) analysis showed that the individual CeO<sub>2</sub> nanoparticles were spherical/round in shape with a diameter of 10–30 nm in size (Figure 1(b) and (c)). EDX analysis showed the presence of cerium and oxygen with weight percentages of approximately 97% and 2%, respectively (Figure 1(d)).

### Exposure to CeO<sub>2</sub> nanoparticle aggregates is associated with increased stress but not death

Compared to untreated worms, we observed CeO<sub>2</sub> particle exposure did not affect nematode longevity irrespective of strain in CL2166 or SJ4005 strains at our chosen dosing concentrations (undocumented). We chose the N2 wild type to verify the survivability results of both GFP transgene strains and still observed no change in longevity with CeO<sub>2</sub> exposure (Figure 2). In an effort to better understand any potential toxicity of the CeO<sub>2</sub> particles, we next investigated whether particle exposure was associated with increased organismal stress using the fluorescent transgenic strains SJ4005 and CL2166. The SJ4005 contains a GFP reporter coupled to HSP-4 production, while the CL2166 strain contains a GFP reporter coupled to GST-4 response genes. Compared to that observed in the unexposed worms, CeO<sub>2</sub> particle exposure appeared to significantly increase HSP-driven fluorescence in a dose- and time-dependent fashion at days 2, 4, and 6 (Figure 3(a) and (c);  $p<0.05$ ). Like that seen with the HSP-driven GFP reporter strain, CeO<sub>2</sub> particle exposure appeared to exhibit a similar effect in the CL2166 animals (Figure 3(b) and (d);  $p<0.05$ ). Taken together, these data suggest that CeO<sub>2</sub> particle exposure is associated with a significant increase in HSP-4 expression and cellular reactive oxygen species (ROS) levels as seen by increased GST-4.

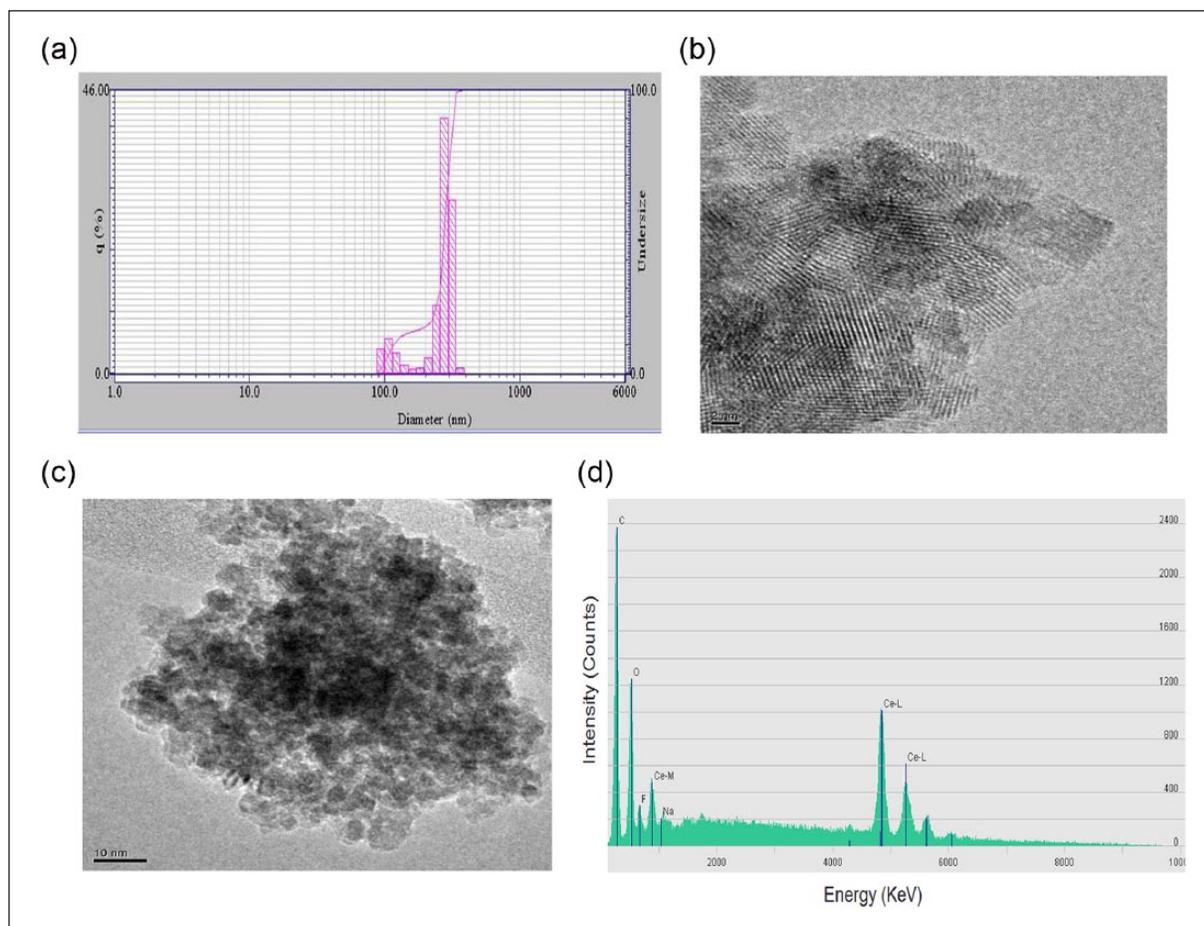
### Exposure to CeO<sub>2</sub> nanoparticle aggregates is associated with diminished egg laying and reduced body length

Age-synchronized worms were isolated in individual NGM plates, and egg production was counted over the entire reproduction period. Compared to that observed in the unexposed worms, exposure to CeO<sub>2</sub> particles significantly decreased the average daily egg production in the CL2166 but not the SJ4005 strain at days 3 and 5 (Figure 4(a) and (c),  $p<0.05$ ) and the total number of eggs produced during the entire reproduction period (Figure 4(b) and (d),  $p<0.05$ ).

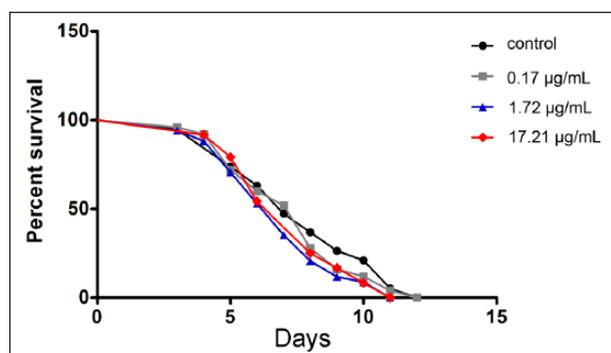
Similar to that seen in egg production, the effects of CeO<sub>2</sub> particle exposure on worm length also appeared to be strain dependent. Specifically, CeO<sub>2</sub> particle exposure appeared to diminish CL2166 body length early in development (Figure 5(b),  $p<0.05$ ), while in the SJ4005 strain, significantly diminished body length was not observed until day 6 (Figure 5(a),  $p<0.05$ ).

### Exposure to CeO<sub>2</sub> nanoparticle aggregates is associated with diminished thermotolerance

To determine whether CeO<sub>2</sub> nanoparticles increase or diminishes stress load during exposure to elevated temperatures, thermotolerance was chosen to further measure the organism's stress response. Our results show that exposure to CeO<sub>2</sub> particles lowered the ability of the SJ4005 strain but not the CL2166 animals to tolerate elevated temperatures (Figure 6,  $p<0.05$ ).



**Figure 1.** Physical characterization of  $\text{CeO}_2$  nanoparticle aggregates by (a) dynamic light scattering (DLS), (b, c) transmission electron microscopy (TEM), and (d) energy dispersive X-ray spectroscopy (EDX).



**Figure 2.** Exposure to  $\text{CeO}_2$  nanoparticle aggregates does not affect *Caenorhabditis elegans* longevity. Strain N2 wild type showed no significant changes in longevity with  $\text{CeO}_2$  particle exposure (0–17.21 µg/mL). Experiments were performed in triplicate ( $n=60$ –100).

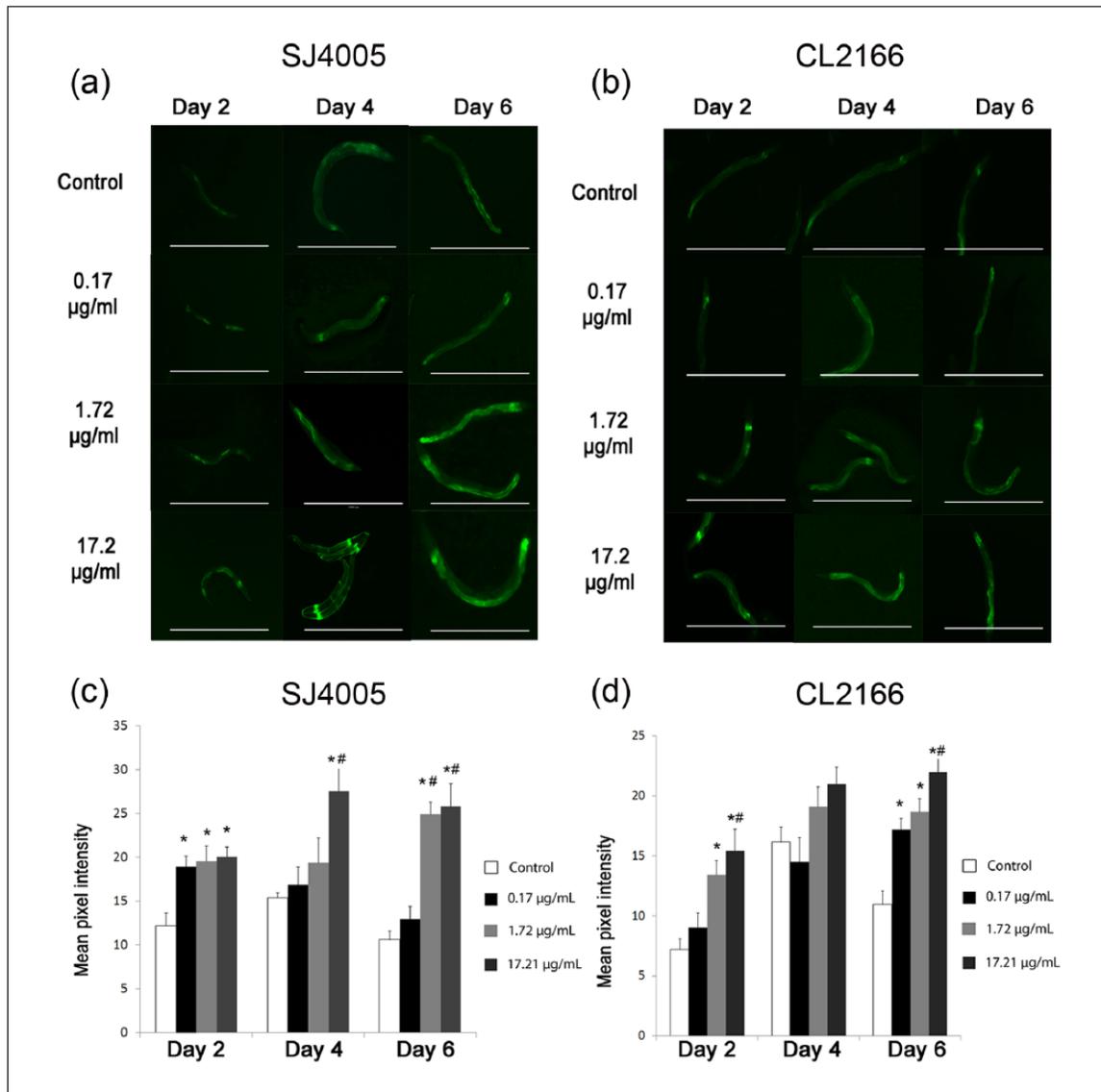
## Discussion

It is thought that engineered nanoparticles may pose a threat to human beings and the environment given their widespread

and growing use in everyday products.<sup>26</sup>  $\text{CeO}_2$  is currently 1 of 14 manufactured nanomaterials on the priority list of nanomaterials under investigation by the Organization for Economic Cooperation and Development (OECD).<sup>27</sup> In contrast to previous reports,<sup>21,28</sup> we examined the effects of exposure to  $\text{CeO}_2$  aggregates given the fact that nanoparticles frequently undergo aggregation in the high ionic strength environments oftentimes observed in environmental and biological fluids.<sup>29</sup> Our data suggest that exposure of *C. elegans* to aggregated  $\text{CeO}_2$  nanoparticles is associated with increased markers of organismal stress, decreased fertility, stunted growth, delays in organismal development, and diminished thermotolerance.

### *Exposure to $\text{CeO}_2$ nanoparticle aggregates is sublethal and increases expression of organismal stress markers*

Exposure to  $\text{CeO}_2$  particles had no significant effect on *C. elegans* lifespan even when used at concentrations as high as 17.21 µg/mL. These results, at first glance, were surprising



**Figure 3.** Exposure to CeO<sub>2</sub> nanoparticle aggregates induces organismal stress. GFP-coupled heat shock production (*HSP-4*) genes from (a) SJ4005 and GFP-coupled reactive oxygen species (ROS) response (*GST-4*) from (b) CL2166 were observed (original images at 4× magnification). Scale bar = 1 mm. CeO<sub>2</sub> caused an increase in GFP-related heat shock protein production in strain (c) SJ4005 and GFP-related ROS expression in strain (d) CL2166. Average mean pixel intensity per unit area is measured in ImageJ software. Data are expressed as mean ± standard error of mean (SEM) relative to controls ( $n = 10-20$ ).

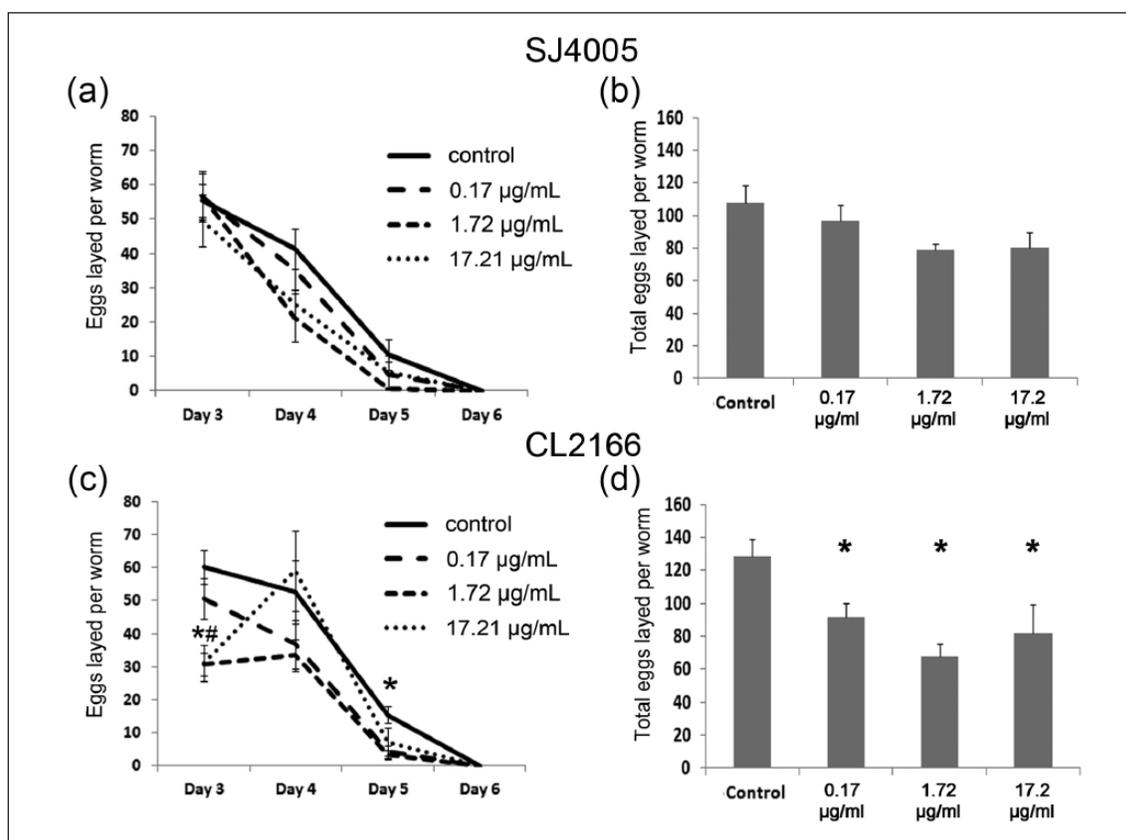
\*Significantly different from control group ( $p < 0.05$ ).

#Significantly different from 0.17 µg/mL CeO<sub>2</sub> group ( $p < 0.05$ ).

given the previous paper of Zhang et al.<sup>21</sup> which demonstrated that exposure to 0.00017 µg/mL was associated with significant increases in the incidence of *C. elegans* mortality. It is possible that differences between this study and previous work may be related to differences in the size of the nanoparticle used. For example, Zhang and co-workers used particles with a mean particle size of  $8.5 \pm 1.5$  nm, whereas in this study, the mean particle size was measured to be  $184 \pm 75$  nm by DLS and 10–30 nm by TEM. It is thought that as particle size increases, the particle becomes generally less permeable and less catalytic due to larger molecular

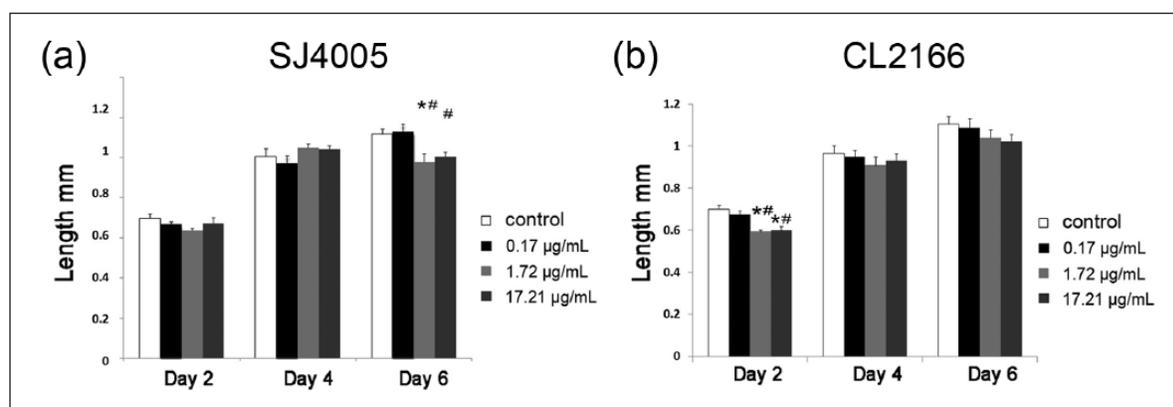
structure hindering exposure to the CeO<sub>2</sub> active site.<sup>4</sup> Multiple factors, such as pH and the ionic strength of the environment, can cause particle aggregation which can result in the loss of nanoscale properties.<sup>30</sup> This has been shown by Arnold et al.<sup>22</sup> who observed that CeO<sub>2</sub> nanoparticles were more toxic than equimolar amounts of “bulk” cerium oxide. Whether the change in particle size is solely responsible for the differences in toxicity observed in this study and previous work is unclear and will require further investigation.

Similar to the work of Zhang and colleagues, we found that exposure to CeO<sub>2</sub> nanoparticle aggregates in *C. elegans*



**Figure 4.** Exposure to CeO<sub>2</sub> nanoparticle aggregates decreases fecundity. Egg production by individual worms was determined daily and then totaled. SJ4005 strain egg production by (a) day and (b) totaled. CL2166 strain egg production by (c) day and (d) totaled ( $n=90$  worms).

\*Significantly different from control group ( $p < 0.05$ ).



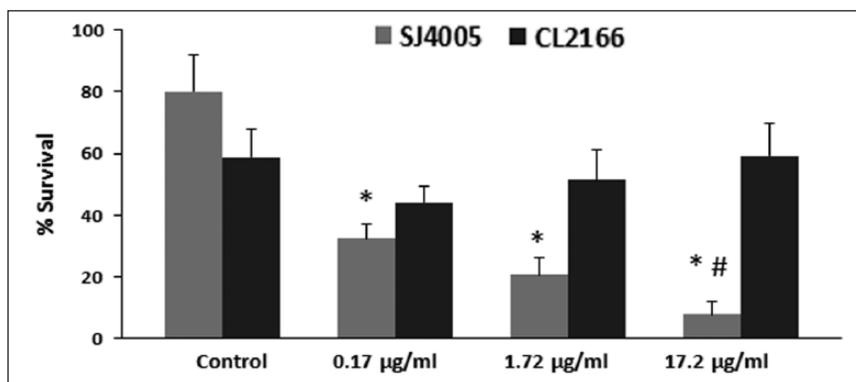
**Figure 5.** Exposure to CeO<sub>2</sub> nanoparticle aggregates affects worm growth and development. Cerium oxide particle exposure decreased length of strain (a) SJ4005 and (b) CL2166.

\*Significantly different from control group ( $p < 0.05$ ).

#Significantly different from 0.17 µg/mL CeO<sub>2</sub> group ( $p < 0.05$ ).

was associated with a toxicological response as demonstrated by increased exposure-induced expression of *GFP* (Figure 3, Panels A–D). Specifically, we found that particle exposure in the SJ4005 strain was associated with an increase in HSP-driven *GFP* expression (Figure 3, Panels A and C)

and that particle treatment in the CL2166 strain induced the ROS-dependent expression of *GFP* in a concentration-dependent manner (Figure 3, Panels B and D). Although beyond the scope of this study, the reason for the observed increase in stress response markers by CeO<sub>2</sub> may be related



**Figure 6.** Exposure to CeO<sub>2</sub> nanoparticle aggregates decreases worm thermotolerance. Age-synchronized SJ4005 or CL2166 worms were exposed to CeO<sub>2</sub> particles (0–17.21 µg/mL) at 35°C for 8h on day 3, and animal survivability was recorded ( $n=60$ ).

\*Significantly different from strain matched control group ( $p < 0.05$ ).

#Significantly different from 0.17 µg/mL CeO<sub>2</sub> group ( $p < 0.05$ ).

not only to its ability to relieve oxidative stress but also to cause it. The ability of CeO<sub>2</sub> to cause oxidative stress has been well documented in cell culture<sup>31,32</sup> and in rats.<sup>13</sup> CeO<sub>2</sub> redox cycling between Ce<sup>3+</sup> and Ce<sup>4+</sup> may play a vital role in the generation of damaging oxygen radicals. Using paramagnetic resonance, previous work has demonstrated that CeO<sub>2</sub> nanoparticles in the presence of hydrogen peroxide can cause the formation of hydroxyl radicals and superoxide anions.<sup>33</sup> Just as the beneficial ROS scavenging properties of CeO<sub>2</sub> rely on the number of oxygen vacancies and the Ce<sup>3+</sup>/Ce<sup>4+</sup> ratio,<sup>34</sup> the oscillatory cycling of giving and taking oxygen appears to work in both directions depending upon the chemical conditions.<sup>33</sup> Whether the creation of hydroxyl and superoxide by CeO<sub>2</sub> explains the increases in organismal stress indices seen in our GFP analysis as well as diminished *C. elegans* fertility, growth, and development observed in this study is currently unclear.

### Exposure to CeO<sub>2</sub> particles attenuates growth and development

It is well known that free radicals can cause deleterious effects on *C. elegans* fertility (fecundity)<sup>35</sup> as well as growth and development.<sup>36</sup> Whether exposure to oxygen radicals, by themselves, is the direct cause of these changes or if such alterations are secondary to these elevations in radical levels is currently unclear. For example, Arnold and colleagues observed similar decrease in *C. elegans* growth following CeO<sub>2</sub> exposure which they suggested was due to diminished food intake that was caused by the interactions of CeO<sub>2</sub> and *E. coli*.<sup>22</sup> Bearing this in mind, it is possible that changes in development and growth may be related to *C. elegans* food intake, as CeO<sub>2</sub> has a strong affinity to bind to *E. coli*<sup>37</sup> which could, in principle, diminish food intake. Restricted dietary intake has been shown to increase lifespan in *C. elegans* at the expense of prolonging time in dauer stages of the development cycle.<sup>38</sup> Although there may be other factors at play,

it is conceivable that the worms exposed to the CeO<sub>2</sub> particles consumed less and that this decrease in food intake may be a contributing factor in the observed decrease in growth and development. Additional experiments, perhaps designed to directly test this assertion, will be useful in proving cause and effect.

It has been previously reported that increased stress plays a role in decreasing growth and development in *C. elegans*.<sup>38,39</sup> In addition to elevations in organismal stress, another potential reason for the decrease in *C. elegans* growth and development seen in this study may be related to the ability of CeO<sub>2</sub> to target and down-regulate nitric oxide synthase (NOS).<sup>40</sup> Nitric oxide (NO) is known to be highly conserved between both invertebrate and vertebrate species, and it is thought that this molecule plays an important role in neurotransmission, water and salt balance, organismal development, and immune function.<sup>41</sup> Although not measured, it is possible that CeO<sub>2</sub> exposure could diminish NOS and NO levels, which one could predict to cause impairments in nervous system function and *C. elegans* development.<sup>42</sup> Further experiments to directly examine this possibility are needed to establish causation.

### Exposure to CeO<sub>2</sub> particles decreases fecundity and ability to endure external stressors and causes strain-specific variations in data

It is thought that the measurement of fecundity is one of the most significant toxicological endpoint assays for assessing toxicity in *C. elegans*.<sup>43</sup> Given the nature of our study design, it is currently difficult to pinpoint the direct mechanism(s) by which exposure to CeO<sub>2</sub> might decrease fertility although we hypothesize that the increased oxidative stress response marked by GST-4 and HSP-4 we observed following CeO<sub>2</sub> exposure is the primary mechanism (Figure 3). Indeed, recent work has demonstrated that nematode stress levels are inversely associated with reproductive capability, along with

worm growth and development.<sup>44</sup> Potentially, increased stress response may also contribute to the diminished thermotolerance we observed following CeO<sub>2</sub> exposure (Figure 6). Why the response to CeO<sub>2</sub> nanoparticle exposure may differ between strains is not clear but may be related to the ability of *C. elegans* to undergo hermaphroditic reproduction which could give rise to spontaneous mutations.<sup>42,45</sup> Additional studies may be warranted to explore this possibility further.

In summary, our data demonstrate that exposure to CeO<sub>2</sub> particle aggregates in *C. elegans* is associated with increased indices of organismal stress, diminished growth, impaired development, and decreased fecundity in both dose- and strain-specific manner. The tendency of nanoparticles to favor aggregation such as that observed during “real world” aquatic exposure suggests that CeO<sub>2</sub> may not be as potentially toxic as previously considered when studied in its non-aggregate form. Additional studies on the effect of aggregated versus non-aggregated CeO<sub>2</sub> nanoparticles at varying concentrations and particle sizes, with both soil and aquatic organisms, will be needed to increase our understanding of the effects of CeO<sub>2</sub> on the environment and those that inhabit it.

### Declaration of conflicting interests

The authors declare that there is no conflict of interest.

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### References

- Mina K, Fritschi L and Knuiman M. A valid semi-quantitative food frequency questionnaire to measure fish consumption. *Eur J Clin Nutr* 2007; 61(8): 1023–1031.
- Aschberger K, Micheletti C, Sokull-Kluttgen B, et al. Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human health—lessons learned from four case studies. *Environ Int* 2011; 37(6): 1143–1156.
- Limbach LK, Li Y, Grass RN, et al. Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. *Environ Sci Technol* 2005; 39(23): 9370–9376.
- Buzeca C, Pacheco II and Robbie K. Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2007; 2(4): MR17–MR71.
- Basu RK, Standage SW, Cvijanovich NZ, et al. Identification of candidate serum biomarkers for severe septic shock-associated kidney injury via microarray. *Crit Care* 2011; 15(6): R273.
- Zholobak NM, Ivanov VK, Shcherbakov AB, et al. UV-shielding property, photocatalytic activity and photocytotoxicity of ceria colloid solutions. *J Photochem Photobiol B Biol* 2011; 102(1): 32–38.
- Cassee FR, van Balen EC, Singh C, et al. Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Crit Rev Toxicol* 2011; 41(3): 213–229.
- Das M, Patil S, Bhargava N, et al. Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. *Biomaterials* 2007; 28(10): 1918–1925.
- Celardo I, Traversa E and Ghibelli L. Cerium oxide nanoparticles: a promise for applications in therapy. *J Exp Ther Oncol* 2011; 9(1): 47–51.
- Korsvik C, Patil S, Seal S, et al. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chem Commun* 2007; 10: 1056–1058.
- Eom HJ and Choi J. Oxidative stress of CeO<sub>2</sub> nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B. *Toxicol Lett* 2009; 187(2): 77–83.
- Horie M, Nishio K, Kato H, et al. Cellular responses induced by cerium oxide nanoparticles: induction of intracellular calcium level and oxidative stress on culture cells. *J Biochem* 2011; 150(4): 461–471.
- Srinivas A, Rao PJ, Selvam G, et al. Acute inhalation toxicity of cerium oxide nanoparticles in rats. *Toxicol Lett* 2011; 205(2): 105–115.
- Wingard CJ, Walters DM, Cathey BL, et al. Mast cells contribute to altered vascular reactivity and ischemia-reperfusion injury following cerium oxide nanoparticle instillation. *Nanotoxicology* 2011; 5(4): 531–545.
- Lanone S, Rogerieux F, Geys J, et al. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. *Part Fibre Toxicol* 2009; 6: 14.
- Ma JY, Zhao H, Mercer RR, et al. Cerium oxide nanoparticle-induced pulmonary inflammation and alveolar macrophage functional change in rats. *Nanotoxicology* 2011; 5(3): 312–325.
- Van Hoecke K, Quik JT, Mankiewicz-Boczek J, et al. Fate and effects of CeO<sub>2</sub> nanoparticles in aquatic ecotoxicity tests. *Environ Sci Technol* 2009; 43(12): 4537–4546.
- Rodea-Palomares I, Boltes K, Fernandez-Pinas F, et al. Physicochemical characterization and ecotoxicological assessment of CeO<sub>2</sub> nanoparticles using two aquatic microorganisms. *Toxicol Sci* 2011; 119(1): 135–145.
- Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics* 1974; 77(1): 71–94.
- Boyd WA and Williams PL. Comparison of the sensitivity of three nematode species to copper and their utility in aquatic and soil toxicity tests. *Environ Toxicol Chem* 2003; 22(11): 2768–2774.
- Zhang H, He X, Zhang Z, et al. Nano-CeO<sub>2</sub> exhibits adverse effects at environmental relevant concentrations. *Environ Sci Technol* 2011; 45(8): 3725–3730.
- Arnold MC, Badireddy AR, Wiesner MR, et al. Cerium oxide nanoparticles are more toxic than equimolar bulk cerium oxide in *Caenorhabditis elegans*. *Arch Environ Contam Toxicol* 2013; 65(2): 224–233.
- Arsenovic PT, Maldonado AT, Colletuori VD, et al. Depletion of the *C. elegans* NAC engages the unfolded protein response, resulting in increased chaperone expression and apoptosis. *PLoS One* 2012; 7(9): e44038.
- Girard LR, Fiedler TJ, Harris TW, et al. WormBook: the online review of *Caenorhabditis elegans* biology. *Nucleic Acids Research* 2007; 35: D472–475.
- Lithgow GJ, White TM, Hinerfeld DA, et al. Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. *J Gerontol* 1994; 49(6): B270–B276.

26. Aitken RJ, Chaudhry MQ, Boxall AB, et al. Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup Med* 2006; 56(5): 300–306.
27. Simonelli F, Marmorato P, Abbas K, et al. Cyclotron production of radioactive CeO(2) nanoparticles and their application for in vitro uptake studies. *IEEE Trans Nanobioscience* 2011; 10(1): 44–50.
28. Roh JY, Park YK, Park K, et al. Ecotoxicological investigation of CeO(2) and TiO(2) nanoparticles on the soil nematode *Caenorhabditis elegans* using gene expression, growth, fertility, and survival as endpoints. *Environ Toxicol Pharmacol* 2010; 29(2): 167–172.
29. Chatterjee S, Bandyopadhyay A and Sarkar K. Effect of iron oxide and gold nanoparticles on bacterial growth leading towards biological application. *J Nanobiotechnology* 2011; 9: 34.
30. Van Hoecke K, De Schampelaere KA, Van der Meeren P, et al. Aggregation and ecotoxicity of CeO<sub>2</sub> nanoparticles in synthetic and natural waters with variable pH, organic matter concentration and ionic strength. *Environ Pollut* 2011; 159(4): 970–976.
31. Park EJ, Choi J, Park YK, et al. Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells. *Toxicology* 2008; 245(1–2): 90–100.
32. Kim IS, Baek M and Choi SJ. Comparative cytotoxicity of Al<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub>, TiO<sub>2</sub> and ZnO nanoparticles to human lung cells. *J Nanosci Nanotechnol* 2010; 10(5): 3453–3458.
33. Heckert EG, Seal S and Self WT. Fenton-like reaction catalyzed by the rare earth inner transition metal cerium. *Environ Sci Technol* 2008; 42(13): 5014–5019.
34. Hardas SS, Butterfield DA, Sultana R, et al. Brain distribution and toxicological evaluation of a systemically delivered engineered nanoscale ceria. *Toxicol Sci* 2010; 116(2): 562–576.
35. Goldstein P and Modric T. Transgenerational, ultrastructural analysis on the antioxidative effects of tocopherol on early gametogenesis in *Caenorhabditis elegans* grown in 100% oxygen. *Toxicol Appl Pharmacol* 1994; 124(2): 212–220.
36. Yasuda K, Hartman PS, Ishii T, et al. Interrelationships between mitochondrial fusion, energy metabolism and oxidative stress during development in *Caenorhabditis elegans*. *Biochem Biophys Res Commun* 2011; 404(3): 751–755.
37. Thill A, Zeyons O, Spalla O, et al. Cytotoxicity of CeO<sub>2</sub> nanoparticles for *Escherichia coli*. Physico-chemical insight of the cytotoxicity mechanism. *Environ Sci Technol* 2006; 40(19): 6151–6156.
38. Fielenbach N and Antebi A. *C. elegans* dauer formation and the molecular basis of plasticity. *Genes Dev* 2008; 22(16): 2149–2165.
39. Fukuyama M, Sakuma K, Park R, et al. *C. elegans* AMPKs promote survival and arrest germline development during nutrient stress. *Biol Open* 2012; 1(10): 929–936.
40. Niu J, Wang K and Kolattukudy PE. Cerium oxide nanoparticles inhibit oxidative stress and nuclear factor-kappaB activation in H9c2 cardiomyocytes exposed to cigarette smoke extract. *J Pharmacol Exp Ther* 2011; 338(1): 53–61.
41. Jacklet JW. Nitric oxide signaling in invertebrates. *Invert Neurosci* 1997; 3(1): 1–14.
42. Hobert O. WormBook. In: Specification of the nervous system (Internet), 2005, <http://www.wormbook.org> (accessed 20 September 2013).
43. Handy RD, Cornelis G, Fernandes T, et al. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. *Environ Toxicol Chem* 2012; 31(1): 15–31.
44. Richards JL, Zacharias AL, Walton T, et al. A quantitative model of normal *Caenorhabditis elegans* embryogenesis and its disruption after stress. *Dev Biol* 2013; 374(1): 12–23.
45. Lipinski KJ, Farslow JC, Fitzpatrick KA, et al. High spontaneous rate of gene duplication in *Caenorhabditis elegans*. *Curr Biol* 2011; 21(4): 306–310.