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## Effects of a nanoceria fuel additive on the physicochemical properties of diesel exhaust particles†

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Nanoceria (*i.e.*, CeO<sub>2</sub> nanoparticles) fuel additives have been used in Europe and elsewhere to improve fuel efficiency. Previously we have shown that the use of a commercial fuel additive Envirox™ in a diesel-powered electricity generator reduced emissions of diesel exhaust particle (DEP) mass and other pollutants. However, such additives are currently not permitted for use in on-road vehicles in North America, largely due to limited data on the potential health impact. In this study, we characterized a variety of physicochemical properties of DEPs emitted from the same engine. Our methods include novel techniques such as Raman spectrometry for analyzing particle surface structure and an assay for DEP oxidative potential. Results show that with increasing Envirox™ concentrations in the fuel (0×, 0.1×, 1×, and 10× of manufacturer recommended 0.5 mL Envirox™ per liter fuel), DEP sizes decreased from 194.6 ± 20.1 to 116.3 ± 14.8 nm; the zeta potential changed from -28.4 mV to -22.65 mV; DEP carbon content decreased from 91.8% to 79.4%; cerium and nitrogen contents increased from 0.3% to 6.5% and 0.2% to 0.6%, respectively; the ratio of organic carbon (OC) to elemental carbon (EC) increased from 22.9% to 38.7%; and the ratio of the disordered carbon structure to the ordered carbon structure (graphitized carbon) in DEPs decreased. Compared to DEPs emitted from 0×, 0.1×, and 1× fuels, DEPs from the 10× fuel had a lower oxidative potential likely due to the increased ceria content because pure ceria nanoparticles exhibited the lowest oxidative potential compared to all the DEPs. Since the physicochemical parameters tested here are among the determinants of particle toxicity, our findings imply that adding ceria nanoparticles into diesel may alter the toxicity of DEPs. The findings from the present study, hence, can help future studies that will examine the impact of nanoceria additives on DEP toxicities.

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### Environmental impact

Nanoceria fuel additives have been used in Europe thanks to their benefits including improved fuel efficiency and reduced emissions of CO<sub>2</sub> and particle mass. However, little is known as to whether and how such additives result in changes in particle physicochemical properties that are determinants of toxicity. This paper presents results from a comprehensive characterization of particles emitted in relation to a range of nanoceria concentrations in a diesel fuel. The additive led to changes in particle size and chemical compositions, elemental to organic carbon ratio, disordered to ordered carbon structure ratio, and oxidative potential. The findings can help future studies aiming to examine the impact of nanoceria additives on the toxicity of emitted particles.

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## 1. Introduction

Diesel exhaust particles (DEPs) have been used in numerous toxicological studies to understand the health effects of particulate air pollution and underlying biological mechanisms of those effects.<sup>1,2</sup> A major reason for using DEPs as a model pollutant is their unique physicochemical properties: carbonaceous core and highly adsorptive surfaces. It is also well recognized, however, that not all DEPs have the same physicochemical properties due to the difference in combustion

conditions and other factors affecting the formation and emission of DEPs.<sup>3,4</sup> Fuel catalyst additives may be a modifying factor of DEP toxicity.

Fuel additives formulated with ceria (CeO<sub>2</sub>) nanoparticles have been used in Europe, Asia Pacific, North America (limited to off-road engines), and South America, as these additives serve as a combustion catalyst leading to improved fuel efficiency.<sup>5</sup> Several previous studies found that nanoceria as a fuel additive is able to shift DEP size distributions toward smaller particle sizes,<sup>6,7</sup> increase their oxidation rate,<sup>6</sup> and alter their chemical composition.<sup>7,8</sup> In an earlier paper, in addition to these findings, we reported that using a commercial fuel additive containing CeO<sub>2</sub> nanoparticles (Envirox™, Energenics Europe Ltd, U.K.) in a diesel power generator resulted in a reduction of fuel consumption per unit of electricity generated, accompanied by reduced emissions of DEP mass, carbon dioxide, carbon monoxide, formaldehyde, acetaldehyde, acrolein, and increased oxides of nitrogen (NO<sub>x</sub>).<sup>9</sup>

These findings point to the potential benefits of using Envirox™ and other nanoceria-based diesel additives in reducing environmental and health impacts. For example, the reduction in the emission of DEP mass and other air pollutants such as aldehydes could reduce cardiorespiratory health risks associated with diesel exhaust exposure.<sup>10</sup> However, this benefit may be reversed if DEPs released from nanoceria-treated fuels are more toxic, *e.g.* due to the decreasing size, than DEPs emitted from untreated fuels.<sup>11</sup> An important question remains as to whether the use of Envirox™ would potentially change the toxicity of DEPs emitted. In order to answer this question, the physicochemical properties of the DEPs, which are the key determinants of toxicity, needed to be assessed. However, previous studies have focused more on reaction mechanisms by which ceria nanoparticles affect the combustion process and pollutant emissions.<sup>6,12,13</sup> Few studies have characterized physicochemical properties of DEPs. The present study aims to conduct a comprehensive analysis of DEP physicochemical properties including the size, morphology, chemical composition, surface charge, crystalline structure, and oxidative potential of DEPs emitted from combusting diesel with varying concentration of Envirox™ in a diesel-powered electric generator.<sup>9</sup> The findings are expected to help future studies that will examine the impact of nanoceria diesel additives on the toxicity of DEPs.

## 2. Materials and methods

The experimental setup is only briefly described below as it has been previously reported.<sup>9</sup>

### 2.1. Diesel engine, fuel additive, and fuels

The “base fuel” used in this study was no. 2 ultra-low (<15 ppm) sulfur diesel purchased at a fuel station in New Jersey in January 2013. The fuel additive, Envirox™, was purchased from Energenics Europe Ltd, U.K., in 2011, and the ceria inside was in the form of 5–10 nm spherical CeO<sub>2</sub> particles.<sup>14</sup> The manufacturer recommends adding 0.5 mL Envirox™ into 1 liter of fuel. We

denote the fuel at this Envirox™ concentration as 1× fuel. We also conducted experiments with the following Envirox™ concentrations in diesel: no Envirox™ added (0×), 0.05 mL Envirox™ per liter diesel (0.1×), and 5 mL Envirox™ per liter base fuel (10×). The theoretical concentrations of nanoceria in 0×, 0.1×, 1×, and 10× fuels were 0, 0.9, 9, and 90 μg mL<sup>-1</sup>, respectively.<sup>9</sup> The diesel engine used in this study was a four-cycle, 406cc electric generator with one cylinder (Model YDG 5500EE, Yanmar Inc., Adairsville, GA), and it was operated under full load to provide 5.5 kW electricity.

### 2.2. Collection and extraction of DEP

A portion of the diesel exhaust exiting the exhaust pipe was directed into a mixing box (50 cm × 50 cm × 60 cm), where it was mixed with purified air (*i.e.*, ambient air that passed through an air purification system consisting of a fiberglass filter followed by a HEPA filter and then an activated carbon filter). The diluted diesel exhaust entered a 25 m<sup>3</sup> stainless steel chamber through a stainless steel air diffuser. Relative humidity and temperature in the chamber were kept at 40–41% and 22–23 °C, respectively. The dilution of the exhaust was controlled so as to result in an airborne particle concentration of 400 ± 80 μg m<sup>-3</sup>, as measured by a calibrated SidePak photometer (AM510, TSI Inc., Shoreview, MN). This concentration was representative of a realistic ambient airborne diesel concentration. Particles were collected on 37 mm Teflon filters with a membrane ring (Pall Life Sciences, Ann Arbor, MI) from the center of the chamber using a pump operating at a flow rate of 10 L min<sup>-1</sup> for 2.5–3 hours. DEPs were also collected on quartz fiber filters (Whatman QM-A 37 mm, part number 1851-037) for analysis of elemental carbon and organic carbon (see Section 2.5 below). Prior to use, the quartz filters were heat treated (at 500 °C for 2 hours) to remove organic impurities.

Particles collected on the Teflon filters were sonicated continuously for 2 hours at 25 °C in 10 mL of high-performance liquid chromatography (HPLC)-grade water (Sigma Co., St Louis, MO) in an ultrasonic machine (3510R-DTH, 100 W, 42 kHz, Branson Ultrasonics Co., Danbury, CT) at 100 W output to suspend the particles into the water. This sonication protocol was finally selected after we had performed a set of optimization experiments, as the selected protocol generated DEP sizes that were the closest to the sizes of airborne DEPs (see the ESI†). At the end of the sonication procedure, the filters were carefully removed and dried, and then weighed to obtain particle extraction efficiency (see Table S2 in the ESI†). DEP suspensions were distributed into different vessels and then stored in a refrigerator at 4 °C before their further use. Just before each use, the DEP suspensions were further sonicated for 5 minutes to ensure uniform particle dispersion. To avoid potential microbiological contamination, all glassware was heat treated at 120 °C in an oven overnight before use.

### 2.3. DEP sizes and zeta potential

Hydrodynamic size and zeta potential of the DEP samples were measured using a Zetasizer dynamic light scattering instrument (ZS Nano, Malvern Instruments, Malvern, UK) at a fixed

temperature of 25 °C and with a detection angle of 90 degrees. Before measurement, DEP water suspensions were diluted to 50  $\mu\text{g mL}^{-1}$  using phosphate-buffered saline buffer (PBS, pH = 7.4) and were sonicated for 5 minutes in a bath sonicator (Branson 5510, Branson Electronics, Danbury, CT). This dilution and sonication procedure was appropriate for making DEP suspension solutions for toxicological testing.<sup>15</sup> The samples were placed in the center of the sonicator to get maximal sonication power. For each batch of the DEP (0 $\times$ , 0.1 $\times$ , 1 $\times$ , and 10 $\times$ ), particles in 3 sets of the suspensions were measured in parallel to obtain averages and standard deviations. For each sample, the size and zeta potential values were averaged over 11 repeated analysis runs.

#### 2.4. DEP morphology

An aliquot of DEP suspensions was dispersed in ethanol by sonication for 1 min. A single drop of the suspension was then dropcast onto 300-mesh holey carbon film transmission electron microscope (TEM) grids (TAAB) and dried under vacuum. High resolution transmission electron microscopy (HRTEM) and high angle annular dark field scanning transmission electron microscopy (HAADF-STEM), combined with energy-dispersive X-ray spectroscopy (EDX) analysis, were carried out using an FEI Titan 80/300 fitted with a Cs (image) corrector, monochromator, and EDX detector (EDAX, Leicester, UK) operated at an accelerating voltage of 300 kV. For STEM experiments, a convergence semi-angle of 14 mrad was used, with an inner and outer HAADF collection angle of 49 and 239 mrad, respectively.

#### 2.5. DEP chemical composition

In parallel to the DEP collection on Teflon filters as described above, diluted DEPs in the test chamber were collected onto quartz fiber filters (Pallflex, Port Washington, NY) at a flow rate of 1 L  $\text{min}^{-1}$ . Prior to use, the quartz fiber filters were heat-treated at 700 °C for one hour to remove any organic impurities. The DEPs collected on these filters were analyzed for elemental carbon (EC) and organic carbon (OC) at a commercial laboratory (Sunset Laboratory Inc., Tigard, OR) using a thermal-optical method.<sup>16</sup> The precision of the method, measured as a relative standard deviation, was 4–6%. The detection limit of this method was in the order of 0.2  $\mu\text{g cm}^{-2}$  filter for both OC and EC. All the DEP samples had EC and OC concentrations at least one order of magnitude higher than the detection limit.

DEPs collected on the Teflon filters were analyzed for carbon, hydrogen, and nitrogen content using an elemental analysis technique at a commercial laboratory (Robertson-Microlit Laboratories, Ledgewood, NJ). The method involved micro-chemical combustion of 1.0 mg of DEPs using a Perkin Elmer 2400 CHN Analyzer (Waltham, MA). High purity helium gas was used as the carrier and oxygen gas (99.9%) was used for the combustion. The operating temperature was 950 °C. Acetanilide (NIST SRM 141d) was used as the calibrating standard. Standard results were within  $\pm 0.3\%$  of theoretical values. Combustion was performed in tin capsules (6 by 4 mm, Elemental Micro-analysis D1006 or equivalent).

The cerium content of the DEP samples was analyzed using an inductively coupled plasma mass spectrometry (ICP-MS) method. In this method, an aliquot of each test DEP suspension was digested in concentrated ultrapure nitric acid in a high-power microwave oven. After dilution with HPLC-grade water, the extract was analyzed on an ICP-MS system (Thermo Elemental Plasma Quad 3, Thermo Scientific, Waltham, MA). NIST standards (NIST SRM3110 and NIST SM1811-002) were used for calibration. The detailed ICP-MS operating and quality control protocols can be found in a previous publication.<sup>17</sup>

#### 2.6. Raman spectroscopy

Raman-mapping spectroscopic characterization was carried out on a Renishaw inVia Confocal Raman microscope (Renishaw plc, Wotton-under-Edge, UK). The system calibration was performed using an integrated silicon wafer prior to measurement. Raman maps were obtained using a red excitation laser (wavelength 633 nm, intensity 100%, scan time 30 seconds) under Streamline™ mode (1800 lines per mm diffraction grating). No obvious damage to the carbon walls was observed after Raman analysis. Baseline correction, peak fitting, and peak integration were analyzed using WiRE 4.1 software.<sup>18</sup>

#### 2.7. Ascorbate depletion assay of oxidative potential

DEP oxidative potential was assessed using an assay in which DEP suspensions were added to an ascorbic acid (an antioxidant) solution. The oxidative potential of DEPs was measured as the depletion rate of ascorbic acid. In addition to the four DEPs (from 0 $\times$ , 0.1 $\times$ , 1 $\times$ , and 10 $\times$  Envirox™), CeO<sub>2</sub> particles (<50 nm in diameter) and a DEP purchased commercially (NIST 2975) were included in this set of test for comparison. Each of these particle samples was incubated with a mixture of ascorbic acid and sodium ascorbate (total ascorbate concentration of 200  $\mu\text{mol L}^{-1}$ , pH 7, 2 hours, 37 °C) in 96-well plates (Greiner Bio-one Ltd, UK). Ascorbate depletion rate ( $\mu\text{M minute}^{-1}$ ) was determined by measuring ascorbate ion concentrations on a UV spectrometer at 265 nm (Spectramax 384, Molecular Devices Ltd, USA) every two minutes over a 2 hour period.

### 3. Results and discussion

#### 3.1. Effects of Envirox™ on DEP size and surface electrical charge

The results on number-weighted particle size and zeta potential values are shown in Table 1. For the size, the two replicate samples were in excellent agreement for 0.1 $\times$ , 1 $\times$ , and 10 $\times$  fuels, where the differences in mean sizes between the two replicates ranged from 0.9 to 9.5%: a larger difference (45%) between the two replicates was observed for the 0 $\times$  fuel. We were not able to ascertain a reason for the differing levels of reproducibility. Nonetheless, based on the average values from the two replicate samples, there is a clear trend that as nano-ceria concentration in the diesel fuel increased, the size of DEPs decreased. From 0 $\times$  to 10 $\times$ , DEP sizes decreased by 40% from 194.6 nm to 116.3 nm. This trend was in agreement with previous findings with airborne DEPs (*i.e.*, DEPs in the air of the

Table 1 Particle size and zeta potential values for DEPs emitted from the four diesel fuels with varying Envirox™ concentration

	Sample #	0×	0.1×	1×	10×
Size (nm)	1	230.3 (±25.9)	143.3 (±12.0)	134.4 (±19.0)	115.8 (±14.4)
	2	158.8 (±11.9)	147.4 (±16.6)	147.2 (±11.4)	116.8 (±15.1)
	Average	194.6 (±20.1)	145.4 (±14.5)	140.8 (±15.7)	116.3 (±14.8)
Zeta potential (mV)	1	-26.2 (±2.5)	-28.4 (±2.7)	-32.2 (±2.2)	-24.6 (±1.8)
	2	-30.6 (±2.0)	-28.4 (±2.6)	-28.1 (±1.8)	-20.7 (±2.3)
	Average	-28.4 (±2.3)	-28.4 (±2.6)	-30.15 (±2.0)	-22.65 (±2.0)

test chamber before undergoing filter collection and subsequent extraction) by our group<sup>9</sup> and others.<sup>6</sup> This trend was mainly due to the fact that addition of ceria nanoparticles increased the soot oxidative process, which led to a reduction in the size of agglomerated particles by breaking them down into smaller particles.<sup>9,19–21</sup> This mechanism also implies that increasing nanoceria in the diesel resulted in increased particle number concentrations and increased particle surface area per unit mass of DEP emitted.<sup>9</sup> In agreement with our previous study, adding Envirox™ resulted in a reduction in DEP mass emissions and a net increase in ultrafine particle number emissions per unit electricity generated.<sup>9</sup>

Reproducibility of zeta potential values, measured as differences in the mean values from the two samples, ranged from 0% to 18% (see Table 1). All DEPs were negatively charged. From 0× to 0.1× and from 0.1× to 1×, the average zeta potential values did not change substantially. However, from 0× to 10×, DEP zeta potential values changed by 20% from -28.4 mV to -22.65 mV. Sarkar and colleagues also reported a similar tendency with zeta potential changing from -40.3 mV to -31 mV as Envirox™ concentration increased from 0× to 10×.<sup>22</sup> Several factors including media pH, particle size, morphology, and composition may affect zeta potentials.<sup>23</sup> In this study all of these factors besides media pH were changed to different degrees, which made the trend in zeta potential as a function of Envirox™ concentration not as clear and not consistent even across the two replicate samples. Our findings suggest that DEP surface charge may be affected by factors more than the fuel content of nanoceria, and we did not identify a definitive role of nanoceria in affecting DEP zeta potential.

There are several studies which report that zeta potential, as a common indicator of surface charge, affects particle bio-reactivity and toxicity such as cytotoxic effects<sup>24</sup> and immune responses in primary human peripheral blood mononuclear cells.<sup>22</sup> The current study did not provide clear evidence that DEP toxicity would be altered *via* altered zeta potential due to the addition of Envirox™ in the fuel.

### 3.2. Effects of Envirox™ on DEP morphology and crystallinity

The cerium content in the DEP was analysed by analytical TEM techniques; and the results are shown in Fig. 1 for DEPs from the 10× fuel. *Z* (atomic number)-contrast HAADF-STEM imaging is useful to indicate atomic number variations within the sample, which is formed by incoherently scattered, high

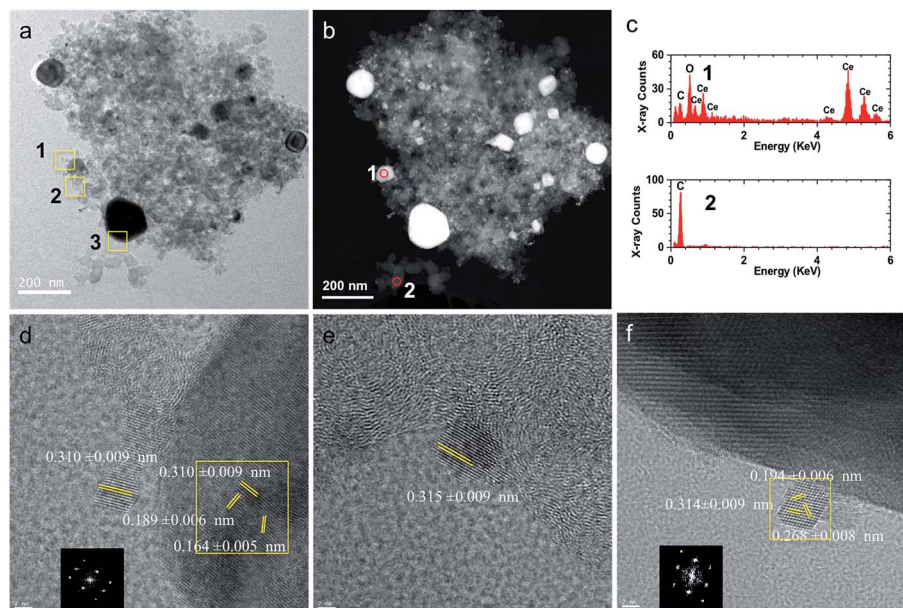
angle electrons, with the intensity approximately proportional to  $Z^2$ . The significant contrast differences as shown by HAADF-STEM in Fig. 1b, *i.e.* particles of high brightness mixed with structures of low brightness, imply variations in chemical composition. Indeed, as indicated by high resolution STEM-EDX analysis, the bright particles (area 1) are Ce- and O- rich in composition (Fig. 1c top), while the structures of lower brightness (area 2) are mainly carbon (Fig. 1c bottom). Cerium in DEPs mainly existed in the form of CeO<sub>2</sub> as revealed by high-resolution TEM (HRTEM). The lattice spacings (Fig. 1d–f) correspond to the (111), (200), (220), and (311) inter-planar spacings of cubic cerium oxide (CeO<sub>2</sub>) (0.312, 0.271, 0.191, and 0.163 nm), respectively. The details of the lattice spacings are presented in Fig. S2 and Table S4 in the ESI.† Images shown in Fig. 1 illustrate that various sizes of CeO<sub>2</sub>, rather than other forms of cerium, were mixed with DEPs. Our findings are consistent with those from previous studies, suggesting that Ce reaches its highest oxidative state (Ce<sup>4+</sup>) and exists as CeO<sub>2</sub> very early during the DEP formation phase.<sup>6,7,12</sup>

The chemical form of cerium may be an important determinant of particle toxicity, as suggested by a recent study reporting that cerium oxide (ceria) nanoparticles are more toxic than other forms of cerium particles.<sup>25</sup> As shown in Fig. 1, ceria in the DEP had a size of 100 to 200 nm, representing particle agglomerates rather than the single ceria particles of 5–10 nm originally added into the fuel.<sup>12</sup> The larger size agglomerates are expected to be less toxic and bio-reactive than smaller non-agglomerated particles.<sup>26</sup>

Furthermore, TEM images showing the morphology of DEPs for three diesel fuels (0×, 1× and 10×) are presented in Fig. 2. The addition of Envirox™ seems to have no effect on the shape of the DEPs, as DEPs from all the fuels looked like spherical (or spheroidal) nano-sized or larger particles comprised of several fused DEPs. The DEP agglomeration status was hard to compare by TEM due to the limitation of small imaged areas and negative effects of drying nanoparticles on the TEM grid. (Note that the ‘negative effects’ here means the formation of nanoparticle aggregates. The standard TEM sample preparation typically consists of dropcasting and drying a drop of nanoparticle suspension on a TEM grid. The drying process results in the formation of nanoparticle aggregation due to surface dewetting and the so-called ‘coffee ring’ effect. Therefore, it is difficult to comment on the aggregation status of nanoparticle samples prepared by this approach. This is a limitation of the method.)

Typical Raman spectra of different DEP samples are shown in Fig. 3a. The D peak (~1350 cm<sup>-1</sup>) and G peak (~1580 cm<sup>-1</sup>),





**Fig. 1** TEM images of DEP for diesel fuel with 10× Envirox™: (a) Bright field TEM (BF-TEM) images of particles; (b) the high angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) image corresponding with (a); (c) STEM-dispersive X-ray spectroscopy (STEM-EDX) spectra taken from corresponding area 1 and area 2 as marked in (b); (d–f) high resolution TEM (HR-TEM) images taken from boxed areas 1–3 in (a); insets are the corresponding fast Fourier transform (FFT) patterns taken from the boxed areas, respectively. The scale of (d–f) is 2 nm.

corresponding to the disordered graphitic lattice and the graphitic lattice of soot particles, respectively,<sup>18</sup> are displayed in the first-order spectra and observed to be strongly overlapped. Further curve fitting revealed more features of the graphitic and disordered lattice of soot carbon walls, and an example for 0× is presented in Fig. 3b. The peak area ratio of G peak to the sum of D1, D3 and D4 peaks provided semi-quantitative characteristics of the level of graphitized carbon (crystallinity). An increasing trend for the G/D ratio implying more graphitized carbon structures, shown in Fig. 4, was found for DEP with increasing concentrations of nano-CeO<sub>2</sub> in the diesel. A previous study found that compared with well-graphitized diesel soot, disordered carbon structures could facilitate oxidation of diesel soot (DEP).<sup>27</sup> Hence, the addition of Envirox™ is expected to decrease the oxidative potential of DEPs by increasing particle crystallinity, one of the mechanisms underlying the effect of nanoceria on the DEP oxidative potential (see Section 3.4 below). On the other hand, the reduction of the oxidative potential of DEPs may also decrease their toxicity.<sup>22</sup>

### 3.3. Effects of Envirox™ on DEP chemical composition

Results of the main elements of DEPs are shown in Table 2. It was found that the addition of Envirox™ at 0.1× concentration did not have much of an effect on the carbon content or nitrogen content, as compared to that in DEPs from the 0× fuel. However, further increase in the concentration of Envirox™ (at 1× and 10×) led to the progressive reduction in carbon content with a concomitant increase in nitrogen content. The hydrogen content varied little across the four DEPs. As expected, cerium content increased with increasing Envirox™ concentrations in the fuel (Table 2). It is interesting to note that the base fuel (0×)

generated DEPs with detectable cerium content. This is due to the use of Ce in catalysts during the production (refinery) of diesel fuel.<sup>34</sup>

Previously we found that increasing nanoceria concentration in the fuel resulted in increasing emissions of gaseous nitrogen species (*e.g.*, NO and NO<sub>2</sub>).<sup>9</sup> It is possible that a fraction of gaseous nitrogen species was converted into condensed-phase nitrogen-containing compounds, which explains the results reported here that increasing nanoceria in the fuel increased DEP nitrogen content (%). For example, nitro-PAHs are well-known constituents of DEPs.<sup>35</sup> Nitro-PAHs and other nitrohydrocarbons usually have lower vapor pressure than the corresponding parent PAHs and the other hydrocarbons.<sup>36</sup> Based on this hypothesis, using cerium-based fuel additives may increase the toxicity of DEPs, since compared with unsubstituted PAHs, nitro-PAHs have far greater toxicity.<sup>37</sup>

The addition of Envirox™ also appears to have facilitated the oxidation of hydrocarbons to oxygenated compounds. As shown in Fig. 5, DEPs from the 10× fuel had the highest OC fraction (corresponding to a decreased EC fraction) of total carbon. However, the OC *versus* EC change was small when Envirox™ was added at 0.1× and 1×. This observation is consistent with the finding of Okuda and colleagues (2009) who reported that adding 7.5 ppm Ce with 0.1 ppm Pt resulted in a larger reduction in EC (54% reduction) than OC (23% reduction).<sup>38</sup> The catalytic and combustion mechanism for these findings is yet to be determined.<sup>39</sup> Based on our observations from the present study (Section 3.3), we propose three possible mechanisms. Firstly, improved oxidation of disordered carbon by nanoceria leaves the DEP core with ordered carbon, which could adsorb more organic carbon. Secondly, more oxidized carbon was

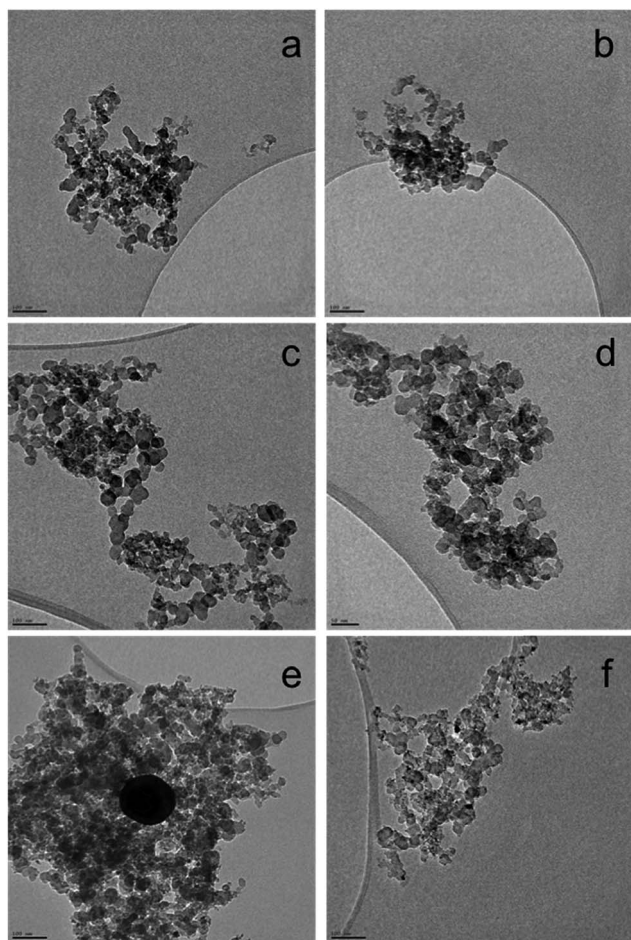


Fig. 2 TEM image of DEPs for diesel fuels with varying Envirox<sup>TM</sup> concentration: a and b (0 $\times$ ); c and d (1 $\times$ ); e and f (10 $\times$ ). The scale is 100 nm for a, b, c, e, and f and 50 nm for d.

produced likely due to the incomplete oxidation of both ordered and disordered carbon. And lastly, with the increase of Envirox<sup>TM</sup> concentration, more ceria nuclei were created, which could cause the condensation of organic gases on their surface.<sup>12</sup> The last mechanism is supported by a previous study;<sup>12</sup> however, whether and how the other two mechanisms are involved need to be addressed in future studies. Since only the proportion of OC and EC was studied in this study without knowing the specific mechanism, how these changes affect the toxicity of DEPs needs to be further investigated.

### 3.4. Effects of Envirox<sup>TM</sup> on DEP oxidative potential

One of the mechanisms by which DEPs (and particles in general) exert toxicity and bioreactivity is the ability of DEP to generate reactive oxygen species (ROS) *in vitro* or *in vivo*.<sup>40</sup> The potency of ROS generation can be assessed using simple, chemistry-based methods. For example, as a surrogate of ROS generation potency, oxidative potentials of the DEPs and two reference materials were determined here through a redox-based assay called the ascorbate depletion test. Ascorbic acid (vitamin C) is a well-known antioxidant, and hence its depletion rate upon reacting with a substance reflects the oxidative potential of the substance.<sup>41</sup> As shown in Fig. 6a, the rate of ascorbic acid depletion (measured as total ascorbate ions) is a function of the concentration of each test material and hence represents a series of “dose-response” curves. A steeper slope in each curve corresponds to the faster depletion of ascorbic acid, and thus higher oxidative potential of the material. Among all the tested particles, pure CeO<sub>2</sub> nanoparticles had the lowest oxidative potential, whereas DEPs from the 0 $\times$  fuel (no Envirox<sup>TM</sup> added) had the highest oxidative potential. The oxidative potential values for DEPs from the 0.1 $\times$ , 1 $\times$ , and 10 $\times$

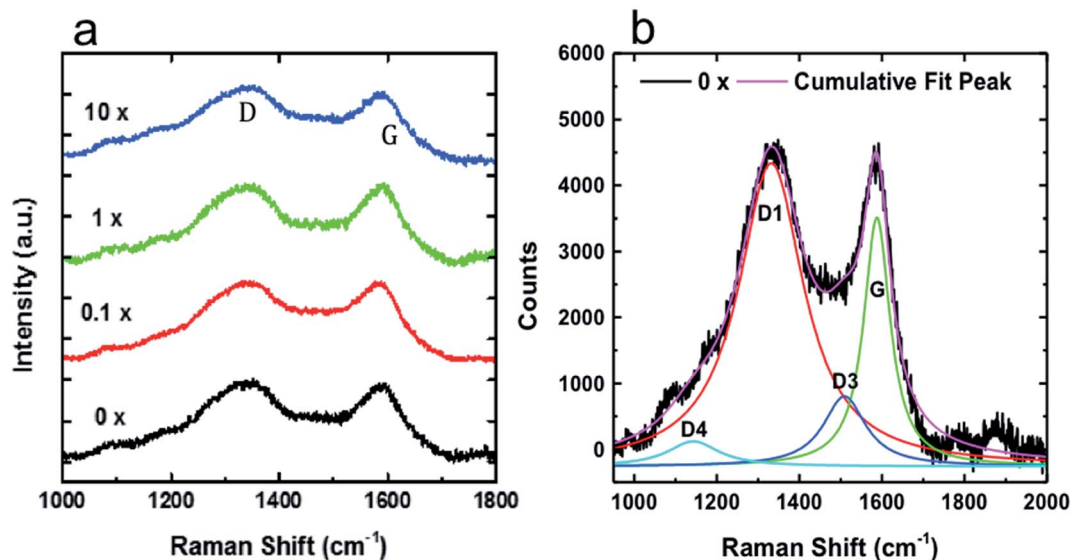


Fig. 3 (a) Raman spectra of DEPs from the four diesel fuels (0 $\times$ , 0.1 $\times$ , 1 $\times$ , and 10 $\times$ ) with an excitation laser wavelength of 633 nm. (b) Curve fitting for a typical Raman spectra of DEP from the 0 $\times$  fuel, fitted peaks included the G band ( $\sim$ 1580  $\text{cm}^{-1}$ , graphitic lattice),<sup>28,29</sup> D1 (D) band ( $\sim$ 1350  $\text{cm}^{-1}$ , disordered graphitic lattice),<sup>28,29</sup> D3 (D') band ( $\sim$ 1500  $\text{cm}^{-1}$ , amorphous carbon)<sup>30,31</sup> and D4 ( $\sim$ 1200  $\text{cm}^{-1}$ , disordered graphitic lattice,<sup>32</sup> polyenes,<sup>30,31</sup> and ionic impurities<sup>33</sup>).

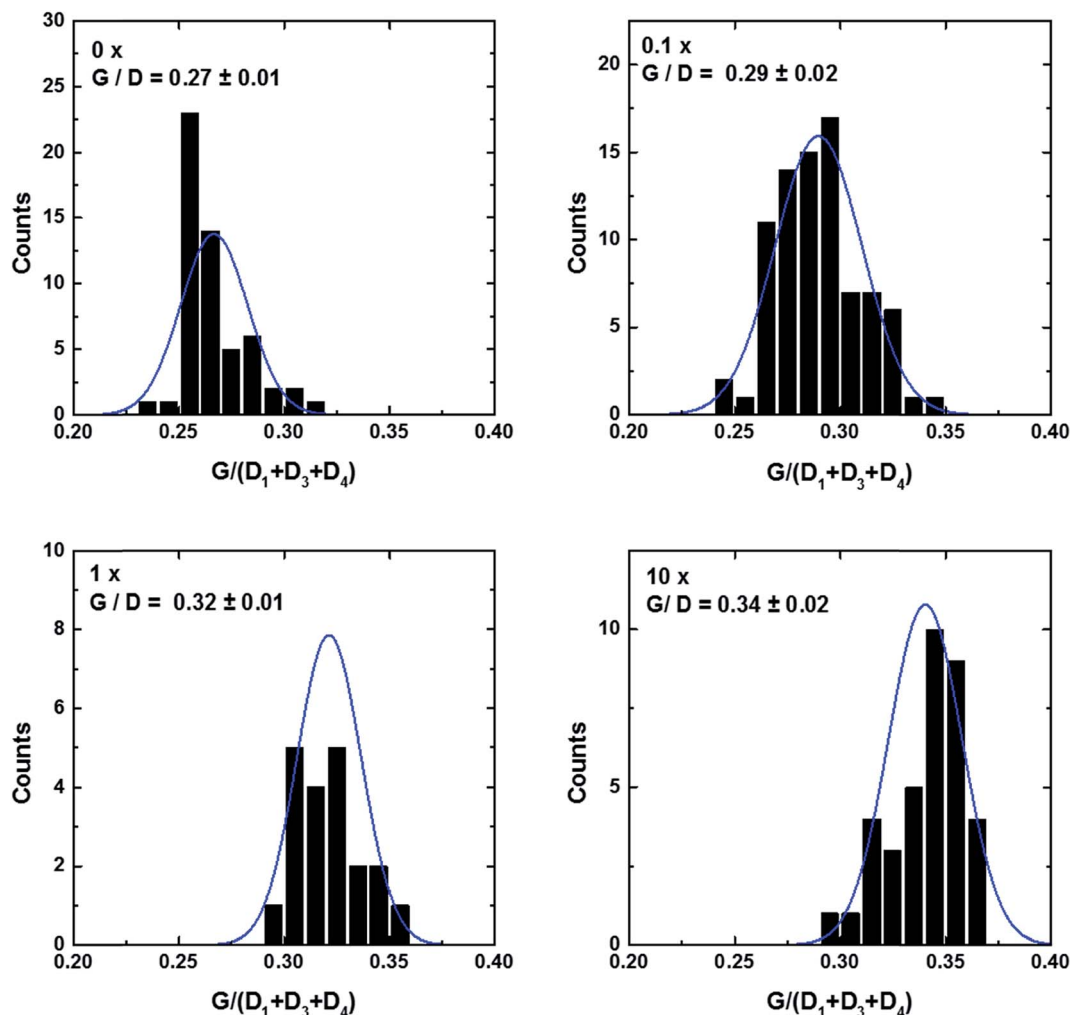


Fig. 4 Distribution of G to D ratios based on Raman spectra of the DEPs emitted from the four diesel fuels with varying concentration of Envirox™ (0×, 0.1×, 1×, 10×).

Table 2 Elemental analysis of the DEPs for the four diesel fuels with varying Envirox™ concentration

Fuel	Carbon (%)	Hydrogen (%)	Cerium (%)	Nitrogen (%)	Other (%)
0×	91.84	2.23	0.30	0.23	5.40
0.1×	91.81	2.12	0.48	0.25	5.34
1×	89.63	2.12	0.73	0.35	7.17
10×	79.40	2.43	6.52	0.61	11.04

were in between those for pure CeO<sub>2</sub> and 0× and they were not different from each other and from that of NIST 2975 (a bulk and aged DEP). In general, adding nanoceria fuel additives might decrease DEP toxicity through a reduction in its ability to generate ROS, but it does not seem to have a clear dose-dependent relationship.

To control the influence of CeO<sub>2</sub>, a single compound with a low oxidative potential, in the DEPs on the reduction of the oxidative potential of the entire sample, we normalized the

ascorbate depletion rates by DEP CeO<sub>2</sub> content. As shown in Fig. 6b, the normalization did not change the overall trend, but the slope for the 10× DEPs became steeper. This might be due to the significant increase of organic carbon content in the 10× group. On the other hand, the significant increase of particle crystallinity for the 10× group was reported by Ivleva *et al.* to have the potential to decrease DEP oxidative potential.<sup>27</sup> Therefore, more study should be conducted in order to understand the mechanisms of how these two parameters combine in influencing the overall oxidative potential of DEP as shown in Fig. 6a.

### 3.5. The role of soot oxidation

Ceria or nanoceria-based diesel catalysts are known to enhance soot oxidation,<sup>42</sup> which may explain some of our findings in the present study. Firstly, the soot oxidation may have facilitated the breakdown of larger particle agglomerates into smaller ones, leading to decreases in particle size.<sup>9,19–21</sup> Secondly, this oxidation process was further revealed by the Raman spectra showing the process of preferentially oxidizing the disordered



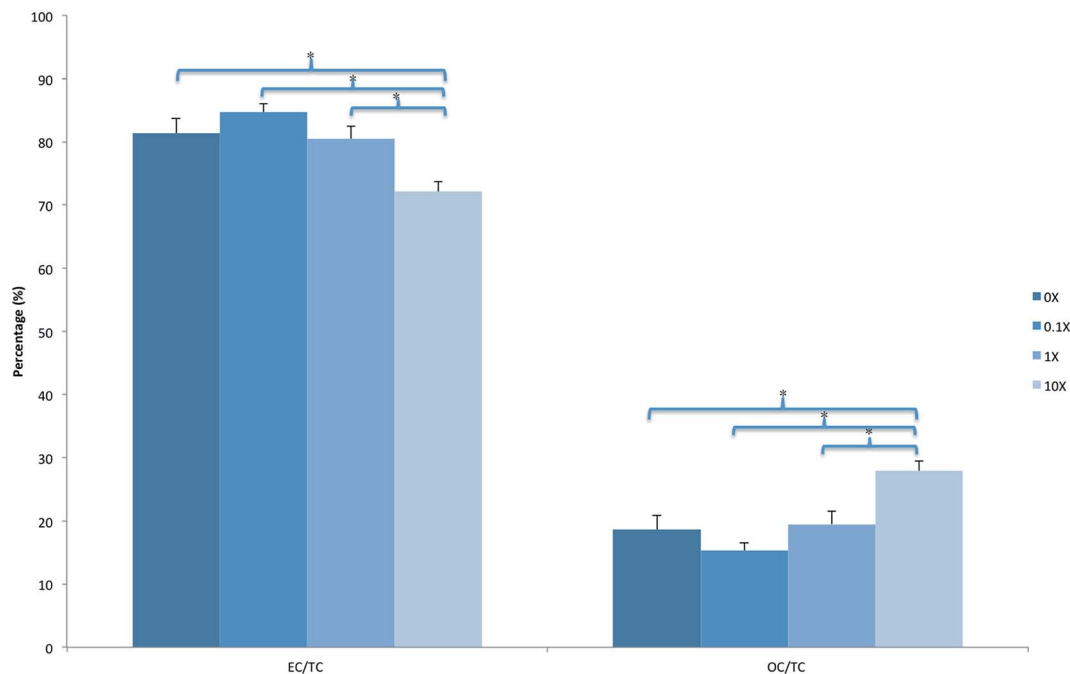


Fig. 5 The ratio of elemental carbon (EC) and organic carbon (OC) to total carbon (TC) of the DEPs emitted from the four diesel fuels with varying Envirox™ concentration (0x, 0.1x, 1x, and 10x). Asterisk (\*) means a significant difference ( $P < 0.05$ ), based on the two-tailed Student  $t$  test. Error bars are constructed based on one standard deviation.

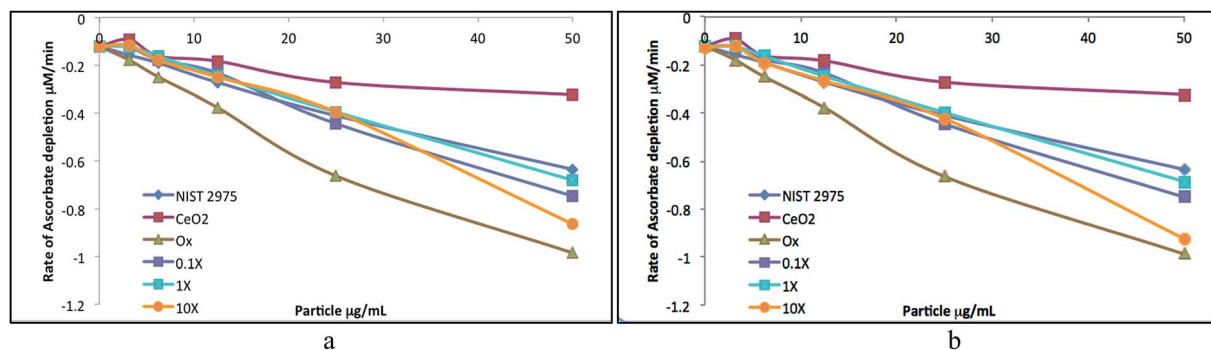


Fig. 6 a. Rate of ascorbic acid (AA) depletion by DEPs (0x, 0.1x, 1x and 10x) and two reference particles ( $\text{CeO}_2$  and NIST 2975). b. Rate of  $\text{CeO}_2$ -normalized AA depletion by DEPs and control particles. NIST 2975 are bulk-generated, aged diesel exhaust particles purchased from the National Institute of Standards and Technology (NIST), Standard Reference Materials #2975.

carbon structure rather than the ordered (graphitized) carbon structure. Thirdly, this oxidation may contribute to the increased organic carbon to elemental carbon ratio for the 10x Envirox™ conditions.<sup>27,39</sup> Finally, the soot oxidation may play a role in altering DEP surface charge and chemical composition (e.g., increasing the nitrogen content). Our experimental data from this study are clearly in support of these mechanisms related to soot oxidation, a fundamental catalytic function of nanoceria added to diesel fuel.

## 4. Conclusions

Using Envirox™ as a diesel additive resulted in changes in certain physicochemical properties of emitted diesel exhaust

particles (DEPs), including reduced particle size, decreased total carbon content (%), decreased disordered carbon structure, decreased elemental carbon fraction (%) in total carbon, increased cerium and nitrogen content, and reduced oxidative potential. These physicochemical properties might be further altered after the DEP is released into the environment,<sup>12</sup> which needs to be addressed in future studies. Given that these physicochemical properties are important determinants of particle toxicity, it is anticipated that using nanoceria additives to diesel fuel would alter the toxicity of the emitted DEPs in various ways.<sup>22,40,43</sup> For example, the increased organized structure and decreased oxidative potential might decrease the bio-reactivity of DEPs, while reduced particle size may increase the diffusion rate of particles within and across the lung and



decrease the clearance rate in the lung, thereby increasing the overall bioreactivity and toxicity.<sup>27,44</sup> Hence, the impact on the overall DEP bioreactivity and toxicity is hard to predict solely based on physicochemical property changes observed in the present study. Our findings, nonetheless, can help future toxicological studies explain the impact of nanoceria additives on DEP toxicity and help link specific physicochemical properties and biological responses.

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