STUDIES ON THE TOXICITY, BIOACCUMULATION, AND BIOPERSISTENCE OF MODEL AND COMMERCIAL CHEMICAL MECHANICAL PLANARIZATION SLURRIES WITH *DAPHNIA MAGNA*

by

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To my family,

For being there every step of the way.

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by

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DISSERTATION

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The University of Texas at Dallas, 2018

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Nanoparticle (NP) abrasives are components of chemical mechanical planarization (CMP) slurries used to polish wafers of semiconducting materials such as silicon. The semiconductor industry is interested in understanding the potential environmental toxicity associated with CMP NPs because the NPs may be discharged to aquatic ecosystems. However, assessing the toxicity of these specialized CMP NPs is challenging because commercial slurries may contain undefined toxic constituents. In response to this challenge, a manufacturer produced four model CMP slurries comprising colloidal or fumed silicon oxide (*c*-SiO_2 or *f*-SiO_2), cerium oxide (CeO_2), or aluminum oxide (Al_2O_3) NPs without known soluble toxicants to permit toxicity assessments of actual NPs used in commercial CMP slurries. This dissertation presents, for the first time, studies on the acute and chronic toxicities, the bioaccumulation, and the biopersistence of model CMP NPs with *Daphnia magna* (*D. magna*) – a fresh-water organism used in ecotoxicity assessments. The major findings presented in chapter two are that different model CMP slurries exert distinct effects on *D. magna* morbidity, growth, and reproductive output, and that the CeO_2 and Al_2O_3 CMP slurries

severely reduced *D. magna* reproduction upon chronic exposure at sub-lethal applied doses, which could have adverse consequences to aquatic ecosystems. The major findings presented in chapter two are that different model CMP slurries exert distinct effects on *D. magna* morbidity, growth, and reproductive output, and that the CeO_2 and Al_2O_3 CMP slurries severely reduced *D. magna* reproduction upon chronic exposure at sub-lethal applied doses, which could have adverse consequences to aquatic ecosystems. The major findings in chapter three are that CeO_2 and Al_2O_3 NPs were accumulated in different amounts by *D. magna*, and that after 48 h of depuration, *D. magna* exposed to 0.10 mg/mL CeO_2 eliminated 85% of the CeO_2 load, and *D. magna* exposed to 0.10 mg/mL of Al_2O_3 eliminated 78% of the Al_2O_3 load. The fourth and the final chapter of this dissertation addresses the question of whether polishing a gallium arsenide (GaAs) wafer can impart added toxicity to CMP NPs. Using a commercial *c*-SiO_2 CMP slurry (Ultra-Sol® 200S), the key findings were that neither the pristine or spent slurry at 0.10 mg/mL *c*-SiO_2 NPs was toxic after a 21-day exposure, but both slurries led to a slight increase in body size and a ~2-fold increase in reproduction, indicative of a hormetic response. Further testing revealed that the effector was a soluble component(s) in the pristine slurry, and not the pristine or spent NPs.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The Semiconductor Research Corporation (SRC) is a non-profit research consortium based in North Carolina that was founded in 1982. Its mission is to fund university projects to generate educated technical talent by addressing the research needs of member companies such as Texas Instruments, IBM, Global Foundries, and Intel. One of their research programs is the Engineering Research Center (ERC) for Environmentally Benign Semiconductor Manufacturing (EBSM) that was founded in 1996. One arm of the ERC-EBSM is a nanotoxicity consortium comprising industrial liaisons from member companies and academic researchers from the University of Arizona, Arizona State University, the University of California at Los Angeles, North Carolina A&T University, and The University of Texas at Dallas (UT Dallas). For the past four years, the main focus of the nanotoxicity consortium has been the assessment of the potential environmental health and safety (EHS) risks of nanoparticle (NP) abrasives used in chemical mechanical planarization (CMP) slurries.

1.2 THE CMP PROCESS

The process of fabrication of integrated circuits used in modern microprocessors and memory chips requires hundreds of complex steps whereby materials are either deposited or removed from a wafer of semiconducting material such as silicon.[1] One of these steps whereby a surface is smoothed to a high tolerance is achieved by CMP. Developed in the 1980s by Klaus D. Beyer at IBM, CMP is a unique process that is a combination of surface reactions with chemical additives

and mechanical polishing with nanoscale abrasives.[2-5] Figure 1.1 shows the CMP tool of our collaborators at North Carolina A&T University. In the CMP process, the slurry is introduced between the rotating polishing pad and a wafer mounted on a carrier (Figure 1.2). The wafer is then pressed on the pad by applying a small pressure between 1–10 psi. The rotating motion of the pad and wafer carrier allows excess material to be removed from the wafer. The slurries used in CMP processes comprise abrasive NPs such as colloidal or fumed silicon oxide (*c*-SiO_2 or *f*-SiO_2), cerium oxide (CeO_2), or aluminum oxide (Al_2O_3), and the chemicals include acids or bases, oxidizers, dispersants, antimicrobials, complexing agents, and corrosion inhibitors (Table 1.1).[6, 7]

Figure 1.1. Image of the IPEC Avanti 472 chemical mechanical planarization (CMP) tool housed at the Joint School of Nanoscience and Nanoengineering–North Carolina A&T University.

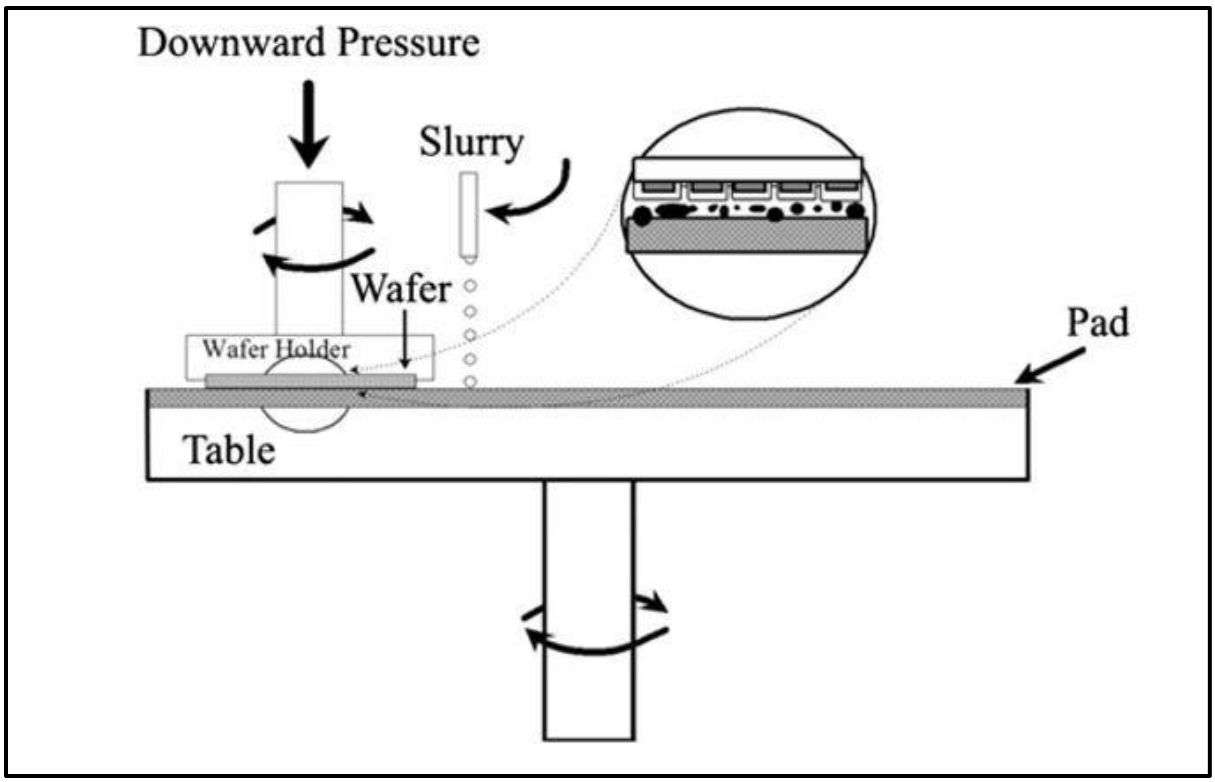

Figure 1.2. Schematic diagram of the key elements of a chemical mechanical planarization tool including a wafer, a rotating polishing pad, a pressurized and rotating wafer holder, and slurry introduction system;[3] reproduced with permission from the *Journal of Colloid and Interface Science* / Elsevier.

The CMP slurry serves multiple purposes: i) it provides lubrication to reduce friction between the pad and the wafer; ii) it acts to dissipate heat generated during the CMP process; iii) it provides the medium for moving reactants and particles to the wafer interface and removing the by-products generated during the CMP process away from the wafer; and most significantly, iv) the NP abrasives in the slurry assist in planarizing the wafer.[2] An example of a CMP process is shown in Figure 1.3 where excess silicon oxide was removed to expose a silicon nitride layer.

Table 1.1. List of typical components in commercial chemical mechanical planarization slurries;[6] reproduced with permission from *Environmental Science: Nano* / Royal Society of Chemistry.

Component	Function	Examples
Abrasive particles	Polish surface	Al_2O_3, CeO_2, amorphous SiO_2
pH adjust	Adjust and buffer pH	HCl, KOH, HNO_3, NH_4OH, H_3PO_4, TMAH, NH_4OH, buffers
Complexing agents	Solubilize dissolved metals	Amino acids (glycine, etc.), carboxylic acids (citric acid, etc.)
Oxidizers	Promote metal removal via oxidative dissolution	H_2O_2, ferric nitrate, KIO_4, $KMnO_4$, etc.
Corrosion inhibitors	Selectivity against removal of certain surfaces, corrosion inhibition	Benzotriazole (BTA), 3-amino-triazole
Surface active organics	Maintain metal oxide particles in a dispersed state	Polyacrylic acid, polyethylene glycol polymer, cetyl trimethyl ammonium bromide, polyethylene cetyl ether
High molecular-weight polymers	Flocculant and/or coat abrasives to "cushion" their abrasiveness	High molecular-weight polyethylene oxides
Biocides	Prevent biological growth	Hydrogen peroxide and others

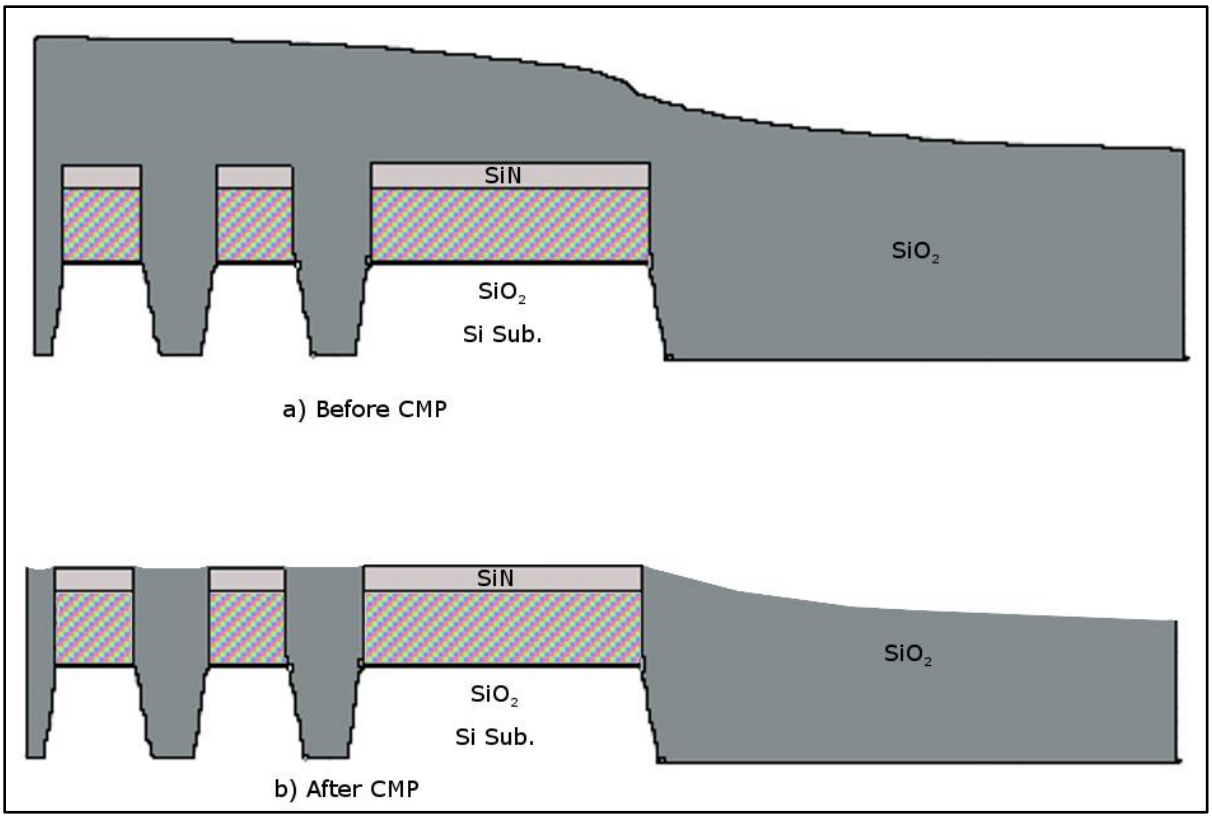

Figure 1.3. Schematic diagram of a chemical mechanical planarization process to expose the SiN layer below the SiO_2 layer;[56] reproduced with permission from *Environmental Science: Nano* / Royal Society of Chemistry.

1.3 CMP WASTE STREAMS

Semiconductor manufacturing sites, or fabs, employ a fleet of CMP tools similar to the one shown in Figure 1.1. A typical wafer requires 0.2–0.8 L of CMP slurry and ~7 L of ultra-pure deionized water for rinsing the wafer and the polishing pad.[7] Tremendous volumes of water are used to clean wafers and the amount of water used in CMP processes can account for 30–40% of the total water used by a single fab.[7, 8] The effluent wastewater from a CMP process can contain particulate matter that is washed off the wafer, slurry components, and any residual material associated with the polishing pad or the wafer.[7] Before this water is returned to a publically-owned

treatment works (POTW), it will be subjected to a number of treatment steps depending on the fab and the municipality it is under (Figure 1.4).[7, 9] While the concentrations of CMP NPs are diluted many-fold in the waste effluent, and while wastewater treatments involving activated sludge can remove as much as 97–99% of metal oxide NPs,[10] it is still possible for CMP NPs to be discharged to a municipal water supply and potentially into an aquatic environment. The semiconductor industry is therefore interested to understand the potential environmental toxicity associated with CMP NPs.

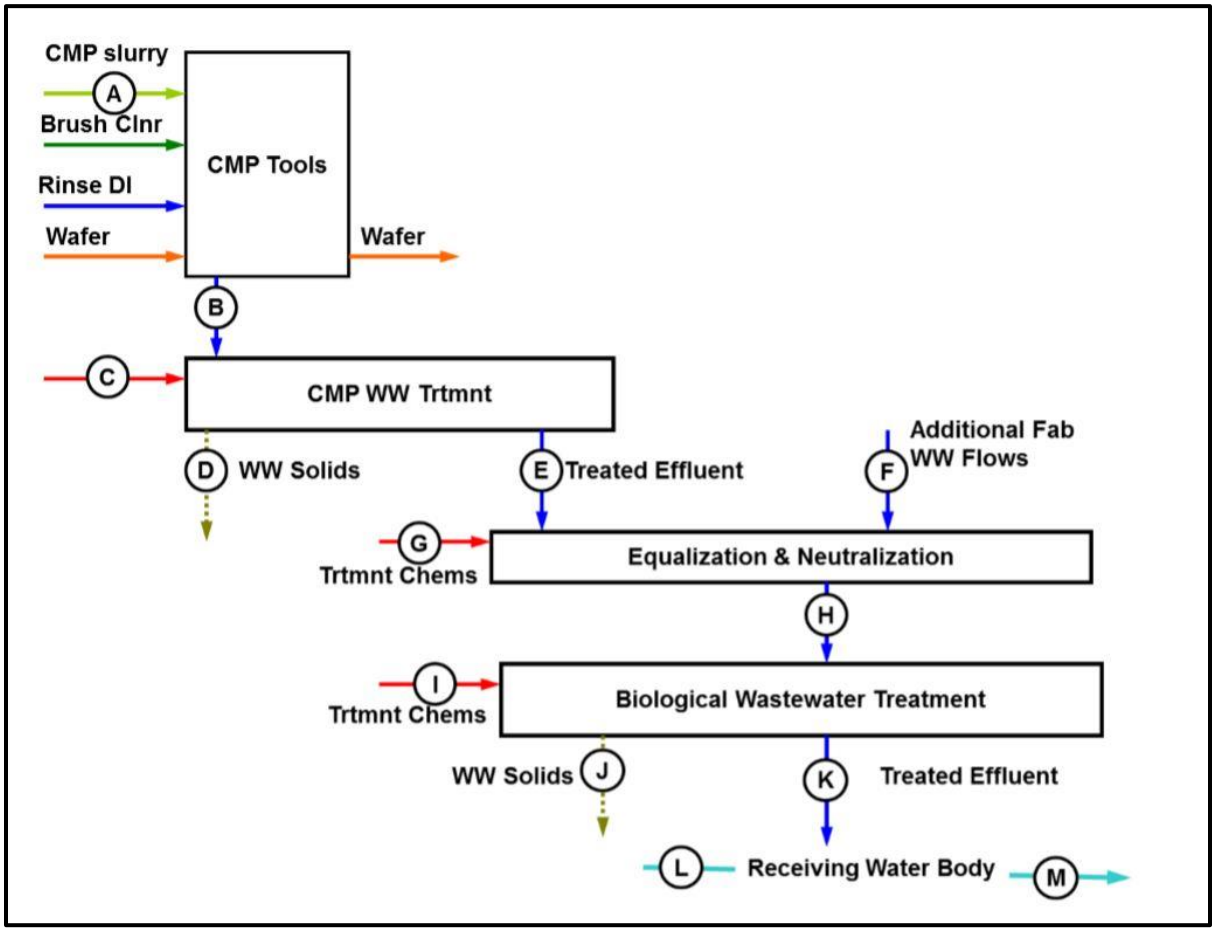

Figure 1.4. Schematic diagram of a chemical mechanical planarization waste stream and the treatments performed before the reclaimed water is delivered to a receiving body;[7] reproduced with permission from *Advances in Chemical Mechanical Planarization* / Elsevier Books.

1.4 MODEL CMP SLURRIES

Assessing the toxicity of CMP NPs is a complex endeavor because subtle differences in metal oxide NP surface reactivity, size, and shape can affect toxicity,[11-22] and because CMP NP assessments are confounded by soluble slurry constituents that are known to be toxic such as oxidants, surfactants, biocides, and corrosion inhibitors. As a result, while a growing number of occupational assessments of commercial CMP slurries inside fabs have been reported,[5, 9, 23, 24] an ecotoxicity assessment of CMP slurries has not been reported. In response to this challenge, a major CMP slurry manufacturer produced four model CMP slurries comprising *c*-SiO_2, *f*-SiO_2, CeO_2, or Al_2O_3 NPs that did not contain proprietary chemicals or known soluble toxic additives to permit toxicity assessments of actual NPs used in commercial CMP slurry formulations. The physical and chemical properties of the four model CMP slurries provided by the manufacturer are shown in Table 1.2. They were verified independently by a thorough physicochemical characterization reported by members of the ERC-EBSM nanotoxicity consortium,[6] using multiple complementary analytical techniques as recommended by the Nano Grand Opportunity consortium of the National Institute of Environmental Health and Sciences.[25]

Table 1.2. Summary of key characteristics of model colloidal silica, fumed silica, ceria and alumina chemical mechanical planarization slurries;[6] reproduced with permission from *Environmental Science: Nano* / Royal Society of Chemistry.

	***c*-SiO_2**	***f*-SiO_2**	**CeO_2**	**Al_2O_3**
<u>Manufacturer Reported</u> Material Composition Additive pH Particle size (nm)	 Colloidal SiO_2 3% SiO_2 <1% acetic acid 2.5 – 4.5 50-60	 Fumed SiO_2 5% SiO_2 <1% KOH 10 120-140	 CeO_2 1% CeO_2 none 3-4 60-100	 Al_2O_3 3% Al_2O_3 <1% nitric acid 4.5-5.0 80-100
<u>Consortium Reported</u> concentration of metal	27 g Si/L	50 g Si/L	9.6 g Ce/L	29 g Al/L
Dissolved organic carbon (DOC; mg/L)	320.5 ± 0.5	4.84 ± 0.03	1.90 ± 0.03	6.77 ± 0.18
Other additives	801.9 ± 1.3 mg acetic acid/L	--	--	134.7 ± 0.8 mg NO_3^-/L
Diameter by SEM (nm)	37 ± 7	38 ± 14	43 ± 16	85 ± 21
Diameter by TEM (nm)	36 ± 9	not detected	39 ± 19	38 ± 16
Diameter by DLS (nm) (Polydispersity Index)	46 ± 0.2 (0.08)	148 ± 5.1 (0.11)	132 ± 0.1 (0.16)	129 ± 1.6 (0.11)
Diameter by NTA (nm)	61 ± 0.9	144 ± 1.8	79 ± 1.3	119 ± 1.1
Single-particle ICP-MS (nm)	not detected	144 ± 26	60 ± 28	66 ± 23
Zeta potential at slurry pH (mV)	-21	-50	43	55

1.5 SILICA (SiO_2) NPS

The element silicon (Si) is a metalloid and a semiconductor. Silicon is the eighth most abundant element in the universe.[26] Figure 1.5 shows the crystal structure of silica, where one atom of Si forms a stable tetrahedron with four oxygen atoms. However, this structure (SiO_4) results in an excess charge of -4. .Therefore, to balance the charge, oxygens are shared by the adjacent tetrahedra to reduce the charge difference. When a silicon tetrahedra shares all four oxygens, the charge is balanced and the chemical formula becomes SiO_2. The molecular weight of amorphous silica is 60.08 g/mol and its density is ~2.65 g/cm^3. The isoelectronic point of SiO_2 is between pH 2–3.[24, 27, 28]

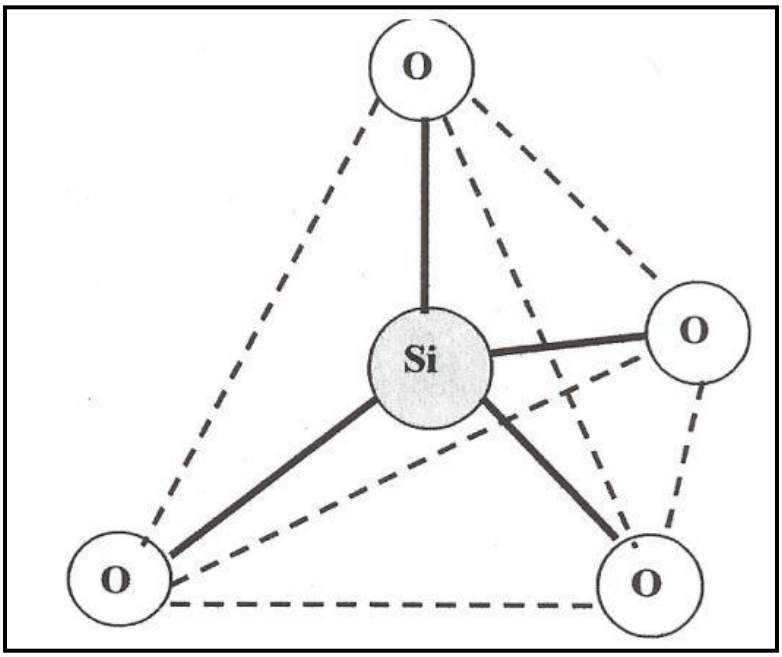

Figure 1.5. Crystal structure of SiO_2;[57] reproduced with permission from the *American Journal of Materials Engineering and Technology* / Science and Education Publishing.

Silica can be crystalline, amorphous, or synthetic amorphous. The SiO_2 NPs used in the CMP process include colloidal silica (*c*-SiO_2) and fumed silica (*f*-SiO_2), which are both synthetic

amorphous materials. Colloidal SiO_2 is a stable dispersion of primary NPs in liquid, typically water. The synthesis of *c*-SiO_2 particles is shown in Figure 1.6. It is synthesized by the hydrolysis of $SiCl_4$ monomers in aqueous solution, followed by condensation of the initial SiO_2 particles. On the other hand, *f*-SiO_2 is produced by pyrolysis of silicon tetrachloride ($SiCl_4$) at high temperatures on the order of 1800 °C as shown in Figure 1.7.[29, 30]

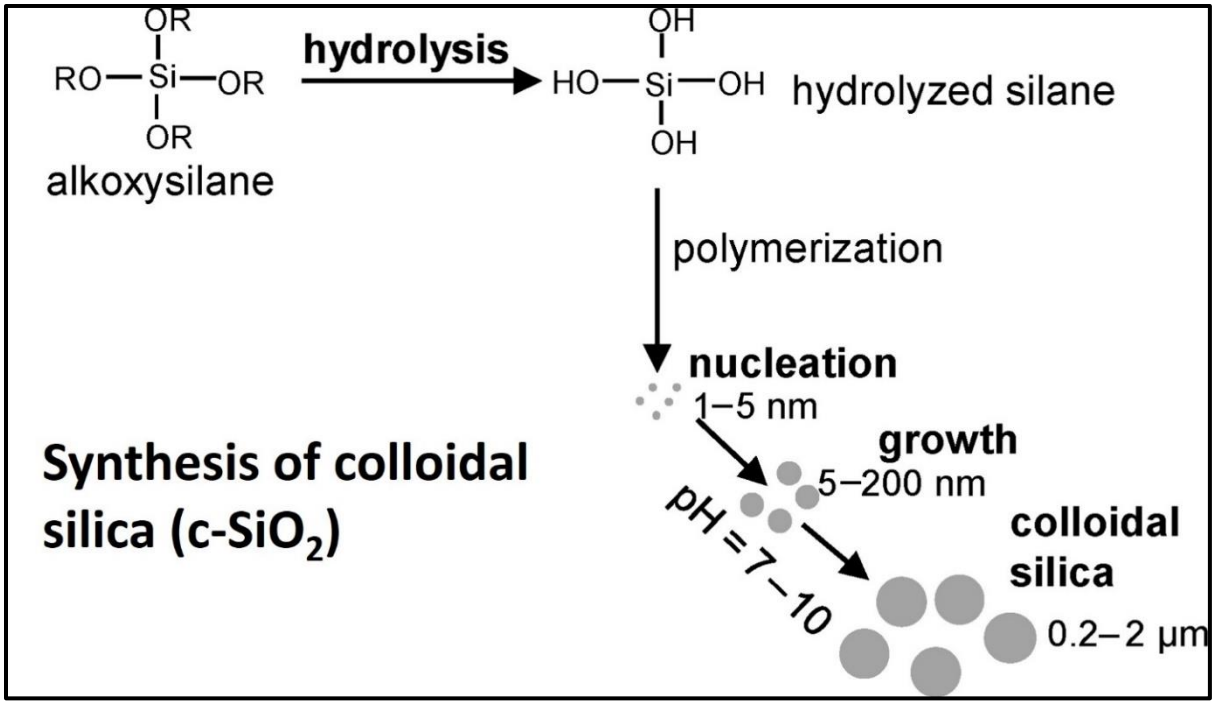

Figure 1.6. Overview of the synthesis of colloidal silica (*c*-SiO_2) particles;[58] reproduced with permission from Wiley Books.

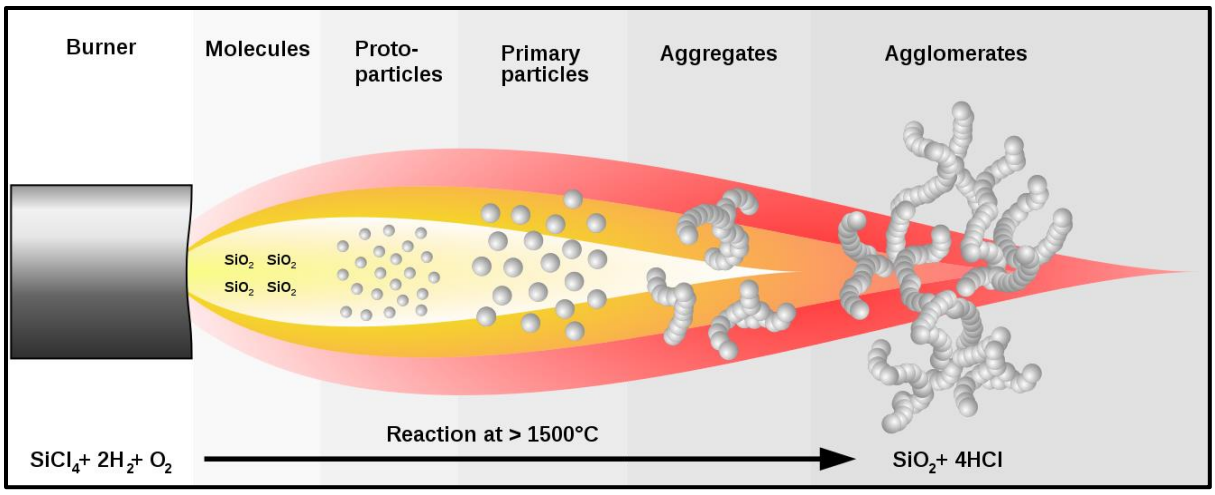

Figure 1.7. Overview of the synthesis of fumed or fused silica (*f*-SiO_2) particles;[59] reproduced with permission from Wikimedia Commons Free Media Repository.

The model *c*-SiO_2 CMP NP slurry used in this thesis was acidic (pH 2.5–4.5) with acetic acid being used as the pH-adjusting agent; it was made to a concentration of 30 mg/mL of *c*-SiO_2 NPs (Table 1.2). A representative transmission electron microscopy (TEM) image of a *c*-SiO_2 CMP NP shows that they were spherical with a size of 50–60 nm (Figure 1.8A). The model *f*-SiO_2 CMP slurry used in this thesis was basic (pH 10) with KOH being used as the pH-adjusting agent; it was made to a concentration of 50 mg/mL of *f*-SiO_2 NPs (Table 1.2). The TEM image of *f*-SiO_2 CMP NPs shows that the NPs were irregularly shaped with sizes of 120–140 nm (Figure 1.8B).

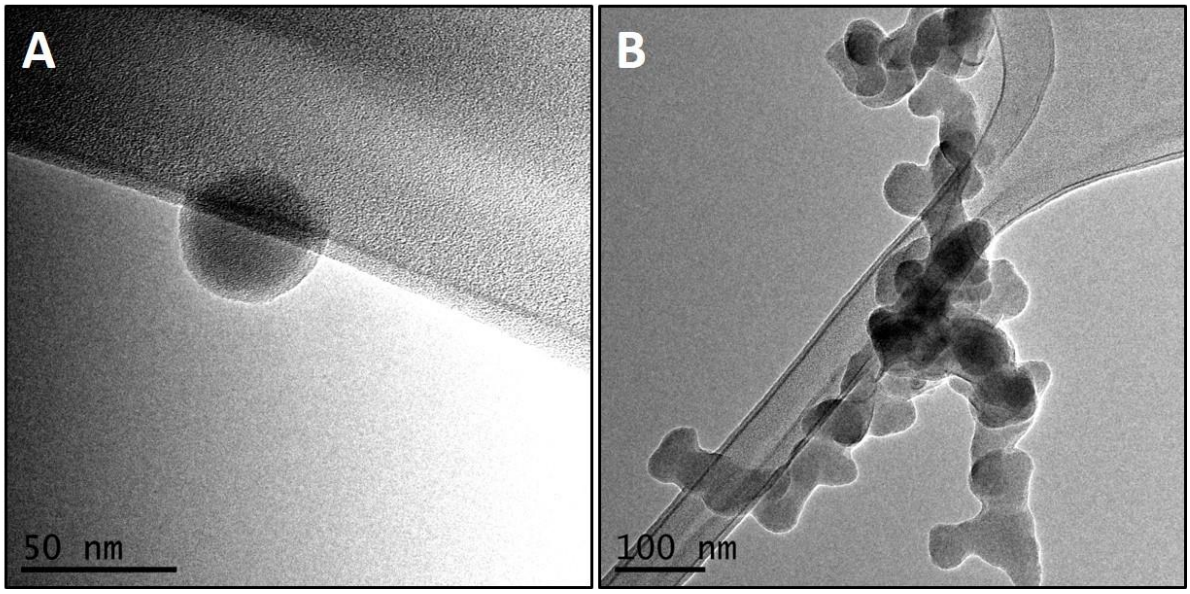

Figure 1.8. Transmission electron micrograph of **(A)** *c*-SiO_2 NP from the model *c*-SiO_2 chemical mechanical planarization slurry;[6] **(B)** *f*-SiO_2 from the model *f*-SiO_2 chemical mechanical planarization slurry [6] reproduced with permission from Environmental Science: Nano / Royal Society of Chemistry.

1.6 CERIA (CeO_2) NPS

Cerium (Ce) is a rare earth metal found in the lanthanide group of the periodic table. Cerium is the most abundant of the rare earth metals, constituting ~0.005 wt% of the earth's crust.[7] Figure 1.9 shows the crystalline structure of face-centered cubic (FCC) CeO_2. It consists of cubic oxygen in the sub-lattice and the cerium ions occupying alternate cube centers. In CeO_2, oxygen anions are tetrahedrally-coordinated to Ce cations and octahedrally-coordinated to surrounding oxygen atoms.[31],[32]

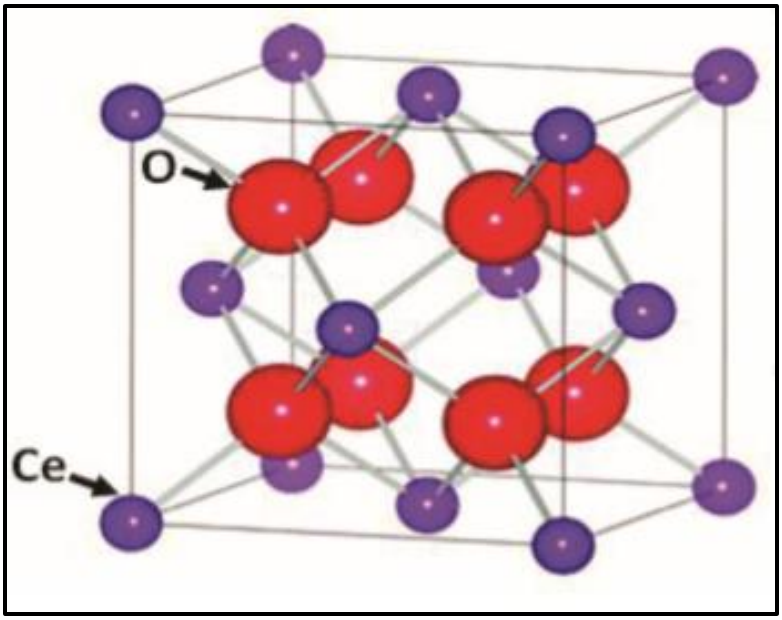

Figure 1.9. Crystal structure of CeO_2;[31] reproduced with permission from InTech Open Access Publishers.

The molecular weight of ceria is 172.115 g/mol and its density is 7.65 g/cm^3. Ceria reaches its isoelectronic point at pH 7.[7, 33] Typically, CeO_2 NPs used in CMP processes are poly–crystalline with a cubic crystal structure.[2] CeO_2 can be synthesized by a number of different precipitation reactions; for example, cerium nitrate salts can be oxidized in a strong base to produce hydrous cerium oxide which precipitates out of solution. The following chemical equations show the synthesis of cerium oxide.[2]

$$Ce^{+3} + 3\ OH^- + (1+x)\ H_2O \rightarrow Ce(OH)_4\ x(H_2O) + \tfrac{1}{2}\ H_2 \quad (1)$$

$$Ce(OH)_4\ x(H_2O) \rightarrow CeO_2 + (x+2)\ H_2O \quad (2)$$

The model CeO_2 CMP slurry used in this thesis was acidic (pH 3–4); it was made to a concentration of 10 mg/mL of CeO_2 NPs (Table 1.2). The TEM image of CeO_2 CMP NPs shows that they were rectangular and irregularly-shaped NPs with sizes of 60–100 nm (Figure 1.10).

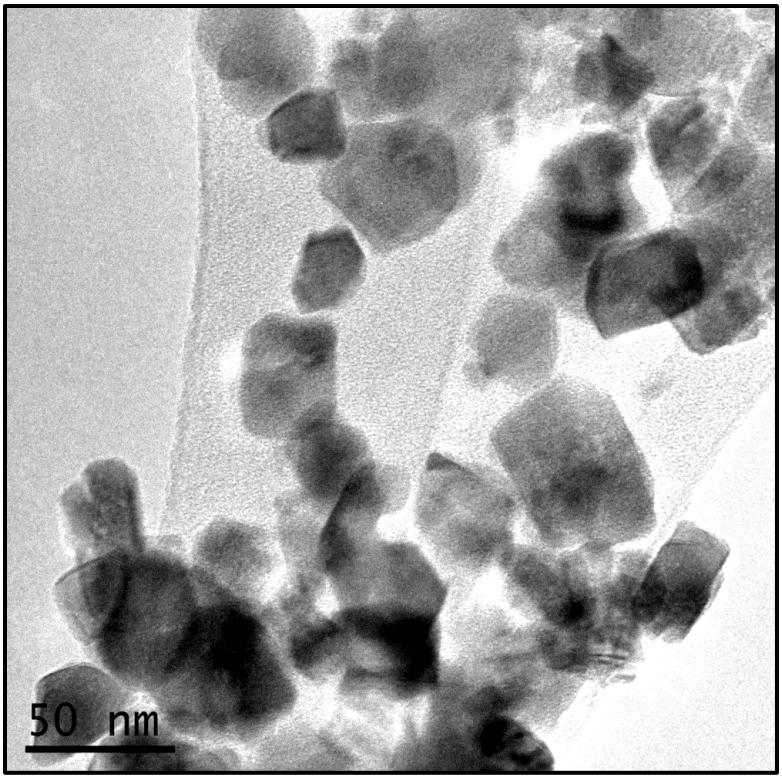

Figure 1.10. Transmission electron micrograph of CeO_2 Nano particles from the model CeO_2 chemical mechanical planarization slurry;[6] reproduced with permission from *Environmental Science: Nano* / Royal Society of Chemistry.

1.7 ALUMINA (Al_2O_3) NPS

Aluminum (Al) is a post-transitional metal and the most abundant metal of the Earth's crust. Alumina is one of the hardest materials on the Moh's hardness scale and has several forms of crystalline structures (α, χ, η, δ, κ, θ, γ, ρ).[2] Except for α-Al_2O_3, the other forms are metastable. These forms are produced by applying heat to aluminum hydroxides or aluminum salts.[34] The crystalline structure of α-Al_2O_3 consists of closely packed planes of large oxygen anions stacked in the sequence shown in Figure 1.11. The Al cation has a charge of +3 and O has a -2 charge. Therefore, only two Al^{3+} ions are required for every three O^{2-} ions to achieve electrical neutrality.

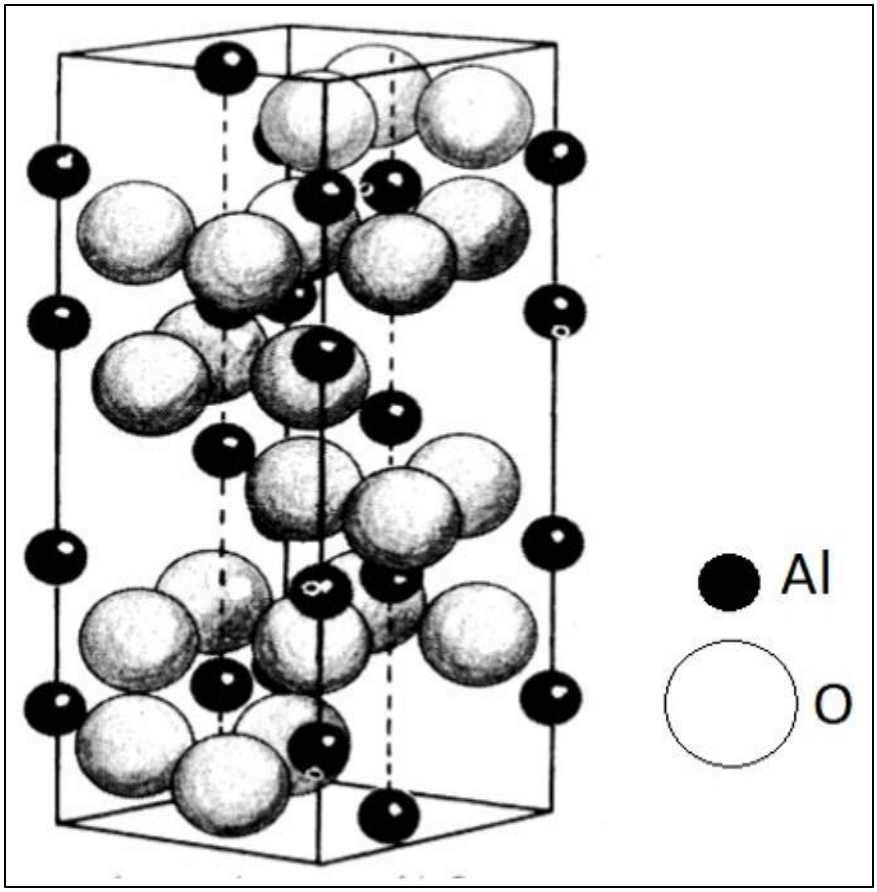

Figure 1.11. Crystal structure of α-Al_2O_3;[34] reproduced with permission from the *Spectrochimica Acta Part B: Atomic Spectroscopy* / Elsevier.

The molecular weight and density of Al_2O_3 are 101.96 g/mol and 3.95 g/cm^3, respectively. The isoelectronic point of Al_2O_3 occurs around pH 9.[7, 33] The Al_2O_3 NPs used in CMP processes are produced by dehydration of boehmite (AlOOH) at high temperatures (500–700 °C).[30] Usually, the structure of Al_2O_3 NPs used in CMP processes are poly-crystalline with an orthorhombic crystal structure.[2] The model Al_2O_3 CMP slurry used in this thesis was acidic (pH 4.5–5.0) with nitric acid being used as the pH-adjusting agent; it was made to a concentration of 30 mg/mL of Al_2O_3 NPs (Table 1.2). The TEM image of Al_2O_3 NPs shows that the particles were rectangular and irregularly-shaped with sizes of 80–100 nm (Figure 1.12).

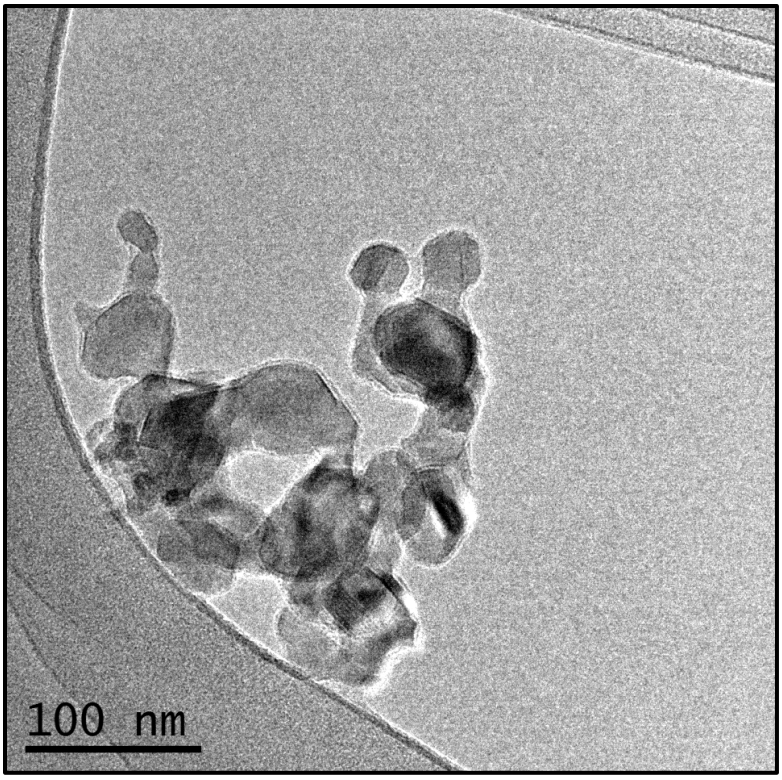

Figure 1.12. Transmission electron micrograph of Al_2O_3 nanoparticles from the model Al_2O_3 chemical mechanical planarization slurry;[6] reproduced with permission from *Environmental Science: Nano* / Royal Society of Chemistry.

1.8 UT DALLAS PROJECT AIMS

The first publication of the ERC-EBSM nanotoxicity consortium contained *in vitro* toxicity assessments of the four model CMP slurries using one marine bacterium, *Aliivibrio fischeri*, and one mammalian cell line, adenocarcinomic human alveolar basal epithelial A549 cells;[6] it did not contain any toxicity assessments using organisms. Accordingly, in January of 2015, the UT Dallas Bionanosciences Group was awarded a three-year grant from the ERC-EBSM to study the toxicity, bioaccumulation, and biopersistence of the four model CMP slurries using RAW 264.7 mouse macrophage cells, the fresh water flea, *Daphnia magna (D. magna)*, and the unicellular protozoa *Tetrahymena pyriformis* (Figure 1.13). The mammalian macrophage cells were chosen because

they are the primary responders to foreign particles that initiate inflammatory responses that can lead to health problems. The fresh-water fleas were chosen because they are an established organism recommended by Environmental Protection Agency (EPA) for ecotoxicity testing. The ciliated protozoa were chosen because they are fast-growing unicellular organisms that reside in natural waters. When I joined the Bionanosciences Group in 2015, my first specific task was to develop and optimize methods for culturing *D. magna*.

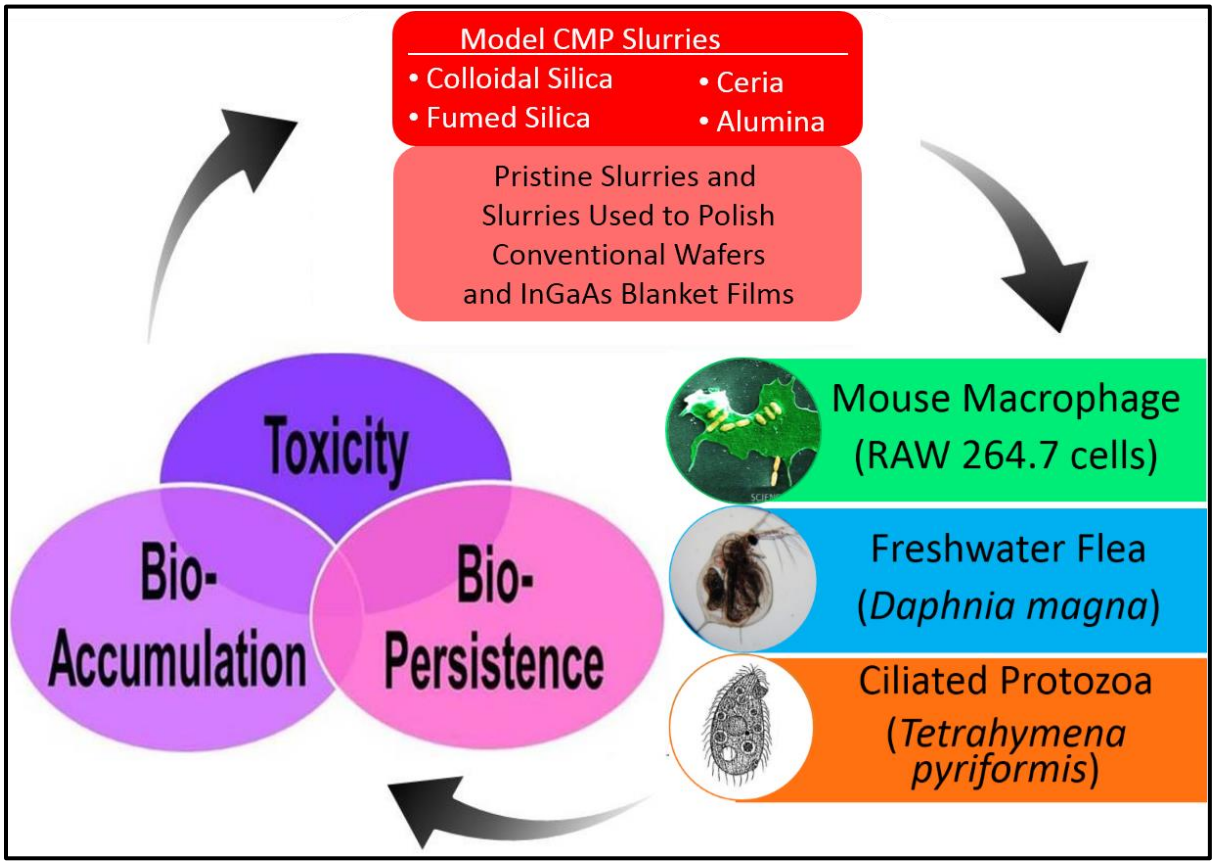

Figure 1.13. Overview of UT Dallas project aims as part of the assessment of the potential environmental health and safety risks of nanoparticle abrasives used in chemical mechanical planarization slurries.

1.9 GENERAL ECOLOGY OF *DAPHNIA*

Daphnia are planktonic crustaceans that belong in the group of Phyllopoda (also known as Branchiopoda). The genus of *Daphnia* consists of more than 100 species of fresh-water fleas. Some of them include *Daphnia pulex*, *Daphnia longispina*, and *D. magna*. In this thesis, *D. magna* were chosen because they are widely used in ecotoxicity studies and they are bigger compared to other *Daphnia* species. Figure 1.14 shows a representative image of an adult *D. magna*. *D. magna* have body sizes that range from 0.5 mm up to 6 mm. Figure 1.15 depicts the detail physiology of *Daphnia*.

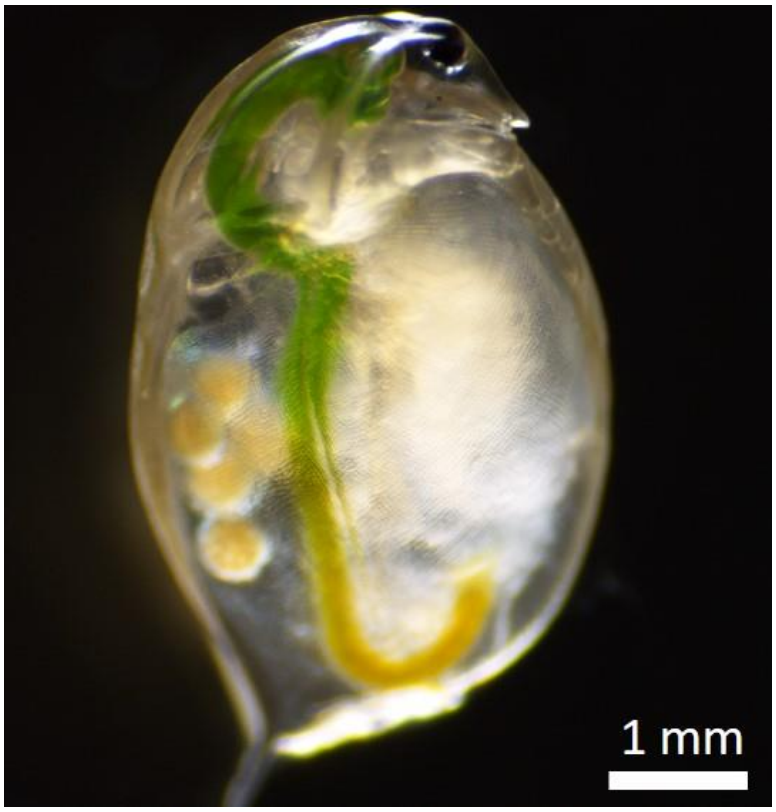

Figure 1.14. Representative optical image (3× magnification) of a daphnid where the scale bar represents 1 mm.

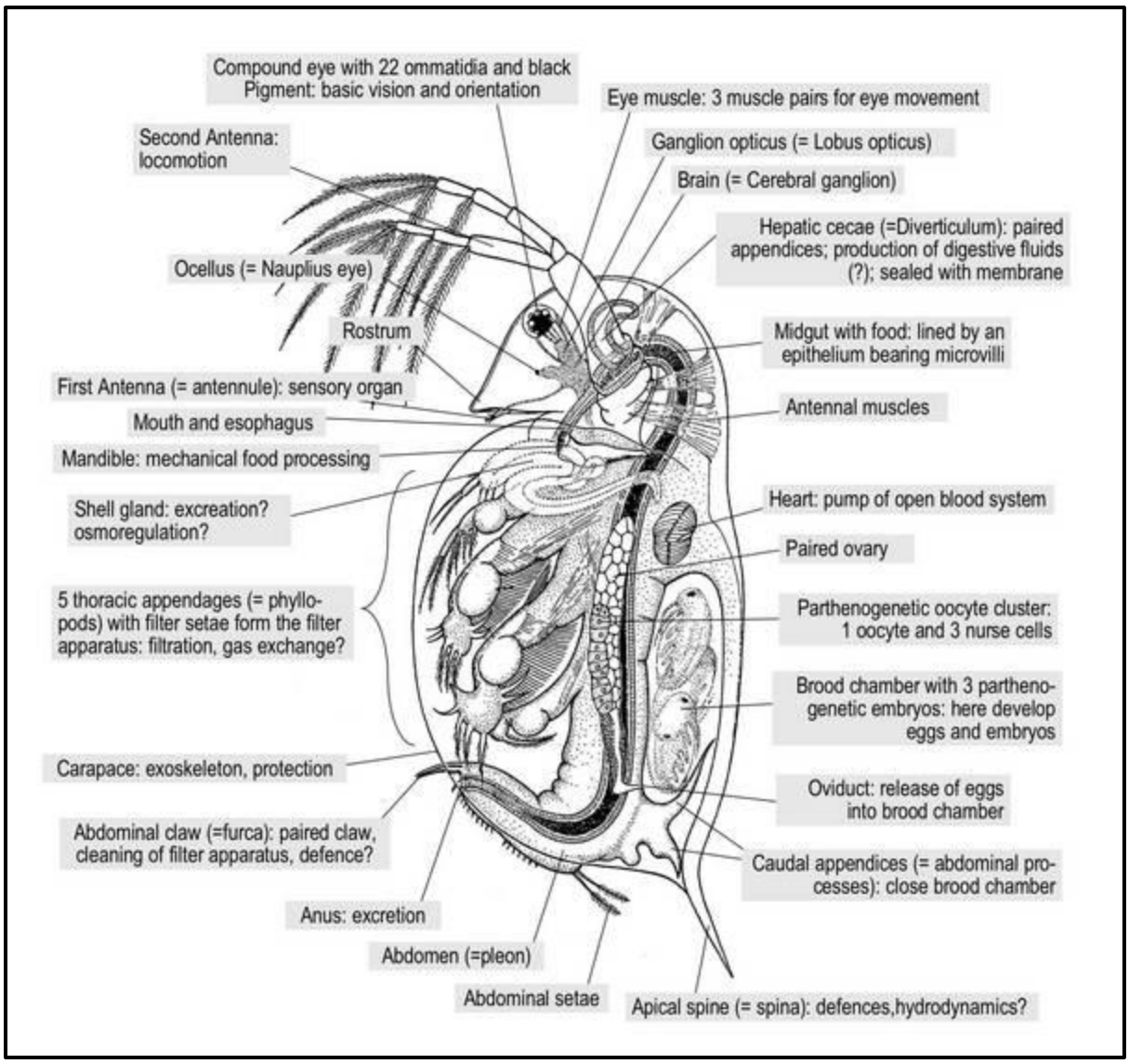

Figure 1.15. The physiology of *Daphnia*;[36] reproduced with permission from the Zoologisches Institut, Universität Basel.

They have flattened and leaf-like legs that are used for swimming and their bodies are made of an un-calcified shell called a carapace. The carapace comprises the polysaccharide, chitin. *D. magna* also have antennae that help with swimming and navigation and leaf-like appendages that help in filtering water and bringing food in with water currents. The size range of particles filtered by *D.*

magna is typically 0.6–40 μm but they can ingest particles up to 70 μm.[35, 36] Algae is the best source of nutrition for *D. magna*, however, they also consume other unicellular organisms such as bacteria and *Tetrahymena*.

D. magna are found in most types of fresh-water bodies with a hardness exceeding 150 mg/L (as $CaCO_3$).[37] In western and northern America, *D. magna* populations are sparse during winter and spring, but when water temperatures are around 6–12 °C their population densities can reach 500 individuals/L. During summer the populations decline to low numbers and in autumn the population rises again.[37, 38] During most of the year, the population of *D. magna* predominantly consists of females. Under stressful conditions, when there is a shortage of food or rapid temperature changes, males appear in the population. Males are easily distinguished from females because they are smaller, have a modified post abdomen, and distinct bulging eyes.[38]

The life spans of *D. magna* depend on several factors such as availability of food, environmental temperature, and water quality. *D. magna* can survive up to 40 days at 25 °C and for 56 days at 20 °C.[38] Under optimal lab conditions, *D. magna* can live for 2 months.[36] Figure 1.16 shows the typical life cycle of *D. magna*. It is one of the few organisms that can reproduce both sexually and asexually. During the growth season, females produce a clutch of parthenogenetic eggs that are deposited in the brood chamber. When environmental conditions are optimal, *D. magna* reproduce asexually and the neonates born are predominantly females. However, under harsher conditions, for example, over population, food shortage, poor quality of water, or extreme temperatures, a different type of egg develops in the brood chamber called a resting egg. These are encapsulated in a protective covering and are called ephippium. These resting eggs are fertilized by male *D. magna* to continue the life cycle.

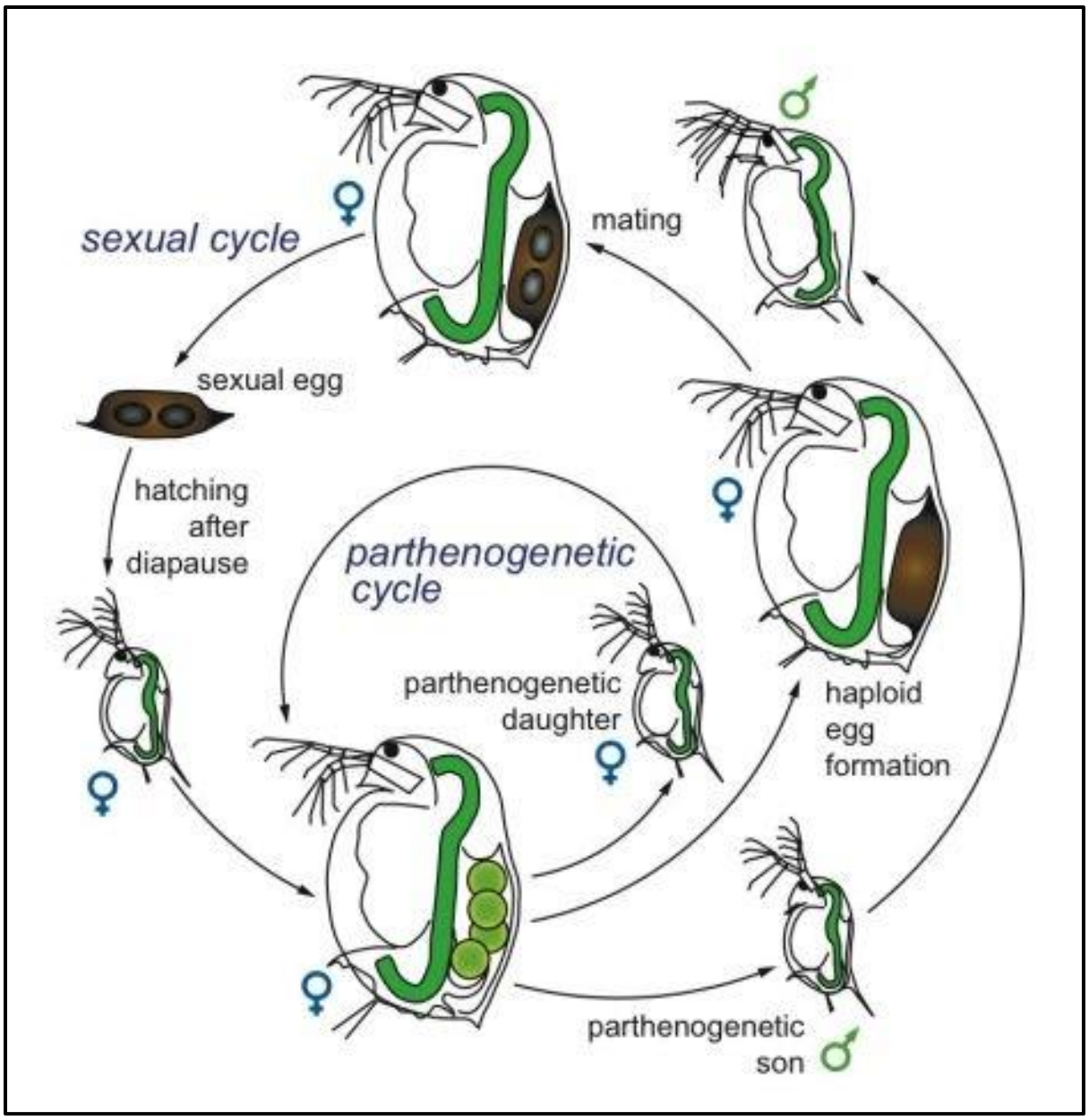

Figure 1.16. Schematic diagram of the sexual and asexual life cycles of a *Daphnia*;[36] reproduced with permission from the Zoologisches Institut, Universität Basel.

1.10 THE ROLE OF *DAPHNIA MAGNA* IN ECOTOXICITY TESTING

Aquatic organism such as *D. magna*, fathead minnows, and green algae are used for toxicity testing because natural bodies of water are the ultimate sinks of any chemical that ends up in our environment.[39] Compared to other testing organisms, *D. magna* are easier to culture and maintain in laboratory conditions, as they do not require great deal of resources.[40-47] Moreover, *D. magna* are recommended by the EPA for ecotoxicity testing because they are sensitive to toxicants and they have a short life span so it is easier to conduct life-cycle studies. *D. magna* are transparent so it is straight-forward using a standard optical microscope to see the morphology of their organs like the heart, gastro intestinal (GI) tract, and brood chamber. Acute, or short term, toxicity assays are usually conducted between 24–96 hours, and they typically involve the measurement of a single endpoint such as mortality. Chronic, or long term, assays are usually conducted between 14–21 days, and they typically involve the measurement of multiple endpoints such as mortality and reproductive output.

1.11 OVERVIEW OF DOCTORAL RESEARCH PROJECT

The goal of my doctoral research was to develop and optimize methods to characterize metal oxide NPs and to assess their effects on *D. magna* in acute and chronic studies. Chapter two represents the first ecotoxicity study of actual NPs used in commercial CMP slurry formulations to *D. magna*. This was important because commercial silica, ceria, and alumina NPs, or laboratory synthesized metal oxide NPs, may be different than the specialized NPs used in CMP processes, and therefore may have different toxicological profiles. In this work, biological endpoints were related to the applied dose of respective metal oxide NPs in each of the four model CMP slurries. The first major finding was that different model CMP slurries exerted distinct and unpredictable

effects on *D. magna* morbidity, growth, and reproductive output. The second major finding was that the CeO_2 and Al_2O_3 CMP slurries severely reduced *D. magna* reproduction upon chronic exposure at sub-lethal applied doses, which could have adverse consequences to aquatic ecosystems.

Chapter three was a continuation of this work where the goals were to determine the bioaccumulation and biopersistence of the model CeO_2 and Al_2O_3 CMP slurries by *D. magna*. This involved developing methods to determine the amounts of CeO_2 and Al_2O_3 NPs associated with *D. magna* using the university's newly-acquired inductively-coupled plasma (ICP)-mass spectroscopy (MS) system (Figure 1.17) and the Bionanosciences Group's new microwave digester (Figure 1.18). This was important so that the toxicity test results based on applied-NP doses could be calibrated to the actual amount of metal oxide NPs accumulated and eliminated by *D. magna*. The major findings were that both CeO_2 and Al_2O_3 were ingested by *D. magna* in a time dependent and dose dependent manner. Specifically, *D. magna* that were exposed for 48 h to 0.1 mg/mL CeO_2 CMP slurry accumulated 120 µg of CeO_2, and those that were exposed to for 48 h to 0.1 mg/mL of Al_2O_3 CMP slurry accumulated 44 µg of Al_2O_3. This shows that different CMP slurry NPs are accumulated in different amounts by *D. magna*. In addition, the biopersistence studies revealed that after 48 h of depuration time, *D. magna* exposed to 0.1 mg/mL CeO_2 successfully eliminated 85% of the initial CeO_2 load, and *D. magna* exposed to 0.1 mg/mL of Al_2O_3 were able to eliminate 78% of the initial Al_2O_3 load. However, this means that metal oxide NP slurries that persist in *D. magna* can be transferred to higher trophic levels and those that are eliminated can be transferred to lower trophic levels.

Figure 1.17. Image of the UT Dallas elemental analysis facility showing (from left to right): argon gas tank, a vented, compressed gas safety cabinet, waste container, a 384-vial auto-sampler, chiller, a vented inductively coupled mass spectrometer, diffusion pump, and personal computer.

Figure 1.18. Image of the Milestone UltraWAVE microwave digestion system at UT Dallas.

Chapter four involved the use of *D. magna* to assess the toxicity of CMP waste effluent containing spent *c*-SiO_2 NPs and potential toxic material from the wafer itself to answer the question of whether polishing a wafer can impart added toxicity to a CMP NP. This work was a collaboration with North Carolina A&T University and first involved identifying a commercial CMP slurry that was not toxic to *D. magna*, and then using it to polish a III-V-containing material with an extreme arm-pressure of 5 psi. Semiconductor manufacturers are interested in III/V-materials because they

are used in the fabrication of optoelectronic devices,[48-50] and because of the well-known toxicity and carcinogenicity of As and GaAs.[51-54] The key findings under chronic 21-day testing conditions were that an Ultra-Sol® 200S CMP slurry containing ~30-nm *c*-SiO_2 NPs at 0.10 mg/mL before (pristine) and after (spent) polishing a gallium arsenide (GaAs) wafer had little effect on *D. magna* morbidity, and that both slurries lead to a significant increase in reproductive output, indicative of a positive hormetic response whereby *D. magna* were under stress. Identical increases in body size and reproductive output were observed with a supernatant of the pristine slurry, in the absence of the *c*-SiO_2 NPs, indicating that the chronic effects were derived from soluble component(s) in the pristine slurry, and not from the *c*-SiO_2 NPs nor from a CMP process that removed ~3 mg of material from the GaAs wafer.

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CHAPTER 2

ACUTE AND CHRONIC TOXICITY OF METAL OXIDE NANOPARTICLES IN CHEMICAL MECHANICAL PLANARIZATION SLURRIES WITH *DAPHNIA MAGNA*

2.1 ABSTRACT

The semiconductor manufacturing industry uses metal oxide nanoparticles (NPs), including colloidal silica (*c*-SiO_2), fumed silica (*f*-SiO_2), ceria (CeO_2), and alumina (Al_2O_3), as abrasives in chemical mechanical planarization (CMP) processes. Assessing the toxicity of NPs used in commercial CMP slurries is difficult because these mixtures may contain undefined toxic constituents. Herein, the fresh water flea *Daphnia magna* (*D. magna)* was used to assess the effects of four model CMP slurries that did not contain known toxic additives. In the acute toxicity assessments, the key findings were that the Al_2O_3 slurry was toxic to *D. magna* with a calculated 96-h LC-50 of 1.1 mg/mL, that the CeO_2 and Al_2O_3 slurries caused severe dose-dependent decreases in body size, and that *c*-SiO_2 slurry caused a modest increase in body size indicative of a hormetic stress response. In the chronic toxicity assessments, the key findings were that the Al_2O_3 slurry lead to a modest increase in morbidity and a significant decrease in body size, that the CeO_2 and Al_2O_3 slurries caused severe dose-dependent decreases in reproductive output, and that the *c*-SiO_2 slurry caused a modest increase in reproduction indicative of a hormetic stress response. The acute and chronic toxicity results demonstrate that different model CMP slurries exert distinct and unpredictable effects on *D. magna* morbidity, growth, and reproductive output. Especially important is that the CeO_2 and Al_2O_3 slurries severely reduced *D. magna* reproduction

upon chronic exposure at low applied doses, which could have adverse consequences to aquatic ecosystems.

2.2 NANO IMPACT

Toxicity assessments of commercial CMP NPs are confounded by slurry constituents that are known to be toxic such as oxidants, surfactants, biocides, and corrosion inhibitors. In response to this challenge, a major CMP slurry manufacturer produced model CMP slurries containing either *c*-SiO_2, *f*-SiO_2, CeO_2, or Al_2O_3 NPs that did not contain known soluble toxic additives. This work reports the first ecotoxicity assessment of these unique model CMP slurries using *D. magna* to study the effects of actual NPs used in commercial CMP slurry formulations.

2.3 INTRODUCTION

The process of fabricating integrated circuits requires hundreds of precise and complex steps whereby materials are deposited and selectively removed from a wafer of semiconducting material such as silicon. One of these critical steps, the removal of excess materials and the smoothing of wafer surfaces to high tolerances, is achieved by a process known as chemical mechanical planarization (CMP). Developed in the early 1980s by Klaus D. Beyer at IBM, CMP involves a combination of surface reactions with chemical additives and mechanical polishing with nanoscale abrasives.[1-3] The chemical additives of a CMP slurry may include acids or bases, buffers, oxidizers, surfactants, biocides, complexing agents, and corrosion inhibitors, as well as, proprietary surface- and redox-active constituents designed to influence NP behavior. The abrasive additives will include an aqueous suspension of metal oxide nanoparticles (NPs) such as

colloidal silica (*c*-SiO_2), fumed silica (*f*-SiO_2), ceria (CeO_2), or alumina (Al_2O_3), depending on the particular application.[4-6] In a typical CMP operation, the slurry is introduced between the wafer and a polishing pad, a small pressure is applied to press the wafer onto the pad, and the two are rotated for a short period until the desired mass of material is removed.[7-10] Large volumes of ultrapure water are then used to wash the wafers to remove slurry components and wafer detritus.[11] Finally, the rinsate is collected and directed to a wastewater treatment facility where it is processed before the reclaimed water is discharged to a municipal water supply.[4]

While the concentrations of CMP NPs are diluted many-fold in the waste effluent, and while wastewater treatments such as the activated sludge treatment method can remove as much as 97–99% of metal oxide NPs,[12] it is still possible for CMP NPs to be discharged to the municipal water supply and potentially into an aquatic environment. The semiconductor industry is therefore interested to understand the potential environmental toxicity associated with CMP NPs. However, assessing the toxicity of CMP NPs is a complex endeavor because subtle differences in metal oxide NP surface reactivity, size, and shape can affect toxicity,[13-24] and because CMP NP assessments are confounded by soluble slurry constituents that are known to be toxic such as oxidants, surfactants, biocides, and corrosion inhibitors. As a result, while a growing number of occupational assessments of commercial CMP slurries inside semiconductor fabrication plants have been reported,[3,4,25,26] to our knowledge an ecotoxicity assessment of CMP slurries has not been reported. In response to this challenge, a major CMP slurry manufacturer produced four model CMP slurries comprising *c*-SiO_2, *f*-SiO_2, CeO_2, or Al_2O_3 NPs that did not contain known soluble toxic additives to permit toxicity assessments of actual NPs used in commercial CMP slurry formulations.[5]

Herein, we study the toxicity of these four model CMP slurries to *Daphnia magna* (*D. magna*). *D. magna* is a filter-feeding fresh-water flea found in ponds and lakes. It is a model planktonic crustacean organism commonly used in ecotoxicity studies because it is sensitive to ecological pollutants and easy to maintain in laboratory cultures.[27-34] For acute toxicity assessments, *D. magna* neonates (<24-h old) were exposed continuously to various concentrations of model CMP slurries for 96 h and the morbidity and the body sizes of the surviving *D. magna* were compared to those of control *D. magna* not exposed to slurries. The *c*-SiO_2 slurry was not lethal up to 4.0 mg/mL but did cause a modest increase in body size. The *f*-SiO_2 slurry was not lethal up to 5.0 mg/mL and did not reduce body size. The CeO_2 slurry was not lethal up to 2.0 mg/mL but did cause a severe dose-dependent decrease in body size. The Al_2O_3 CMP slurry was the only one among the four model CMP slurries that showed acute lethal toxicity to *D. magna* with a calculated 96-h LC-50 of 1.1 ± 0.2 mg/mL in addition to a severe dose-dependent decrease in *D. magna* body size. Supernatants of the CeO_2 and Al_2O_3 slurries, in the absence of the NPs, had no acute effects on *D. magna* mortality or body size indicating that the observed adverse effects were derived from CeO_2 and Al_2O_3 NPs and not from soluble component(s) in the slurries. In addition, results are presented suggesting that ingested CeO_2 or Al_2O_3 CMP NPs may restrict subsequent food consumption, which impairs growth. For chronic toxicity assessments, *D. magna* neonates were exposed continuously to sub-lethal concentrations of model CMP slurries for 21 d and the morbidity, body sizes, and the numbers of offspring produced were compared to those of the control group. The *c*-SiO_2 slurry was not lethal at 0.1 mg/mL, did not reduce body size, but did lead to a modest increase in reproductive output. The *f*-SiO_2 slurry was not lethal at 0.1 mg/mL, did not reduce body size, and did not decrease reproductive output. The CeO_2 slurry had no impact

on morbidity or body size at 0.2 mg/mL, but did cause a severe dose-dependent decrease in reproductive output. The Al_2O_3 slurry, however, lead to a modest increase in morbidity at 0.1 mg/mL, a significant decrease in body size, and a severe dose-dependent decrease in reproductive output. The acute and chronic toxicity results demonstrate that different model CMP slurries exert distinct and unpredictable effects on *D. magna* morbidity, growth, and reproductive output. Of particular concern are the observations that the CeO_2 and Al_2O_3 CMP slurries caused significant reduction in *D. magna* reproduction upon chronic exposure at low applied doses, which could have adverse consequences to aquatic ecosystems.

2.4 EXPERIMENTAL SECTION

2.4.1 Materials and solutions

The model CMP slurries were custom-synthesized without any proprietary chemical additives by a major CMP slurry manufacturer for the Semiconductor Research Corporation (SRC) Engineering Research Center for Environmentally Benign Semiconductor Manufacturing (ERC). The physical and chemical properties of the four model CMP slurries provided by the manufacturer are shown in Table A1 and were verified independently by a thorough physicochemical characterization reported by members of the SRC-ERC nanotoxicity consortium.[5] The *c*-SiO_2 CMP slurry was prepared with acetic acid and contained highly-spherical 50–60 nm NPs while the *f*-SiO_2 CMP slurry was prepared with potassium hydroxide and contained irregular-shaped 120–140 nm NPs. The CeO_2 slurry was prepared without additives and contained rectangular-shaped 60–100 nm NPs. The Al_2O_3 CMP slurry was prepared in nitric acid and contained irregular-shaped 80–100 nm NPs. All CMP slurries were stored in high density polyethylene (HDPE) bottles at

room temperature away from direct light and were vortexed thoroughly before use. Deionized water (18.2 MΩ-cm) was obtained using a Milli-Q® Advantage A10 water purification system (Millipore; Billerica, MA). Blue polystyrene beads (5.0% w/v in deionized water) with a reported mean diameter of 0.47 µm were purchased from Spherotech Inc. (Lake Forest, IL). Unless otherwise specified, all other chemicals were of the highest grade available and were purchased from VWR International (Radnor, PA).

Daphnia medium (i.e., moderately hard reconstituted water) was made in accordance with U.S. Environmental Protection Agency (EPA) guidelines by mixing: 192 mg/L $NaHCO_3$, 120 mg/L $CaSO_4 \cdot 2H_2O$, 120 mg/L $MgSO_4$, and 8 mg/L KCl in well-aerated deionized water.[35] The hardness of *daphnia* medium was maintained between 250–425 mg/L $CaCO_3$ as measured using a HACH® aqua check water quality test strip. The pH was maintained between 7.4–8.4 as measured using a Fisher Scientific model 25 pH/ion meter and an Accumet® combination Ag/AgCl reference pH electrode. Dissolved oxygen was maintained between 8–10 mg/L as measured using a colorimetric method (CHEMets® kit K-7512; Midland, VA). The *daphnia* diet comprised a commercially-prepared mixture of yeast, CEROPHYLL®, and Troutchow (YCT) supplemented with green microalgae (*Pseudokirchnerilla subcapitata*), both purchased from Marinco Bioassay Laboratory, Inc. (Sarasota, FL).

2.4.2 Preparation of model CMP slurries for acute toxicity tests

Stock CMP slurries were diluted with *daphnia* medium to contain the following concentrations of NPs: 2.0, 3.0, and 4.0 mg/mL for the *c*-SiO_2 CMP slurry; 2.0, 4.0, and 5.0 mg/mL for the *f*-SiO_2 CMP slurry; 0.50, 1.0, and 2.0 mg/mL for the CeO_2 CMP slurry; and 0.75, 1.5, and 3.0 mg/mL for the Al_2O_3 CMP slurry. To ensure that the pH of the *daphnia* medium

remained neutral, the pH of the acidic *c*-SiO_2 and the basic *f*-SiO_2 model CMP slurries were adjusted to 7.0–7.5 using minimal amounts of 0.1 M NaOH or 1 M HCl, respectively. No pH adjustments were required for the model CeO_2 and Al_2O_3 CMP slurries following dilution with *daphnia* medium. Supernatants of a 2.0 mg/mL CeO_2 CMP slurry and a 3.0 mg/mL Al_2O_3 CMP slurry were collected after samples prepared in *daphnia* medium were left undisturbed for 24 h.

2.4.3 Preparation of model CMP slurries for chronic toxicity tests

Stock CMP slurries were diluted in *daphnia* medium to contain the following concentrations of NPs: 0.10 mg/mL for the *c*-SiO_2 CMP slurry; 0.10 mg/mL for the *f*-SiO_2 CMP slurry; 0.050, 0.10, and 0.20 mg/mL for the CeO_2 CMP slurry; and 0.025, 0.050, and 0.10 mg/mL for the Al_2O_3 CMP slurry. pH adjustments were not required owing to the small volumes of slurries added to the *daphnia* medium.

2.4.4 Dynamic light scattering (DLS) analyses of model CMP slurry NPs

The particle size distributions of CMP slurry NPs were analyzed by DLS using a Zetasizer Nano-ZS 3600 DLS analyzer (Malvern Instruments; Worcestershire, UK) with a 633-nm laser at a fixed angle of 173°. Stock CMP slurries were diluted with pH 7.4 *daphnia* medium to a final NP concentration of 4.0 mg/mL for the *c*-SiO_2 slurry, 5.0 mg/mL for the *f*-SiO_2 slurry, 2.0 mg/mL for the CeO_2 slurry, and 3.0 mg/mL for the Al_2O_3 slurry. Aliquots (500 µL) of each sample were placed in disposable polystyrene cuvettes and ten consecutive 30-s runs were acquired per measurement at 25 °C. Three separate measurements were obtained for each sample and the mean particle size in terms of hydrodynamic diameter was calculated. Particle size distributions were measured periodically during the course of this work.

2.4.5 Culture methods

All *D. magna* were acquired from Marinco Bioassay Laboratory, Inc. Cultures were maintained according to established guidelines and recommendations.[27,35,36] Critical variables such as *D. magna* suppliers, test container materials, test container volumes, number of *D. magna* per test container, *D. magna* food sources, amounts of food, feeding frequency, light luminosity, room temperature, and the pH and dissolved oxygen content of the *daphnia* medium were optimized prior to toxicity testing. In brief, *D. magna* cultures were maintained in 100-mL polypropylene beakers containing 80 mL of *daphnia* medium and 5 animals per beaker. *D. magna* cultures were maintained in a temperature-controlled room at 20 ± 1 °C with a natural photoperiod of 8-h darkness and 16-h light provided by an incandescent fluorescent lamp. *D. magna* were fed three times a week on Monday, Wednesday, and Friday. The food comprised 1 mL of YCT and 1 mL of green algae (3.5×10^7 cells). Once a week *D. magna* neonates were removed and the culture medium was replaced with fresh medium, and every two weeks the culture beakers were additionally cleaned of debris. The *daphnia* medium was also replaced with fresh medium the day before *D. magna* neonates were collected for toxicity tests.

2.4.6 Acute toxicity assays

The standard conditions for 96-h toxicity tests with *D. magna* are summarized in Table A2; they are based on established guidelines for acute toxicity testing of materials on aquatic organisms and on recommendations for working with metal oxide NPs in aqueous environments.[27,35,37-41] A static test method was chosen because it was less labor intensive and it required less CMP slurry material.[13,33,35] Test solutions containing CMP slurries were prepared immediately prior to use as described above. In brief, five neonates (<24-h old) were randomly selected from a pool of

neonates and placed in 8 mL of control or test solution in a 10-mL well of a 6-well culture plate that was covered with a clear lid to minimize the evaporation of water;[42] a total of 30 *daphnids* were used for each sample in an independent trial. Culture plates were kept in an incubator maintained at constant temperature of 20 ± 1 °C with a photoperiod of 16-h light and 8-h darkness.[39,43] Culture plates were continuously rotated on an orbital shaker (model Back-to-Basics; Bellco Biotechnology, Vineland, NJ) with a constant rpm setting of 60 to minimize NP sedimentation and to mimic currents in natural waters; this method was chosen since approaches involving sonication or aeration have been reported to stress *D. magna*.[34,44,45] Unless otherwise specified, *D. magna* were fed daily with 48 µL of YCT and 32 µL of green algae (1.1×10^6 cells/well). The pH of control and test media was measured at the beginning and at the end of each trial, and the dissolved oxygen was measured at the end of each trial. The morbidity of individual *D. magna* was visually determined every 24 h; *D. magna* that were unable to swim within 10 s of gentle agitation were considered dead. While it was the norm for ≥93% of *D. magna* in control containers to survive and appear healthy for 96 h, trials were discarded if ≤90% of control *D. magna* survived. Morbidity was reported as the LC-50 (i.e., the lethal concentration which kills 50% of a *D. magna* population). The body sizes of individual *D. magna* were determined by acquiring images of surviving *D. magna* using a Nikon SMZ745T stereomicroscope with Nikon NIS-Elements D software to outline bodies (sans antennae and tail) and to calculate areas.[46] Statistical significance was determined using either a two-tail student's t-test with equal variances or single-variable ANOVA where $p < 0.05$ was considered significant.

2.4.7 Chronic toxicity assays

The standard conditions for 21-day toxicity tests with *D. magna* are summarized in Table A2; they are based on established guidelines for chronic toxicity testing of materials on aquatic organisms and on recommendations for working with metal oxide NPs in aqueous environments.[27,37,38,46-53] In brief, ten neonates (<24-h old) were randomly selected from a pool of neonates and placed in 100 mL of control or test solution in 250-mL polypropylene beakers that were covered with a thin sheet of clear plastic to minimize evaporation of water; a total of 30 *daphnids* in three beakers were used for each sample in an independent trial. The beakers were continuously rotated on a Bellco orbital shaker with a constant rpm setting of 60 in an incubator maintained at constant temperature of 20 ± 1 °C with a photoperiod of 16-h light and 8-h darkness. Test solutions containing CMP slurries were prepared immediately prior to use as described above. The media of the control and test groups were replaced with freshly prepared media and slurries every 4 d, and food (500 µL of YCT and 2 mL of green algae (7.0×10^7 cells)) was added to each beaker every other day. The morbidity of individual *D. magna* was determined every 24 h as described previously. If $\leq$80% of control *D. magna* survived, the entire trial was discarded. The body sizes of individual *D. magna* were determined as described above every two days for the first ten days and then on days 14, 18, and 21, subsequently. The reproductive output of adult *D. magna* was determined by daily counting the number of neonates produced; all neonates were removed from control and test beakers after being counted. If resting eggs (ephippia) were noticed in control *D. magna* at any stage during the trial (an indicator of stress), the entire trial was discarded. Statistical significance was determined using either a two-tail student's t-test with equal variances or single-variable ANOVA where $p < 0.05$ was considered significant.

2.5 RESULTS

2.5.1 Model CMP NP aggregation states in daphnia medium

The aggregation states of the four model CMP slurries were previously characterized following dilution with deionized water.[5] To assess the aggregation states of model CMP NPs in pH 7.4 *daphnia* medium, DLS analyses were performed. The mean hydrodynamic diameter of the *c*-SiO_2 CMP NPs in *daphnia* medium was 47 ± 1 nm, which closely resembled the value of the dry primary NP size reported by the manufacturer (Table A1). The mean hydrodynamic diameter of the *f*-SiO_2 CMP NPs in *daphnia* medium was 701 ± 112 nm (~5× greater than the size of the dry NPs), indicating a slight degree of *f*-SiO_2 NP aggregation in this relatively low ionic strength medium. While metal oxide NPs tend to aggregate as functions of the pH and ionic strength of the aqueous medium,[54] the *c*-SiO_2 and *f*-SiO_2 NPs did not aggregate significantly in *daphnia* medium because the isoelectronic point (i.e., the pH at which a NP shows no net charge and has a zeta potential of 0 mV) of SiO_2 is between pH 2 and 3.[55,56] In contrast, significant NP aggregation was observed visually within minutes for the CeO_2 and the Al_2O_3 CMP NPs in *daphnia* medium, and hydrodynamic diameters could not be accurately determined since the dimensions of the largest aggregates exceed the DLS working range. This was expected because the isoelectronic points of CeO_2 and Al_2O_3 NPs are pH 6.71 and pH 7.06, respectively, which are both close to the neutral pH of the media.[57]

2.5.2 Acute effects of model CMP slurries on Daphnia magna morbidity and body size

For acute toxicity assessments, *D. magna* neonates (<24-h old) were exposed to various concentrations of model CMP slurries for 24, 48, 72, and 96 h. Under these conditions, *D. magna* swam randomly around all regions of the test container except at the highest applied slurry

concentrations when their swimming rates appeared slower, notably in the presence of the highly-aggregated CeO_2 and Al_2O_3 CMP slurries. This is important to note since the settling of highly-aggregated metal oxide NPs leads to *D. magna* being exposed to a higher regional concentration of NPs when they swam near the bottom of test containers.[58] Figure 2.1 shows the morbidity and body size results for *D. magna* exposed to each of the four model CMP slurries. With the *c*-SiO_2 CMP slurry (Figure 2.1A), no morbidity was observed after 96 h even with the highest applied NP dose of 4.0 mg/mL, and modest increases in body sizes (17–24%) were observed compared to unexposed controls. With the *f*-SiO_2 CMP slurry (Figure 2.1B), a slight degree of morbidity (14%) was observed at 96 h only with the highest applied NP dose of 5.0 mg/mL, and no differences in body sizes were observed compared to unexposed controls. With the CeO_2 CMP slurry (Figure 2.1C), no morbidity was observed after 96 h even with the highest applied NP dose of 2.0 mg/mL. However, there was a severe dose-dependent decrease in *D. magna* body size; specifically, surviving *D. magna* exposed to 2.0 mg/mL of the CeO_2 CMP slurry for 96 h were 46% smaller compared to unexposed controls. With the Al_2O_3 CMP slurry (Figure 2.1D), severe morbidity was observed with a calculated 72-h LC-50 of 2.6 mg/mL and a calculated 96-h LC-50 of 1.1 mg/mL. Furthermore, there was a severe dose-dependent decrease in *D. magna* body size such that the surviving *D. magna* were 53% smaller than the unexposed controls after a 96-h exposure to the Al_2O_3 CMP slurry at 1.5 mg/mL, the highest concentration tested.

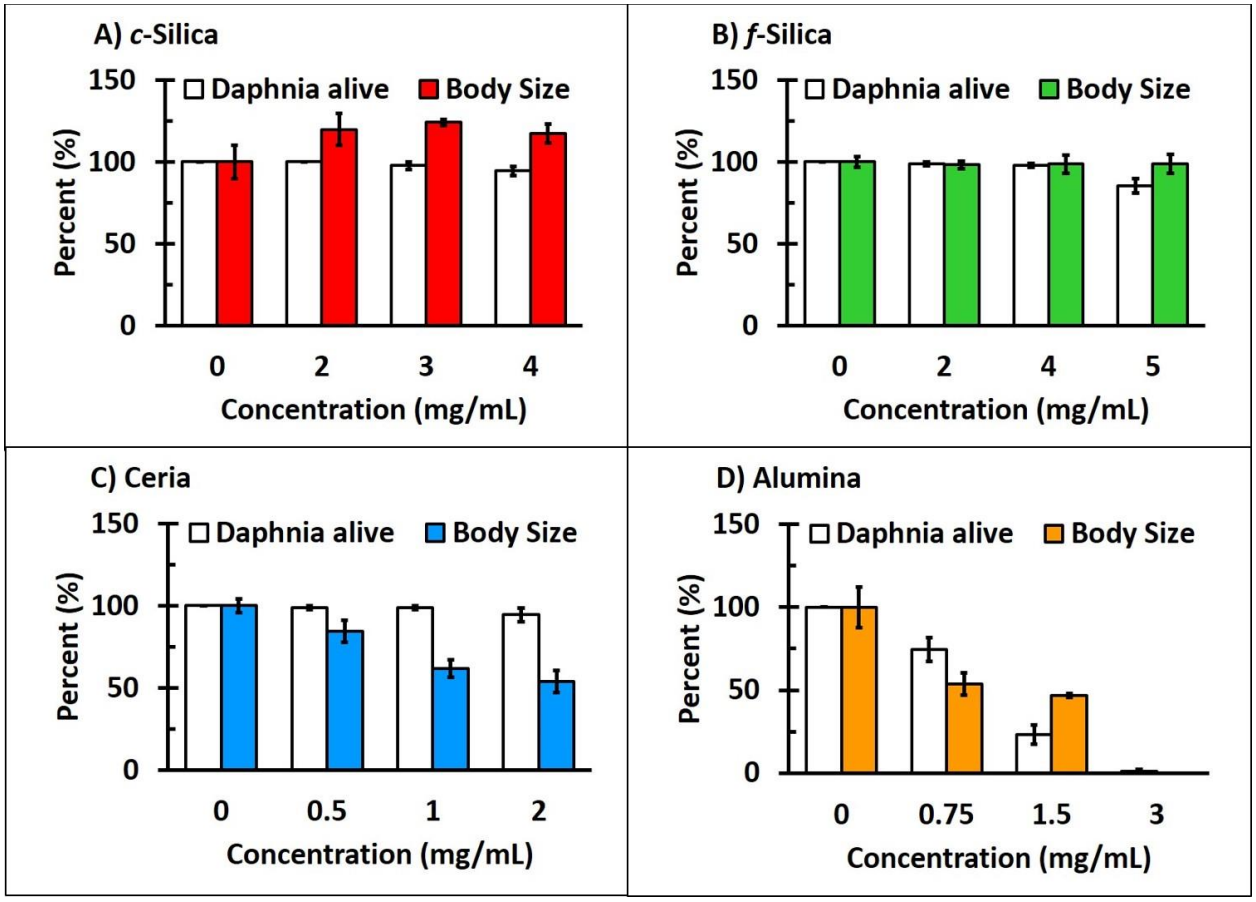

Figure 2.1. Acute morbidity and effects on *D. magna* size after a 96-h exposure to model CMP slurries. Percent survival (clear bars) and body sizes (shaded bars) of *D. magna* exposed to various concentrations of the: **(A)** colloidal silica (*c*-SiO_2), **(B)** fumed silica (*f*-SiO_2), **(C)** ceria (CeO_2), or **(D)** alumina (Al_2O_3) CMP slurry measured after 96 h relative to control *D. magna* not exposed to a CMP slurry (set to 100%). All data points are the mean of at least three independent trials and the error bars represent the standard error of mean (SEM).

Comparative images of *D. magna* exposed to model CMP slurries for 72 h are shown in Figure 2.2. There was a modest increase in body size after exposure to the *c*-SiO_2 CMP slurry at 4.0 mg/mL compared to unexposed controls (Figures 2.2A and 2.2B). The body sizes of *D. magna* exposed to the *f*-SiO_2 CMP slurry were not significantly different compared to unexposed controls (Figures 2.2A and 2.2C) even at 5.0 mg/mL. However, *D. magna* exposed to the CeO_2 slurry at 2.0 mg/mL or the Al_2O_3 slurry at 1.5 mg/mL (Figures 2.2D and 2.2E) were significantly smaller compared to unexposed *D. magna*. Besides the striking differences in body sizes, another key

observation concerned the appearances of the digestive tracts. Since green algae is the major food source in the diet of filter-feeding *D. magna*,[59] the digestive tracts of fed daphnids were accordingly green, as shown in the image of a control *D. magna* not exposed to a slurry (Figure 2.2A). The same green coloration was observed in the images of *D. magna* exposed to the *c*-SiO_2 and *f*-SiO_2 CMP slurries (Figures 2.2B and 2.2C), whereas the digestive tracts of *D. magna* exposed to the CeO_2 and Al_2O_3 CMP slurries were not obviously green (Figures 2.2D and 2.2E). Instead, the smaller digestive tracts of these *D. magna* appeared cream colored, similar to the natural colors of these two slurries. This observation suggests that the presence of highly-aggregated CeO_2 or Al_2O_3 NPs in *D. magna* digestive tracts might impair the organism's ability to take up food such as algae, as further discussed in a later section. Altogether, the results of the acute toxicity assessment demonstrate that different model CMP slurries have distinct effects on *D. magna* morbidity, body size, and digestive tract appearances.

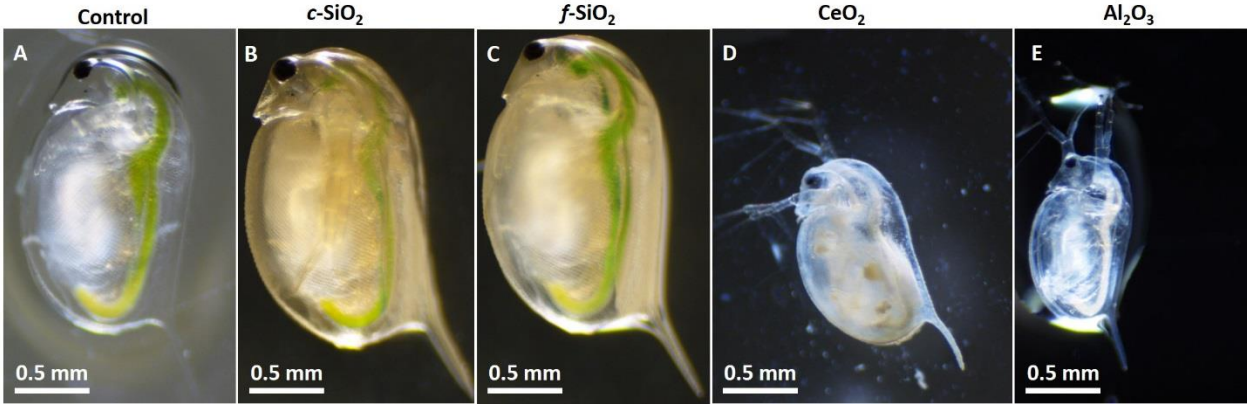

Figure 2.2. Sizes of *D. magna* after a 72-h exposure to model CMP slurries. Representative optical images (5× magnification) of: **(A)** control *D. magna* not exposed to a CMP slurry; **(B)** *D. magna* exposed to 4.0 mg/mL of the *c*-SiO_2 CMP slurry; **(C)** *D. magna* exposed to 5.0 mg/mL of the *f*-SiO_2 CMP slurry; **(D)** *D. magna* exposed to 2.0 mg/mL of the CeO_2 CMP slurry; and, **(E)** *D. magna* exposed to 1.5 mg/mL of the Al_2O_3 CMP slurry.

2.5.3 Acute effects of the supernatants of model CeO_2 and Al_2O_3 CMP slurries on Daphnia magna morbidity and body size

To determine if soluble components of the model CeO_2 and Al_2O_3 CMP slurries contributed to the severe acute effects observed with *D. magna,* supernatants of the 2.0 mg/mL CeO_2 slurry and the 3.0 mg/mL Al_2O_3 slurry prepared in *daphnia* media were presented to *D. magna*. Figure A1 shows the acute morbidity and body size of *D. magna* exposed to the supernatants of the CeO_2 and Al_2O_3 CMP slurries. There was no morbidity nor significant differences in body sizes observed after 96 h compared to unexposed controls. The results therefore indicate that the soluble component(s) of these CMP slurries were not responsible for the effects observed with *D. magna*. Instead, the toxic effects partition with insoluble component(s) associated with the particulate matter in these slurries, namely CeO_2 and Al_2O_3 NPs. In addition, these results indicate that Ce(III), Ce(IV), or Al(III) ions that may have dissociated from the respective NPs were not present in the supernatants at levels high enough to cause any observable effects with *D. magna* under these test conditions.

2.5.4 Acute effects of model CeO_2 and Al_2O_3 CMP slurries on Daphnia magna body size under different feeding conditions

A series of experiments were performed to test the hypothesis that the presence of CeO_2 or Al_2O_3 NPs in the digestive tracts of *D. magna* might impaired the organism's ability to take up food such as algae. The first experiment compared the body sizes of surviving *D. magna* that were starved for 72 h to those of fed *D. magna* that were exposed to model CeO_2 or Al_2O_3 CMP slurries for 72 h. As shown in Figure A2, the body sizes of starved *D. magna* were 57% smaller compared to fed *D. magna* controls that were not treated with a CMP slurry, and were similar to the body

sizes of fed *D. magna* exposed to either the CeO_2 CMP slurry at 2.0 mg/mL or the Al_2O_3 CMP slurry at 1.5 mg/mL. Moreover, less green coloration (from ingested algae) was observed in the digestive tracts of the starved and the slurry-treated *D. magna* (Figures A2B, A2C, and A2D) relative to fed controls (Figure A2A). Hence, these results support the hypothesis that smaller *D. magna* body sizes (i.e., stunted growth) might be due to ingested CeO_2 or Al_2O_3 CMP NPs somehow impairing the organism's ability to consume food, such as green algae, that is needed to sustain normal growth.

The next experiment to explore this hypothesis further involved exposing *D. magna* to increasing amount of nutrients in the absence (controls) and the presence of the Al_2O_3 CMP slurry. Figure 2.3 shows the acute effects on *D. magna* body sizes after a 96-h exposure to 0.1 mg/mL of the Al_2O_3 CMP slurry under normal (1X), double (2X), and quadruple (4X) feeding conditions. For all feeding regimens, increased availability of food lead to readily observed increases in body sizes for the controls and the Al_2O_3 slurry-treated *D. magna* populations. However, for a given feeding regimen, *D. magna* exposed to the Al_2O_3 CMP slurry remained significantly smaller than the corresponding controls. Analogous trends were observed for *D. magna* treated with 1.0 mg/mL of the CeO_2 CMP slurry (data not shown). So, while increasing the amount of food did promote *D. magna* growth, the adverse effects of the CeO_2 and Al_2O_3 slurries on *D. magna* growth were not fully mitigated by excess food alone.

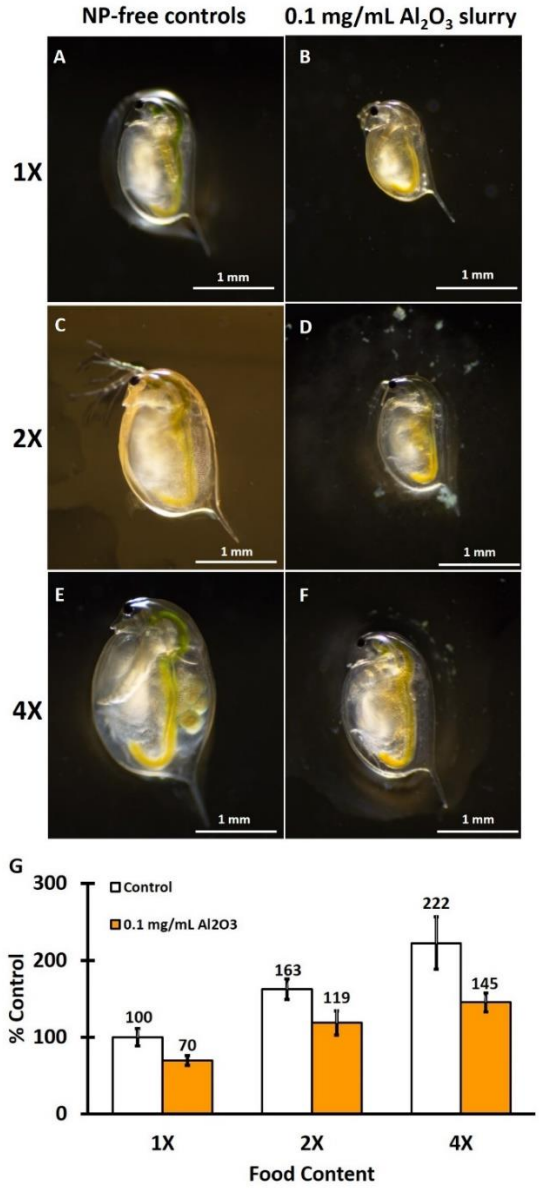

Figure 2.3. Acute effects on *D. magna* size after a 96-h exposure to the model Al_2O_3 CMP slurry under different feeding conditions. Representative optical images (3× magnification) of: **(A)** control *D. magna* fed 1X food (normal feeding regimen) and not exposed to a CMP slurry; **(B)** *D. magna* fed 1X food and exposed to 0.1 mg/mL of the Al_2O_3 CMP slurry; **(C)** *D. magna* fed 2X food and not exposed to a CMP slurry; **(D)** *D. magna* fed 2X food and exposed to 0.1 mg/mL of the Al_2O_3 CMP slurry; **(E)** *D. magna* fed 4X food and not exposed to a CMP slurry; and, **(F)** *D. magna* fed 4X food and exposed to 0.1 mg/mL of the Al_2O_3 CMP slurry. **(G)** Summary plot where the body sizes of *D. magna* exposed to the Al_2O_3 CMP slurry (shaded bars) and *D. magna* not exposed to CMP slurries (clear bars) are shown relative to the 1X-control *D. magna* not exposed to a CMP slurry (set to 100%). The numbers above the bars are the mean values for at least three independent trials and the error bars represent the SEM.

As filter-feeders, it has been shown that *D. magna* can retain suspended particles larger than 450 nm in diameter on their filtering appendages and that such particles can be ingested and accumulated in their digestive tracts.[59,60] Therefore, the next experiment tested whether Al_2O_3 slurry-treated *D. magna* could ingest food just as well as untreated controls by adding blue polystyrene beads with a hydrodynamic diameter of 463 nm in *daphnia* medium and visualizing their accumulation in the digestive tracts of *D. magna*. In brief, control *D. magna* were fed a normal diet for 96 h in the absence of a CMP slurry, and as was the norm, their digestive tracts appeared green due to the presence of algae (data not shown but similar to what is observed in Figure 2.2A). Subsequently, these *D. magna* were placed in a food-free aqueous solution containing 0.005 w/v% blue polystyrene beads for 30 min. As shown in Figure 2.4A, blue coloration was observed throughout the digestive tract and there was little if any green observed. These results indicate that healthy control *D. magna* can rapidly consume a significant and visually-observable amount of blue beads that accumulate throughout their entire digestive tract. The same protocol was repeated on two sets of Al_2O_3 slurry-treated *D. magna*. Specifically, *D. magna* were fed for 96 h in the presence of 0.1 mg/mL or 0.2 mg/mL of the Al_2O_3 CMP slurry, removed from the food and slurry, and then exposed to a food- and slurry-free aqueous solution containing only the blue polystyrene beads for 30 min. In viewing the images of the Al_2O_3 slurry-treated *D. magna* in Figures 2.4B and 2.4C, (i) blue coloration was indiscernible throughout their digestive tracts, (ii) cream coloration was observed near the beginning of the digestive tracts, and (iii) yellow-green coloration was observed near the end of the digestive tracts. Analogous trends were observed for *D. magna* treated with 1.0 mg/mL of the CeO_2 CMP slurry (data not shown).

These observations demonstrate that *D. magna* treated with the CeO_2 or Al_2O_3 slurries were not able to ingest blue beads since their digestive tracts were filled with indigestible metal oxide NPs.

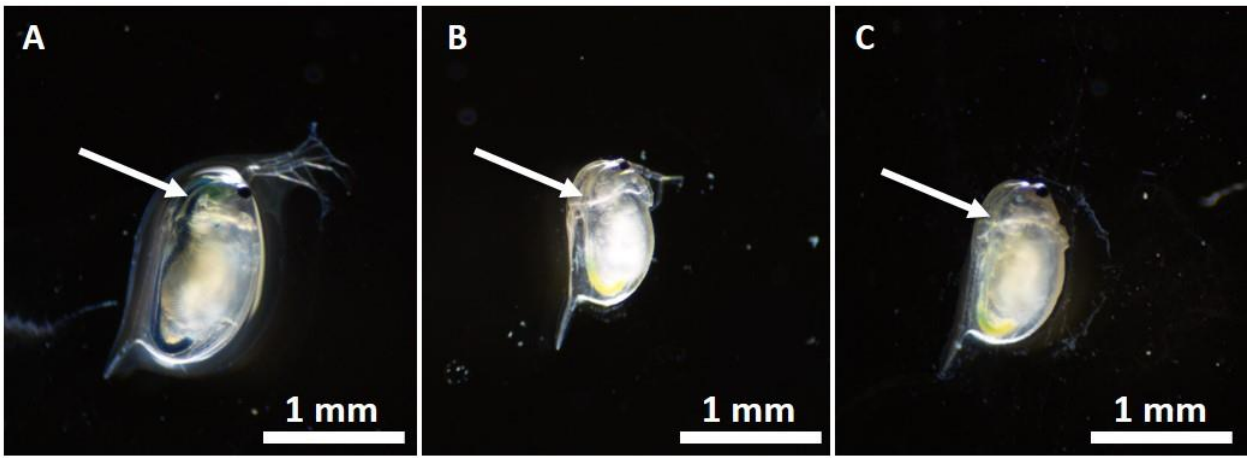

Figure 2.4. Acute effects on *D. magna* size after a 96-h exposure to the model Al_2O_3 CMP slurry followed by a 30-min exposure to blue polystyrene NPs. Representative optical images (3× magnification) of: **(A)** *D. magna* that were fed but not exposed to a CMP slurry for 96 h followed by a 30-min exposure to a food-free solution containing 463-nm diameter blue polystyrene beads; **(B)** *D. magna* fed and exposed to 0.1 mg/mL of the Al_2O_3 CMP slurry for 96 h followed by a 30-min exposure to blue polystyrene beads in the absence of food and slurry; and, **(C)** *D. magna* fed and exposed to 0.2 mg/mL of the Al_2O_3 CMP slurry for 96 h followed by a 30-min exposure to blue polystyrene beads in the absence of food and slurry. The white arrows denote the midgut region.

In summary, the combined results support the hypothesis that highly-aggregated CeO_2 or Al_2O_3 CMP NPs accumulate in the digestive tracts of *D. magna* and that this impairs the consumption of nutrients, which leads to a reduction in growth, as evidenced by the smaller body sizes of CeO_2 and Al_2O_3 slurry-treated *D. magna* compared to controls. Conversely, the model slurry containing the least aggregated metal oxide NPs, the *c*-SiO_2 CMP slurry, lead to a modest increase in *D. magna* body sizes. To achieve a more comprehensive understanding of the acute effects, chronic toxicity assessments were performed as described next.

2.5.5 Chronic effects of model CMP slurries on Daphnia magna morbidity, body size, and reproductive output

For chronic toxicity assessments, *D. magna* neonates (<24-h old) were continuously exposed for 21 d to lower (sub-lethal) concentrations of model CMP slurries than those used in the acute studies, and three biological endpoints were monitored (morbidity, body size, and reproductive output). Under these test conditions, *D. magna* swam actively around all regions of the test container except when exposed to highest applied doses of slurry when their swimming rates appeared slower, most notably in the presence of the highly-aggregated CeO_2 and Al_2O_3 CMP slurries. Figure A3 shows the morbidity, body size, and reproductive output of *D. magna* exposed for 21 d to 0.1 mg/mL of the *c*-SiO_2 or *f*-SiO_2 CMP slurry. A slight degree of morbidity (10%), a slight increase in body size (10%), and a modest increase in reproductive output (18%) were observed with the *c*-SiO_2 CMP slurry, and a slight degree of morbidity (13%), a slight increase in body size (10%), and a slight increase in reproductive output (10%) were observed with the *f*-SiO_2 CMP slurry. Even though the morbidity of *D. magna* was not severely affected by chronic exposure to either of the two model silica slurries, the slight increases in body size and the slight (*f*-SiO_2) and modest (*c*-SiO_2) increases in reproductive output suggest the onset of a hormetic process – an adaptive response characterized by a beneficial effect to a moderate environmental stress.[61-63]

In contrast to the two model silica slurries, significant adverse effects were exerted on *D. magna* by chronic exposures to sub-lethal doses of the CeO_2 and Al_2O_3 CMP slurries. Figure 2.5 shows the morbidity, body size, and reproductive output of *D. magna* exposed to various concentrations of the CeO_2 CMP slurry. A slight degree of morbidity (10%) and a slight decrease

in body size (13%) was observed relative to controls after a 21-d exposure to the highest applied NP dose of 0.2 mg/mL (Figures 2.5A and 2.5B). However, there was a severe dose-dependent decrease in the reproductive output of *D. magna*; specifically, the number of offspring produced by *D. magna* exposed for 21 d to the highest applied dose of the CeO_2 CMP slurry (0.2 mg/mL) was 81% less compared to controls (Figure 2.5C). Moreover, as shown in Figures 2.5E–G, the digestive tracts of *D. magna* exposed to CeO_2 CMP slurries appeared cream colored and lacked the predominate green coloration that was observed in control *D. magna* (Figure 2.5D). Finally, it was observed that the reproductive activity for *D. magna* exposed to the CeO_2 CMP slurries was delayed by 3–4 d compared to controls (Figure A4). Overall, this suggests that the long-term exposure of the model CeO_2 CMP slurry at a concentration that imposes no life-threatening danger to *D. magna* may nevertheless cause a serious reduction in reproduction, which may endanger the *D. magna* population in an ecosystem.

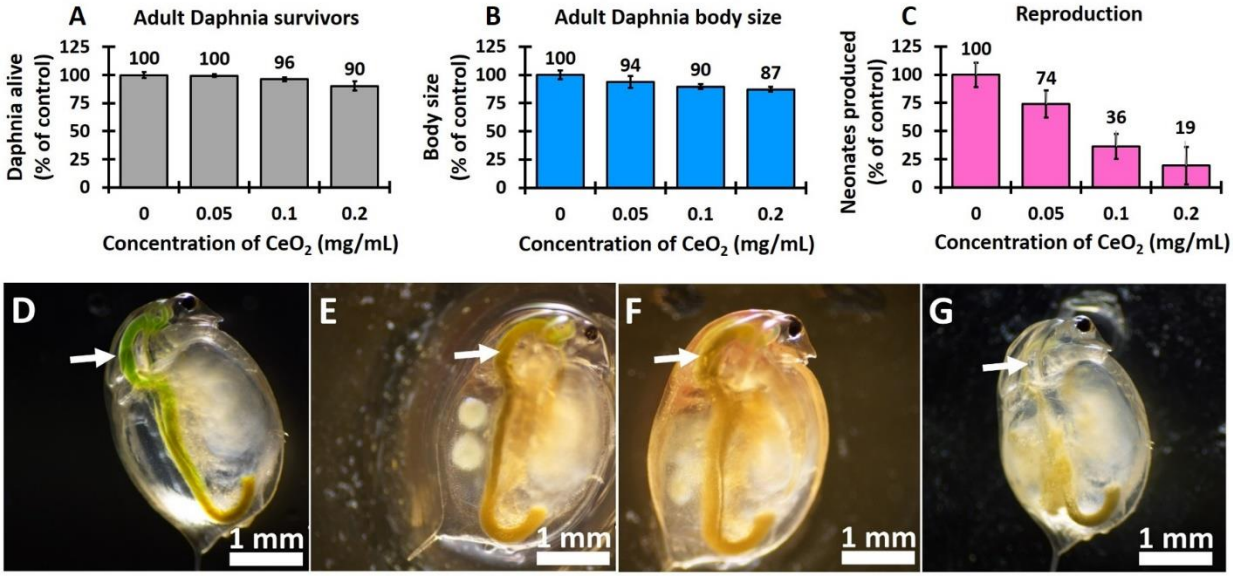

Figure 2.5. Chronic morbidity and effects on *D. magna* size and reproduction after a 21-day exposure to the model CeO_2 CMP slurry. **(A)** Percent survival, **(B)** body size, and **(C)** reproductive output of adult *D. magna* exposed to various concentrations of the CeO_2 CMP slurry measured after 21 d and plotted relative to control *D. magna* not exposed to a CMP slurry (set to 100%). The numbers above the bars are the mean of three independent trials and the error bars represent the SEM. Representative optical images (3× magnification) after 21 d showing *D. magna* sizes and morphologies for: **(D)** control *D. magna* not exposed a CMP slurry; **(E)** *D. magna* exposed to 0.05 mg/mL of the CeO_2 CMP slurry; (F) *D. magna* exposed to 0.10 mg/mL of the CeO_2 CMP slurry; and, **(G)** *D. magna* exposed to 0.20 mg/mL of the CeO_2 CMP slurry. The white arrows denote the midgut region.

Figure 2.6 shows the morbidity, body size, and reproductive output of *D. magna* exposed to various concentrations of the Al_2O_3 CMP slurry. A modest degree of morbidity (20%) and a significant decrease in body size (27%) was observed relative to controls after a 21-d exposure to the highest applied NP dose of 0.1 mg/mL (Figures 2.6A and 2.6B). Additionally as shown in Figure A5, the reduction in body size was apparent early (starting at day 4) for *D. magna* exposed to 0.1 mg/mL of the Al_2O_3 CMP slurry. Furthermore, there was a severe dose-dependent decrease in the reproductive output of *D. magna*; specifically, the number of offspring produced by *D. magna* exposed for 21 d to the highest applied dose of the Al_2O_3 CMP slurry (0.1 mg/mL) d was

45% less compared to controls (Figure 2.6C). Moreover, the digestive tracts of *D. magna* exposed to Al_2O_3 CMP slurries appeared cream colored (Figures 2.6E–G), similar to those of *D. magna* exposed to CeO_2 CMP slurries (Figures 2.5E–G), and lacked the green coloration that was observed in control *D. magna* (Figure 2.6D). Finally, as shown in Figure A6, the reproductive activity for *D. magna* exposed to Al_2O_3 CMP slurry was also delayed compared to controls. Overall, this suggests that long-term exposure to the model Al_2O_3 CMP slurry at a concentration that has a modest effect on morbidity and growth may adversely impact *D. magna* populations by also reducing reproduction.

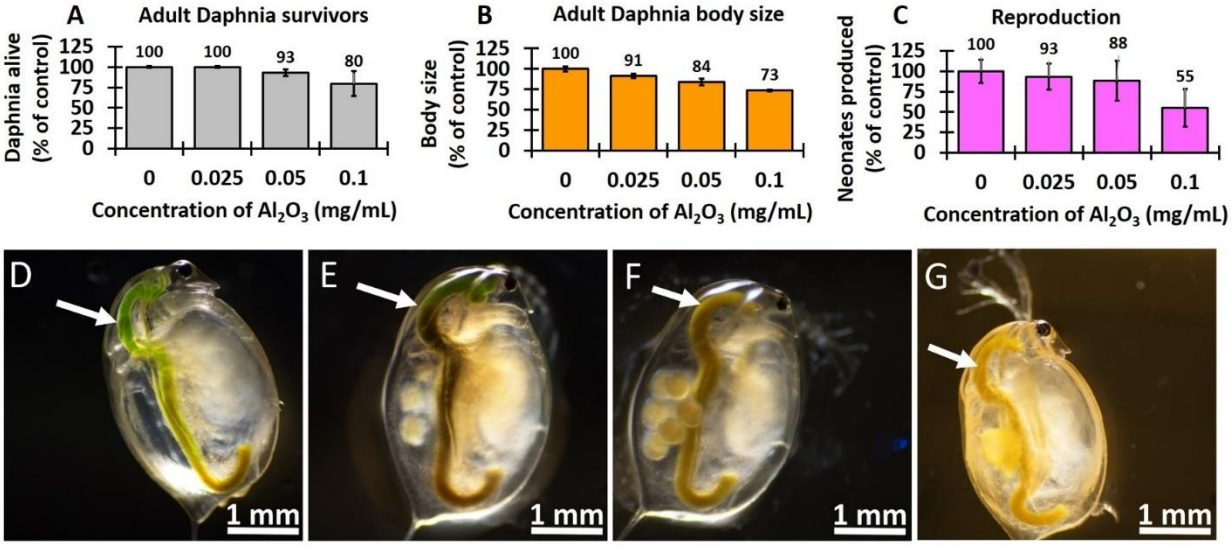

Figure 2.6. Chronic morbidity and effects on *Daphnia magna* size and reproduction after a 21-day exposure to the model Al_2O_3 CMP slurry. **(A)** Percent survival, **(B)** body size, and **(C)** reproductive output of adult *D. magna* exposed to various concentrations of the Al_2O_3 CMP slurry measured after 21 d and plotted relative to control *D. magna* not exposed to a CMP slurry (set to 100%). The numbers above the bars are the mean of three independent trials and the error bars represent the SEM. Representative optical images (3× magnification) after 21 d showing *D. magna* sizes and morphologies for: **(D)** control *D. magna* not exposed to CMP slurry; **(E)** *D. magna* exposed to 0.025 mg/mL of the Al_2O_3 CMP slurry; **(F)** *D. magna* exposed to 0.050 mg/mL of the Al_2O_3 CMP slurry; and, **(G)** *D. magna* exposed to 0.100 mg/mL of the Al_2O_3 CMP slurry. The white arrows denote the midgut region.

2.6 DISCUSSION

A number of works have studied the acute and chronic effects of SiO_2, CeO_2, and Al_2O_3 NPs on *D. magna* (detailed below), and the key effectors reported for these metal oxide NPs have been the primary sizes of individual NPs, aggregation state of the NPs, and their surface chemistries and coatings.[13,45,54,64] Nonetheless, direct comparisons of works are hampered by differences in experimental arrangements and test conditions such as applied NP doses, exposure times, and incomplete NP characterizations, most notably, NP aggregation states in the test medium used.

2.6.1 Acute effects of SiO_2 NPs on Daphnia magna morbidity and body size

Three groups have reported acute effects of SiO_2 NPs suspended in water on *D. magna* although only one group clearly denoted whether *c*-SiO_2 or *f*-SiO_2 NPs were used. Specifically, Clement et al. studied the acute toxicity of 14 nm *f*-SiO_2 NPs and reported a calculated 72-h EC-50 of 0.0297 mg/mL with *D. magna*.[65] In studies conducted by Xu and co-workers, a dose-dependent acute toxicity of nano-sized (10–20 nm) SiO_2 NPs was reported with a calculated 24-h LC-50 of 0.661 mg/mL, whereas micron-sized (5–10 µm SiO_2 particles) showed no adverse effects on *D. magna* morbidity.[66,67] Finally, Lee et al. assessed the acute toxicity of nano-sized SiO_2 and observed slight increases in *D. magna* morbidity (15% and 10% for 7 nm and 10 nm SiO_2 NPs, respectively) following a 96-h exposure to an applied NP dose of 0.001 mg/mL.[68] In general, it appears that only SiO_2 NPs with primary particle sizes $\leq$20 nm pose adverse effects on *D. magna* morbidity. The primary particle sizes of the *c*-SiO_2 and *f*-SiO_2 NPs in the model slurries used in the current study ranged from 50–60 nm and 120–140 nm, respectively, and we did not observe significant *D. magna* morbidity after a 96-h exposure with either slurry, even at high,

environmentally-irrelevant applied NP doses of 4.0 mg/mL (*c*-SiO_2; Figure 2.1A) or 5.0 mg/mL (*f*-SiO_2; Figure 2.1B). In conclusion, both model silica CMP slurries can be classified as "practically nontoxic" (i.e., showing no adverse effects at levels ≤0.1 mg/mL) according to the EPA's ecotoxicity classification for aquatic organisms under acute morbidity testing conditions.[69]

In addition to mortality, Lee et al. also used body size as an endpoint and observed that *D. magna* exposed to 7 nm and 10 nm SiO_2 NPs were not significantly different in size relative to controls after 96 h.[68] This was similar to our observations with the *f*-SiO_2 CMP slurry, where there were no differences in body sizes compared to controls (Figures 2.1B and 2.2C). However, this was contrary to our observations with the *c*-SiO_2 CMP slurry (Figures 2.1A and 2.2B), where body sizes increased modestly by 17–24% compared to controls, which is indicative of a hormetic process by the organism to adapt to a moderate environmental stress.

2.6.2 Chronic effects of SiO_2 NPs on Daphnia magna morbidity, body size, and reproductive output

Two studies have previously reported chronic effects of SiO_2 NPs on *D. magna*. Lee et al. studied the chronic toxicity of 7 nm and 10 nm SiO_2 NPs to *D. magna* and reported no effects on reproductive output or DNA damage,[68] while Lillicrap et al. studied 150 nm *f*-SiO_2 NPs and reported no morbidity up to an applied NP dose of 0.1 mg/mL, a slight dose-dependent increase in reproductive output and a slight dose-dependent increase in the lengths of *D. magna* (which they attributed to a positive hormetic response).[70] In our chronic toxicity assessment of the model *c*-SiO_2 and *f*-SiO_2 CMP slurries, both at an applied dose of 0.1 mg/mL, we observed slight morbidity (10% and 13%) and a slight increase in body size (10% and 10%), respectively, after a 21-d exposure relative to controls (Figure A3). We also observed a increases in reproductive output

(18% and 10%) with the *c*-SiO_2 and *f*-SiO_2 CMP slurries, respectively, relative to controls (Figure A3). It is therefore interesting to note that our chronic toxicity observations with the model *c*-SiO_2 and *f*-SiO_2 CMP slurries are similar to those reported by Lillicrap et al.[70] In summary, *D. magna* were not adversely affected by chronic exposure to either of the two model SiO_2 slurries, instead, an adaptation mechanism in response to the presence of the model silica slurries can lead to a *D. magna* population with higher reproduction and larger body sizes.

2.6.3 Acute effects of CeO_2 NPs on Daphnia magna morbidity and body size

A number of studies have previously reported the acute effects of CeO_2 NPs on *D. magna*. Gaiser et al. reported that 25 nm CeO_2 NPs were not toxic to *D. magna* at an applied dose of 0.010 mg/mL and minor adverse effects were observed only at high, environmentally-irrelevant concentrations.[43,72,73] Manier et al. also studied 25 nm CeO_2 NPs and reported insignificant morbidity at concentrations up to 1.0 mg/mL.[74] Similarly, Van Hoecke et al. studied the acute toxicity of 14 nm, 20 nm, and 29 nm CeO_2 NPs at an applied NP dose of 1.0 mg/mL and reported insignificant morbidity with *D. magna*.[75] On the other hand, Lee et al. reported 10% morbidity to *D. magna* with 15 nm CeO_2 NPs and insignificant morbidity with 30 nm CeO_2 NPs at an applied NP dose of 0.001 mg/mL.[68] So, while there is not a total consensus with respect to the acute morbidity of 14–30 nm CeO_2 NPs to *D. magna*, in all cases, there were no severe effects reported. On the contrary, Garcia et al. studied a smaller CeO_2 NP, with a primary particle size of 6.5 nm, and reported a calculated 48-h LC-50 of 0.012 mg/mL with *D. magna*.[38] Interestingly, we observed no morbidity to *D. magna* at applied NP doses up to 2.0 mg/mL (Figure 2.1C) with the model CMP slurry which contain CeO_2 NPs with a primary particle size of 60–100 nm. In conclusion, according to the EPA's ecotoxicity classification for aquatic organisms, the model

ceria CMP slurry can also be classified as "practically nontoxic" under acute morbidity testing conditions.[69]

In contrast to the morbidity results, a severe dose-dependent decrease in *D. magna* body size was observed with the model CeO_2 CMP slurry where the body sizes of *D. magna* exposed to 2.0 mg/mL of CeO_2 CMP NPs for 96 h were 46% smaller compared to controls (Figures 2.1C and 2.2D). In addition, *D. magna* treated with the model CeO_2 CMP slurry showed an absence of green algae nutrients in their smaller digestive tracts (Figure 2.2D), and the reduced body sizes of *D. magna* exposed to the model CeO_2 CMP slurry was similar to those of starved *D. magna*. This suggests that the ingested CeO_2 NPs may have restricted subsequent food consumption, which in turn lead to impaired growth. Indeed, similar effects of CeO_2 NPs on *D. magna* body size was previously reported by Gaiser et al. where growth inhibition caused by aggregated CeO_2 NPs (with a primary NP size of 25 nm) at applied NP doses up to 0.01 mg/mL was observed.[43,73] In addition, they noted an absence of green algae in the digestive tracts of CeO_2-treated *D. magna*, which was believed to be indicative of reduced feeding, increased metabolism, and/or excretion rates.[43] Furthermore, they hypothesized that adverse effects observed at high applied doses of CeO_2 NPs were caused by the physical presence of the NPs in the digestive tract that inhibited feeding and movement and that overwhelmed the organism. It is therefore reasonable to assume that highly-aggregated CeO_2 CMP NPs consumed by *D. magna* accumulated in the digestive tract, and that this impaired the consumption of nutrients, which in turn led to a reduction in growth, as evidenced by their smaller body sizes compared to controls.

2.6.4 Chronic effects of CeO_2 NPs on Daphnia magna morbidity, body size, and reproductive output

Two studies have previously reported chronic effects of CeO_2 NPs on *D. magna*. Van Hoecke et al. studied the chronic exposure of various sized CeO_2 NPs and reported that all *D. magna* died when exposed to 0.056 mg/mL of 14 nm CeO_2 NPs or 0.100 mg/mL of 20 nm CeO_2 NPs.[75] Additionally, reduced growth of *D. magna* was observed with 14 nm, 20 nm, or 29 nm CeO_2 NPs at 1.0 mg/mL, and it was suggested that the stunted growth was due to a reduced intake of food since algae and CeO_2 NPs formed aggregates, which depleted the amount of food available for *D. magna* to eat.[75] Moreover, the calculated EC-50 values for reproductive output were reported to be 0.021, 0.025, and 0.043 mg/mL for CeO_2 NPs with sizes of 14, 20, and 29 nm, respectively, versus 0.182 mg/mL for bulk CeO_2.[75] Conversely, Lee et al. studied the chronic exposure of 15 nm and 30 nm CeO_2 NPs and reported no significant effects on growth and reproductive output, however significant DNA damage was observed.[68] The lack of complete agreement of these reports has been addressed by Collin et al. who concluded in a recent review that the high variability in toxicity results for similarly-sized CeO_2 NPs could be because *D. magna* are not particularly sensitive to CeO_2 NPs relative to other aquatic organisms.[71] In our work, there was slight morbidity (10%) and a slight decrease in body size (13%) after a 21-d exposure to the highest applied dose of 0.2 mg/mL of the CeO_2 CMP slurry relative to controls (Figures 2.5A and 2.5B), which were similar to the observations of Lee et al.[68] However, reproductive output was decreased severely by 81% after a 21-d exposure to 0.2 mg/mL of the CeO_2 CMP slurry relative to controls (Figure 2.5C), which was akin to the observations of Van Hoecke et al.[75] In conclusion, the severely reduced capability in reproduction observed with *D. magna* exposed to CeO_2 CMP

NPs demonstrates an environmental concern of this model CMP slurry towards the *D. magna* population in an aquatic ecosystem.

2.6.5 Acute effects of Al_2O_3 NPs on Daphnia magna morbidity and body size

The effects of micron-sized and nano-sized Al_2O_3 NPs on *D. magna* was previously investigated by Zhu et al. who reported that while micron-sized Al_2O_3 was not toxic to *D. magna*, 80-nm Al_2O_3 NPs were toxic to *D. magna* with a calculated 48-h LC-50 of 0.162 mg/mL.[34] Furthermore, they noted the adherence and accumulation of Al_2O_3 NPs in *D. magna* bodies and concluded that the observed morbidity associated with high applied doses of aggregated Al_2O_3 NPs was caused by the physical presence of the NPs, which inhibited movement, caused mechanical disruption of *D. magna* feeding appendages, and basically overwhelmed the organism.[34] In our work, the Al_2O_3 CMP slurry had a severe acute effect on *D. magna* morbidity with a calculated 72-h LC-50 of 2.6 ± 0.2 mg/mL and a calculated 96-h LC-50 of 1.1 ± 0.2 mg/mL (Figure 2.1D). Nonetheless, even with the fairly high applied doses of highly-aggregated Al_2O_3 NPs, the model Al_2O_3 CMP slurry can also be classified as "practically nontoxic" under acute morbidity testing conditions according to the EPA's ecotoxicity classification for aquatic organisms.[69]

In addition to acute morbidity, a severe dose-dependent decrease in *D. magna* body size was also detected under acute test conditions with the Al_2O_3 CMP slurry; specifically, the body sizes of *D. magna* exposed to 1.5 mg/mL of Al_2O_3 CMP slurry for 96 h were 53% smaller compared to controls (Figures 2.1D and 2.2E) and green algae nutrients were not observed in their smaller digestive tracts (Figure 2.2E). To investigate the possible underlying cause of this effect, a series of experiments were conducted and the results provided support to the hypothesis that the

presence of Al_2O_3 NPs in the digestive tracts of *D. magna* impaired the organism's ability to take up nutrients such as algae. First, starved *D. magna*, were shown to have similar smaller body sizes to fed *D. magna* that were treated with the Al_2O_3 CMP slurry (Figure A2); second, this problem was not averted by the addition of extra food (Figure 2.3); and third, as demonstrated by the inability to take up blue polystyrene beads, the digestive tracts of *D. magna* pre-exposed to the Al_2O_3 CMP slurry were clogged with Al_2O_3 NPs (Figure 2.4). Therefore, a logical explanation is that highly-aggregated Al_2O_3 NPs in *D. magna* digestive tracts impaired the organism's ability to take up nutrients such as algae, which could be responsible in-part for the severe morbidity and reduction in growth observed with *D. magna* by the model Al_2O_3 CMP slurry under acute test conditions.

2.6.6 Chronic effects of Al_2O_3 NPs on Daphnia magna morbidity, body size, and reproductive output

To our knowledge, there have not been any reports concerning the chronic effects of Al_2O_3 NPs on *D. magna*. We found, for the first time, a modest degree of chronic morbidity (20%) and a significant decrease in body size (27%) after a 21-d exposure to the highest applied dose of 0.1 mg/mL of the Al_2O_3 CMP slurry relative to controls (Figures 2.6A and 2.6B). Most importantly, a severe dose-dependent decrease in reproductive output was detected in *D. magna* exposed to the Al_2O_3 CMP slurry for 21 d; specifically, reproductive output was decreased by 45% upon exposure to the highest applied dose of 0.1 mg/mL of the Al_2O_3 CMP slurry relative to controls (Figure 2.6C). In conclusion, similar to the chronic exposure results with the model CeO_2 slurry, the severely reduced capability in reproduction observed in *D. magna* exposed to the model Al_2O_3

slurry causes a long-term environmental concern towards the *D. magna* population in an aquatic ecosystem.

2.7 CONCLUSIONS

The four model CMP slurries used in this *D. magna* ecotoxicity study are unique in that they represent the simplest formulations to generate stable aqueous suspensions of four metal oxide NPs that are actually used in complex proprietary commercial slurries. Herein, we studied, for the first time, the toxicity of these four model CMP slurries to *D. magna*. In the acute 4-d toxicity assessments, the key findings were that the Al_2O_3 slurry was lethal with a calculated 96-h LC-50 of 1.1 mg/mL, and that the slurries with highly-aggregated NPs (i.e., the CeO_2 and Al_2O_3 slurries) caused a severe dose-dependent decrease in body sizes. Interestingly, this contrasted a modest increase in body size observed with the slurry comprising the least-aggregated NPs (i.e., the *c*-SiO_2 slurry), which is indicative of a positive hormetic process. In the chronic 21-d toxicity assessments with non-lethal NP doses, the key findings were that the Al_2O_3 slurry lead to a modest increase in morbidity and a significant decrease in body size, and that the CeO_2 and Al_2O_3 CMP slurries caused a severe dose-dependent decrease in reproductive output. Interestingly, this contrasted a modest increase in reproduction observed with the *c*-SiO_2 CMP slurry, which was credited to a positive hormetic response. In conclusion, distinct and unpredictable adverse effects were observed with different model CMP slurries on *D. magna* morbidity, growth, and reproductive output. Especially important is that the CeO_2 and Al_2O_3 CMP slurries severely reduced *D. magna* reproduction upon chronic exposure at low applied doses, which could have adverse consequences to aquatic ecosystems and warrants further study.

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APPENDIX A

Table A1. Chemical and physical properties of pristine model CMP slurries provided by the manufacturer.

	Colloidal Silica (*c*-SiO_2)	Fumed Silica (*f*-SiO_2)	Ceria (CeO_2)	Alumina (Al_2O_3)
Additive	<1% acetic acid	<1% KOH	none	<1% nitric acid
pH	2.5–4.5	10.0	3.0–4.0	4.5–5.0
Composition	3% SiO_2	5% SiO_2	1% CeO_2	3% Al_2O_3
Particle size (nm)	50–60	120–140	60–100	80–100

Table A2. Summary of conditions for acute and chronic toxicity tests with *Daphnia magna*.

Test parameter	Conditions	
Test type	Acute toxicity assessment, Static non-renewal	Chronic toxicity assessment, Static renewal
Test duration	4 d	21 d
Temperature	20 ± 1°C	20 ± 1°C
Photoperiod	16-h light / 8-h dark	16-h light / 8-h dark
Test chamber size	10 mL	250 mL
Test solution volume	8 mL	100 mL
Age of test organisms	<24-h old	<24-h old
Organisms/test chamber	5	10
Replicate test chambers	6	3
Number of organisms/trial	30	30
Feeding regime	48 µL of YCT and 32 µL of Pseudokirchnerilla subcapitata (1.1×10^6 cells)	500 µL of YCT and 2 mL of Pseudokirchnerilla subcapitata (7.0×10^7 cells)
Renewal of test solutions	Not required	After 96 h
Test chamber cleaning	Not required	After 96 h
End points	Morbidity and body size	Morbidity, body size, and reproductive output
Test acceptability	≥90% survival of controls	≥80% survival of controls and no ephippium (resting eggs) in controls

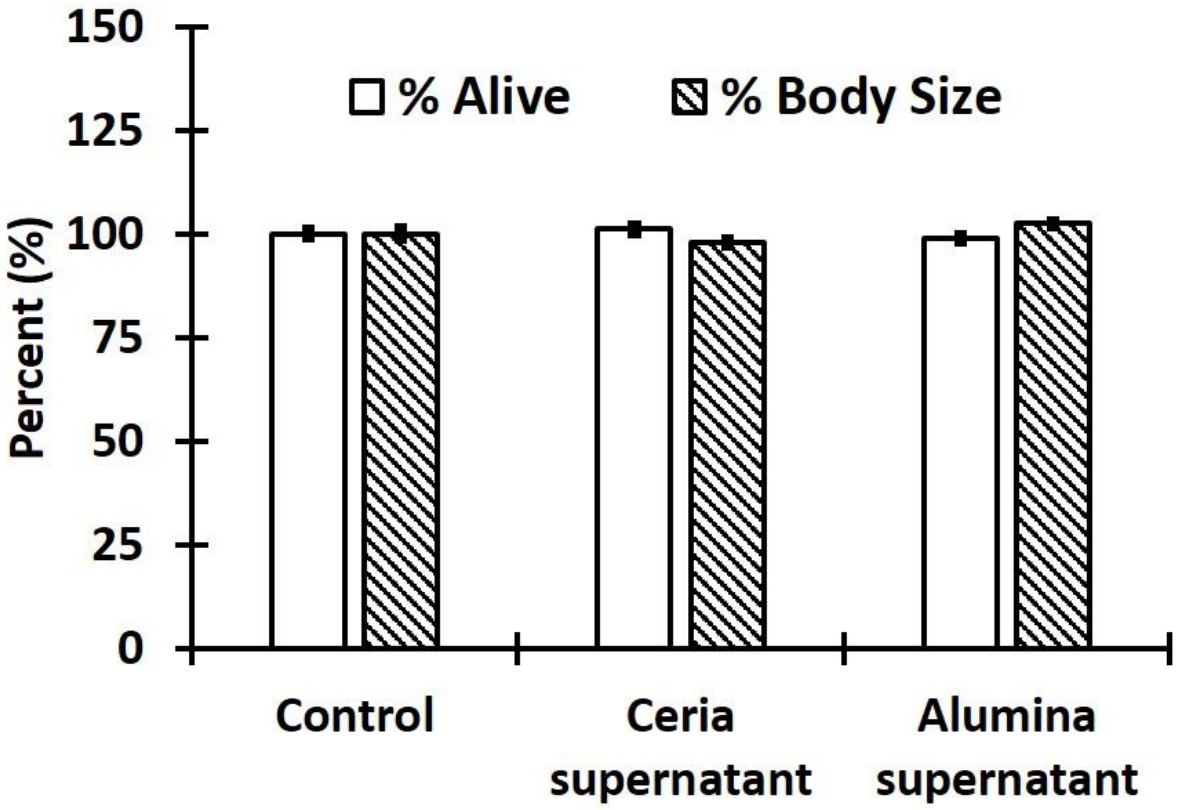

Figure A1. Acute morbidity and effects on *D. magna* size after a 96-h exposure to the supernatant collected from a 2.0-mg/mL model CeO_2 CMP slurry or a 3.0-mg/mL model Al_2O_3 CMP slurry. Clear bars: Percent survival of control *D. magna* not exposed to a CMP slurry supernatant (set to 100%), *D. magna* exposed to the CeO_2 CMP slurry supernatant, or *D. magna* exposed to the Al_2O_3 CMP slurry supernatant. Patterned bars: Percent body sizes of control *D. magna* not exposed to a CMP slurry supernatant (set to 100%), *D. magna* exposed to the CeO_2 CMP slurry supernatant, or *D. magna* exposed to the Al_2O_3 CMP slurry supernatant. Each data point is the mean of at least three independent trials and the error bars represent the standard error of the mean (SEM).

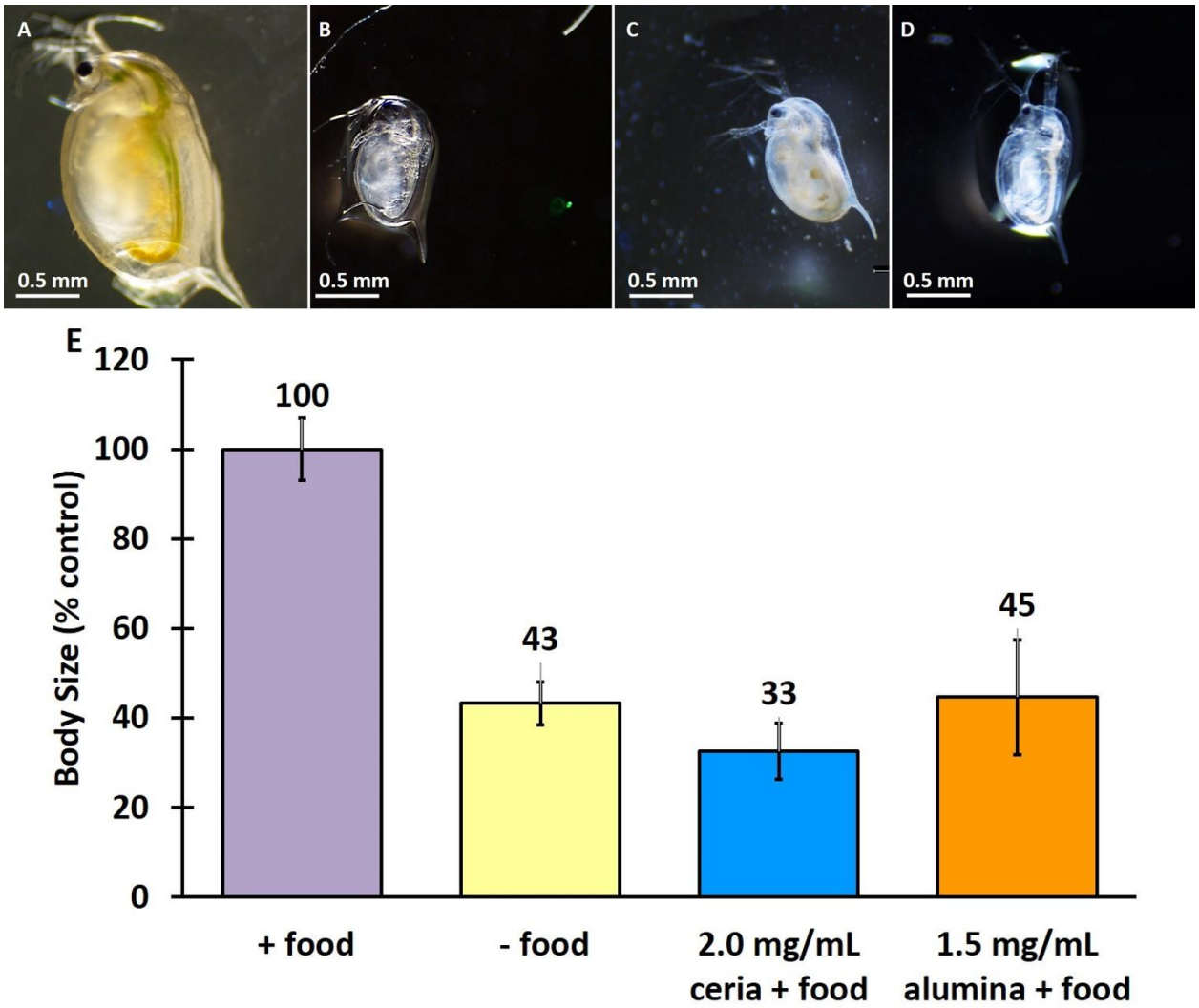

Figure A2. Sizes of starved *D. magna* are similar to those that are fed and exposed to model CeO_2 or Al_2O_3 CMP slurries for 72 h. Representative optical images (5× magnification) of: (A) control *D. magna* that were fed but not exposed to a CMP slurry; (B) *D. magna* that were starved but not exposed to a CMP slurry; (C) *D. magna* that were fed and exposed to 2.0 mg/mL of the CeO_2 CMP slurry; and, (D) *D. magna* that were fed and exposed to 1.5 mg/mL of the Al_2O_3 CMP slurry. (E) Summary plot where the body sizes of the starved *D. magna* and fed *D. magna* exposed to the CeO_2 or Al_2O_3 CMP slurries are shown relative to control *D. magna* that were fed but not exposed to a CMP slurry (set to 100%). The numbers above the bars represent the mean of at least three independent trials and the error bars represent the SEM.

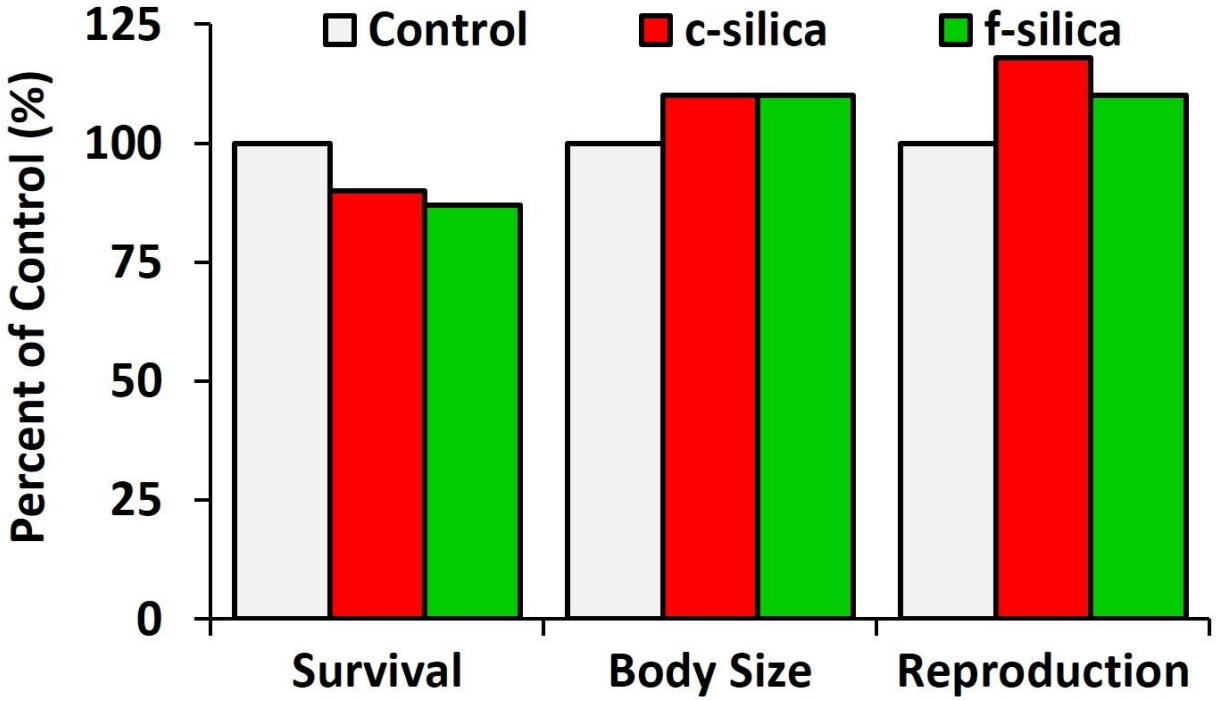

Figure A3. Chronic morbidity and effects on *D. magna* size and reproduction after a 21-d exposure to the model *c*-SiO_2 or *f*-SiO_2 CMP slurries. Percent survival, body size, and reproductive output of adult *D. magna* exposed to 0.1 mg/mL of either the *c*-SiO_2 (red bars) or the *f*-SiO_2 (green bars) CMP slurry for 21 d are plotted relative to the control *D. magna* that were not exposed to a CMP slurry (set to 100%; clear bars). The data for each endpoint are from two independent trials.

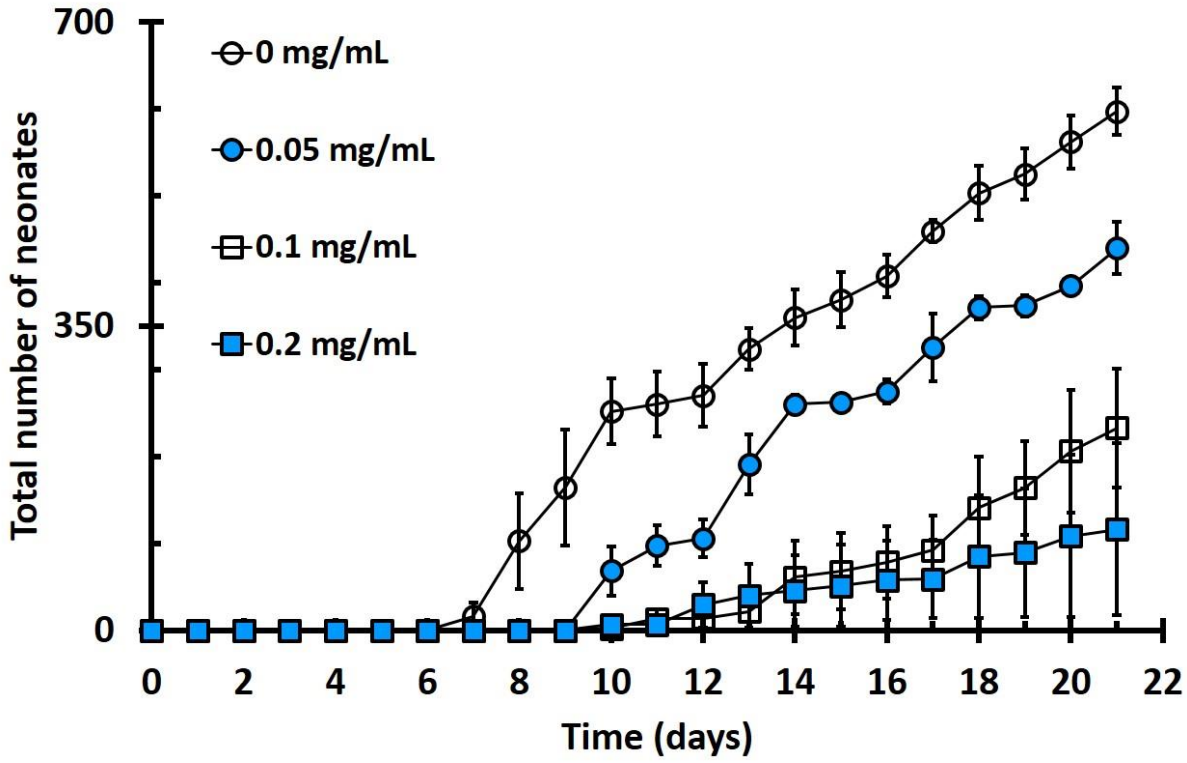

Figure A4. Chronic effects on *D. magna* reproduction during a 21-d exposure to various concentrations of the model CeO_2 CMP slurry. Clear circles show the reproductive output of control *D. magna* not exposed to a CMP slurry; blue circles show the reproductive output of *D. magna* exposed to 0.05 mg/mL of the CeO_2 CMP slurry; clear squares show the reproductive output of *D. magna* exposed to 0.10 mg/mL of the CeO_2 CMP slurry; and, blue squares show the reproductive output of *D. magna* exposed to 0.20 mg/mL of the CeO_2 CMP slurry. All data points are the mean of at least three independent trials and the error bars represent the SEM.

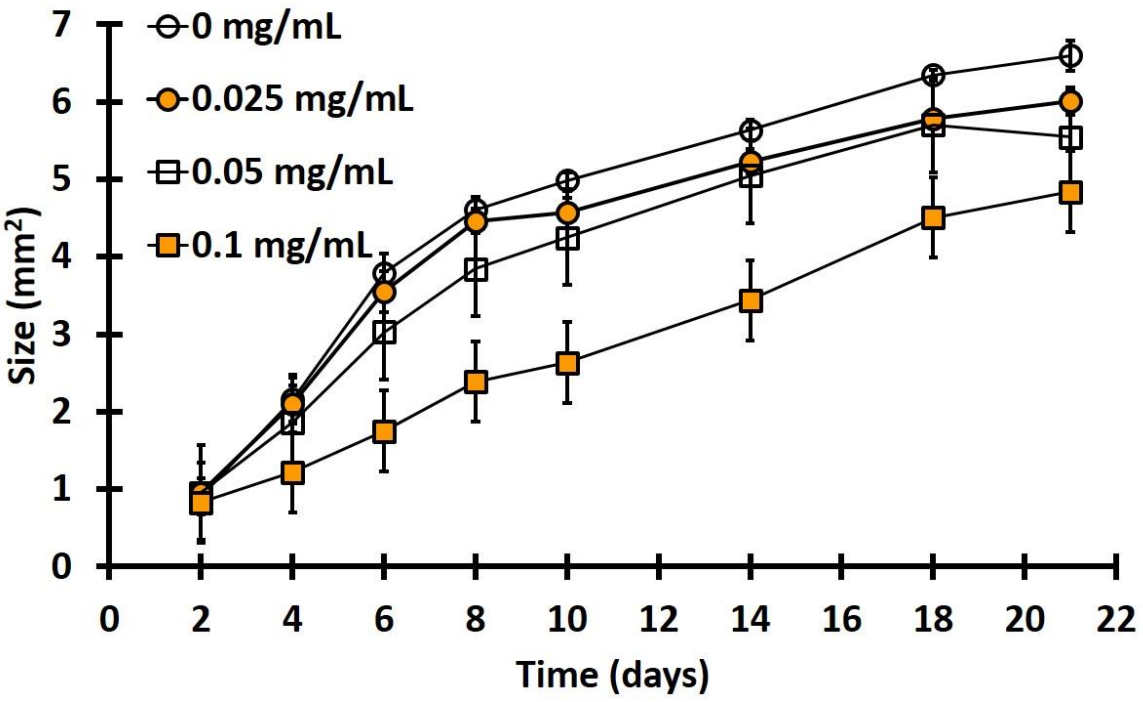

Figure A5. Chronic effects on *D. magna* size during a 21-d exposure to various concentrations of the model Al_2O_3 CMP slurry. Control *D. magna* not exposed to a CMP slurry (clear circles) reached a mean body size of 6.6 ± 0.2 mm^2 by day 21. *D. magna* exposed to 0.025 mg/mL of the Al_2O_3 CMP slurry (orange circles) reached a mean body size of 6.0 ± 0.2 mm^2 by day 21. *D. magna* exposed to 0.05 mg/mL of the Al_2O_3 CMP slurry (clear squares) reached a mean body size of 5.5 ± 0.3 mm^2 by day 21. *D. magna* exposed to 0.1 mg/mL (orange squares) reached a mean body size of 4.8 ± 0.0 mm^2 by day 21. All data points are the mean of at least three independent trials and the error bars represent the SEM.

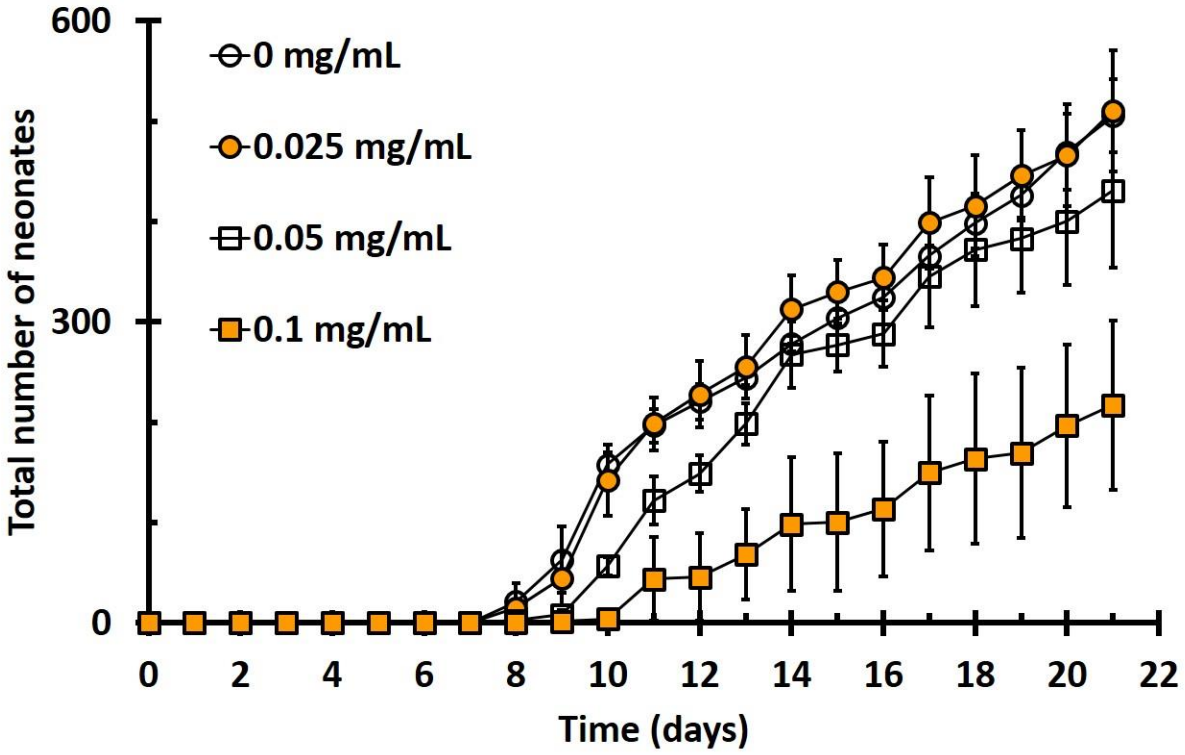

Figure A6. Chronic effects on *D. magna* reproduction during a 21-d exposure to various concentrations of the model Al_2O_3 CMP slurry. Clear circles show the reproductive output of control *D. magna* not exposed to a CMP slurry; orange circles show the reproductive output of *D. magna* exposed to 0.025 mg/mL of the Al_2O_3 CMP slurry; clear squares show the reproductive output of *D. magna* exposed to 0.50 mg/mL of the Al_2O_3 CMP slurry; and, orange squares show the reproductive output of *D. magna* exposed to 0.10 mg/mL of the Al_2O_3 CMP slurry. All data points are the mean of at least three independent trials and the error bars represent the SEM.

CHAPTER 3

BIOACCUMULATION AND BIOPERSISTENCE OF CERIA AND ALUMINA FROM MODEL CHEMICAL MECHANICAL PLANARIZATION SLURRIES IN *DAPHNIA MAGNA*

3.1 ABSTRACT

The purpose of this study was to determine the bioaccumulation and biopersistence in *Daphnia magna* (*D. magna*) of model nanoparticle (NP) slurries containing either ceria (CeO_2) or alumina (Al_2O_3). The semiconductor manufacturing industry uses these NP slurries in the chemical mechanical planarization (CMP) process to polish wafer surfaces. Semiconductor manufacturers generate high volumes of the slurries and are interested in the impact they might have on aquatic organisms. In the present study, *D. magna* were exposed to a CeO_2 or Al_2O_3 model CMP slurry at 0.01 mg/mL and 0.10 mg/mL for various time points. The key findings were that both CeO_2 and Al_2O_3 were ingested by *D. magna* in a time dependent and dose dependent manner. *D. magna* that were exposed for 48 h to 0.10 mg/mL CeO_2 CMP slurry accumulated 120 µg of CeO_2, and those that were exposed for 48 h to 0.10 mg/mL of Al_2O_3 CMP slurry accumulated 44 µg of Al_2O_3. This shows that different metal oxide NPs in slurries are accumulated to different amounts by *D. magna*. In addition, biopersistence studies revealed that after 48 h of depuration time, *D. magna* exposed to 0.10 mg/mL CeO_2 successfully eliminated 85% of the initial CeO_2 load, and *D. magna* exposed to 0.10 mg/mL of Al_2O_3 eliminated 78% of the initial Al_2O_3 load.

3.2 INTRODUCTION

The process of manufacturing a layered integrated circuit (IC) used in data storage and memory chips requires several complex steps whereby materials are deposited and selectively removed from a semiconducting wafer.[1] One of the processes involved in IC manufacturing is called chemical mechanical planarization (CMP) in which a semiconducting wafer is polished to high tolerances and excess material is removed. The CMP process uses a slurry containing abrasive nano-particles (NPs) such as cerium oxide (CeO_2), aluminum oxide (Al_2O_3), and silicon oxide (SiO_2). Large amounts of water are used to rinse the wafer and slurry components from the wafer following CMP processes.[2] NP slurries can be discharged in waste water streams where they may potentially enter and affect the aquatic ecosystem. However, CMP NP assessments can be confounded by slurry constituents that are known to be toxic such as oxidants, surfactants, biocides and corrosion inhibitors. In response to this challenge, a major CMP slurry manufacturer produced model pristine CMP slurries containing either colloidal silica (*c*-SiO_2), fumed silica (*f*-SiO_2), ceria (CeO_2) or alumina (Al_2O_3) NPs that did not contain toxic additives, which facilitates toxicity, bioaccumulation, and biopersistence assessments of actual NPs used in commercial CMP slurry formulations.

Previously we reported acute and chronic toxicity of *c*-SiO_2, *f*-SiO_2 CeO_2 and Al_2O_3 slurries on *D. magna*. For acute toxicity studies, the *c*-SiO_2 slurry (up to 4 mg/mL) and the *f*-SiO_2 slurry (up to 5 mg/mL) were not lethal to *D. magna* and did not reduce body sizes. The CeO_2 slurry showed no morbidity up to 2 mg/mL; however, by 96 h of exposure time, *D. magna* body sizes decreased by 46% compared to controls. The Al_2O_3 slurry showed acute lethal toxicity to *D. magna* with a 96-h LC-50 of 1.1 mg/mL. Moreover the body sizes of *D. magna* exposed to 1.5

mg/mL of Al_2O_3 slurry decreased by nearly 53%. In chronic toxicity assays, the *c*-SiO_2 and *f*-SiO_2 at 0.1 mg/mL did not cause a significant mortality, did not reduce body size, and did not compromise *D. magna* reproductive output over a 21-day exposure. Chronic exposure to CeO_2 slurry at 0.1 mg/mL did not cause significant mortality but body size was reduced slightly by 10% and reproduction was reduced by 64%. The most significant adverse impacts were observed on *D. magna* with chronic exposure to Al_2O_3 at 0.1 mg/mL, where 20% mortality, 27% reduction in body size, and a 45 % decrease in *D. magna* reproductive capacity. These results prompted us to investigate the bioaccumulation and biopersistence in *D. magna* of the CeO_2 and Al_2O_3 CMP slurries since they had lethal and sub lethal effects on *D. magna*. Several studies have documented the bioaccumulations and biopersistence in *D. magna* of nano materials such as titanium dioxide, gold, silver, carbon nanotubes, fullerene, graphene, and quantum dots.[3-9] However, we are not aware of reports on the accumulation of CeO_2 and Al_2O_3 NP by *D. magna*.

In the present study, *D. magna* were exposed to CeO_2 or Al_2O_3 CMP slurry at 0.01 mg/mL and 0.1 mg/mL for various time points. The key findings were that both CeO_2 and Al_2O_3 were ingested by *daphnia* in a time dependent and dose dependent manner. *D. magna* that were exposed for 48 h to 0.1 mg/mL CeO_2 CMP slurry accumulated 120 μg of CeO_2, and those that were exposed to for 48 h to 0.1 mg/mL of Al_2O_3 CMP slurry accumulated 44 μg of Al_2O_3. This shows that different metal oxide slurries are accumulated in different amounts by *D. magna*. In addition biopersistence studies revealed that after 48 h of depuration time, *D. magna* exposed to 0.1 mg/mL CeO_2 successfully eliminated 85% of the initial CeO_2 load, and *D. magna* exposed to 0.1 mg/mL of Al_2O_3 were able to eliminate 78% of the initial Al_2O_3 load.

3.3 EXPERIMENTAL

3.3.1 Materials and solutions

The pristine model CeO_2 and Al_2O_3 CMP slurries were custom-synthesized without any proprietary chemical additives by a major CMP slurry manufacturer who provided them to the Semiconductor Research Corporation (SRC) Engineering Research Center for Environmentally Benign Semiconductor Manufacturing (ERC). Thorough characterization of pristine model CeO_2 and Al_2O_3 CMP slurries was published previously.[2] The hydrodynamic sizes and zeta potentials of CeO_2 and Al_2O_3 in pH 7.4 *daphnia* media were presented in Karimi et al.[3] *Daphnia* medium (i.e., moderately hard reconstituted water) was made in accordance with U.S. Environmental Protection Agency (EPA) guidelines by mixing: 192 mg/L $NaHCO_3$, 120 mg/L $CaSO_4 \cdot 2H_2O$, 120 mg/L $MgSO_4$, and 8 mg/L KCl in well-aerated deionized water.[11] The hardness of *daphnia* medium was maintained between 250–425 mg/L $CaCO_3$ as measured using a HACH® aqua check water quality test strip. The pH was maintained between 7.4–8.4 as measured using a Fisher Scientific model 25 pH/ion meter and an Accumet® combination Ag/AgCl reference pH electrode. Dissolved oxygen was maintained between 8–10 mg/L as measured using a colorimetric method (CHEMets® kit K-7512; Midland, VA). *Daphnia* food comprising yeast, CEROPHYLL®, and Troutchow (YCT) and *Pseudokirchnerilla subcapitata* was purchased from Marinco Bioassay Laboratory, Inc. (Sarasota, FL). Deionized water (18.2 MΩ-cm) was obtained using a Milli-Q® Advantage A10 water purification system (Millipore; Billerica, MA). Trace-metal grade nitric acid (69%) and hydrochloric acid (37%) were purchased from Fisher Scientific (Hampton, NH), and Inductively coupled mass spectrometer (ICP-MS) calibration standard and internal standards were purchased from Inorganic Ventures (Christiansburg, VA).

3.3.2 Culturing D. magna for bioaccumulation and biopersistence assays

The *D. magna* neonates less than 24-h old were placed in 100 mL of *daphnia* media in a 250-mL polypropylene beaker, 10 per beaker. Food was 0.5 mL of YCT and 2 mL of algae added every other day for 10 days. After 4 days, *daphnia* media was changed and the plastic beakers which housed the daphnids were thoroughly rinsed with Milli-Q deionized water to remove debris and waste products. 10-day old female *D. magna* were used for bioaccumulation and biopersistence assay. Their size was measured using a Nikon SMZ745T stereomicroscope with Nikon NIS-Elements D software. Only female *daphnia* that were ~3 mm in core body length (longest dimension of *daphnia* exclusive of spine) were used for the experiments.[12,13]

3.3.3 Bioaccumulation experiments

Test solutions containing pristine model CeO_2 or Al_2O_3 CMP slurries were prepared by diluting a stock pristine model CeO_2 or Al_2O_3 CMP slurry with pH 7.4 *daphnia* medium to 0.01 and 0.10 mg/mL of CeO_2 or Al_2O_3 NP. In all cases, significant NP aggregation was observed visually within minutes for the CeO_2 and the Al_2O_3 CMP NPs in *daphnia* medium. This was expected because the isoelectronic points of CeO_2 and Al_2O_3 NPs are pH 6.71 and pH 7.06, respectively, which are both close to the neutral pH of the media.[14] The conditions established for *D. magna* bioaccumulation assessments were followed from current literature.[10, 14, 16] Triplicates and controls used 30 daphnids per experiment. *D. magna* treated with 0.01 or 0.10 mg/mL of CeO_2 CMP slurry were exposed for 0, 0.5, 4, 24 and 48 h in 100 mL of test solution in a 250-mL polypropylene beaker. At the same time *D. magna* treated with 0.01 or 0.1 mg/mL of Al_2O_3 CMP slurry were exposed for 0, 0.5, 2, 4, 24 and 48 h in 100 mL of test solution in a 250-mL polypropylene beaker. All experiments were conducted in an incubator maintained at 20 ± 1 °C

with a photoperiod of 16-h light and 8-h darkness.[17, 18] The beakers were continuously rotated on an orbital shaker (model Back to Basics; Bellco Biotechnology, Vineland NJ) with a constant rpm setting of 60 to minimize NP sedimentation.[19] The beakers were covered with a thin layer of clear plastic to minimize evaporation. *D. magna* were not fed during the exposure time to reduce interference of CeO_2 or Al_2O_3 NP with food.[20] After each exposure, *D. magna* were thoroughly rinsed with 100 mL of Milli-Q deionized water for 10 min. This step was repeated three times for a total of three rinses. Then *D. magna* were placed in Eppendorf tubes and dried in an oven over night at 70 °C. Each experiment was conducted three times and their average and standard error of mean (SEM) was used to plot graphs.

3.3.4 Acid digestion and ICP-MS

To achieve reliable and consistent recoveries of the model pristine CMP NP slurries following acid digestion, acid chemistries and micro wave digestion parameters were optimized. An UltraWAVE microwave digester was used to digest model pristine CeO_2 and Al_2O_3 CMP NPs. To reduce background contamination, Teflon vials were used as disposable glass vials inherently have some percent of alumina. To digest CeO_2 NPs concentrated HCl (37%) and concentrated HNO_3 (69%) were used in the ratio of 3:1. Al_2O_3 NPs were digested using only concentrated HNO_3 (69%). Samples of *daphnia* exposed to CeO_2 or Al_2O_3 slurries digested in 1 mL of concentrated HNO_3 (69%) over night to ensure complete breakdown of dried *daphnia*. Both CeO_2 and Al_2O_3 CMP slurries were digested using the same microwave parameters: ramp time to 250 °C was 20 min and hold time at 250 °C was 20 min. These parameters along with appropriate combination of acid(s) led to complete digestion of both NP slurries and *D. magna*. Once the vials were cooled, samples were removed from the digester and diluted with milli-Q water for ICP-MS

analysis. All samples and the method blanks were prepared in metal-free centrifuge tubes (VWR® Metal-Free Centrifuge Tubes, Propylene, Sterile).

Elemental analyses were conducted using an Agilent 7900 Quadrupole ICP-MS. The element Ce was calibrated using blanks and standards of 10 ng/mL, 100 ng/mL, 1000 ng/mL, 10000 ng/mL, and 25000 ng/mL concentrations prepared from a 100-µg/mL standard solution (Inorganic Ventures, Christiansburg, VA). The measured amounts of Ce were used to calculate the concentration of CeO_2 NP in samples. Similarly the element Al was calibrated using blanks and standards of 10 ng/mL, 100 ng/mL, 1000 ng/mL, and 10000 ng/mL concentrations prepared from 100 µg/mL standard solution. The measured amounts of Al were used to calculate the concentration of Al_2O_3 NP in samples. The internal standard used was indium 115 that was made to a final concentration of 50 µg/L.[21] The recovery of CeO_2 and Al_2O_3 CMP NPs was greater than 95% (data not shown).

3.3.5 Lysates from untreated daphnia

Lysates from *daphnia* that were untreated with slurries were examined to see if the *D. magna* matrix interfered with ICP-MS elemental detection of Al and Ce. Thirty adult *D. magna* were lysed using concentrated HNO_3 (69%) at 250 °C in an UltraWAVE single reaction chamber microwave digester (Milestone, Shelton, CT). The lysate was diluted to 1.0 M HNO_3 using Milli-Q deionized water and spiked with known amounts of Al (III) or Ce (IV) ion ICP-MS standards. The results from spiking the *daphnia* lysate with a Ce or Al calibration standard indicated that lysate did not cause any physical interference with the detection of Ce or Al ions (data not shown).

3.3.6 Association of NPs with daphnia at 4° C

To access the amount of NP associated with the outer carapace of *D. magna*, bioaccumulation experiments were conducted at 4 °C. Briefly, ten day old female *D. magna* were placed in a cold room overnight at 4 °C to ensure they were dormant. The dormant *daphnids* were exposed to 0.01 mg/mL and 0.10 mg/mL of pristine model CeO_2 or Al_2O_3 CMP slurry for 4 h. Food was not added during this exposure time. After 4 h, *daphnia* were rinsed three times with Milli-Q deionized water as described previously. *D. magna* were dried in the oven over night at 70° C.

3.3.7 Biopersistence experiments

The conditions established for *D. magna* biopersistence assays were followed from existing literature. [10, 15, 16] Ten day old female *daphnia* were exposed to 0.1 mg/mL of pristine model CeO_2 or Al_2O_3 CMP slurry for 48 h. Food was not added during the exposure to the slurries. Triplicates were used with 30 daphnids per experiment. After the exposure to the CMP NP slurry, *D. magna* were allowed to depurate for 0, 0.5, 24 and 48 h. During depuration time, fresh *daphnia* media was added supplemented with 0.5 mL of YCT and 2 mL of algae. *Daphnia* media was changed and food was replenished every 24 h. After the depuration process, *D. magna* were rinsed thoroughly with Milli-Q deionized water as described before, then placed in Eppendorf tubes and dried overnight in an oven at 70 °C. Samples were acid digested then analyzed using ICP-MS as described above. Each experiment was conducted three times and their average and SEM was used to plot graphs.

3.4 RESULTS

3.4.1 Bioaccumulation of pristine model CeO_2 and Al_2O_3 CMP slurry

Sub-lethal doses of 0.01 and 0.10 mg/mL CeO_2 and Al_2O_3 were tested in this study as the objective was to determine the accumulated NPs responsible for their previously observed sub-lethal chronic effects [3]. Figure 3.1 shows the bioaccumulation results of *D. magna* exposed to model CeO_2 and Al_2O_3 CMP slurries. *D. magna* ingested quantifiable levels of CeO_2 and Al_2O_3 NP when exposed to both 0.01 and 0.10 mg/mL of CeO_2 or Al_2O_3 CMP slurry and the amount of CeO_2 or Al_2O_3 accumulated was concentration- and time-dependent. The results indicate that the rate of accumulation was fastest in the initial 30 min of exposure, but then slowed and by 24 h reached a plateau, suggesting a steady state balance of uptake and loss (Figure 3.1)

D. magna exposed for 48 h to 0.01 and 0.10 mg/mL of CeO_2 accumulated 45 ± 7 µg and 120 ± 9 µg of CeO_2, respectively (Figure 3.1A). The amounts of Al_2O_3 accumulated by *D. magna* exposed to 0.01 and 0.10 mg/mL of Al_2O_3 CMP for 48 h were 22 ± 3 µg and 44 ± 1 µg of Al_2O_3, respectively (Figure 3.1B). It is interesting that even though the difference between exposure concentrations was 10 fold, the amount accumulated by 48 h at the higher concentration of 0.10 mg/mL of CeO_2 was only ~2.7 times more than was observed with the lower concentration. Similarly, the amount of Al_2O_3 accumulated at 0.10 mg/mL by 48 h was only 2 times more than amount accumulated at 0.01 mg/mL of Al_2O_3. This could be because both CeO_2 and Al_2O_3 aggregate in *daphnia* media, and *daphnia* are not exposed to a homogenous solution [14]. Due to aggregation the concentration of CeO_2 and Al_2O_3 NPs at the bottom is higher than at the other regions of the beaker, and *daphnia* are exposed to higher concentrations at the bottom of the beaker than the applied nominal concentration.

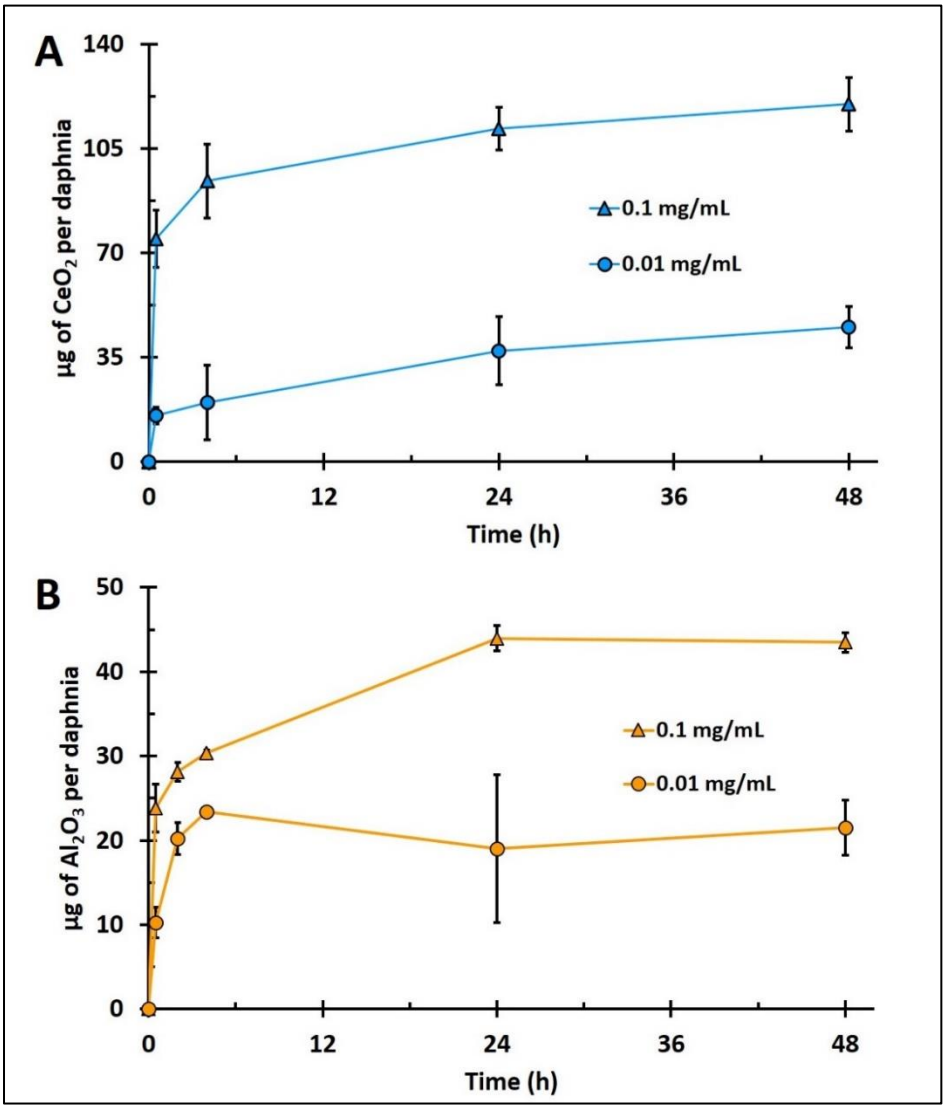

Figure 3.1. Bioaccumulation of CeO_2 and Al_2O_3 in pristine model CMP slurries at 20 °Cas a function of time. **(A)** Bioaccumulation in *D. magna* exposed CeO_2 CMP slurries at 0.01 mg/mL (blue circles) and 0.10 mg/mL (blue triangles). **(B)** Bioaccumulation in *D. magna* exposed Al_2O_3 CMP slurries at 0.01 mg/mL (orange circles) and 0.10 mg/mL (orange triangles). All data points are the mean of at least three independent trials and the error bars are the SEM.

3.4.2 Association of CeO_2 and Al_2O_3 with D. magna at low temperature

To determine the background association of NPs with daphnids, experiments were done under conditions where daphnids were not expected to ingest. This was a control experiment to help interpret accumulation data shown in figure 3.1. Figure 3.2 shows results for *D. magna* exposed to 0.01 and 0.10 mg/mL of pristine model CeO_2 and Al_2O_3 NP slurries for 4 h at 4 °C and 20 °C. At lower temperatures, *D. magna* accumulated significantly less CeO_2 NPs. When *daphnia* were exposed to 0.01 mg/mL of CeO_2 NPs at 4 °C the amount CeO_2 measured was 95% less than that measured at 20 °C. At higher exposure concentration of 0.10 mg/mL of CeO_2 NP, the amount of CeO_2 measured was 86% less than that measured at 20 °C. When *daphnia* were exposed to 0.01 mg/mL of Al_2O_3 NP at 4 °C the amount of Al_2O_3 per *D. magna* was 96% less than the amount at 20 °C. Similarly *daphnia* exposed to higher concentration of 0.10 mg/mL of Al_2O_3 NP had 90% less Al_2O_3 compared to *D. magna* at 20 °C. This indicates that accumulation is concentration- and temperature-dependent and that at the lower temperature *D. magna* do not accumulate as much as they do at higher temperatures. Moreover, this experiment suggests that metabolically inactive *D. magna* do not actively ingest NPs and hence any amount of NPs measured would be associated to the outside surface of *D. magna*. The results indicated that at low temperature there was little association of NPs with dormant *daphnids*. This provides evidence that accumulation at higher temperature is due to *daphnids* ingesting NPs and not just NPs binding on the outside carapace or surface of the test organism.

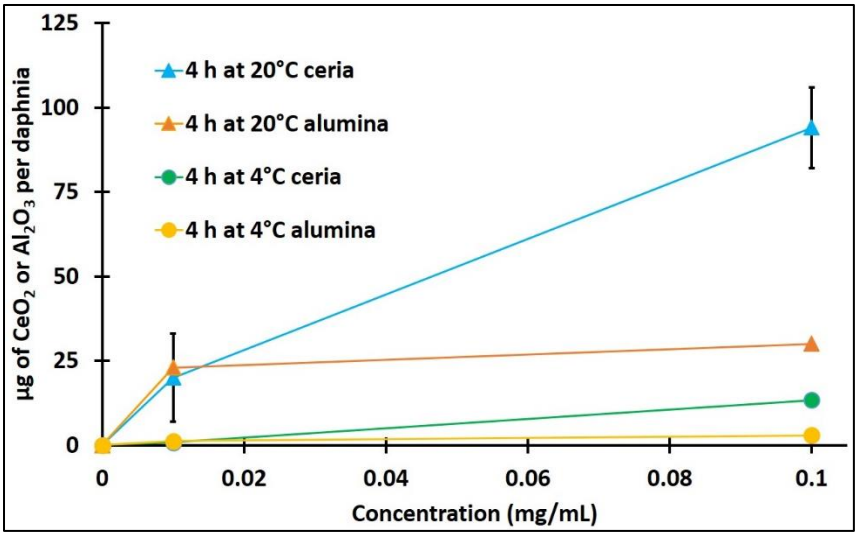

Figure 3.2. Temperature dependent association of CeO_2 and Al_2O_3 in pristine model CMP slurries as a function of concentration. Bioaccumulation in *D. magna* exposed CeO_2 CMP slurries for 4 h at 4°C (green circles) and 20 °C (blue triangles). Bioaccumulation in *D. magna* exposed Al_2O_3 CMP slurries for 4 h at 4 °C (yellow circles) and 20 °C (orange triangles). All data points are the mean of at least three replicates from one trial and the error bars are the SEM.

3.4.3 Biopersistence of pristine model CeO_2 and Al_2O_3 CMP slurry in D. magna

D.magna exposed to clean *daphnia* media and food after being treated with 0.1 mg/mL of CeO_2 and Al_2O_3 NP slurries for 48 h steadily eliminated most of the initial accumulated load of CeO_2 and Al_2O_3 NPs (Figure 3.3A). In the first 24 h of the depuration phase, *D. magna* eliminated 77 ± 7 µg (71%) of the initial CeO_2-NP load. In the following 24 h, *D. magna* eliminated another 15 ± 6 µg (15%) of CeO_2 NP from their system. However, 16 ± 9 µg (15%) of CeO_2 NPs persisted in *D. magna* even after 48 h of depuration. Figure 3.3B shows an optical image of *D. magna* exposed to 0.10 mg/mL of CeO_2 NPs for 48 h. The white arrow denotes the mid gut which is filled with CeO_2 NP and appears cream colored. After depuration for 24 h in the absence of NPs, the *daphnids* appear to be recovering and consuming food as normal, as green algae can be seen in the mid gut region (Figure 3.3C). This provides a visual indication that *D. magna* were able to remove

CeO_2 from their gut as the cream colored CeO_2 was replaced by green algae. Overall, by 48 h of depuration time, *D.magna* successfully eliminated 92 ± 10 µg (85%) of the CeO_2 NPs.

Similarly, *D.magna* exposed to fresh *daphnia* media and food after being treated with 0.10 mg/mL of Al_2O_3 NP slurry for 48 h successfully eliminated significant amounts of the initially accumulated Al_2O_3 NPs (Figure 3.3A). In the first 24 h of the depuration phase, *D. magna* eliminated 26 ± 1 µg (57%) of the initial Al_2O_3-NP load. In the next 24 hours *D. magna* eliminated another 10 ± 2 µg (22%) of Al_2O_3 NPs from their system. However, 10 ± 2 µg (22%) of Al_2O_3 NPs persisted in *D. magna* even after 48 h of depuration. Figure 3.3D shows an optical image of *D. magna* exposed to 0.10 mg/mL of Al_2O_3 NP for 48 h. The white arrow denotes the mid gut that is filled with Al_2O_3 NPs and appears beige colored. However, *D. magna* 24 h after depuration appear to be recovering, as green algae can be seen in the mid gut region (Figure 3.3E). This provides a qualitative measurement of *D. magna* road to recovery as green algae replaced the beige colored Al_2O_3 over time. Overall, by 48 h of depuration time, *D. magna* successfully eliminated 36 ± 2 µg (78%) of the Al_2O_3 NPs.

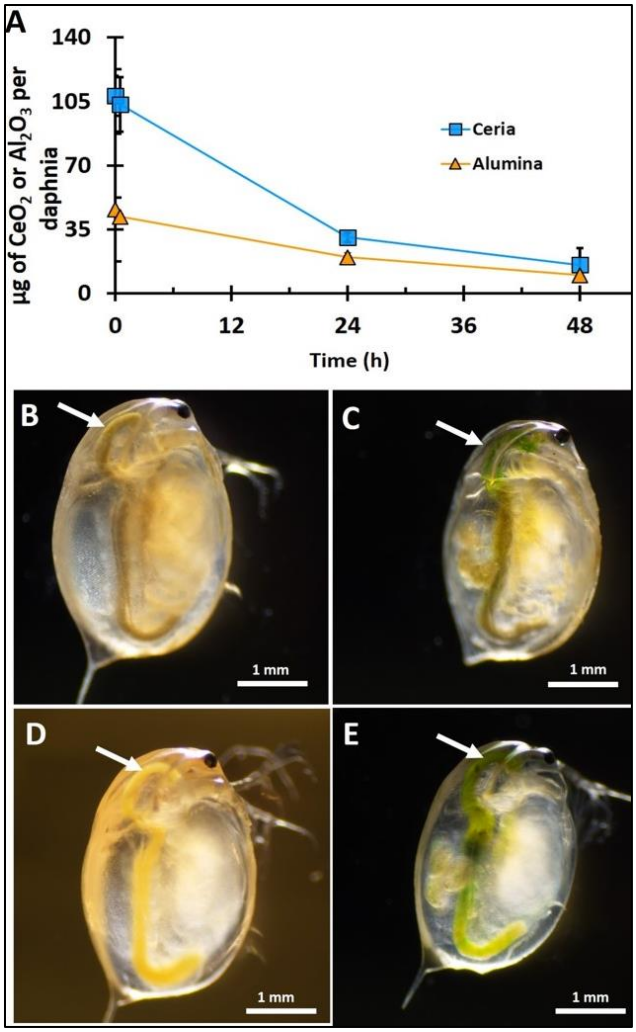

Figure 3.3. Biopersistence of CeO_2 and Al_2O_3 CMP in pristine model CMP slurries at 20 °C as a function of time. **(A)** Depuration in *D. magna* exposed CeO_2 CMP slurry at 0.10 mg/mL (blue squares) and *D. magna* exposed Al_2O_3 CMP slurry (orange triangles). All data points are the mean of at least three independent trials and the error bars are the SEM. Representative optical images (3× magnification) of: **(B)** *D. magna* exposed for 48 h to CeO_2 in pristine model slurry; **(C)** *D. magna* depurated for 24 h after being exposed to CeO_2 in pristine model slurry for 48 h; **(D)** *D. magna* exposed for 48 h to Al_2O_3 in pristine model slurry; and **(E)** *D. magna* depurated for 24 h after being exposed to Al_2O_3 in pristine model slurry for 48 h. The white arrows denote the midgut region.

3.5 DISCUSSION

3.5.1 Bioaccumulation of CeO_2 and Al_2O_3 NP CMP slurry by D. magna

In previous chronic toxicity studies, we showed that prolonged exposure effects on *D. magna* to a CeO_2 or Al_2O_3 CMP slurry were measured as function of applied dose.[3] which raised concerns about the potential long-term biopersistence of CeO_2 and Al_2O_3 NPs within *D. magna*. Since both CeO_2 and Al_2O_3 aggregate at physiological pH, it is difficult to determine the actual concentration that *D. magna* are exposed to. To address this difficulty, we studied the bioaccumulation and biopersistence to *D. magna* of CeO_2 and Al_2O_3 NP slurries.

The size of the NP aggregate is of importance for accumulation.[22,23] *D. magna* are filter feeders that sieve large quantities of water to collect suspended particles. They filter particulates indiscriminately and will ingest any particles that can be retained by their filtering appendages. The size range of particles filtered by *D. magna* is typically 0.6–40 µm but they can ingest particles up to 70 µm.[24, 25] Food particles are captured and transported to the food groove. In search of food, *D. magna* browse at the sediment-water interface and stir up the sediment. There is evidence that sediment-associated metal contaminants are ingested by *D. magna.*[8, 26, 27] Studies have shown that larger aggregates ~1 µm are retained in *D. magna* gut compared to smaller particles.[5, 15, 28] Lewinski et al. studied the accumulation of CdSe/ZnSe quantum dots larger than 0.1 µm by *D. magna* and observed increased uptake in *D. magna* exposed to aggregated quantum dots.[5] Another study conducted by Rosenkraz et al. found that 1 µm polystyrene beads were taken up 28 times more than 0.020 µm polystyrene beads. They concluded that since *D. magna* are capable of ingesting up to 70 µm particles, 1 µm falls under the lower range and hence *D. magna* actively accumulated the 1 µm NP. On the other hand the uptake of 0.020 µm NPs was considered to be

passive and unintentional since the size of the particles was too small.[28] Another study conducted by Xiao et al. on Cu and ZnO NPs found that both of these NPs aggregated up to 1 µm and were ingested by *D. magna* from the sediment.[15] Therefore the size of the NP aggregate is of importance for accumulation studies.

In this study we measured the amount of CeO_2 or Al_2O_3 bioaccumulated by *D. magna* when exposed to 0.10 mg/mL CeO_2 or Al_2O_3 CMP slurry and found that the amount accumulated began to plateau by 48 h. If one makes the assumption that the approximate plateau accumulation of 120 ± 9 µg of CeO_2 and 44 ± 1 µg of Al_2O_3 per *D. magna* is a reasonable estimate of the amount of CeO_2 or Al_2O_3 found in *D. magna* when exposed to 0.10 mg/mL of CeO_2 or Al_2O_3 for prolonged times, then the plateau amounts of CeO_2 and Al_2O_3 per *D. magna* may be responsible for the known sub-lethal effects with CeO_2 and Al_2O_3. It is important to note that both CeO_2 and Al_2O_3 aggregate at physiological pH; therefore, the exact concentration that causes toxic effects is difficult to determine. Hence the bioaccumulated amounts provide a bench mark for toxicity in the wild for *D. magna* that are exposed to a non-uniform concentration of CeO_2 or Al_2O_3 NPs. The bioaccumulation values reported here are pertinent to the feeding habits of *D. magna* as they browse at the sediment water interface where they would be exposed to aggregated materials such as CeO_2 and Al_2O_3 NPs. The average weight of a 3 mm long adult *D. magna* is 250 µg [29] and the amount of CeO_2 accumulated is almost 48% of *D. magna's* weight. This could explain the slow/lethargic movement of *D. magna* exposed to CeO_2 CMP slurry compared to controls observed in Karimi et al.[3] At an exposure concentration of 0.1 mg/mL, the amount of Al_2O_3 accumulated is 17.6% of *D. magna's* weight.

3.5.2 *Biopersistence of CeO_2 and Al_2O_3 NP CMP slurry by D. magna*

In this study, *D. magna* eliminated 85% of the total initial CeO_2-NP load by the end of the 48 h depuration. The rate of elimination was fastest in the first 24 h during which 71% of the CeO_2 was removed. In the first 24 h the rate of removal of CeO_2 was 3.2 µg/hr. However, in the last 24 h the rate of removal slowed down to 0.625 µg/hr. Similarly, *D. magna* removed 78% of the total initial Al_2O_3 NP load by the end of 48 h of depuration. The rate of removal was 1.08 µg/hr for the first 24 h, however, the rate slowed down to 0.42 µg/hr. This could be because in the first 24 h the *daphnia* rapidly eliminated materials from the gut, followed by a slower elimination phase, which may represent removal of material that was internalized or adsorbed to the *daphnia's* gut.[20]

Unlike passive filters that accumulate until they get clogged, the filtering apparatus of *D. magna* is active and self-cleaning.[24] Even though Gillis et al. reported that it takes *D. magna* 8 h to clear their gut completely,[26] most depuration studies report various degrees of elimination of a variety of NP from *D. magna*.[4, 5, 7, 9] Petersen et al. found that *D. magna* removed 50-85% of an initial load of carbon nanotubes when depurated in the presence of algae.[4] Guo et al. reported that more than 90% of the initial load of graphene was removed from the intestine of *daphnia* after being fed algae.[9] Zhu et al. reported that 80% of titanium dioxide was removed from *daphnia* after a 72 h depuration.[7] Lewinski et al. studied the elimination of quantum dots and reported 47-64% removal of the initial load of quantum dots from *daphnia*.[5] These studies show that different NPs require different lengths of depuration time

In our study *D. magna* did not completely eliminate CeO_2 or Al_2O_3 even after 48 h of depuration time. This is consistent with other studies done by Zhu et al. on titanium oxide NPs where they reported that it took 74 h to eliminate 50% of the NPs from the gut.[7] We observed that

D. magna exposed to 0.1 mg/mL of CeO_2 and Al_2O_3 accumulated 55% (24 μg) and 62.3% (75 μg) of the initial load for CeO_2 and Al_2O_3 respectively, within the first 30 minutes of exposure. However, depuration was slower for both CeO_2 and Al_2O_3. In the first 30 minutes of depuration, *D. magna* exposed to 0.1 mg/mL of CeO_2 eliminated only 4.6% (5 μg) of the initial load. It took *D. magna* at least 24 h to eliminate 71% of the initial CeO_2 load. Similarly, in the first 30 minutes of depuration, *D. magna* exposed to 0.1 mg/mL of Al_2O_3 eliminated only 8% (4 μg) of the initial load. It took *D. magna* at least 24 h to remove 57% of the initial Al_2O_3 load. This shows that both CeO_2 and Al_2O_3 NP were not completely eliminated from *D. magna*, and would likely require longer than 48 h of depuration to fully purge CeO_2 and Al_2O_3 NP from their intestines.

It is interesting to note that for the same exposure concentration of 0.1 mg/mL for both CeO_2 and Al_2O_3, *D. magna* accumulated ~2.7 times more CeO_2 than Al_2O_3. However, by the end of the depuration phase only ~1.6 times more CeO_2 than Al_2O_3 remained. The rate of removal of CeO_2 is ~3 times faster compared to rate of removal of Al_2O_3. This could be why Al_2O_3 is more toxic to *D. magna* (acute LC50 96h 1.1 mg/mL) than CeO_2. *D. magna* exposed to Al_2O_3 are sick and tend to accumulate less Al_2O_3 compared to CeO_2.[3] Further, the rate of removal is also slower for *D. magna* exposed to Al_2O_3 compared to *D. magna* exposed to CeO_2.

3.6 CONCLUSION

The two model CMP slurries used in this *D. magna* uptake and elimination study are unique in that they represent the simplest formulations to generate stable aqueous suspensions of the CeO_2 and Al_2O_3 NP that are actually used in complex commercial slurries. Herein we studied for the first time the bioaccumulation and biopersistence of the two model slurries in *D. magna.* The key

findings were that both CeO_2 and Al_2O_3 were ingested by *D. magna* as a function of time and dose. *D. magna* that were exposed for 48 h to 0.1 mg/mL CeO_2 CMP slurry accumulated 120 µg of CeO_2, and those that were exposed to for 48 h to 0.1 mg/mL of Al_2O_3 CMP slurry accumulated 44 µg of Al_2O_3. This shows that different metal oxide slurries are accumulated in different amounts by *D. magna*. In addition, biopersistence studies revealed that after 48 h of depuration time, *D. magna* exposed to 0.1 mg/mL CeO_2 successfully eliminated 85% of the initial CeO_2 load, and *D. magna* exposed to 0.1 mg/mL of Al_2O_3 were able to eliminate 78% of the initial Al_2O_3 load. However, the amount metal oxide NP slurries that persist in *D. magna* might be available for transfer to higher trophic level and the amount of metal oxide eliminated might be available to lower trophic level.

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CHAPTER 4

ACUTE AND CHRONIC TOXICITY TO *DAPHNIA MAGNA* OF COLLOIDAL SILICA NANOPARTICLES IN A CHEMICAL MECHANICAL PLANARIZATION SLURRY AFTER POLISHING A GALLIUM ARSENIDE WAFER

4.1 ABSTRACT

Semiconductor manufacturers use slurries of metal oxide nanoparticles (NPs) as abrasives in chemical mechanical planarization (CMP) processes on wafers containing films of III/V semiconducting materials. Assessing the toxicity of these specialized NPs is challenging not only because commercial slurries may contain undefined toxic constituents, but because CMP processes can change the physical and chemical properties of the NPs. Herein, the fresh water flea *Daphnia magna* (*D. magna)* was used to assess the effects of Ultra-Sol® 200S CMP slurry containing ~30-nm colloidal silica (*c*-SiO_2) NPs before (pristine) and after (spent) a GaAs wafer was polished with an extreme arm-pressure of 5 psi. In the acute 96-hour toxicity assessments, both the pristine and spent slurries at 4.0 mg/mL *c*-SiO_2 NPs had little effect on *D. magna* morbidity and body sizes. In the chronic 21-day toxicity assessments, neither slurry at 0.10 mg/mL *c*-SiO_2 NPs was toxic, but both slurries lead to a slight (9–10%) increase in body size and a significant (~2-fold) increase in reproductive output, indicative of a positive hormetic response whereby *D. magna* were under stress. Identical increases in body size and reproductive output were observed with a supernatant of the pristine slurry, in the absence of the *c*-SiO_2 NPs, indicating that the chronic effects were derived from soluble component(s) in the pristine slurry, and not from the *c*-SiO_2 NPs nor from the CMP process that removed ~3 mg of material from a GaAs wafer.

4.2 INTRODUCTION

Semiconductor manufacturers use chemical mechanical planarization (CMP) tools to remove materials from wafers of semiconducting material such as silicon and to smooth the resulting surfaces to high tolerances (Zantye et al., 2004; Oliver, 2004; Krishnan et al., 2010). The process involves a combination of surface reactions with chemical additives and mechanical polishing with abrasive nanoparticles (NPs) such as colloidal silica (*c*-SiO_2) (Matijevic and Babu, 2008; Wang et al., 2011). These components are sold in the form of a slurry where the concentration of suspended NPs can be as high as 50 wt%. The chemical additives of a CMP slurry may include acids or bases, buffers, oxidizers, surfactants, complexing agents, biocides, and corrosion inhibitors, as well as, proprietary surface- and redox-active constituents designed to influence NP behavior (Speed et al., 2015; Speed, 2016). The basic operations of a CMP tool involve pressing a rotating wafer on to a rotating polishing pad, and delivering slurry, ultrapure water, and other chemicals such as pad conditioners at various stages of the process. Large volumes of ultrapure water are also used to wash a wafer to remove components and their reaction products, as well as, any pad and wafer detritus (Corlett, 2000). Depending on the particular semiconductor manufacturing site or "fab", the resulting CMP effluent can undergo CMP-specific treatments, be blended and neutralized with waste from other fab sources, and be further diluted and processed in a biological wastewater treatment facility before the reclaimed water is discharged to a municipal water supply (Shepard and Brenner, 2014; Speed, 2016).

Assessing the potential ecotoxicity of CMP slurry NPs is a complex endeavor for three main reasons. First, subtle differences in metal oxide NP surface reactivity, size, and shape can affect toxicity (Love et al., 2012; Misra et al., 2012; Ma and Lin, 2013; Maurer-Jones et al., 2013;

Herd et al., 2013; Petersen et al., 2014), and so laboratory-synthesized NPs may be different than the specialized NPs used in CMP processs (Flatherty et al., 2015). Second, toxicity assessment can be confounded by slurry constituents other than the NPs that are known to be toxic. Third, the CMP process can modify pristine NPs such that species present in the post-CMP effluent can absorb to them (Matovu et al., 2013; Bi and Westerhoff, 2016), and potentially impact their toxicity. In response to the first two challenges, a major CMP slurry manufacturer produced a model CMP slurry representing the simplest formulation to generate a stable aqueous suspension of actual *c*-SiO_2 NPs used in commercial slurries without the addition of known soluble toxicants (Speed et al., 2015). Recently, we reported the first ecotoxicity assessement of this unique model CMP slurry using *Daphnia magna* (*D. magna)* as the test organism (Karimi et al., 2018). *D. magna* is a filter-feeding fresh-water flea found in ponds and is a model organism commonly used in ecotoxicity studies because it is sensitive to ecological pollutants and easy to maintain in the laboratory (Adema, 1978; Guilhermino et al., 2000; Miner et al., 2012; Seitz et al., 2013; Romer et al., 2013). Acute and chronic studies on the effects of the model *c*-SiO_2 CMP slurry on *D.magna* revealed no major toxic biological responses (Karimi et al., 2018); however, the model slurry is not suitable for polishing wafers and cannot be used to assess whether polishing modifies the toxicity of a CMP slurry.

Herein, we extend this work to the study of a commercial *c*-SiO_2 CMP slurry before (pristine) and after (spent) polishing a composite allium arsenide (GaAs) wafer. The slurry used was Ultra-Sol® 200S CMP containing ~30-nm *c*-SiO_2 NPs and it was chosen because control studies showed that the pristine material was not overtly toxic to *D. magna* prior to polishing. The GaAs wafer was chosen because semiconductor manufacturers use this material in the fabrication

of optoelectronic devices and as a substrate for the epitaxial growth of other III/V-materials (Banerjee, 2014; Løvik et al., 2015; Gupta and Gupta, 2016). In addition, As is a well-known toxicant and carcinogen, is an EPA priority pollutant under the U.S. Clean Water Act and GaAs is classified by the European Chemical Agency as a category-1B carcinogen (Sharma and Sohn, 2009; Chitambar, 2010; Flora and Dwivedi, 2012; Bomhard et al., 2013; Fan et al., 2015). Samples of the spent Ultra-Sol® 200S CMP slurry were obtained by using deionized water to rinse material off of a GaAs wafer that was polished with an arm pressure of 5 psi – a harsh condition not used in typical operations because it can scratch wafer surfaces. For comparative analyses, the pristine and spent slurries were diluted accordingly based on the determined levels of *c*-SiO_2 NPs that they contained. In the acute toxicity assessments, *D. magna* neonates were exposed continuously for 96 h to pristine or spent CMP slurries containing up to 4.0 mg/mL of *c*-SiO_2 NPs, and the morbidity and the body sizes of the surviving *D. magna* were compared to those of control *D. magna* not exposed to slurries. Under these test conditions, neither the pristine nor spent slurries significantly affected *D. magna* morbidity or body size. For chronic toxicity assessments, *D. magna* neonates were exposed continuously to a lower concentration (0.10 mg/mL of *c*-SiO_2 NPs) of pristine or spent CMP slurries for 21 d and the morbidity, body sizes, and the numbers of offspring produced were compared to those of the control group. Under these test conditions, neither slurry was toxic but both slurries lead to a slight (9–10%) increase in body size and a significant (~2-fold) increase in reproductive output, indicative of positive hormetic responses whereby *D. magna* were under stress. Identical increases in body size and reproductive output were observed with a supernatant of the pristine slurry, in the absence of the *c*-SiO_2 NPs, indicating that the chronic effects were

derived from soluble component(s) in the pristine slurry, and not from the *c*-SiO_2 NPs nor from the CMP process that removed ~3 mg of material from a GaAs wafer.

4.3 MATERIALS AND METHODS

4.3.1 Chemical and solutions

Ultra-Sol® 200S (lot # ETI-19217) was purchased from Eminess Inc. (Monroe, NC). This is a high-purity, alkaline (pH 9.5) colloidal silica slurry (24 wt% solids) containing proprietary non-drying additives for reducing problems associated with the caking or crystallizing of silica on exposed surfaces. The small particle size (~30 nm *c*-SiO_2 NPs) is designed for the critical polishing of precision optoelectronic materials such as GaAs. Stock and diluted CMP slurries were stored in high density polyethylene (HDPE) bottles at room temperature away from direct light and were vortexed briefly before use. *Daphnia* medium was made in accordance with U.S. Environmental Protection Agency (EPA) guidelines by mixing: 192 mg/L $NaHCO_3$, 120 mg/L $CaSO_4 \cdot 2H_2O$, 120 mg/L $MgSO_4$, and 8 mg/L KCl in well-aerated deionized water (EPA, 2002). The hardness of *daphnia* medium was maintained between 250–425 mg/L $CaCO_3$ as measured using a HACH® aqua check water quality test strip. The pH was maintained between 7.4–8.4 as measured using a Fisher Scientific model 25 pH/ion meter and an Accumet® combination Ag/AgCl reference pH electrode. Dissolved oxygen was maintained between 8–10 mg/L as measured using a colorimetric method (CHEMets® kit K-7512; Midland, VA). The *daphnia* diet comprised a commercially-prepared mixture of yeast, CEROPHYLL®, and Troutchow (YCT) supplemented with green microalgae (*Pseudokirchnerilla subcapitata*), both purchased from Marinco Bioassay Laboratory, Inc. (Sarasota, FL). Deionized water (18.2 MΩ-cm) was obtained using a Milli-Q®

Advantage A10 water purification system (Millipore; Billerica, MA). Unless otherwise specified, all other chemicals were of the highest grade available and were purchased from VWR International (Radnor, PA).

4.3.2 CMP tool and GaAs wafer polishing

An IPEC Avanti 472 CMP tool with a conditioned 50-mil IC1000 K-groove polishing pad was used to polish 6" GaAs wafers (Qorvo Inc.) for 110 s using Ultra-Sol® 200S CMP slurry, as described previously (Crawford and Aravamudhan, 2017). Briefly, the CMP conditions were set as follows: the arm pressure was 5.0 psi, the platen speed was 72 rpm, the carrier speed was 80 rpm, the back pressure was 2.0 psi, and the CMP slurry flow rate was 200 mL/min preceded by a 10-s pre-wet step. The waste effluent containing spent Ultra-Sol® 200S CMP slurry was collected from the tool by plugging the tub basin drain and by vacuum aspirating waste effluent into clean HDPE collection containers. The amount of material removed from the wafer was determined following ellipsometry analyses performed with a Gaertner Scientific LSE-WS ellipsometer.

4.3.3 Dynamic light scattering (DLS) and zeta potential analyses

The hydrodynamic sizes and zeta potentials of pristine and spent Ultra-Sol® 200S CMP slurry NPs were determined using a Zetasizer Nano-ZS 3600 analyzer (Malvern Instruments; Worcestershire, UK) with a 633-nm laser at a fixed angle of 173°. Pristine and spent slurries were diluted with pH 7.4 *daphnia* medium to a final *c*-SiO_2 NP concentration 4.0 mg/mL. Aliquots (500 µL) of each sample were placed in a capillary cell and ten consecutive 30-s runs were acquired per measurement at 25 °C. Three independent DLS and zeta potential measurements were acquired per sample, and the average particle size distribution in terms of hydrodynamic diameter

(HDD) and polydispersity index (PDI), and the mean net surface charges, were calculated. Particle size distributions were measured periodically during the course of this work.

4.3.4 Transmission electron microscopy (TEM)

The sizes and morphologies of NPs before and after the CMP process were measured using a Zeiss Libra 120 transmission electron microscope operated at 120 kV. Diluted slurry samples were drop-cast and air-dried on a TEM.

4.3.5 Elemental analyses

All elemental analyses were performed by Precilab, Inc. (Carrollton, TX, USA). Elements were calibrated using blanks and standards of 0.050 ppb, 0.100 ppb, 0.250 ppb, and 0.500 ppb concentrations of the respective elements prepared from 1000 ppm standard solutions (Inorganic Ventures, Christiansburg, VA); the internal standard was rhodium 103. The samples and standard solutions were aspirated through a nebulizer into a torch chamber and then injected into the ~10,000 K plasma through argon gas flow. The determination of Ga and As in pristine and spent slurries was performed using a Varian 820MS inductively coupled plasma (ICP) mass spectrometer (MS) in hot mode (a 1400 W plasma). Filtrates and eluates of spent slurry samples were also evaluated for the presence of Ga and As after the samples were passed through a 3,000 MWCO centrifugal filter unit. The determination of Si was performed using a Thermo iCAP 7400 ICP-optical emission spectrometer (OES), and the measured amounts of Si were used to calculate the concentrations of *c*-SiO_2 NPs in the as-received, pristine (214 mg/mL) and the collected, spent (40 mg/mL) Ultra-Sol® 200S CMP slurries.

4.3.6 Arsenic speciation

An Agilent 1260 Infinity Bio-Inert high performance liquid chromatography (HPLC) system using ammonium phosphate buffer (pH 6.0, 10 mM, 0.9 mL/min) flowing through a Hamilton PRX100 (5-µm pore size, 4.1 × 150 mm) column was used separate organic and inorganic As(III) and As(V) species, and an Agilent 7500cx ICP-MS was used to quantify As amounts.

4.3.7 Culture methods

All *D. magna* were acquired from Marinco Bioassay Laboratory, Inc. Cultures were maintained according to established guidelines and recommendations (Green, 1954; Adema, 1978; EPA, 2002), and critical variables such as *D. magna* suppliers, test container materials, test container volumes, number of *D. magna* per test container, *D. magna* food sources, amounts of food, feeding frequency, light luminosity, room temperature, and the pH and dissolved oxygen content of the *daphnia* medium were optimized prior to toxicity testing (Karimi et al., 2018). Briefly, *D. magna* cultures were maintained in 100-mL polypropylene beakers containing 80 mL of *daphnia* medium and 5 animals per beaker in a temperature controlled room at 20 ± 1 °C with a natural photoperiod of 8-h darkness and 16-h light provided by an incandescent fluorescent lamp. *D. magna* were fed three times a week on Monday, Wednesday, and Friday. The food comprised 1 mL of YCT and 1 mL of *Pseudokirchnerilla subcapitata* (~3.5×10^7 cells). Once a week *D. magna* neonates were temporarily removed and the *daphnia* medium was replaced with fresh medium, and every two weeks the culture beakers were additionally cleaned. The *daphnia* medium was also replaced with fresh medium the day before *D. magna* neonates were removed for toxicity tests.

4.3.8 Acute toxicity assays

The standard conditions for 96-h toxicity tests with *D. magna* were detailed previously (Karimi et al., 2018) and were based on established guidelines for acute toxicity testing of materials on aquatic organisms, adhering to recommendations for working with metal oxide NPs in aqueous environments (Anderson, 1932; Adema, 1978; Masters et al., 1991; EPA, 2002; OECD, 2004; Baun et al., 2008; Lazorchak et al., 2009; Chowdhury et al., 2010; Handy et al., 2012; Petersen et al., 2015; Gong et al., 2016). A static test method was chosen because it was less labor intensive and it required less CMP slurry material (EPA, 2002; Baun et al., 2008; Seitz et al., 2013). Test solutions containing CMP slurries were prepared immediately prior to use by diluting either the pristine or spent Ultra-Sol® 200S CMP slurry with pH 7.4 *daphnia* medium to contain 2.0 or 4.0 mg/mL of *c*-SiO_2 NPs. Briefly, five neonates (<24-h old) were randomly selected from a pool of neonates and placed in 8 mL of control or test solution in a 10-mL well of a 6-well culture plate that was covered with a clear lid to minimize the evaporation of water (Baumann et al., 2014); a total of 30 daphnids were used for each sample in an independent trial. Culture plates were kept in an incubator maintained at constant temperature of 20 ± 1 °C with a photoperiod of 16-h light and 8-h darkness (Gaiser et al., 2011; Lazorchak et al., 2009). Culture plates were continuously rotated on an orbital shaker (model Back-to-Basics; Bellco Biotechnology, Vineland, NJ) with a constant rpm setting of 60 to minimize NP sedimentation and to mimic currents in natural waters; this method was chosen since approaches involving sonication or aeration have been reported to stress *D. magna* (Zhu et al., 2009; Chowdhury et al., 2010; Handy et al., 2012). Unless otherwise specified, *D. magna* were fed daily with 48 µL of YCT and 32 µL of green algae (~1.1×10^6 cells/well). The pH of control and test media was measured at the beginning and at the end of each

trial, and the dissolved oxygen was measured at the end of each trial. The morbidity of individual *D. magna* was visually determined every 24 h; *D. magna* that were unable to swim within 10 s of gentle agitation were considered dead. While it was the norm for ≥93% of *D. magna* in control containers to survive and appear healthy for 96 h, trials were discarded if ≤90% of control *D. magna* survived. Morbidity was reported as the LC-50 (i.e., the lethal concentration which kills 50% of a *D. magna* population). The body sizes of individual *D. magna* were determined by acquiring images of surviving *D. magna* using a Nikon SMZ745T stereomicroscope with Nikon NIS-Elements D software to outline bodies (sans antennae and tail) and to calculate areas (Ranta et al., 1993). Statistical significance was determined using either a two-tail student's t-test with equal variances or single-variable ANOVA where $p < 0.05$ was considered significant.

4.3.9 Chronic toxicity assays

The standard conditions for 21-day toxicity tests with *D. magna* were detailed previously (Karimi et al., 2018); they were based on established guidelines for chronic toxicity testing of materials on aquatic organisms and on recommendations for working with metal oxide NPs in aqueous environments (Anderson, 1932; Adema, 1978; Stephenson and Watts, 1984; EPA, 1986; EPA, 1987; Persoone et al., 1989; Enserink et al., 1990; Ranta et al., 1993; Chowdhury et al., 2010; Handy et al., 2012; ASTM, 2012; OECD, 2012; Petersen et al., 2015; Gong et al., 2016). Test solutions containing CMP slurries were prepared immediately prior to use by diluting either the pristine or spent Ultra-Sol® 200S CMP slurry with pH 7.4 *daphnia* medium to contain 0.10 mg/mL of *c*-SiO_2 NPs. Supernatants of pristine Ultra-Sol® 200S slurry were prepared by centrifuging a pristine slurry containing 4.0 mg/mL of *c*-SiO_2 NPs for 2 h at 100,000×g using a Beckman Optima TLX ultracentrifuge with a TLA100.3 rotor. In all cases, test solutions were replaced with fresh

medium and slurry every 4 d. Briefly, ten neonates (<24-h old) were randomly selected from a pool of neonates and placed in 100 mL of control or test solution in 250-mL polypropylene beakers that were covered with a thin sheet of clear plastic to minimize evaporation of water; a total of 30 daphnids in three beakers were used for each sample in an independent trial. The beakers were continuously rotated on a Bellco orbital shaker with a constant rpm setting of 60 in an incubator maintained at constant temperature of 20 ± 1 °C with a photoperiod of 16-h light and 8-h darkness. Test solutions containing CMP slurries were prepared immediately prior to use as described above. The media of the control and test groups were replaced with freshly prepared medium and slurries every 4 d, and food (500 µL of YCT and 2 mL of green algae (~7.0×10^7 cells)) was added to each beaker every other day. The morbidity of individual *D. magna* was determined every 24 h as described in the previous section. If ≤80% of control *D. magna* survived, the entire trial was discarded. The body sizes of individual *D. magna* were determined every two days for the first ten days and subsequently on days 14, 18, and 21. The reproductive output of adult *D. magna* was determined by daily counting the number of neonates produced, which were removed from control and test beakers after being counted. If resting eggs (ephippia) were noticed in control *D. magna* at any stage during the trial (an indicator of stress), the entire trial was discarded. Statistical significance was determined using either a two-tail student's t-test with equal variances or single-variable ANOVA where $p < 0.05$ was considered significant.

4.4 RESULTS AND DISCUSSION

4.4.1 *Characterization of pristine and spent Ultra-Sol® 200S c-SiO_2 CMP NPs*

Figure 4.1A shows a representative TEM image of *c*-SiO_2 NPs in the as-received pristine Ultra-Sol® 200S slurry. The NPs are largely spherical with sizes on the order of 30 nm, which

closely agreed with the 30-nm value reported by the manufacturer. DLS and zeta potential analyses of the pristine Ultra-Sol® 200S slurry were performed to assess the aggregation state of *c*-SiO_2 NPs in pH 7.4 *daphnia* media (Table 4.1).

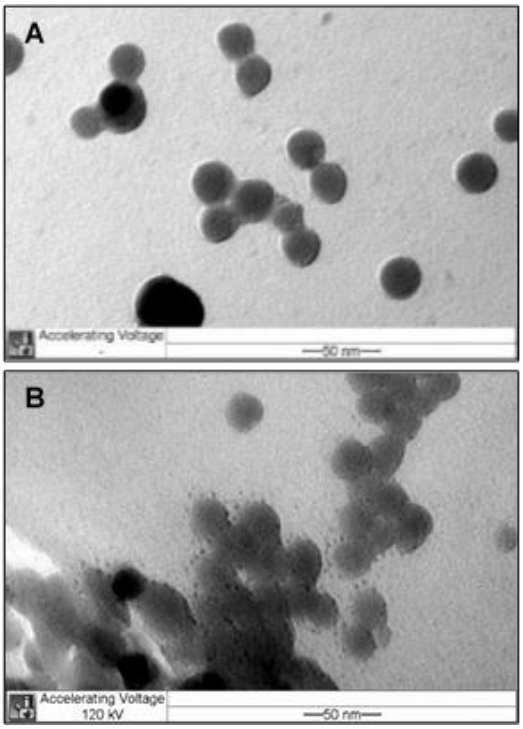

Figure 4.1. TEM image of a pristine and spent Ultra-Sol® 200S slurries. Representative TEM images of *c*-SiO_2 NPs in: **(A)** a pristine Ultra-Sol® 200S slurry, and **(B)** a spent Ultra-Sol® 200S slurry that was used to polish a GaAs wafer.

Table 4.1. DLS and zeta potential analyses of pristine and spent Ultra-Sol® 200S slurries diluted with pH 7.4 *daphnia* medium to contain equivalent concentrations of *c*-SiO_2 NPs.

Parameters	Pristine Ultra-Sol® 200S	Spent Ultra-Sol® 200S
c-SiO_2 NPs (mg/mL)	4.0	4.0
Hydrodynamic diameter (nm)	31.86	32.99
Polydispersity index	0.06	0.12
Zeta potential (mV)	-25.3	-26.8

The mean HDD of *c*-SiO_2 NPs in the pristine Ultra-Sol® 200S slurry was 31.86 nm and the zeta potential was -25.3 mV, indicative of stably-suspended NPs that did not aggregate. The *c*-SiO_2 NPs did not aggregate in *daphnia* medium because the isoelectronic point of SiO_2 is between pH 2 and 3 (Schwarz et al., 2000; Liu et al., 2011; He et al., 2015), resulting in a substantial negative charge at neutral pH that stabilizes the NPs. The amount of elemental Si present in the pristine Ultra-Sol® 200S slurry was determined using an ICP-OES, and the calculated amount of SiO_2 was 214,389 ppm or 21.4 wt%, close to the value of 24 wt% solids reported by the manufacturer. An ICP-MS was used to determine that there was 44 ppb Ga and 26 ppb As in the pristine Ultra-Sol® 200S slurry, whose presence was likely due to impurities in the seed particles used to grow *c*-SiO_2 NPs (Crawford and Aravamudhan, 2017). Following dilution with *daphnia* medium, the concentrations of Ga and As that *D. magna* were exposed to from the pristine 2.0-mg/mL *c*-SiO_2 NP slurry sample were 0.41 ppb and 0.24 ppb, respectively, and 0.82 ppb and 0.48 ppb, respectively, from the 4.0-mg/mL *c*-SiO_2 NP sample.

Standard solid 6" GaAs wafers were polished with Ultra sol® 200S slurry using K-groove pads on an IPEC Avanti 472 CMP tool, as described previously (Crawford and Aravamudhan, 2017). The CMP conditions used were purposely chosen to be harsh compared to normal operations in a fab to represent an upper limit scenario for GaAs material removal. The removal rate was measured to be 160 Å/min and the amount of GaAs material removed was ~3 mg in 110 seconds, and optical imaging revealed dozens of visible scratches across the surface of the GaAs wafer post-CMP (Figure 4.2). Incident particles and arsine gas were not detected in the air samples above the CMP tool under these conditions, using previously described methods (Crawford and Aravamudhan, 2017).

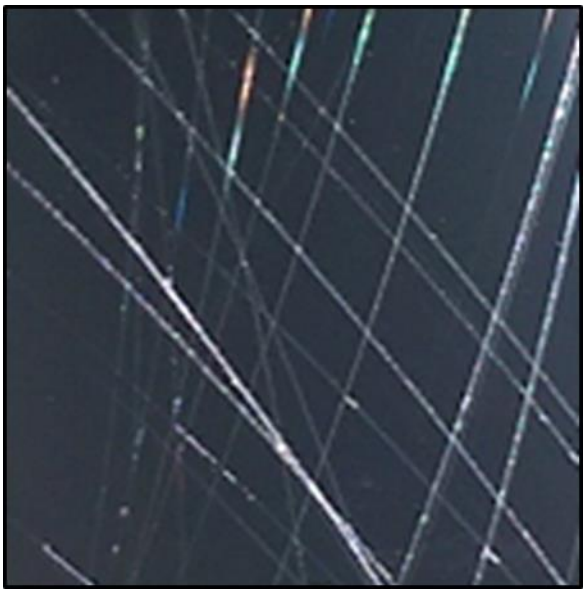

Figure 4.2. Representative image of a Gallium Arsenide wafer polished with Ultra-Sol® 200S slurry. Optical image of a 2.25 square inch region of a GaAs wafer showing numerous scratches from *c*-SiO_2 NPs under harsh polishing conditions.

A minimum amount of deionized water was used to clean the GaAs wafer surface to maximize the amount of collected material in the spent CMP slurry. Figure 4.1B shows a representative TEM image of *c*-SiO_2 NPs in the spent Ultra-Sol® 200S slurry. Like the pristine material, the spent *c*-SiO_2 NPs are also largely spherical with sizes on the order of 30 nm, indicating the shapes and sizes of the NPs were not changed significantly by the CMP process. Pad debris was not observed in spent slurry samples; however, in some of the TEM images, small (~5 nm) dark particulates were observed, which are undoubtedly insoluble Ga species, as described below. DLS and zeta potential analyses of the spent Ultra-Sol® 200S slurry were performed to assess the aggregation state of *c*-SiO_2 NPs in pH 7.4 *daphnia* media (Table 4.1). The mean HDD of *c*-SiO_2 NPs in the spent Ultra-Sol® 200S was 32.99 nm and the zeta potential was -26.8 mV, indicative of stably-suspended NPs that did not aggregate. The amount of elemental Si present in the spent Ultra-Sol® 200S slurry was determined using an ICP-OES, and the calculated amount of SiO_2 was 40 ppm,

indicating the spent slurry was diluted ~5.4-fold relative to the pristine slurry. An ICP-MS was used to determine that there was 165 ppb Ga and 429 ppb As in the spent Ultra-Sol® 200S slurry, representing increases of ~4× and ~15×, respectively, relative to the pristine slurry. Following dilution with *daphnia* medium, the concentrations of Ga and As that *D. magna* were exposed to from the spent 2.0-mg/mL *c*-SiO_2 NP slurry samples were 8.25 ppb and 21.50 ppb, respectively, and 16.50 ppb and 42.00 ppb, respectively, from the 4.0-mg/mL *c*-SiO_2 NP samples. Note, the highest concentrations of Ga and As presented to *D. magna* in these slurry samples were four orders and one order of magnitude lower, respectively, than previously reported half-maximal response concentrations for *D. magna* exposed to Ga and As. For example, a 48-h LC-50 of 237,000 ppb was calculated for Ga (III) (Zeng et al., 2017), a 48-h LC-50 of 2,400 ppb was calculated for As(III) (Okamoto et al., 2015), and 24-h EC-50s of 3,500 ppb and 10,000 ppb were calculated for As(III) and As(V), respectively (He et al., 2016). Spent slurry samples were filtered and the concentrations of Ga and As in the filtrates and eluates were determined using an ICP-MS. Nearly all (99.94%) of the Ga was captured on the filter, as expected due to the insolubility of Ga_2O_3 particles formed following the oxidation of Ga in water (Matovu et al., 2013); conversely, more than 94.50% of the As was detected in the eluate, as anticipated due to the water solubilities of the As(III) and As(V) species formed under basic conditions (Torrance et al., 2010; Bi and Westerhoff, 2016). Speciation analyses of these eluates revealed the spent slurry comprised 45.4% As(III) and 54.6% As(V) species, and that organic arsenic compound in the form of either dimethylarsinic acid (DMA), monomethyl arsenic acid (MMA), or arsenobetaine (AB), were not detected.

4.4.2 Acute toxicity assays

For acute toxicity assessments, *D. magna* neonates were exposed to various concentrations of *c*-SiO_2 CMP NPs for 24, 48, 72, and 96 h. Under these conditions, *D. magna* swam randomly around all regions of the test container except at the highest applied slurry concentrations when their swimming rates appeared somewhat slower. Figure 4.3 shows the morbidity results for *D. magna* exposed to pristine and spent Ultra-Sol® 200S slurries and that no significant morbidity was observed after 96 h with either slurry even at the highest applied *c*-SiO_2 NP dose of 4.0 mg/mL. Three groups have previously reported acute morbidity results for SiO_2 NPs with *D. magna*. In studies conducted by Xu and co-workers, a dose-dependent toxicity of nano-sized (10–20 nm) SiO_2 NPs was reported with a calculated 24-h LC-50 of 0.661 mg/mL (Bing et al., 2009; Yang et al., 2014). Second, Choi and co-workers assessed the acute toxicity of nano-sized SiO_2 and observed slight increases in *D. magna* morbidity (15% and 10% for 7 nm and 10 nm SiO_2 NPs, respectively) following a 96-h exposure to an applied NP dose of 0.001 mg/mL (Lee et al., 2009). Finally, in our previous work with 50–60 nm *c*-SiO_2 NPs in a model CMP slurry, no morbidity was observed after 96 h even with the highest applied NP dose of 4.0 mg/mL (Karimi et al., 2018). It therefore appears that only SiO_2 NPs with primary particle sizes $\leq$20 nm pose any significant effects on *D. magna* morbidity under acute testing conditions.

Figure 4.3 also shows the body size results for *D. magna* exposed to pristine and spent Ultra-Sol® 200S slurries. The body sizes of *D. magna* exposed to 4.0 mg/mL of *c*-SiO_2 NPs in the pristine slurry for 96 h increased slightly by ~9% ($p = 0.35$) compared to control *D. magna* that were not exposed to slurry (Figure 4.3A), while those of *D. magna* exposed to 4.0 mg/mL of *c*-SiO_2 NPs in the spent slurry for 96 h decreased slightly by ~6% ($p = 0.42$) compared to controls

(Figure 4.3B). Since neither result was statistically significant, they are similar to the work of Lee et al., who observed no differences in the body sizes of *D. magna* exposed to 7 nm and 10 nm SiO_2 NPs relative to controls after 96 h (Lee et al., 2009).

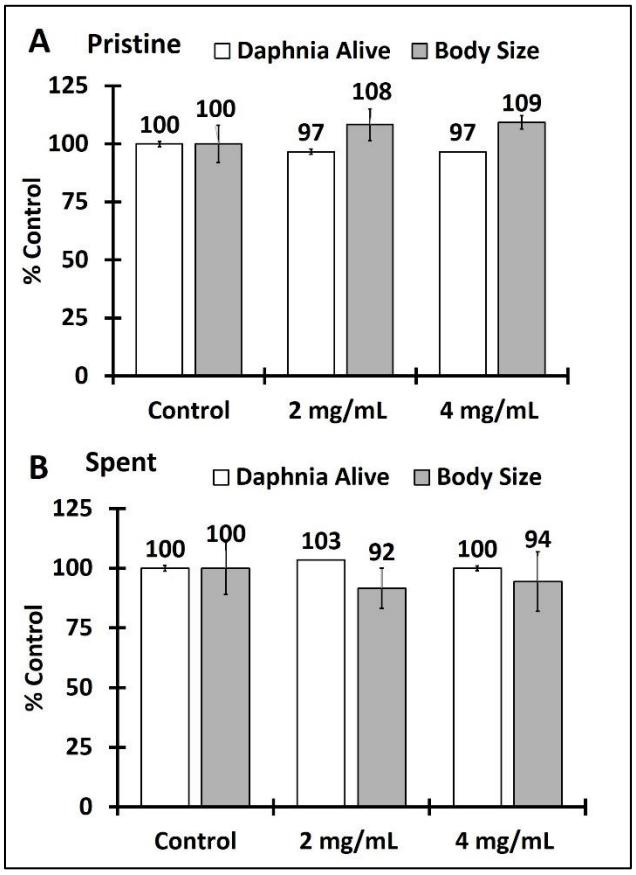

Figure 4.3. Acute morbidity and effects on *D. magna* body size after a 96-h exposure to pristine and spent Ultra-Sol® 200S slurries. Percent survival **(clear bars)** and body sizes **(shaded bars)** of *D. magna* exposed for 96 h to various concentrations of *c*-SiO_2 NPs in: **(A)** a pristine Ultra-Sol® 200S slurry, and **(B)** a spent Ultra-Sol® 200S slurry that was used to polish a GaAs wafer, plotted relative to control D. magna not exposed to a CMP slurry (set to 100%). All data points are the mean of at least three independent trials and the error bars represent the standard error of the mean (SEM).

However, these results are contrary to our previous observations with *D. magna* exposed to 4.0 mg/mL of 50–60 nm *c*-SiO_2 NPs in a model CMP slurry (Karimi et al., 2018), where body sizes

increased modestly by 17–24% compared to controls, which is indicative of a hormetic process – an adaptive response characterized by a beneficial effect to a moderate environmental stress (Mattson, 2008; Rabus and Laforsch, 2011; Stanley et al., 2013).

Images of *D. magna* exposed to the commercial Ultra-Sol® 200S slurry are shown in Figure 4.4. Since green algae is the major food source in the diet of filter-feeding *D. magna* (Gillis et al., 2006), the mid gut regions of daphnids were accordingly green, as shown in the image of a control *D. magna* not exposed to a slurry (Figure 4.4A). Interestingly, the same green coloration was observed in the images of *D. magna* exposed to the pristine and spent Ultra-Sol® 200S slurries (Figures 4.4B and 4.4C). The presence of green algae in the guts of treated and control daphnids indicates continuous and uninterrupted uptake of nutrients and that *c*-SiO_2 NPs were not physically clogging the gut, as was observed previously with *D. magna* that were exposed to model ceria and alumina CMP NPs (Karimi et al., 2018).

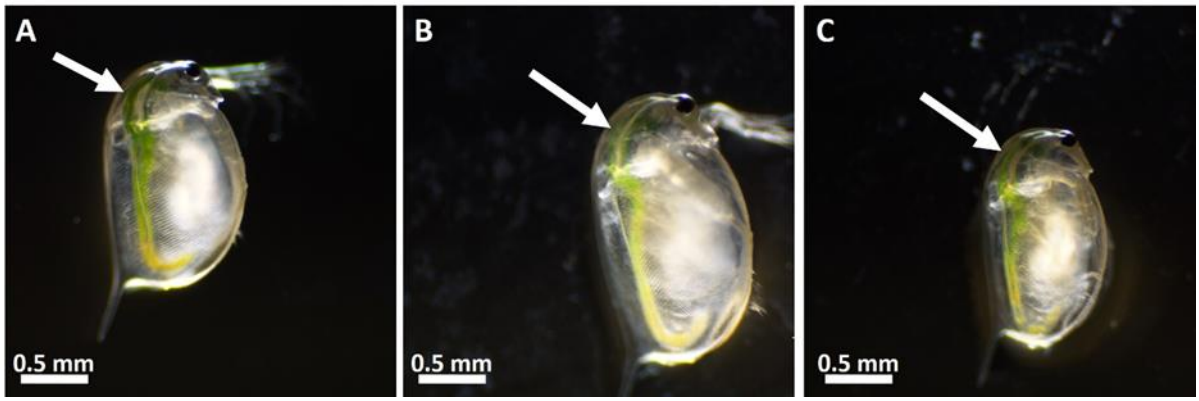

Figure 4.4. Sizes of *D. magna* after a 96-h exposure to pristine and spent Ultra-Sol® 200S slurries. Representative optical images (5× magnification) of: **(A)** control *D. magna* not exposed to Ultra-Sol® 200S slurry, **(B)** *D. magna* exposed to 4.0 mg/mL of *c*-SiO_2 NPs in the pristine Ultra-Sol® 200S slurry, and **(C)** *D. magna* exposed to 4.0 mg/mL of *c*-SiO_2 NPs in the spent Ultra-Sol® 200S slurry that was used to polish a GaAs wafer. The white arrows denote the midgut region.

In conclusion, both the pristine and spent Ultra-Sol® 200S CMP slurries can be classified as "practically nontoxic" (i.e., showing no adverse effects at levels ≤0.10 mg/mL) according to the EPA's ecotoxicity classification for aquatic organisms under acute morbidity testing conditions (EPA, 2016).

4.4.3 Chronic toxicity assays

For chronic toxicity assessments, *D. magna* neonates were continuously exposed for 21 d to lower (sub-lethal) concentrations of *c*-SiO_2 CMP NPs than those used in the acute studies, and three biological endpoints were monitored (morbidity, body size, and reproductive output). Under these test conditions, *D. magna* swam actively around all regions of the test container. Figure 4.5A shows the morbidity results for *D. magna* exposed for 21 d to 0.10 mg/mL of *c*-SiO_2 NPs in the two Ultra-Sol® 200S slurries. There was no significant morbidity observed for either the pristine or spent Ultra-Sol® 200S slurry. In a 21-d chronic toxicity assessment of the 50–60 nm *c*-SiO_2 NPs in a model CMP slurry at an applied dose of 0.10 mg/mL, we previously observed a slight degree of morbidity (10%) relative to controls (Karimi et al., 2018).

Figure 4.5B shows that the body sizes of *D. magna* exposed for 21 d to 0.10 mg/mL of *c*-SiO_2 NPs in the Ultra-Sol® 200S pristine slurry and in the spent slurry increased by ~9% (p = 0.000007) and by ~10% (p = 0.0000006), respectively, compared to control *D. magna* that were not exposed to slurry. In a 21-d chronic toxicity assessment of the 50–60 nm *c*-SiO_2 NP in a model CMP slurry at an applied dose of 0.1 mg/mL, we also observed a 10% increase in body size relative to controls (Karimi et al., 2018).

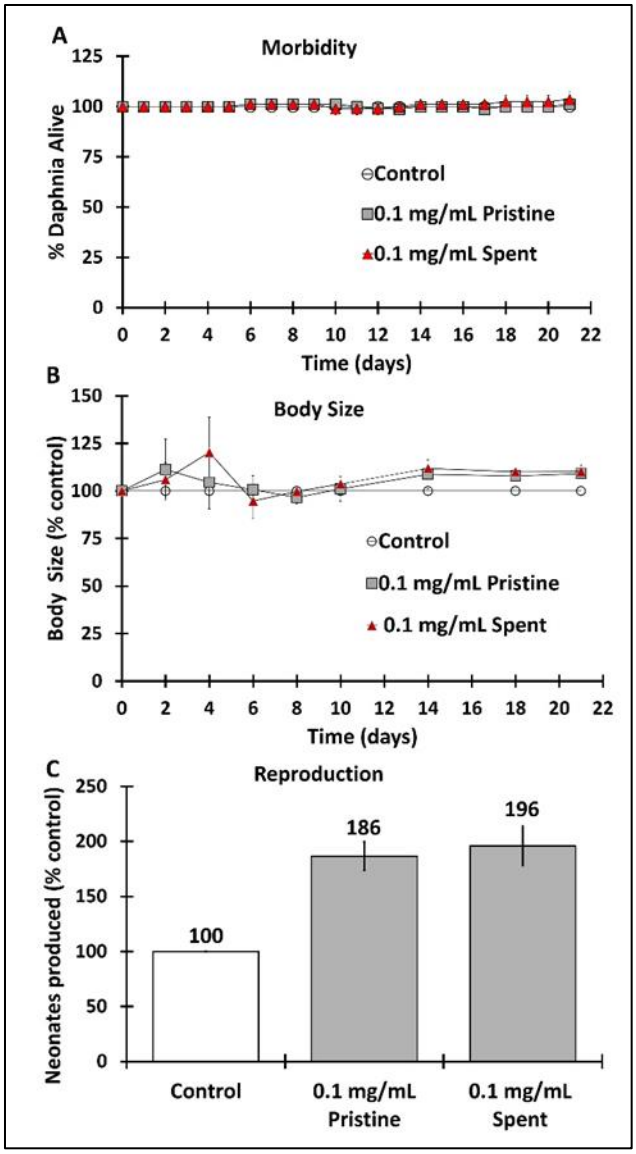

Figure 4.5. Chronic morbidity and effects on *D. magna* body size and reproduction after a 21-day exposure to pristine and spent Ultra-Sol® 200S slurries. (A) Percent survival, **(B)** body size, and **(C)** reproductive output of adult *D. magna* exposed for 21 d to 0.10 mg/mL of *c*-SiO_2 NPs in a pristine or a spent Ultra-Sol® 200S slurry that was used to polish a GaAs wafer, plotted relative to control *D. magna* not exposed to a CMP slurry (set to 100%). The numbers above the bars are the mean of three independent trials and the error bars represent the SEM.

Images of *D. magna* exposed to the commercial Ultra-Sol ® 200S slurries are shown in Figure 4.6. Again, the presence of green algae in the guts of treated and control daphnids indicates

continuous and uninterrupted uptake of nutrients and that *c*-SiO_2 NPs were not physically clogging the gut.

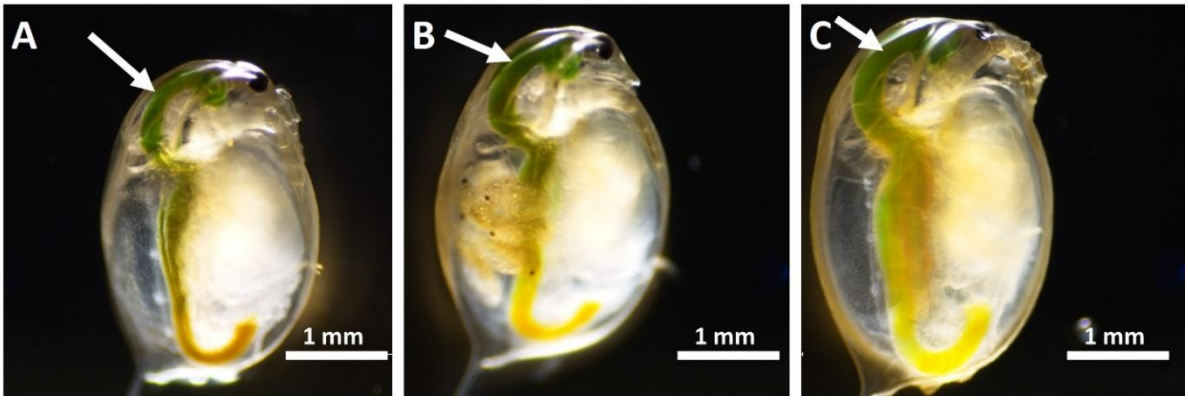

Figure 4.6. Sizes of *D. magna* after a 21-d exposure to pristine and spent Ultra-Sol® 200S slurries. Representative optical images (3× magnification) of: **(A)** control *D. magna* not exposed to Ultra-Sol® 200S slurry, **(B)** *D. magna* exposed to 0.10 mg/mL of *c*-SiO_2 NPs in the pristine Ultra-Sol® 200S slurry, and **(C)** *D. magna* exposed to 0.10 mg/mL of *c*-SiO_2 NPs in the spent Ultra-Sol® 200S slurry that was used to polish a GaAs wafer. The white arrows denote the midgut region.

The most striking chronic results were the significant 1.86-fold and 1.96-fold increases in reproductive output of *D. magna* exposed for 21 d to 0.10 mg/mL of *c*-SiO_2 NPs in the pristine and spent Ultra-Sol® 200S slurries, respectively (Figure 4.5C), both of which are indicative of a positive hormetic response process. In a 21-d chronic toxicity assessment of the 50–60 nm *c*-SiO_2 NPs in a model CMP slurry at an applied dose of 0.10 mg/mL, we previously observed a modest (18%) increase in reproductive output relative to controls (Karimi et al., 2018), which contrasts the work of Lee et al., who studied the chronic effects of 7 nm and 10 nm SiO_2 NPs to *D. magna* and reported no effects on reproductive output (Lee et al., 2009).

To determine if a soluble component of the pristine Ultra-Sol® 200S slurry contributed to the chronic effects observed with *D. magna* a the pristine slurry containing 214 mg/mL *c*-SiO_2

NPs was centrifuged at 100,000×g to pellet the NPs and *D. magna* were exposed to the supernatant. After 21 days, there was no significant morbidity observed, there was a ~12% ($p = 0.0046$) increase in body size compared to control *D. magna* ($p = 0.0046$), and there was a significant 1.84-fold increase in reproductive output of *D. magna* that were exposed to the supernatant (data not shown). Since the results for *D. magna* exposed to the supernatant of the pristine slurry were essentially identical to those observed with *D. magna* exposed pristine Ultra-Sol® 200S slurry, we conclude that the pellet of solid material (>99% of which was *c*-SiO_2 NPs) remaining in the centrifugation tube was not responsible for the chronic effects observed with *D. magna*, and instead, that the chronic effector(s) stemmed from soluble component(s) in the as-received pristine slurry. Since the highest concentrations of Ga and As presented to *D. magna* in these slurry samples were four orders and one order of magnitude lower, respectively, than previously reported half-maximal response concentrations for *D. magna* exposed to Ga and As (Okamoto et al., 2015; He et al., 2016; Zeng et al., 2017), it is unlikely that these III-V materials are the effectors. While speculative, a possible candidate is an oxidizer such as hydrogen peroxide, sodium hypochlorite, sodium iodate, sodium periodate, or dibromine, since oxidizers are well-known components of *c*-SiO_2 slurries designed for polishing GaAs substrates (Torrance et al., 2010; Matovu et al., 2013).

4.5 CONCLUSIONS

III-V semiconducting materials are increasingly being used in the fabrication of electronic devices, which can contribute to their potential release into the environment. Herein, *D. magna* was used to address the question of whether polishing a GaAs wafer can impart added toxicity to ~30-nm *c*-SiO_2 NPs contained within an Ultra-Sol® 200S CMP slurry. Under acute test conditions, there were no major differences between the results observed with either the pristine

or spent slurries, both had little effect on *D. magna* morbidity and body sizes. Under chronic test conditions, there were no major differences between the results observed with either the pristine or spent slurries, neither was toxic but both lead to a slight (9-10%) increase in body sizes and a significant (~2-fold) increase in reproductive output, indicative of positive hormetic responses. Identical increases in body size and reproductive output were observed with a supernatant of the pristine slurry, in the absence of the *c*-SiO_2 NPs, suggesting that the chronic effects were derived from soluble component(s) in the pristine slurry, and not from the *c*-SiO_2 NPs nor from the CMP process that removed ~3 mg of material from a GaAs wafer. While CMP waste streams in actual fabs are more complex mixtures than the effluent collected from a single CMP tool in the present work, the results presented here may help to guide decisions on the ecological risks of pristine and spent *c*-SiO_2 slurries used to CMP GaAs wafers.

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CHAPTER 5

CONCLUSIONS

In this dissertation we investigated the effects on *D. magna* of four model CMP slurries and two commercially available CMP slurries. The four model slurries are unique in that they represent the simplest formulations to generate stable aqueous suspensions of four metal oxide NPs that are actually used in complex proprietary commercial slurries. Herein, we reported, for the first time, the toxicity of these four model CMP slurries to *D. magna*. In the acute 4-d toxicity assessments, the key findings were that the Al_2O_3 slurry was lethal with a calculated 96-h LC-50 of 1.1 mg/mL, and that the slurries with highly-aggregated NPs (i.e., the CeO_2 and Al_2O_3 slurries) caused a severe dose-dependent decrease in body sizes. Interestingly, this contrasted a modest increase in body size observed with the slurry comprising the least-aggregated NPs (i.e., the *c*-SiO_2 slurry), which is indicative of a positive hormetic process. In the chronic 21-d toxicity assessments with non-lethal NP doses, the key findings were that the Al_2O_3 slurry lead to a modest increase in morbidity and a significant decrease in body size, and that the CeO_2 and Al_2O_3 CMP slurries caused a severe dose-dependent decrease in reproductive output. Interestingly, this contrasted a modest increase in reproduction observed with the *c*-SiO_2 CMP slurry, which was credited to a positive hormetic response. In conclusion, distinct and unpredictable adverse effects were observed with different model CMP slurries on *D. magna* morbidity, growth, and reproductive output.

Furthermore, we studied the bioaccumulation and biopersistence of the two model CMP slurries CeO_2 and Al_2O_3 since these slurries had significant adverse effects on *D. magna*. The key findings were that both CeO_2 and Al_2O_3 were ingested by *D. magna* as a function of time and dose.

D. magna that were exposed for 48 h to 0.1 mg/mL CeO_2 CMP slurry accumulated 120 µg of CeO_2, and those that were exposed to for 48 h to 0.1 mg/mL of Al_2O_3 CMP slurry accumulated 44 µg of Al_2O_3. This shows that different metal oxide slurries are accumulated in different amounts by *D. magna*. In addition biopersistence studies revealed that after 48 h of depuration time, *D. magna* exposed to 0.1 mg/mL CeO_2 successfully eliminated 85% of the initial CeO_2 load, and *D. magna* exposed to 0.1 mg/mL of Al_2O_3 were able to eliminate 78% of the initial Al_2O_3 load. However, the amount metal oxide NP slurries that persist in *D. magna* might be available for transfer to higher trophic level and the amount of metal oxide NP slurries eliminated might be available to other trophic levels.

Finally we investigated effects on *D. magna* exposed to a commercially available CMP slurry Ultra-Sol® 200S used to polish wafers containing III-V semiconducting materials. Herein, *D. magna* was used to address the question of whether polishing a GaAs wafer can impart added toxicity to ~30-nm *c*-SiO_2 NPs contained within an Ultra-Sol® 200S CMP slurry. Under acute test conditions, there were no major differences between the results observed with either the pristine or spent slurries, both had little effect on *D. magna* morbidity and body sizes. Under chronic test conditions, there were no major differences between the results observed with either the pristine or spent slurries, neither was toxic but both lead to a slight (9-10%) increase in body sizes and a significant (~2-fold) increase in reproductive output, indicative of positive hormetic responses. Identical increases in body size and reproductive output were observed with a supernatant of the pristine slurry, in the absence of the *c*-SiO_2 NPs, suggesting that the chronic effects were derived from soluble component(s) in the pristine slurry, and not from the *c*-SiO_2 NPs nor from the CMP process that removed ~3 mg of material from a GaAs wafer. While CMP waste

streams in actual fabs are more complex mixtures than the effluent collected from a single CMP tool in the present work, the results presented here may help to guide decisions on the ecological risks of pristine and spent *c*-SiO_2 slurries used to CMP GaAs wafers.

BIOGRAPHICAL SKETCH

Sarah Karimi was born in Karachi, Pakistan on January 1, 1990. After completing her schoolwork at Karachi Grammar School in 2009, Sarah entered Southern Methodist University in Dallas, Texas. In May 2013, she graduated magna cum laude with a Bachelor of Science in Chemistry and Environmental Sciences, and minors in Biology and Mathematics from Southern Methodist University. In August 2013 Sarah began her graduate studies at The University of Texas at Dallas where she joined the UT Dallas Bionanosciences Group under the tutelage of Dr. Rockford K. Draper and Dr. Paul Pantano. In 2016, Sarah received her Master of Science in Chemistry from The University of Texas at Dallas. During her time at The University of Texas at Dallas, she worked as graduate research assistant for the Department of Chemistry under the direction of Paul Pantano and Rockford K. Draper. After earning her Ph.D. she will move to Herndon, VA where she plans on working as a postdoc.

CURRICULUM VITAE

SARAH KARIMI
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EDUCATION

Doctor of Philosophy, Chemistry August 2018
University of Texas at Dallas
Studies on the toxicity, bioaccumulation, and biopersistence of model and commercial chemical mechanical planarization slurries with Daphnia magna
Cumulative GPA: 3.72/4.00

Masters of Science, Chemistry May 2016
University of Texas at Dallas
Acute Toxicity Assessments to Daphnia magna of Model Chemical Mechanical Planarization Slurries
Cumulative GPA: 3.72/4.00

Bachelor of Science, Chemistry and Environmental Science May 2013
(Minor in Biology and Mathematics)
Southern Methodist University
Cumulative GPA: 3.85/4.00 (*magna cum laude*) Phi Beta Kappa

ACADEMIC HONORS

- Simon Karecki Award for Research in Environmentally Benign Semiconductor Manufacturing 2017
- American Chemical Society Award for Outstanding Senior in Inorganic Chemistry 2013
- Inducted into the National Honor Society Phi Beta Kappa 2013
- Outstanding Academic Achievement Award 2011
- Academic Excellence Award 2009
- Scholarships at Southern Methodist University
- Distinguished Scholar Award 2009
- Rotunda Scholarship Award 2009
- Jesky Scholarship Award from the Department of Chemistry 2010
- Lezenby Scholarship Award from the Department of Chemistry 2010
- Foscue Scholarship Award from the Huffington Department of Earth Science 2010
- Student Senate Scholarship for International Students 2010
- Hamilton Undergraduate Research Scholar 2011

PROFESSIONAL MEMBERSHIPS

- Society of Toxicology
- American Chemical Society
- Phi Beta Kappa, Gamma of Texas
- Alpha Lambda Delta Honor Society
- Mortar Board National College Senior Honor Society
- Golden Key Honour Society
- National Society of Colligate Scholars
- Southern Methodist University Geo Club

PROFESSIONAL EXPERIENCE

University of Texas at Dallas 2015
Graduate Research Assistant – Bionanosciences Group
•Started and managed an aquatic toxicology project funded by Semiconductor Research Corporation (SRC), successfully performed lab experiments on model fresh water organisms to assess the toxicity of chemicals of interest to the semiconductor industries. Presented research results at several national conferences.
•Learned project management skills, learned how to operate sophisticated instrumentation, wrote standard operating procedures for ICP-MS and microwave digesters, maintained instruments, and participated in lab safety workshops. Assisted in writing annual reports for the SRC funding agency.

University of Texas at Dallas 2013
Graduate Teaching Assistant – Department of Chemistry and Biochemistry
•Taught 1 semester of Quantitative Analysis laboratory and 3 semesters of Organic Chemistry laboratory. Conducted lectures for laboratory sections, graded lab reports, tutored students, conducted quizzes and prepared the chemicals and instrumentation required for the experiments.

Southern Methodist University
Undergraduate Research Assistant – Department of Chemistry 2010
•Assisted Professor John Buynak for two years in synthesizing and optimizing carbapenem antibiotics. I was successful in synthesizing several new molecular entities which can be used as reagents for further reactions. Also co-authored two poster presentations and one peer-reviewed publication.

Undergraduate Research Assistant – Department of Geology 2011
•Assisted Professor Bonnie Jacobs for six months with the construction of paleo-climate patterns by establishing a relationship between leaf stomatal frequencies and atmospheric carbon dioxide concentrations. I succeeded in showing an inverse relationship between the stomatal frequency on the leaf and the content of carbon dioxide surrounding the leaf.

Undergraduate Research Assistant – Department of Chemistry 2012
•Assisted Professor Michael Lattman for four months by successfully synthesizing complex organic compounds such as developing calix[5]arenes using recycling techniques in an effort to reduce the process' environmental impact.

PEER-REVIWED PUBLICATIONS

Sarah Karimi**,** Meiline Troung, Ru-hung Wang, Rockford Draper and Paul Pantano. Acute and Chronic Toxicity of metal oxide nanoparticles in chemical mechanical planarization slurries with *Daphnia magna*, manuscript submitted November 2017 to *Environmental Science: Nano*.

Sarah Karimi, Meiline Troung, Shayam Aravamudhan, Steven Crawford, Ru-hung Wang, Carole Mikoryak, Rockford Draper and Paul Pantano. Acute and Chronic Toxicity to *Daphnia magna* of colloidal silica nanoparticles in a commercial chemical mechanical planarization slurry after polishing a gallium arsenide wafer, manuscript to be submitted to *NanoImpact*.

Sarah Karimi, Meiline Troung, Ru-hung Wang, Paul Pantano and Rockford Draper. Bioaccumulation and Biopersistence of metal oxide nanoparticles in chemical mechanical planarization slurries with *Daphnia magna*, manuscript to be submitted to *Environmental Science and Technology*.

Weirui Chai, Emily Nguyen, Jennifer Doran, Katie Han, Alexander Weatherbie, Daniel Fernandez, Sarah Karimi, Ritwick Mynam, Caroline Humphrey, Sara Rana, and John D. Buynak. Urazole Synthesis Part 2: Facilitating N4 Substitution. *Tetrahedron Letters*, 2013, 54, 2308–2310.

SCIENTIFIC PRESENTATIONS

Bioaccumulation and Biopersistence of Model Chemical Mechanical Planarization Slurries in Daphnia (poster)

Society of Toxicology, 57th Annual Meeting, March 2018

Bioaccumulation, Biopersistence, and Toxicity of CMP Nanoparticles in Mammalian and Aquatic Models (oral)

SRC Engineering Research Center, 21st Annual Industrial Liaison Meeting, April 2017

Acute and Chronic Toxic Effects of Model Chemical Mechanical Planarization Slurries on Morbidity, Body Size, and Reproduction of Daphnia magna (poster)

Society of Toxicology, 56th Annual Meeting, March 2017

Acute and Chronic Toxic Effects of Model Chemical Mechanical Planarization Slurries on Morbidity, Body Size and Reproduction of Daphnia magna (poster)

International Conference on the Environmental Effects of Nanoparticles and Nanomaterials, 11th Annual Meeting, August 2016

Bioaccumulation, Biopersistence, and Toxicity of CMP Nanoparticles in Mammalian and Aquatic Models (oral)

SRC Engineering Research Center, 20th Annual Industrial Liaison Meeting, April 2016

Toxicity Assessment of Model Chemical Mechanical Planarization Slurries using Daphnia magna (poster)

Society of Toxicology, 55th Annual Meeting, March 2016

Bioaccumulation, Biopersistence, and Toxicity of CMP Nanoparticles in Mammalian and Aquatic Models (oral)

SRC Engineering Research Center, 19th Annual Industrial Liaison Meeting, April 2015

OTHER EXPERIENCE

Publicity Officer – American Assoc. of Petroleum Geologists at Southern Methodist University Aug 2011 – May 2012

Volunteer Teacher – Catholic Church Community Center, Dallas, Texas Feb 2010 – May 2010

Volunteer Teacher – Behbood Association School, Karachi, Pakistan Dec 2008 – May 2009

Volunteer Teacher – Garage School, Karachi, Pakistan Dec 2007 – Dec 2008