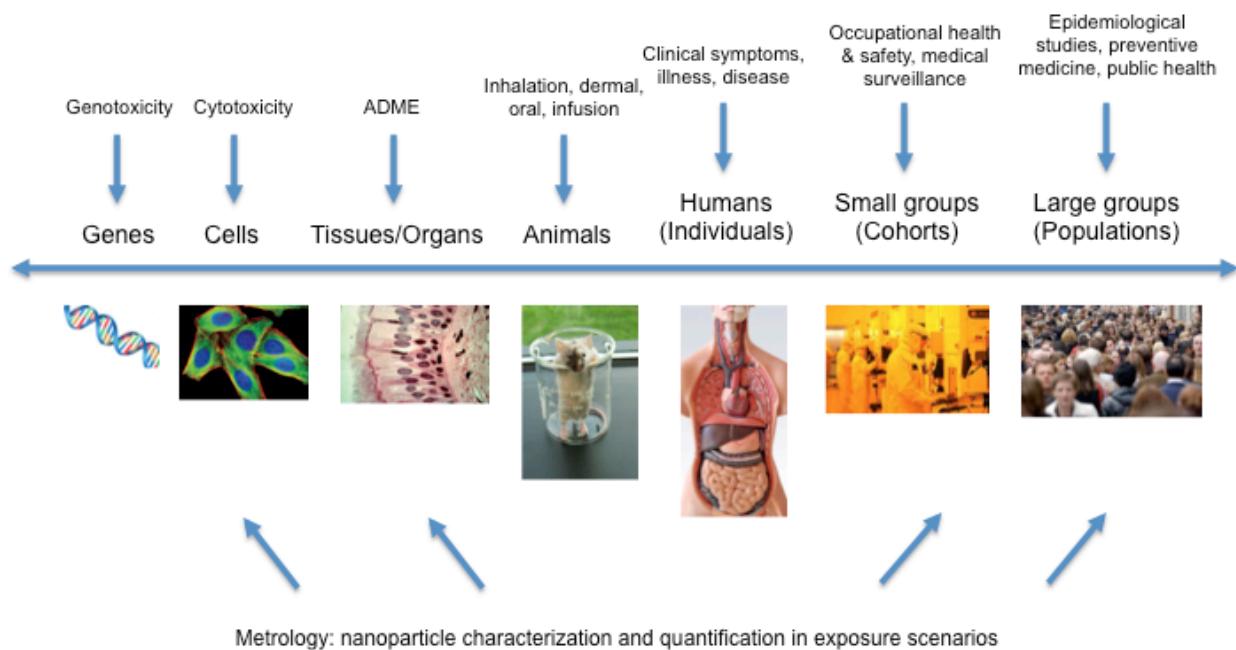


Occupational and Environmental Health and Safety of Engineered Nanomaterials

Sara Brenner, MD, MPH

Scope: To proactively address the emerging needs of health and safety research related to engineered nanomaterials, seeking to develop in real-time the innovative technologies and methodologies needed to assess, monitor, and safely accelerate nanotechnology.



Stebounova, L, Morgan H, Grassian V, **Brenner S.** Health and Safety Implications of Occupational Exposure to Engineered Nanomaterials. *WIREs Nanomedicine & Nanobiotechnology* 2012, 4, 310-321.

My research focuses on the health and safety of individuals, workers, populations, and the environment as they relate to engineered nanomaterials. Through research projects across a broad portfolio, I serve as a link between basic life science research, clinical translational research, direct patient care, and public health interventions aimed at improving population and individual health outcomes. Over the past seven years, I was involved in the formation and operation of the NanoHealth and Safety Center (NSC) at CNSE, a public-private partnership that addressed gaps in our understanding of the safety and risks associated with the unique characteristics of nanoscale materials used in the semiconductor industry. With a background in basic science, internal medicine, and preventive medicine, I oversee a research team that assesses the health and safety of individuals, workers, and populations through the lens of occupational risks, hazards, and exposures. The disciplines of occupational medicine, environmental health, epidemiology, public policy, health care administration, and social and behavioral health intersect at critical points in the design and implementation of appropriate health and safety research relevant to nanotechnology in the workplace, consumer market place, and environment. I aim to address these considerations as well as the public policy, social, and ethical implications of nanotechnology in order to guide the vision and direction of occupational and environmental health and safety research and regulation.

2017 RESEARCH PROJECTS

Occupational Exposure Assessment of Engineered Nanoparticles During Chemical Mechanical Planarization (CMP) Operation and Maintenance

Engineered nanoparticles are used in the semiconductor industry during chemical mechanical planarization (CMP), a process that occurs multiple times at different stages of the integrated circuit (IC) fabrication process for silicon complementary metal-oxide-semiconductor transistors (Fig. 1). Because of their size and novel chemical physical properties, certain nanoparticles (NPs) could be more toxic than their bulk material, with the potential that existing occupational control measures and exposure limits may not be sufficiently protective. Currently, there is little published data available on potential occupational exposures to the engineered nanoparticles used in CMP (nanoscale alumina, silica, ceria). Additional studies are needed to evaluate potential nanoparticle exposures in the workplace.

Our research assesses potential worker exposures to engineered nanomaterials (ENMs) and agglomerates during CMP tool operation, maintenance and related tasks. We obtained air samples from the task area and workers' personal breathing zones to assess the potential for inhalation exposure to ENMs. This was achieved by using multiple complementary instruments and approaches to compare particle number concentrations during tasks with potential exposure to nanoparticles with background levels, and to characterize airborne particulate by size, morphology, and chemical composition. Microvacuum samples of selected surfaces in the workplace were also collected and analyzed for the presence of the materials of interest to assess for potential cutaneous exposure.

Based on findings from 2011-2013, different areas at the sampling location where workers handle ENMs were identified for further testing based on the likelihood of inhalation or cutaneous exposures. To date, results from air and surface samples collected suggest that ENMs used or generated by CMP become aerosolized during various tasks and may be accessible for inhalation or cutaneous exposures by workers (Fig. 2). A deeper investigation and further exposure assessments have been prioritized based on risk. Additional research is needed to further quantify the level of exposure and determine the potential human health impacts. Furthermore, it is important to interpret this exposure assessment data alongside hazard (toxicological) data in order to assess risk to workers. Risk assessments should also be

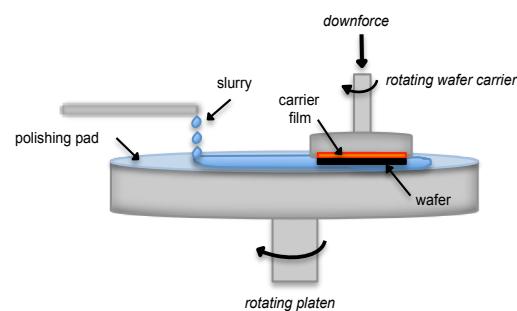


Figure 1. CMP polishing mechanism. (Roth GA, Neu-Baker NM, Brenner SA. SEM analysis of particle size during conventional treatment of CMP process wastewater. *Sci. Tot. Environ.* 2015, 508:1-6. doi:10.1016/j.scitotenv.2014.11.075.)

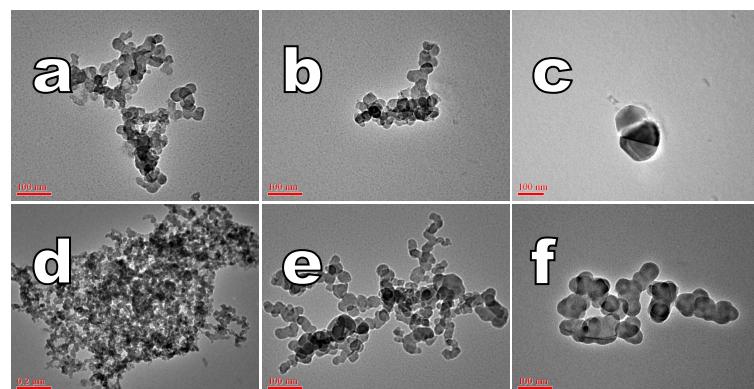


Figure 2. TEM images of air samples obtained in the cleanroom, subfab, and WWT. (a)-(b) Agglomerates of amorphous silica from WWT task area. Scale bars = 100nm. (c) Alumina from worker PBZ in WWT. Scale bar = 100nm. (d) Mixed Al-Si agglomerate from WWT background. Scale bar = 0.2um. (e) Agglomerate of amorphous silica from subfab task area. Scale bar = 100nm. (f) Agglomerate of alumina from the cleanroom task area. Scale bar = 100nm. (Brenner SA, Neu-Baker NM, Eastlake AC, Beaucham CC, Geraci CL. NIOSH Field Studies Team assessment: worker exposure to aerosolized metal oxide nanoparticles in a semiconductor fabrication facility. *J. Occup. Environ. Hyg.* 2016, 13(11): 871-880. doi: 10.1080/15459624.2016.1183015.)

conducted in conjunction with information available regarding the effectiveness of personal protective equipment and other controls in order to reduce exposures to ENMs.

The most significant hurdle holding back exposure science, risk assessment, and the development of safety guidelines for the nanotechnology workforce is the lack of validated analytical techniques that accurately identify and characterize ENMs captured in occupational settings. The associated costs, time, and lack of standardization of existing methods make it impossible for industries to implement exposure assessment programs or comply with new or forthcoming recommended exposure limits for ENMs. This research team seeks to advance the state of the science by developing and testing a new protocol for analysis of ENMs on filters by: further developing a novel hyperspectral imaging (HSI) method for high-throughput screening; evolving best-known methods for direct visualization of filter-captured ENMs by developing and incorporating advanced techniques into the new protocol; and testing the new protocol on real-world samples obtained during occupational exposure scenarios.



Relevant publications:

- 1) Brenner SA, Neu-Baker NM, Eastlake AC, Beaucham CC, Geraci CL. NIOSH Field Studies Team assessment: worker exposure to aerosolized metal oxide nanoparticles in a semiconductor fabrication facility. *Journal of Occupational and Environmental Hygiene* 2016, 13(11): 871-880. doi: 10.1080/15459624.2016.1183015.
- 2) Brenner SA, Neu-Baker NM, Caglayan C, Zurbenko IG. An occupational exposure assessment for airborne metal oxide nanoparticles in a semiconductor fab and subfab. *Journal of Occupational and Environmental Hygiene* 2016, 13(9): D138-D147. doi: 10.1080.15459624.2016.1183012.
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- 7) Shepard MN, Brenner S. An occupational exposure assessment for engineered nanoparticles used in semiconductor fabrication. *Annals of Occupational Hygiene* 2014, 58(2):251-265. doi: 10.1093/annhyg/met064.

Development of Advanced Imaging and Analytical Techniques for Occupational Exposure to Nanomaterials

This project assists the U.S. National Institute for Occupational Safety and Health (NIOSH) by advancing risk assessment and reduction strategies for occupational exposures, monitoring of materials that may impact population health and public safety, and the development of industrial practice standards for

product safety. Monitoring and surveillance techniques are being developed with NIOSH and other collaborators and partners to assess the occupational impact of exposure to nanomaterials. A framework will be built to employ custom-tailored strategies to mitigate potential risks associated with nanotechnology-based manufacturing and formulation processes that are currently in use to create nano-enabled products already on the market as well as those under development. NIOSH, in dialogue with SUNY Poly CNSE and other research centers, has identified a fundamental hurdle in advancing exposure science for the nanotechnology workforce: the lack of validated analytical techniques that consistently, reliably, and accurately identify and characterize ENMs captured in occupational settings. Exploring new or alternate visualization techniques is critical for addressing exposure assessment needs.

Hyperspectral imaging (HSI) is a versatile technique that has seen use in geological and ecological studies due to its ability to map materials based on their characteristic spectral profile. These profiles on a macroscopic scale are heavily indicative of the material's composition. The Brenner Research Team has employed an Olympus BX-43 microscope equipped with a darkfield camera, a hyperspectral camera, and a CytoViva® light source to enable the same materials analysis on nanoscale materials. Spectral profiles for nine commercial CMP slurries have been assembled into a reference library for comparison to unknown samples. This library has been verified to successfully map pixels in identifiable particles while excluding background pixels. Reference spectral libraries will be developed for each ENM involved in the study (Fig. 3).

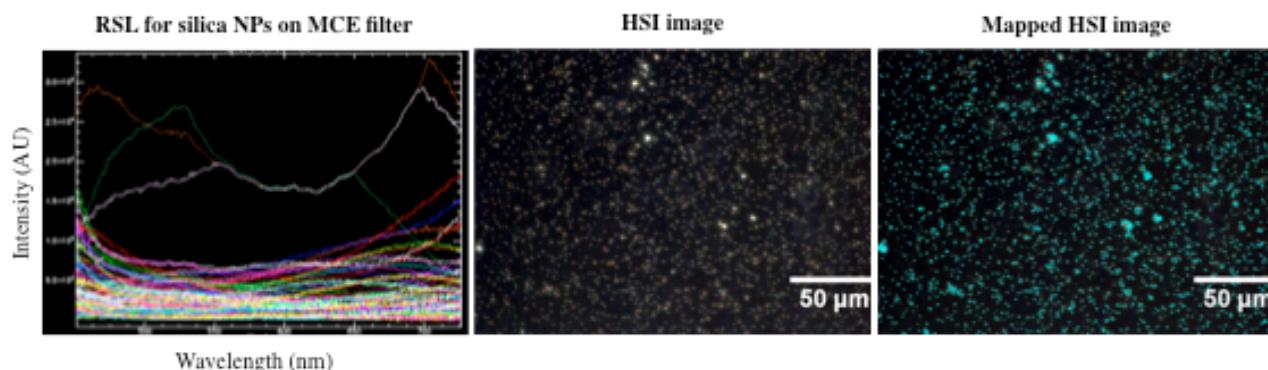


Figure 3. Silica reference spectral library (RSL), hyperspectral image, and mapped hyperspectral image. Left: RSL for silica NPs (Sigma Aldrich) on MCE filter. Center: 40x hyperspectral image of silica NPs on MCE filter. Right: mapped hyperspectral image of silica NPs on MCE filter. Aqua map indicates pixels with spectra that positively match spectra in the RSL, indicating presence and location of silica NPs. (Neu-Baker NM, Smith D, Segrave A, Beach J, Zurbenko IG, Dunn K, Brenner SA. Protecting the nanotechnology workforce: a new protocol for characterization of filter-captured nanomaterials from occupational exposure assessments. *TechConnect World Innovation Conference & Expo Proceedings*. Accepted March 2017.)



Relevant publications:

- 1) Neu-Baker NM, Smith D, Segrave A, Beach J, Zurbenko IG, Dunn K, Brenner SA. Protecting the nanotechnology workforce: a new protocol for characterization of filter-captured nanomaterials from occupational exposure assessments. *TechConnect World Innovation Conference & Expo Proceedings*. Accepted March 2017.
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- 5) Guttenberg M, Bezerra L, Neu-Baker NM, Sosa Peña MP, Elder A, Oberdörster G, Brenner SA. Biodistribution of inhaled metal oxide nanoparticles mimicking occupational exposure: a preliminary investigation using enhanced darkfield microscopy. *Journal of Biophotonics* 2016, 9(10):987-993. doi:10.1002/jbio.201600125.
- 6) Sosa Peña MP, Gottipati A, Tahiliani S, Neu-Baker NM, Frame MD, Friedman AJ, Brenner SA. Hyperspectral imaging of nanoparticles in biological samples: simultaneous visualization and elemental identification. *Microscopy Research and Technique* 2016, 79(5):349-358. doi:10.1002/jemt.22637.
- 7) Roth GA, Sosa Peña MP, Neu-Baker NM, Tahiliani S, Brenner SA. Identification of metal oxide nanoparticles in histological samples by enhanced darkfield microscopy and hyperspectral mapping. *Journal of Visualized Experiments* 2015, 106:53317. doi:10.3791/53317.
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Advancing Nanoscale Imaging Technology for Environmental and Biological Applications: Development and Commercialization of Customized Software Plugins to Expedite Hyperspectral Data Acquisition and Image Analysis

Collaborators: James Beach, PhD, CytoViva, Inc.; Brian Northan, True North Intelligent Algorithms, LLC

Enhanced darkfield microscopy and hyperspectral imaging (EDFM-HSI) is an emerging technique for direct visualization and hyperspectral analysis of ENMs in a variety of matrices. HSI combines spectrophotometry and imaging to capture spectral data in the visible and near-infrared (VNIR) wavelengths for each pixel in a hyperspectral image. Spectral data can be used to identify ENMs of interest using a mapping algorithm, such as the spectral angle mapper (SAM). The CytoViva (CytoViva, Inc., Auburn, AL) EDFM-HSI system has broad-ranging applications across numerous scientific disciplines for the visualization and identification of nanoscale materials. Dr. Brenner and her research team are at the forefront of methods development for EDFM-HSI of engineered NPs in animal tissues from toxicology collaborations, NPs in industrial wastewater, and NPs captured on filter media from occupational exposure assessments. Based on research conducted by the Brenner Research team utilizing this tool since 2012 and other CytoViva users around the world, it is clear that the existing HSI analysis software (ENVI 4.8, Harris Geospatial Solutions) does not allow for high-throughput analysis and is missing essential functions which greatly hinders data acquisition, timely dissemination of research results, and widespread use of EDFM-HSI for biological and environmental applications. This project seeks to break through these existing limitations by developing a new suite of low-level, open source hyperspectral data analysis and visualization routines, which will be used to build proprietary plugins using ImageJ (NIH) for use with the CytoViva EDFM-HSI system. The overarching goal of this project is to develop a series of low-level libraries that can be shared with other researchers (short-term) and commercialize a full suite of high-level automated analysis plugins that will facilitate applications for HSI research and development for a broad range of scientific and industrial applications (long-term).



Relevant publications:

- 1) Neu-Baker NM, Smith D, Segrave A, Beach J, Zurbenko IG, Dunn K, Brenner SA. Protecting the nanotechnology workforce: a new protocol for characterization of filter-captured nanomaterials from occupational exposure assessments. *TechConnect World Innovation Conference & Expo Proceedings*. Accepted March 2017.
- 2) Neu-Baker NM, Eastlake AC, Brenner SA. Developing a rapid screening method for direct visualization of nanoparticles captured on filter media during occupational exposure assessments. *TechConnect World Innovation Conference & Expo Proceedings*. Accepted March 2017.
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- 6) Sosa Peña MP, Gottipati A, Tahiliani S, Neu-Baker NM, Frame MD, Friedman AJ, Brenner SA. Hyperspectral imaging of nanoparticles in biological samples: simultaneous visualization and elemental identification. *Microscopy Research and Technique* 2016, 79(5):349-358. doi:10.1002/jemt.22637.
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- 8) Roth GA, Tahiliani S, Neu-Baker NM, Brenner SA. Hyperspectral microscopy as an analytical tool for nanomaterials: advanced review. *WIREs Nanomedicine & Nanobiotechnology*. Available online January 2015. doi:10.1002/wnan.1330.

Acute and Subchronic Effects Following Inhalation Exposure to Engineered Metal Oxide Nanoparticles in a Rat Model

Collaborators: Günter Oberdörster, DVM, PhD and Alison Elder, PhD, University of Rochester

Inhalation has been identified as the most prominent potential route of human exposure during manufacturing, distribution and use of engineered nanomaterials. There are, however, major challenges to performing a realistic risk assessment for these materials due to a lack of accurate occupational exposure data and of health effects-related data using realistic exposure conditions. Until now, *in vitro* and *in vivo* studies have identified hazard using bolus type delivery methods and unrealistically high doses in order to induce an inflammatory response. Furthermore, bolus type delivery methods are limited in determining chronic health effects that may result from prolonged exposure to very low concentrations. This study investigates the extent to which dose, dose rate and duration of exposure to slurries containing metal oxide nanoparticles influences associated health outcomes. Specifically, this study evaluates dose-related outcomes following inhalation exposures in rats to SiO₂, Al₂O₃ and CeO₂ NP-containing slurries, as well as traditional bolus delivery methods for comparison. This study also aims to characterize the retention of inhaled slurry NPs in different regions of the respiratory tract and translocation to secondary organs.

A goal of the study is to identify and characterize nanoparticle retention in the lungs and potential translocation to other organs. Organ sections from the acutely exposed rats were prepared for brightfield and darkfield microscopy. In this way, we are able to elucidate the biodistribution of these metal oxide nanoparticles following inhalation exposure. To date, we have observed materials of interest in each organ type sectioned and prepared from these studies (Fig. 4). We have discovered that nanoparticles reach the circulatory system, likely through the lungs where they have been observed in the blood vessels. Additionally, nanoparticles have been located in other organs as well, though it is as of yet uncertain whether they are the same particles as the exposure. Identifying contaminant at this time remains a major issue, but one that is slowly getting resolved as the ENMs used for exposure become better characterized to allow for easier exclusion of others. This is currently under investigation, as well as determining a method for semi-quantitative analysis of biodistribution using EDFM.

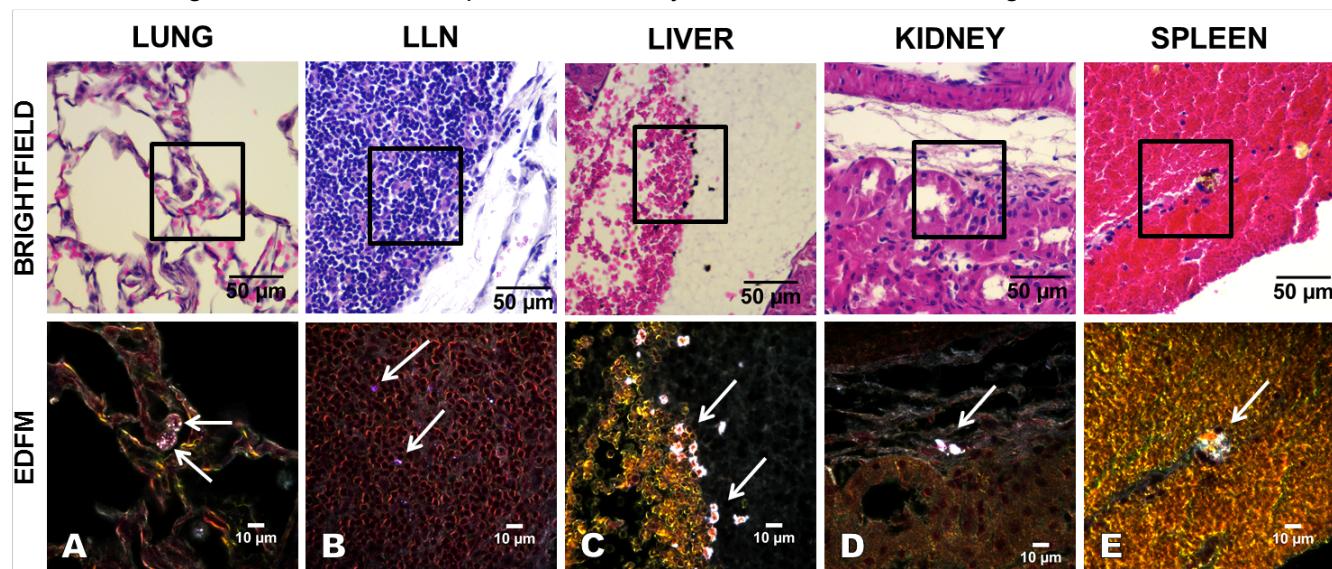


Figure 4. Biodistribution of NPs in organs of rats exposed via inhalation to ceria NPs. The top row shows brightfield (BF) images taken at 40x magnification, with their respective EDFM images at 100x magnification shown in the bottom row. From left to right, (A) Multiple NPs (arrows) are seen within two alveolar macrophages in the alveolar space of the lung. This rat was exposed to a medium concentration ($7.4\text{mg}/\text{m}^3$) of ceria NP-containing slurry aerosol for 4 hours, and sacrificed at 7 days post-exposure. (B) Multiple NPs (arrows) are seen among the white blood cells in the lymph node. This rat was exposed to a high concentration ($9.5\text{mg}/\text{m}^3$) of ceria slurry aerosol for 6 hours, and sacrificed at 24 hours post-exposure. (C) A central vein in the liver shows a dilated lumen congested with red blood cells and multiple NPs (arrows). This rat was exposed to a medium concentration ($7.4\text{mg}/\text{m}^3$) of ceria slurry aerosol for 4 hours, and sacrificed at 7 days post-exposure. (D) Two clusters of NPs (arrow) are seen over the connective tissue adjacent to several tubules in the renal cortex. This rat was exposed to a high concentration ($9.5\text{mg}/\text{m}^3$) of ceria slurry aerosol for 6 hours, and sacrificed at 24 hours post-exposure. (E) Multiple agglomerated NPs (arrow) are shown in the red pulp of the spleen surrounded by numerous red blood cells. This rat was exposed to a low concentration ($3.5\text{mg}/\text{m}^3$) of ceria slurry aerosol for 4 hours, and sacrificed at 24 hours post-exposure. (Guttenberg M, Bezerra L, Neu-Baker NM, Sosa Peña MP, Elder A, Oberdörster G, Brenner SA. Biodistribution of inhaled metal oxide nanoparticles mimicking occupational exposure: a preliminary investigation using enhanced darkfield microscopy. *J. Biophoton.* 2016, 9(10):987-993. doi:10.1002/jbio.201600125.)



Relevant publications:

- Dillon JCK, Bezerra L, Sosa Peña MP, Neu-Baker NM, Brenner SA. Hyperspectral data influenced by sample matrix: the importance of building relevant reference spectral libraries to map materials of interest. *Microscopy Research and Technique.* 2017. doi:10.1002/jemt.22816

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Identification and Determination of Fate of SiO₂ and Metal Oxide Nanoparticles During Conventional Wastewater Treatment

Characterization of ENMs used in or generated by industrial processes, their fate, and their health effects will help to inform employee and environmental health and safety policy. We were able to fully develop a scanning particle mobility particle sizing (SMPS) protocol, which enabled accurate characterization of CMP slurries while minimizing the non-trivial background effects, and should be amenable to other aqueous samples, such as wastewater. We also examined these materials by single-particle inductively-coupled plasma mass spectrometry (SP-ICP-MS) and inductively-coupled plasma optical emission spectroscopy (ICP-OES). Results from ICP-OES and SP-ICP-MS were able to determine the mass and had a high degree of similarity, and indicate that safety data sheets (SDS) provided by slurry manufacturers may be highly inaccurate, which is a safety concern. While SMPS provided some excellent information on particle counts and size, agreeing reasonably with other measurement techniques, SP-ICP-MS yielded unusually low counts and anomalous sizes, and thus it may not be amenable to examining certain materials, such as silica.

We studied the fate of ENMs used in the CMP process throughout the semiconductor fabrication wastewater treatment (WWT) system. The goal of this study was to assess whether the WWT processes resulted in size-dependent filtration of particles in the nanoscale range by analyzing wastewater samples using scanning electron microscopy (SEM). Statistical analysis demonstrated no significant differences in particle size between sampling points, indicating low or no selectivity of WWT methods for NPs based on size (Fig. 5). This work suggests that NPs could be released to the municipal waste stream from industrial sources.

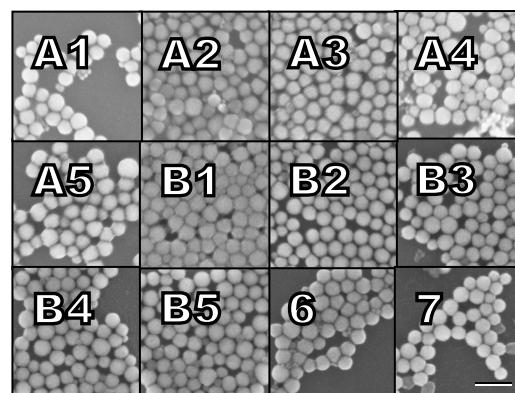


Figure 5. Representative SEM images of nanoparticles in wastewater samples. Representative SEM images from a single sampling event. Since acid and base WWT systems are independent and parallel, letters "A" and "B" denote samples drawn from each of these systems, respectively. Note the relative uniformity of nanoparticles across each sample point in size and morphology (scale bar equals 100nm; all images are the same scale). (Roth GA, Neu-Baker NM, Brenner SA. SEM analysis of particle size during conventional treatment of CMP process wastewater. *Sci. Tot. Environ.* 2015, 508:1-6.)

In addition, we began examining the impact of these materials on primary cell lines. It was clear that silica-based slurries had, by two different metrics, a much more pronounced negative effect on viability, as they did on cancer lines. Curiously, alumina- and ceria-based slurries had positive effects on viability at low concentration.

The Brenner Research Team is also currently investigating the utility of EDFM-HSI (CytoViva) for the identification and characterization of slurry NPs in wastewater.

Relevant publications:

- 1) Roth GA, Neu-Baker NM, Brenner SA. Comparative characterization methods for nanoparticle abrasives. *Journal of Chemical Health and Safety* 2015, 22(6):26-32. doi:10.1016/j.jchas.2015.02.001.
- 2) Roth GA, Neu-Baker NM, Brenner SA. SEM analysis of particle size during conventional treatment of CMP process wastewater. *Science of the Total Environment* 2015, 508:1-6. doi:10.1016/j.scitotenv.2014.11.075.