As stated in Part 1 of this article (Bhattacharyya et al. 2017), nanotechnology has been applied in the last 10 to 15 years across a wide and increasing range of sectors including healthcare, electronics, food production, and cosmetics. Nanotechnology could also improve water and wastewater treatment and use, including enabling use of traditionally unconventional water sources. Just as with any new technology, there are risks associated with nanotechnology use and as such, strategic and wise application is necessary.

This two-part article is an overview of nanotechnology applications and implications within the water industry. Part 1 provided a basic background on nanotechnology and literature survey on nanoparticle use, fate, and effects in various types of water systems. Part 2 describes the toxicological and ecological impacts of nanoparticles (NPs), reviews analytical measurement techniques, and assesses where novel platforms would be most beneficial.

**TOXICOLOGY/ECOLOGY IMPACTS**

With rapidly increasing use of engineered nanomaterials, environmental release of these contaminants is likely already occurring, perhaps on a significant scale. Approximately 6–8% of annual funding from the National Nanotechnology Initiative is for assessing environmental health and safety of nanomaterials (NNI n.d.). A significant amount of those resources are by necessity concerned with occupational human exposure and many ecological
questions are unaddressed (Servin & White 2016). For both terrestrial and aquatic systems, there are numerous potential release and exposure pathways (Gottschalk & Nowack 2011), including intentional release of nano-enabled agrichemicals and agents/substances for soil remediation (Servin et al. 2015). Given the widespread use of NPs in consumer products, presence of engineered nanoparticles (ENPs) in the waste stream is anticipated (Peijnenburg et al. 2015), and land application of resulting biosolids represents an indirect but potentially significant pathway for ENP introduction into agriculture. Ecotoxicological impacts of NPs and the potential for bioaccumulation in plants and microorganisms is a subject of current research, as NPs are considered to be generated as a result of environmental effects and thus have novel impacts (Figure 1). There is consensus in the scientific community that understanding nanomaterial fate and effects in the environment is insufficient. Given the size-specific toxicity/risk concerns and the lack of a robust nanospecific regulatory framework for ENP-containing consumer products, the discipline of nanotoxicology has developed to address these critical knowledge gaps.

There has been incremental progress in this area recently, including some comprehensive literature reviews that also highlight major topics of concern (Schwab et al. 2016, Gardea-Torresdey et al. 2014, Garner & Keller 2014, Batley et al. 2013, Gottschalk et al. 2013). Most research in nanotoxicology has focused on detrimental effects of the NPs themselves, with the vast majority of work focusing on short-term, high-dose assays in model systems. Consideration of chronic, low, or environmentally realistic dose scenarios in more complex systems is widely recognized as a more likely exposure pathway for sensitive receptors, but to date, these scenarios have remained largely unexplored (Servin & White 2016). Only after these types of investigations have been completed can true exposure to human and nonhuman receptors be determined for an accurate risk assessment (Holden et al. 2016).

Some early reviews were published in an attempt to evaluate nanomaterial effects in terrestrial systems (Handy et al. 2008, Klaine et al. 2008). Much of the focus was on identifying strengths and weaknesses of published studies and, more importantly, on highlighting the many areas in need of study. Inclusion of appropriate controls, such as bulk and ionic treatments, is needed for robust experimental design and meaningful analysis of the results (Bar-Ilan et al. 2012). Studies have been published that address most aspects of the biotic community; however, because many of the exposure concentrations are quite (likely unrealistically) high, the literature is dominated by reports of toxicity. The measured parameters as a function of exposure range from basic physiological assays, such as growth or oxidative stress response, to highly sensitive molecular and “omic” endpoints. For example, Jiang et al. (2009) examined the toxicity of bulk and NP aluminum, silicon, titanium dioxide (TiO2), and zinc oxide (ZnO) to three bacterial species; with the exception of TiO2, NPs were significantly more toxic than their bulk counterparts. Gong et al. (2011) reported that NP nickel oxide was severely toxic to Chlorella vulgaris, causing thylakoid dysfunction and membrane breakage. With regard to animal species, Zhang et al. (2011) noted that at relatively low concentrations (1–100 nM), exposure to NP cerium oxide (CeO2) induced oxidative damage in C. elegans and reduced overall life span by 12%. Hu et al. (2010) observed that exposure to NP TiO2 and ZnO caused significant toxicity to the redworm.
Several difficulties are apparent when reviewing literature on nanomaterial effects in the environment. Particle type-specific and species-specific response is common. In addition, growth/exposure conditions and overall system complexity dramatically impact observed effects. Many published studies involve short-term, high-dose exposures in media that may have little environmental relevance (Servin & White 2016). Although this experimental approach was a valid and necessary first step in addressing NP toxicity, the data generated are not sufficient for determining true risk to sensitive receptors in the environment. Lastly, the lack of robust detection platforms for NPs in complex environmental media (soils, sediments, biotic tissues) remains a major hurdle. Although techniques to determine effects are mature and range from the biochemical to the molecular (the various “omics”), thoroughly characterizing the nature of the toxicant at the time of exposure remains problematic.

LABORATORY METHODS FOR NP DETECTION

A variety of analytical techniques are used for detecting nanomaterials, depending upon type of physiochemical property or other information sought. Several technologies have been developed to characterize either bulk nanomaterials or individual NPs (including the subset of materials termed “quantum dots”). While the list of potential physical and chemical properties is expansive, a short list of most commonly sought properties includes (1) size distribution, (2) shape, (3) composition, (4) physiochemical structure, and (5) agglomeration state. For a more complete list, including analytical techniques for measuring properties, see Table 1.

Recently nanomaterial characterization has seen considerable growth by application of induced coupled plasma–mass spectrometry (ICP–MS) to analyze single particles (sp–ICP–MS) (Lee & Chan 2015). The benefit of this approach is a simplified sample preparation or even a direct, dilute-and-shoot approach. After in-line dilution, sp–ICP–MS can be used to analyze each separate NP as it is aerosolized and ionized in the plasma, resulting in a signal intensity that is directly proportional to particle size. The mass-to-charge ratio of resulting ions can subsequently be used to provide elemental analysis. Taken together, the analysis from an sp–ICP–MS can therefore provide elemental composition of most nanomaterials (although only a single isotope can be determined at a time), size distribution, and concentration of the nanomaterial in the test solution. The typical lower size limit of detection for sp–ICP–MS is 20 nm. This technique may be applied to complex or biological matrices, such as processing of organic material via enzymatic digestion (Linsinger et al. 2013, Loeschner et al. 2013) or acidic digestion (Loeschner et al. 2011).

This application of ICP–MS to the characterization of nanomaterial fate began in the early 2000s, with initial focus on leaching silver (Ag) NPs from implanted medical devices (Furno et al. 2004). Concern has also been raised about the increased

With rapidly increasing use of engineered nanomaterials, environmental release of these contaminants is likely already occurring, perhaps on a significant scale.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Abbreviation</th>
<th>Concentration</th>
<th>Size</th>
<th>Size Distribution</th>
<th>Surface Charge</th>
<th>Surface Area</th>
<th>Shape</th>
<th>Agglomeration</th>
<th>Crystallinity</th>
<th>Chemical Properties</th>
<th>Mechanical Properties</th>
<th>Thermal Properties</th>
<th>Electrical Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acoustic spectroscopy</td>
<td>—</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Atomic force microscopy</td>
<td>AFM</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Aerosol particle mass analyzer</td>
<td>AMS</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Brunauer-Emmett-Teller</td>
<td>BET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Condensation particle counter</td>
<td>CPC</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Differential electric mobility analyzing system</td>
<td>DMAS</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Differential scanning calorimetry</td>
<td>DSC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Dynamic light scattering</td>
<td>DLS</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Electron back-scatter diffraction</td>
<td>EBSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Electron energy loss spectroscopy</td>
<td>EELS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Energy dispersive X-ray spectrometry</td>
<td>EDX/EDS</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fluorescence spectroscopy</td>
<td>FL</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fourier transform infrared spectroscopy/imaging</td>
<td>FTIR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Induced grating method</td>
<td>IG</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Inductively coupled plasma–mass spectrometry and single particle ICP–MS</td>
<td>ICP–MS</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Laser diffraction</td>
<td>—</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Liquid chromatography–mass spectrometry</td>
<td>LC–MS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Particle tracking analysis</td>
<td>PTA</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Optical absorption spectroscopy</td>
<td>UV-Vis-NIR</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Quartz microbalances</td>
<td>QCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Raman spectroscopy/imaging</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Scanning electron microscopy</td>
<td>SEM</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Secondary ion mass spectrometry</td>
<td>SIMS</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Selected area electron diffraction</td>
<td>SAED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Small angle X-ray scattering</td>
<td>SAXS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Static light scattering photometer</td>
<td>SLS</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Thermogravimetric analysis</td>
<td>TGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Transmission electron microscopy</td>
<td>TEM</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>X-ray diffraction</td>
<td>XRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>X-ray photoelectron spectroscopy</td>
<td>XPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Adapted from Salmon et al. 2010

Checkmarks indicate parameters that can be measured by each technique.
prevalence of Ag NP suspensions used in hospitals for their bactericidal properties (Chen & Schluesener 2008), as well as often incorporated into other surgical materials for slow release (Nangmenyi et al. 2009). Long-term exposure to these Ag NPs has been shown to result in bioaccumulation in the environment (Judy et al. 2011), and accumulation in rats has been linked to neurological lesions (Tang et al. 2009). Once exposed to such nanomaterials, rats can retain the particles for up to six months, during which time they are slowly excreted (e.g., via urine) (Sadauskas et al. 2009). The US Environmental Protection Agency has led research on the environmental transformation and fate of several types of nanomaterials, and has concluded that common disinfectants are a significant source of potentially toxic nanomaterials in the environment. The rise in use of nanomaterials and their potential toxic effects stresses the urgency for a nanomaterial biomonitoring effort.

Another useful technique for the characterization of nanomaterials at low concentrations and for simultaneous, multiple NP composition analysis is the field-flow fractionation, or FFF, to ICP–MS (Meermann & Laborda 2015). The size of the nanomaterial is characterized according to its size-specific mobility within a channel, resulting in a distribution of particles, called a fractogram. Figure 2 shows a screenshot from an ICP–MS run for nanoparticles. The benefit of this added separatory technique is that many particles may be measured at the same time, including different isotopes, although the throughput is more restrictive than sp–ICP–MS. The typical lower size limit of detection of FFF is 1 nm.

Scanning electron microscopy (SEM) is another well-established method for assessing individual NP size. A finely focused electron beam is used to scan the surface of a material in a vacuum chamber, characterizing the surface (or material on the surface) according to shape and structure, with a resolution of tens of nanometers. The addition of an energy dispersive spectrometer can further permit characterization of larger NPs, useful for measuring the spectroscopy of backscatter X-rays from the initial electron beam scan (thus providing compositional analysis).

With the advent of transmission electron microscopy (TEM), the minimum size resolved can be <10 nm, and is often coupled to X-ray spectroscopy techniques to further characterize NP composition (X-ray energy dispersive spectrometer) (Pyrz & Buttrey 2008). Additionally, the TEM penetrates the sample, providing additional material characteristics. Information for an individual or bulk nanomaterial using a TEM can
include structure, crystallography, and chemical composition of the material. Recent work has compared TEM with other methods for NP characterization, including dynamic light scattering, sedimentation flow-field fractionation, asymmetrical flow-field fractionation, and centrifugal liquid sedimentation (Cascio et al. 2014).

A limitation of both SEM and TEM has been the requirement of placing samples in a vacuum for analysis. This limitation has recently been addressed by the emerging technique called environmental transmission electron microscopy, whereby a thin, electron-transparent window separates the beam and the sample (Takeda & Yoshida 2013).

There have been recent advances in the use of surface enhanced Raman spectroscopy (SERS) to detect Ag NPs in complex matrices, including consumer products and food (Guo et al. 2016, 2015; Zhang et al. 2016). Here the approach is to use indicator molecules (ferbam, 4-mercapto-benzoic acid) that bind specifically to the Ag NP (not ion or bulk), resulting in a complex that generates a unique SERS signal that can be readily detected. Current efforts are focused on expanding the list of various engineered NPs detectable by this procedure as well as lowering detection limits in a range of additional complex matrices.

Common environmental and industrial nanomaterials that may accumulate in humans include TiO₂, associated with neurological lesions (Wang et al. 2008a, 2008b), CuO, associated with kidney and liver lesions (Liu et al. 2009), TiO₂/ZnO/ CeO₂, associated with oxidative stress (Xia et al. 2008), and cadmium selenide/zinc sulfide, associated with acute toxicity (Pace et al. 2010). In the latter case, not only is the nanomaterial itself toxic, but any solubilized cadmium that leaches from the nanomaterial is also toxic.

Morphology of the nanomaterial is also a significant characteristic—smaller NPs are generally more toxic than larger ones (Liu et al. 2010), and the more aspherical NPs are more toxic than spherical (Stoehr et al. 2011, Sun et al. 2011). Thus, in developing a clinical assessment for nanomaterials in specimens, one needs to focus on composition and morphology as well as the presence of dissolved toxic elements.

ICP–MS technology has been specifically used to detect the presence of suspect toxic elements (not specifically NPs) in biological specimens, including TiO₂ (Schmidt & Vogelsberger 2006), CeO₂ (Patil et al. 2007), beryllium (Huang et al. 2011), gold (Scheffer et al. 2008, Kerr & Sharp 2007), platinum (Gehrke et al. 2011), and silicon dioxide (Gonzalez et al. 2010). Use of ICP–MS with SEM or TEM orthogonally has been beneficial in demonstrating that composition, concentration, and morphology all play roles in assessing overall NP toxicity (Beltrami et al. 2011, Chen et al. 2010, Sonavane et al. 2008).

CONCLUSION
Assessment of future use of nanotechnology-based water treatment must take account of both the risks and opportunities. The regulatory, ethical, legal, and social implications of nanotechnology require additional research in developed as well as developing countries. There are reports on the potential health and environmental risks of using nanotechnology for water treatment (Kumar et al. 2014), but the full effects of exposure to nanomaterials at water treatment plants or in drinking water are yet to be determined. While nanotechnologies have tremendous potential to address global water problems, water professionals and scientists need to work constructively to discuss and engage in dialogues with industries and local communities to understand the problems and opportunities for applying nanotechnology to water improvements. The lack of regulatory aspects for the use of nanotechnology demands partnering among researchers, communities, and industry in public and private sectors to encourage and support novel research and innovative applications, develop positions for related policies, and develop appropriate business models to sustainably exploit the potential in field of nanotechnology.

ACKNOWLEDGMENT
This article was developed under Assistance Agreement No. 83483301 awarded by the US Environmental Protection Agency (USEPA); it has not been formally reviewed by the agency. The views expressed in this article are solely those of the Association of Public Health Laboratories (APHL) and/or the authors and USEPA does not endorse any products or commercial services mentioned in this publication. This project was entirely funded with funds from a federal program of $1,400,000. We thank Julienne Nassif at APHL for her valuable comments on the manuscript.

ABOUT THE AUTHORS
Sanjib Bhattacharyya (to whom correspondence may be addressed) is laboratory director of the City of Milwaukee Health Department, 841 N. Broadway, Rm. 205, Milwaukee, WI 53202 USA; sbhatt@milwaukee.gov. He is an adjunct faculty member at the University of Wisconsin–Milwaukee Zilber School of Public Health and clinical associate professor, College of Health Sciences. He has a PhD in medical microbiology and postdoctorate from the Medical College of Wisconsin, Milwaukee. He also has BS, MS, and PhD degrees from the University of Kolkata, Kolkata, West Bengal, India. Bhattacharyya has more than 20 years of relevant experience in the fields of chemistry, microbiology, molecular biology, and laboratory management in the areas of clinical and environmental health science. Jack Bennett is section chief of environmental chemistry at the Connecticut Department of Public Health.
Beltrami, D.; Calestani, D.; Maffini, M.; Suman, Bar-Ilan, O.; Louis, K.M.; Yang, S.P.; Pedersen, Angel, B.M.; Batley, G.E.; Jarolimek, C.V.; & Conn. Health Laboratory, Rocky Hill, Conn. Luke C. Short is unit manager at the DC Public Health Laboratory, Department of Forensic Sciences, Washington, D.C. Tamara S. Theisen is laboratory director at the Saginaw County Department of Public Health, Saginaw, Mich. Michael D. Wichman is an adjunct assistant professor at the University of Iowa, Coralville, Iowa. Jason C. White is vice-director at the CT Agricultural Experiment Station, New Haven, Conn. Sarah Wright is environmental laboratories senior specialist at the Association of Public Health Laboratories, Silver Spring, Md.

https://doi.org/10.5942/jawwa.2017.109.0154

REFERENCES


AWWA RESOURCES


These resources have been supplied by *Journal AWWA* staff. For information on these and other AWWA resources, visit www.awwa.org.