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JES JOURNAL OF ENVIRONMENTAL SCIENCES

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A new technique helps to uncover unknown peptides and disinfection by-products in water

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ARTICLE INFO

Available online 30 March 2016

Keywords:
Disinfection by-products (DBPs)
Drinking water
Halogenated peptides
Liquid chromatography
Natural organic matter (NOM)
Non-targeted analysis
Tandem mass spectrometry
Water contaminants

Environmental water samples can be extremely complex, with potentially thousands of molecules that can derive from natural organic matter (NOM) and thousands that derive from anthropogenic contaminants. As complex as these samples are, drinking water can be even more complex. Due to disinfectants that are used to treat drinking water (*e.g.*, chlorine, chloramines, ozone, or chlorine dioxide), NOM and contaminant molecules can transform into many new disinfection by-products (DBPs). For example, the contaminant triclosan, which is used in many antibacterial hand soaps, can transform in the presence of chlorine to form six DBPs, including chloroform, three chlorophenoxyphenols, and two chlorophenols (Rule et al., 2005). And, NOM can transform into thousands of DBPs. Thus, the complexity of the water greatly magnifies.

In addition to being highly complex, there are also concerns over the toxicity of DBPs that are formed in drinking water. Disinfected drinking water has been associated with such adverse effects as bladder cancer, miscarriage, and birth defects (Waller et al., 1998; Nieuwenhuijsen et al., 2000; Bove et al., 2002; Villanueva et al., 2004; Savitz et al., 2005). And, many DBPs have been found to be cytotoxic, mutagenic, genotoxic, teratogenic, or carcinogenic (Richardson et al., 2007; Plewa et al., 2008). While a small number of DBPs are regulated in many countries (*e.g.*, 11 are regulated in the U.S.), toxicology data point to the possibility that DBPs other than those regulated may be responsible for the human health effects observed. As a result, it is important to thoroughly characterize and identify DBPs formed in drinking water.

Most efforts to this end have used gas chromatography (GC)-mass spectrometry (MS) with electron ionization (EI), largely because it is easier to identify unknown molecules this way. The availability of large mass spectral library databases, along with easy-to-spot chromatographic peaks and the lack of matrix effects, has made GC–MS a tool of choice for uncovering new DBPs over the last several years (Richardson, 2002, 2012). To-date, nearly 700 DBPs have been identified (Richardson, 1998, 2011).

However, GC–MS is limited to volatile and semi-volatile compounds with low molecular weights (<~800 Da), and many compounds are likely missed. In fact, the measurement of total organic halogen in chlorinated drinking water indicates that >50% of the halogenated DBPs are still unaccounted for (Krasner et al., 2006). And, this surrogate measurement only captures halogenated compounds.

A new paper published by Tang et al. (2016) introduces an entirely new strategy to comprehensively identify compounds in source waters and finished drinking water (Fig. 1). This strategy combines multiple solid phase extraction (SPE), liquid chromatography (LC) with 2 complementary columns, high resolution-MS/MS, and a new technique called precursor ion elimination (PIE). First, through the use of multiple SPE, a much broader range of compounds can be extracted.

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http://dx.doi.org/10.1016/j.jes.2016.03.008

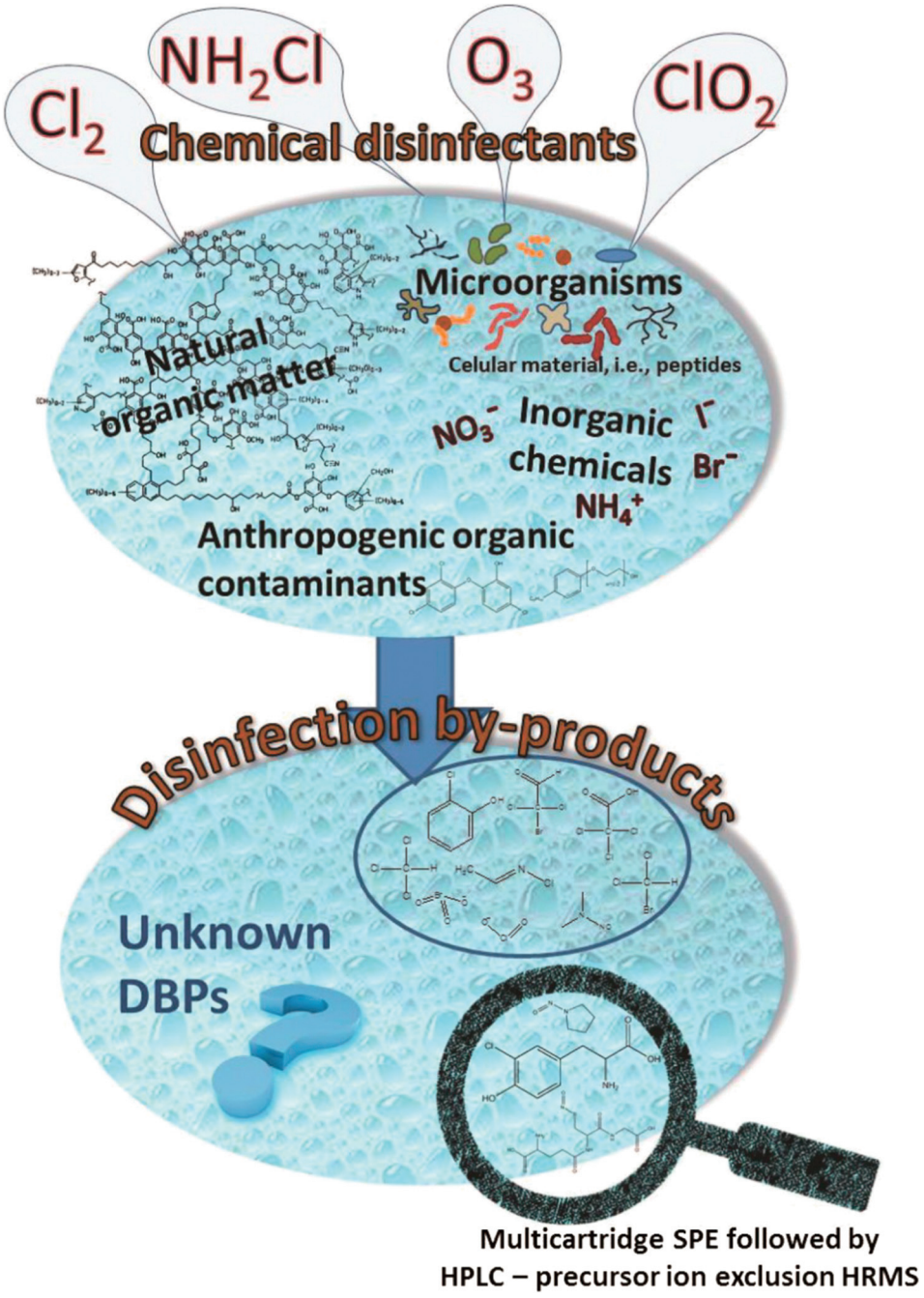

Fig. 1 – New approach to comprehensively identify DBPs and other compounds in water.

Researchers typically use a single sorbent phase to extract organic molecules from water, which is not able to effectively extract all types of compounds. However, by using 3 different SPE phases (Oasis HLB, Bond Elut C18, or Bond Elut ENV), >5000 putative organic compounds were extracted, with ~40% unique compounds identified with each SPE.

Next, rather than using GC–MS, the authors used LC–MS, which allowed the capture of a much broader range of compounds. And, while researchers will generally choose a single LC column (typically C18), the authors used two different complementary ones (C18 and a hydrophilic interaction liquid chromatography (HILIC) column), which dramatically increased the number of compounds that could be detected and resolved, allowing 50% unique identifications with each. The novel PIE strategy then further extended the number of quality MS/MS spectra that could be obtained. This technique involved an initial MS/MS scan of the high abundance ions, followed by a second MS/MS scan, which excluded these high abundance ions and focused on the ions at lower abundance. This process allowed 30%–40% more compounds to be detected than with a traditional single LC–MS/MS approach.

In addition, because a high resolution mass spectrometer was used (a triple-TOF), these MS/MS spectra contained accurate mass information that allowed formulas for the

molecular ions and the fragment ions to be obtained. Finally, the LC–MS/MS spectra were searched against the Human Metabolome Database (HMDB) and structures were proposed. Because the HMDB contains many peptides and other biomolecules, it was particularly handy for identifying a number of peptides (>600) in these samples. Interestingly, not all of these peptides were only in the raw source waters, but >100 of the peptides and amino acids were unique to the finished drinking water. Twenty-five of them were confirmed with authentic standards, including a few chlorinated and nitrosated peptides that had not been reported previously. Finally, the authors were able to determine three different disinfection reaction pathways that converted the peptides into toxic DBPs. This was a radically different approach to identify compounds in water, and in the end, it allowed the identification of hundreds of compounds in a single study.

While this new strategy was specifically applied to drinking water, it also has tremendous utility for other types of environmental samples. For example, an intense area of research for a number of years has been in trying to understand and characterize different types of NOM in environmental waters (Barrett et al., 2000; Huang et al., 2016; Zheng et al., 2016). It is likely that this more comprehensive identification approach will shed new light on NOM structure and function. This new strategy could also be used to identify new emerging contaminants and their transformation products in water. While environmental pollutants likely will not have many successful matches in the HMDB, the expanded SPE-PIE-LC-high resolution-MS/MS approach should allow the identification of many more contaminants in a much shorter time.

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